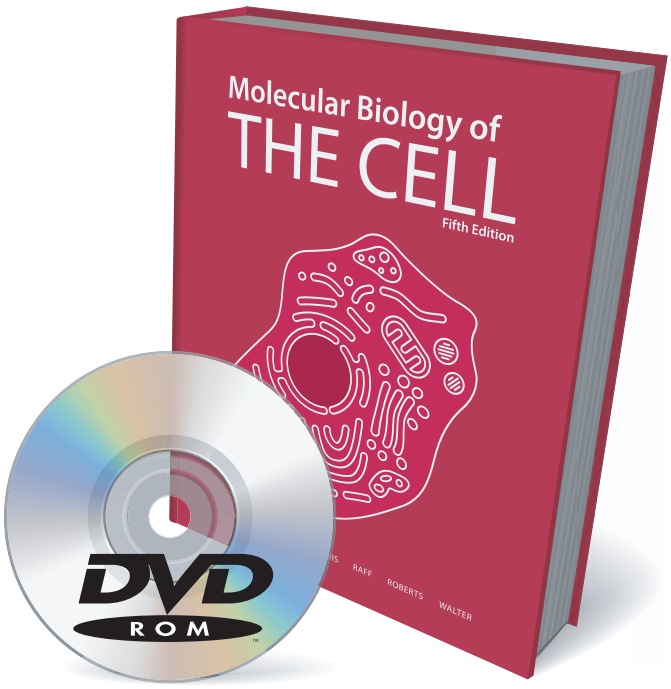
DVD MEDIA GUIDE



Molecular Biology of THE CELL

Fifth Edition

Bruce Alberts
Alexander Johnson
Julian Lewis
Martin Raff
Keith Roberts
Peter Walter

The DVD-ROM contains the following media for students and instructors:

- Chapters 21–25 of *Molecular Biology of the Cell,* Fifth Edition
- *The Art of Molecular Biology of the Cell,* Fifth Edition
- The *Cell Biology Interactive* media player

This document contains an overview of the contents of the DVD-ROM that accompanies *Molecular Biology of the Cell,* Fifth Edition. It also contains the "Viewing Guide" for the *Cell Biology Interactive* media player on the DVD. The "Viewing Guide" contains transcripts of the voice-over narration for the videos, animations, and molecular models, as well as credits for the scientists and artists.

Garland Science
Taylor & Francis Group

Chapters 21 to 25 of *Molecular Biology of the Cell*, Fifth Edition

The following chapters, covering multicellular systems, are available on the DVD in PDF format:

- Chapter 21: Sexual Reproduction: Meiosis, Germ Cells, and Fertilization
- Chapter 22: Development of Multicellular Organisms
- Chapter 23: Specialized Tissues, Stem Cells, and Tissue Renewal
- Chapter 24: Pathogens, Infection, and Innate Immunity
- Chapter 25: The Adaptive Immune System

The chapters are located in the "Chapters 21-25" folder. The files can be opened with the Adobe® Acrobat® Reader or other PDF software.

The Art of Molecular Biology of the Cell, Fifth Edition

The figures, tables, and micrographs from the book are available on the DVD and are located in the "Art of MBoC5" folder. They have been pre-loaded into PowerPoint® presentations, one for each chapter of the book. A separate folder contains individual versions of each figure, table, and micrograph in JPEG format. The folders are called "PowerPoint" and "JPEGs." The panels from the book are available in PDF format and located in the "Panels" folder.

The *Cell Biology Interactive* Media Player

The *Cell Biology Interactive* media player is located in the "Cell Biology Interactive" folder. There is both a Windows® and Macintosh OS X® version of this application, and folders for each are labeled accordingly. *Cell Biology Interactive* requires installation of the Apple QuickTime® Player, as well as Adobe Acrobat Reader or a similar PDF reader.

Cell Biology Interactive contains over 150 animations, videos, molecular models, and interactive electron micrographs. The media is organized by the table of contents of *Molecular Biology of the Cell, Fifth Edition*, or can be sorted by media type. It is also directly linked to specific sections of the book by "media codes." Use of the media codes is explained in both the "Note to the Reader" at the front of the book, as well as the introduction to the "*Cell Biology Interactive* Viewing Guide."

The movies and micrographs used in the *Cell Biology Interactive* media player can also be accessed as individual files in their native formats from the "media" folder located in the "Cell Biology Interactive" folder.

The "Viewing Guide" for the *Cell Biology Interactive* media player follows. It contains a table of contents, transcripts of the voice-narration for the individual movies, and credits for the scientists and artists.

Cell Biology Interactive Viewing Guide

Artistic and Scientific Direction by **Peter Walter**

Narrated by **Julie Theriot**

Production Design and Development by **Michael Morales**

INTRODUCTION

Welcome to the *Cell Biology Interactive* Media Player

As never before, new imaging and computer technologies have increased our access to the inner workings of living cells. We have tried to capture some of the excitement of these advances in *Cell Biology Interactive*. The media player contains over one hundred and fifty video clips, animations, molecular structures, and high-resolution micrographs—all designed to complement the material in the individual book chapters. Nearly all items are accompanied by a short narration that introduces and explains key concepts. Our intent is to provide students and instructors with an opportunity to observe living cells and molecules in action, since words and static images simply cannot do justice to the remarkable dynamics of the cellular and molecular world.

One cannot watch cells crawling, dividing, segregating their chromosomes, or rearranging their surface without a sense of wonder at the molecular mechanisms that underlie these processes. We hope that *Cell Biology Interactive* will motivate and intrigue students while reinforcing basic concepts covered in the text, and thereby will make the learning of cell biology both easier and more rewarding. We also hope that instructors can use these visual resources in the classroom to illuminate, not only the course material, but also the beauty and wonder of this microcosm. We designed animations to bring to life some of the more complicated figures in the book. Many of the videos provide visual demonstrations of topics that can be difficult to appreciate, such as membrane fluidity, and the high-resolution micrographs allow students to explore some magnificent cell images in detail. We have also created three-dimensional models of some of the most interesting molecules, presented in short tutorials.

The contents of *Cell Biology Interactive* represent the work of numerous laboratories around the world that provided video clips from original research, animation segments, micrographs, and molecular data. We are deeply indebted to the scientists who generously made this material available to us.

Using the Media Codes

Molecular Biology of the Cell, Fifth Edition contains "media codes" that directly link material in the book to movies on *Cell Biology Interactive*. The media codes are integrated throughout the book to indicate when relevant videos and animations are available on *Cell Biology Interactive*. The four-letter codes are enclosed in brackets and highlighted in color, like this. **<ATCG>** The interface for *Cell Biology Interactive* contains a media-code window where you enter the 4-letter code. When the code is typed into the interface, and the "go" button is pushed, the corresponding media item will load into the movie player.

The media codes for all the movies and electron micrographs are also listed in this "Viewing Guide." We hope this feature will facilitate use of *Cell Biology Interactive* and help integrate the dynamic world of the media player with the printed text.

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V = video, A = animation, EM = electron micrograph

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V = video, A = animation, EM = electron micrograph

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V = video, A = animation, EM = electron micrograph

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V = video, A = animation, EM = electron micrograph

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Appendix

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V = video, A = animation, EM = electron micrograph

1.1 Keratocyte Dance <GTTA>

Keratocytes, found on the scales of fish, are specialized for very rapid motility in order to heal scratches.

Video editing and concept: Justin Reichman
Music: Freudenhaus Audio Productions (www.fapsf.com)

Mark S. Cooper
University of Washington

1.2 Crawling Amoeba <ATGG>

This single-celled amoeba crawls around by using actin polymerization to push out pseudopods, or false feet, to explore new territory. At the same time, organelles move in complex patterns within the cell.

CELLS alive!
www.cellsalive.com

1.3 Swimming Eutreptiella <TCGC>

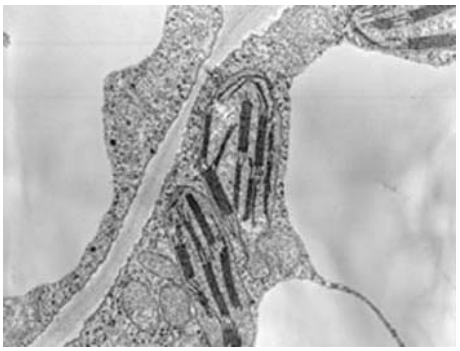
Some cells use rather peculiar ways to move, such as this eutreptiella flagellate, which uses both flagella and pronounced cell shape changes to swim.

Richard E. Triemer
Rutgers, State University of New Jersey

1.4 Plant Cells <AACA>

Find me:

- plasma membranes and cell wall
- vacuoles
- chloroplasts
- thylakoids
- mitochondria



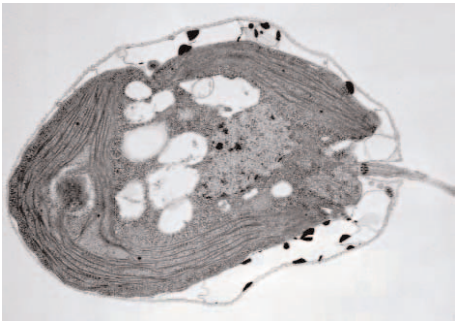
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

1.5 *Chlamydomonas* <TTTA>

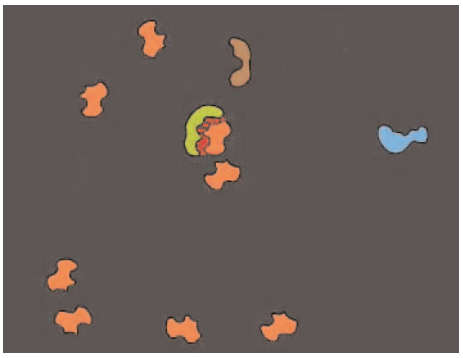
Find me:

- nucleus
- chloroplast
- vacuoles
- flagella
- cell membrane



Doug Bray
The University of Lethbridge, Canada

2.1 Noncovalent Interactions <TGCG>



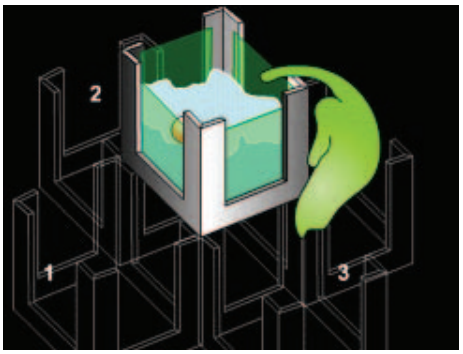
Molecules in solution undergo random thermal movements and may encounter each other frequently if the concentration is sufficiently high. If two molecules with poorly matched surfaces collide, only a few weak bonds will form between them. Thermal motion of the molecules rapidly breaks these bonds apart, and the molecules separate.

If the surfaces of two molecules are well matched, many weak bonds will form between the two. The bonds hold the molecules together for a long time before thermal jolting tears them apart.

Tightly bound molecules will spend most of their time associated although they will go through cycles of association and dissociation. The affinity of the two molecules for one another is a measure of the relative time they spend bound together.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

2.2 Analogy of Enzyme Catalysis <TAAA>



Envision a molecule, here symbolized by the ball, that, in principle, can react in four different ways.

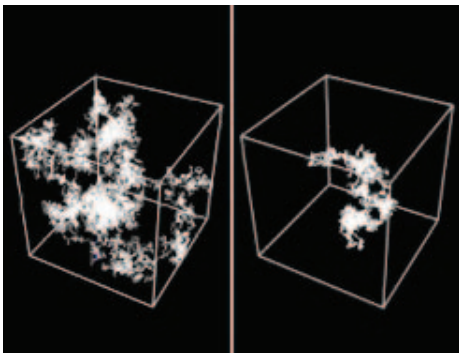
To undergo any of these reactions, the molecule must overcome an activation energy barrier that is of a characteristic height for each of the possible reactions.

This can be achieved, for example, by putting more energy into the system, such as when heat is added. In the example shown here, the molecule will enter many different reaction paths by overcoming similar activation energy barriers.

An enzyme, in contrast, reduces the activation energy barrier of only one specific reaction path. An enzyme therefore allows reactions to proceed at normal temperatures and directs them into one desired pathway.

Original illustrations and storyboard:
Nigel Orme and Christopher Thorpe

2.3 Brownian Motion <GGTA>



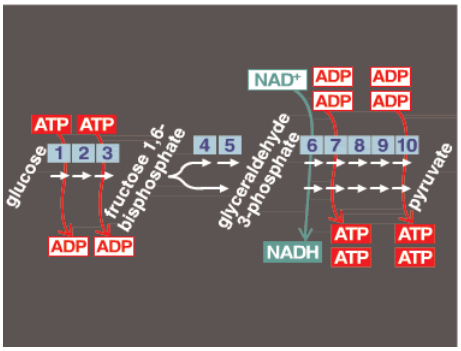
Powered by thermal energy, molecules are constantly in motion—called Brownian motion—which allows them to diffuse through cells in a random walk. Our simulation shows the degree to which a small sugar molecule on the left and a larger protein on the right explore the interior space of a cell, here shown as a cube with a 10 micrometer side. The animation represents one second in real time.

Note that the paths of the molecules in the second simulation are different from those in the first, thus showing the randomness of Brownian motion.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

Julian Lewis
Imperial Cancer Research Fund

2.4 Glycolysis <GGGC>



Cells break down food molecules, such as glucose, through multi-step pathways. In the process of glycolysis, the breakdown of one glucose molecule into two three-carbon molecules produces a net gain of energy that is captured by the molecules ATP and NADH. In eucaryotes, the breakdown product, pyruvate, is imported into mitochondria, where it ultimately feeds into the citric acid cycle and the electron transport chain.

Glycolysis involves a sequence of 10 steps. In the first three steps, energy in the form of ATP is invested to be recouped later. In the fourth and fifth steps, this energy allows glucose to be split into two smaller molecules from which energy can be harnessed efficiently. And in the last four steps, energy is released stepwise as ATP and NADH. The elegant chemistry that evolved to catalyze these reactions ensures that energy is released in small portions that can be efficiently captured. Less controlled combustion reactions would release most of the energy as heat.

In the first step, the enzyme hexokinase uses ATP to phosphorylate glucose. This investment of energy primes glucose for energy-releasing reactions later in glycolysis.

The resulting molecule is glucose 6-phosphate. ADP is released. This first step of glycolysis is irreversible.

In the second step of glycolysis, the enzyme phosphoglucose isomerase catalyzes the opening of the ring form of glucose 6-phosphate to the open chain form.

The same enzyme then performs a reversible reaction in which the carbonyl group of glucose 6-phosphate changes position from the first carbon to the second carbon in the chain.

This reaction involves a water molecule, which donates a hydrogen ion to the carbonyl oxygen.

A hydrogen ion is then retrieved from the hydroxyl group on the second carbon, creating a new water molecule. In the process, fructose 6-phosphate is formed.

The same enzyme, phosphoglucose isomerase, catalyzes the formation of fructose 6-phosphate into its ring form.

In the third step of glycolysis, the enzyme phosphofructokinase uses ATP to phosphorylate fructose 6-phosphate. ADP is released and the molecule fructose 1,6-bisphosphate is formed.

This third step, in which the second phosphorylation event occurs, is irreversible and is a major regulatory point in the commitment to glycolysis. The phosphorylations in steps 1 and 3 represent an investment of energy that will be paid back in the later stages of the pathway.

Step 4 of glycolysis begins with the opening of the ring form of fructose 1,6-bisphosphate into its open chain form.

In this step, the enzyme aldolase cleaves fructose 1,6-bisphosphate into two molecules.

One molecule that is formed is the 3-carbon glyceraldehyde 3-phoshate. The enzyme performs additional reactions on the second 3-carbon molecule. The second molecule is dihydroxyacetone phosphate.

In step 5 of glycolysis, the enzyme triose phosphate isomerase catalyzes the isomerization of dihyroxyacetone phosphate into glyceraldehyde 3-phosphate. The catalytic mechanism of this enzyme is very similar to that of phosphoglucose isomerase, back in step 2. The result is two molecules of glyceraldehyde 3-phosphate.

All of the subsequent steps of glycolysis will occur twice—once for each molecule of glyceraldehyde 3-phosphate. These are the energy generation steps of gylcolysis.

In step 6, the enzyme glyceraldehyde 3-phosphate dehydrogenase uses NAD^+ to oxidize glyceraldehyde 3-phosphate. The resulting molecule is connected to the enzyme by a high-energy thioester bond.

A molecule of inorganic phosphate displaces the high-energy thioester bond, forming a high-energy acyl-anhydride bond. The resulting molecule is 1,3-bisphosphoglycerate.

In the seventh step, the enzyme phosphoglycerate kinase dephosphorylates 1,3-bisphosphoglycerate. The high-energy phosphate is transferred to ADP, forming ATP. The 3-carbon molecule is now 3-phosphoglycerate. Because this reaction occurs twice, once for each 3-carbon molecule, a total of 2 ATPs are generated. At this point the energy investment from the first three steps has been paid back.

In the eighth step, 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is transformed by the enzyme phosphoglycerate mutase into 2-phosphoglycerate.

In the ninth step, the enzyme enolase removes a water molecule from 2-phosphoglycerate, creating phosphoenolpyruvate. The loss of water redistributes energy within the molecule, creating a phosphate group with an extremely high free-energy of hydrolysis.

In the tenth and last step of glycolysis, the enzyme pyruvate kinase transfers the high-energy phosphate group to ADP, forming ATP and pyruvate.

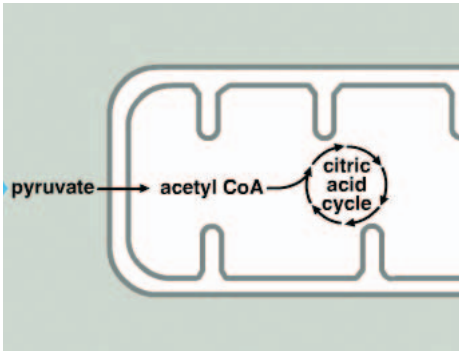
In the second half of glycolysis, many of the reactions release energy, captured in the form of ATP and NADH. Overall the net energy produced in glycolysis from a single molecule of glucose is two molecules of ATP and two molecules of NADH.

The chemistry of glycolysis is conserved all the way from bacteria to animal cells.

Chemistry Consultant: Patricia S. Caldera-Muñoz

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

2.5 Citric Acid Cycle <TAGT>



Cells break down food molecules, such as glucose, through multi-step pathways. For example, in the process of glycolysis, breakdown of glucose molecules releases energy that is captured by the energy carrier molecules, such as ATP and NADH. A breakdown intermediate, pyruvate, is imported into mitochondria, where it is converted into acetyl CoA and fed into the citric acid cycle. Acetyl CoA can also be generated by breakdown of fats or amino acids.

In this circular reaction path of the citric acid cycle, carbon atoms are "burned"—that is, oxidized—and released one-by-one as the waste product carbon dioxide. In this way, energy is released stepwise and captured by energy carriers, including NADH. NADH funnels energy to the electron transport chain in the inner mitochondrial membrane. This fuels the proton gradient that is then used for the production of ATP, the cell's primary energy currency.

The molecule that enters the citric acid cycle is the 2-carbon compound acetyl CoA. Acetyl CoA joins with the 4-carbon oxaloacetate to create the 6-carbon citrate.

We'll track the carbons from acetyl CoA with a red color. The two carbon atoms from oxaloacetate marked in blue will be released during this cycle to form carbon dioxide.

In the next step of the cycle, citrate rearranges to form isocitrate. Note that the hydroxyl group is in a different position in these two molecules.

In the next step, energy is captured by an NADH molecule, and a molecule of carbon dioxide is released. In this reaction, isocitrate is converted to aketoglutarate. The hydroxyl-bound carbon is stripped of its hydrogen atoms, resulting in a carbonyl group. One of these hydrogen atoms is picked up by NAD^+ to form NADH, and another is released as a proton. The carbon and 2 oxygen atoms are then released as CO_2, creating the 5-carbon α-ketoglutarate.

The next reaction also produces NADH and releases CO_2. The α-ketoglutarate from the previous reaction is converted to succinyl CoA by the addition of the coenzyme A. The enzyme for this reaction adds a high-energy thioester bond to coenzyme A, releasing the carbon and 2 oxygen atoms and converting NAD^+ to NADH.

The next reaction releases enough energy to form GTP, an energy-carrying molecule related to ATP. In this reaction, succinyl CoA is converted into succinate. The release of the coenzyme A group provides the energy to combine GDP and inorganic phosphate into GTP.

Note that succinate is symmetrical molecule. The two end carbons are chemically identical, and the two carbons in the middle are chemically identical. For convenience we will continue tracing only the 2 carbons depicted in the upper half of the molecule.

In the next step, a molecule of $FADH_2$ is produced. $FADH_2$, like NADH, is an energy carrier that feeds high-energy electrons to the electron transport chain. In this reaction, succinate is converted to fumarate. Hydrogen atoms from succinate are stripped off and donated to FAD to produce $FADH_2$.

In the next reaction, fumarate combines with a water molecule. The resulting molecule is malate, with the water molecule added across the two central carbons.

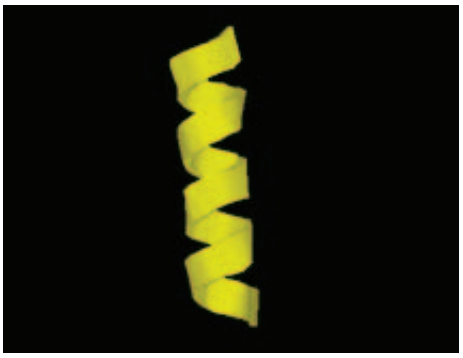
The next step produces the final NADH molecule. In this reaction, malate is converted to oxaloacetate. The carbon carrying the hydroxyl group is converted to a carbonyl group. This reaction releases hydrogen atoms and converts NAD^+ to NADH, releasing a proton, and producing the four-carbon oxaloacetate.

Oxaloacetate is thus replenished and can take part in another cycle, returning to step 1. Note the new position of the red carbon atoms, which originated from the acetyl CoA in the previous cycle. In subsequent cycles, these carbons will eventually be lost as CO_2. The green labels indicate the positions of the new carbons added during this new cycle.

Chemistry Consultant: Patricia S. Caldera-Muñoz

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

3.1 α Helix <GTAG>



PDB ID source: Glactone

The α helix is one of the most common secondary structures in proteins. Amino acid side chains project outwards from the polypeptide backbone that forms the core of the helix. The chain is stabilized in this conformation by hydrogen bonds between the backbone amino group of one amino acid and the backbone carbonyl group of another amino acid that is four positions away. These interactions do not involve side chains. Thus many different sequences can adopt an α helical structure.

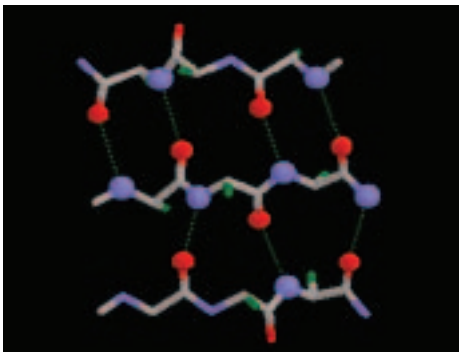
α helices are regular cylindrical structures. Amino acid side chains project outwards from the peptide backbone that forms the core of the helix. One full turn occurs every 3.6 residues and extends the length of the helix by approximately 0.5 nm.

Secondary structures are often represented in cartoon form to clarify the underlying structure of a protein. In this representation, the twisted 3D ribbon follows the path of the peptide backbone.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

Source: Glactone (www.chemistry.gsu.edu/glactone)

3.2 β Sheet <TGCT>



PDB ID number *: 1PGB

The β sheet is another common secondary structure. In contrast to an α helix, it is formed by hydrogen bonds between backbone atoms on adjacent regions of the peptide backbone, called β strands. These interactions do not involve side chains. Thus, many different sequences can form a β sheet.

A β sheet is a regular and rigid structure often represented as a series of flattened arrows. Each arrow points towards the protein's C-terminus. In the example shown here the two middle strands run parallel—that is, in the same direction—whereas the peripheral strands are antiparallel.

The amino acid side chains from each strand alternately extend above and below the sheet, thereby allowing each side to have distinct properties from the other. β sheets are usually twisted and not completely flat.

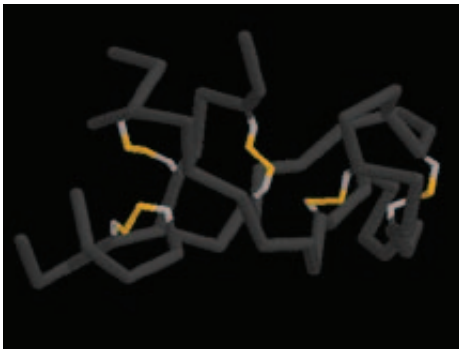
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

3.3 Disulfide Bonds <ATAC>

Disulfide bonds stabilize the structure of many proteins by forming intramolecular bridges. In this example, five disulfide bonds "zip up" the center of a protease inhibitor. Most extracellular proteins contain disulfide bonds.

Disulfide bonds are formed by oxidation of two cysteine residues. In this reaction, the hydrogen atoms are removed from their sulfur atoms to allow formation of the sulfur–sulfur bond.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: 1BI6

3.4 Proline Kinks <CAGT>

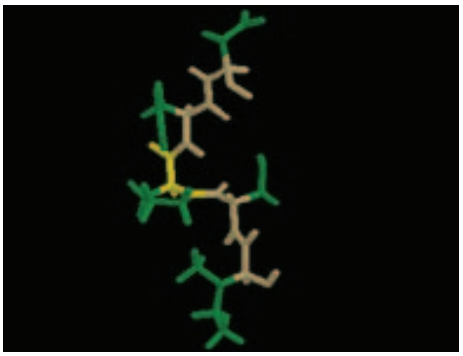
Because of the geometry of its side chain, proline cannot readily fit into the regular structure of an α helix. Prolines are therefore often found in loops at the ends of α helices, acting as "helix breakers."

Of the twenty naturally occurring amino acids, proline is the only one that has a cyclic side chain, forming a five-membered ring that includes the backbone nitrogen. This geometry severely limits the flexibility of the backbone.

Because of this restricted conformational flexibility, many proline residues introduce sharp kinks into the path of a polypeptide backbone.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

Source: Klotho: Biochemical Compounds Declarative Database (www.ibc.wustl.edu/klotho/)



PDB ID number *: Bovine α-chymotrypsin-eglin c complex (1ACB); L-proline (Klotho); Gal4 complex with DNA (1D66)

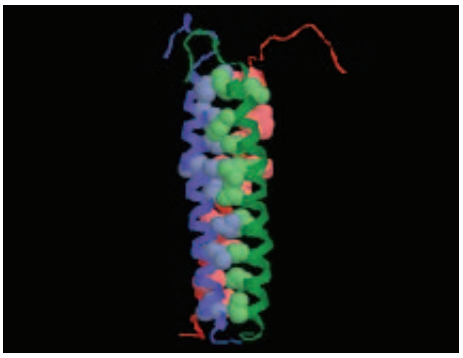
3.5 Coiled-Coil <CGGA>

In a typical coiled-coil two α helices wrap around each other to form a stable structure. One side of each helix contains mostly aliphatic amino acids, such as leucines and valines, while the other side contains mostly polar residues. Helices containing distinct hydrophobic and polar sides are called amphipathic. In a coiled-coil, two amphipathic helices are aligned so their hydrophobic sides snuggle tightly together in the center, with their polar faces exposed to the solvent.

A triple coiled-coil is another stable structure formed by α helices. In this case, three amphipathic helices twist around a central axis. The hydrophobic sides of all three helices face the center of the coil, creating a stable hydrophobic core.

Coiled-coils are often found in elongated, fibrous proteins. A triple coiled-coil is the major structural theme in fibrinogen, a protein involved in blood clotting. The fibrous nature of this protein is intimately related to its ability to form clots.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: GCN4 leucine zipper (2ZTA); Trimeric coiled-coil domain of chicken cartilage matrix protein (1AQ5); Native chicken fibrinogen (1EI3)

3.6 SH2 Domain <GTGA>

The SH2 domain of the tyrosine kinase and oncogene Src is used here to demonstrate the different ways in which a protein structure can be displayed. The *backbone view* shows the path of the polypeptide chain. The chain is colored blue at its C terminus.

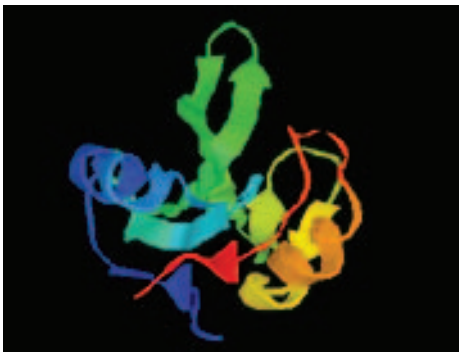
The *ribbons view* accents α helices and β sheets. These secondary structure elements determine the fold of most polypeptide chains. β strands are shown as arrows pointing from the N- to the C-terminus, and α helices are shown as twisted cylinders.

In a *wireframe* presentation, the covalent bonds between all of the atoms in the polypeptide are shown as sticks.

A *spacefill* view depicts each atom in the polypeptide as a solid sphere. The radius of the sphere represents the van der Waals radius of the atom. The coloring scheme follows the same rainbow spectrum used before with the N terminus red and the C terminus blue.

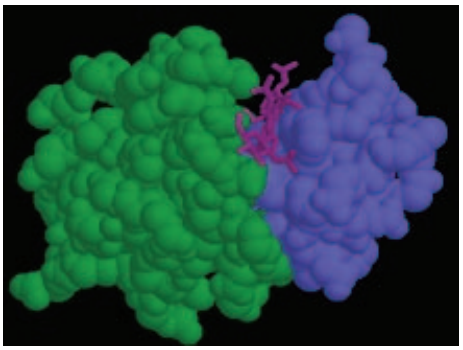
In this spacefill view different atoms in the polypeptide chain are colored according to element. By convention, carbon is colored gray, nitrogen blue, oxygen red, and sulfur yellow.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: 1SHA

3.7 Lysozyme I <AGCA>



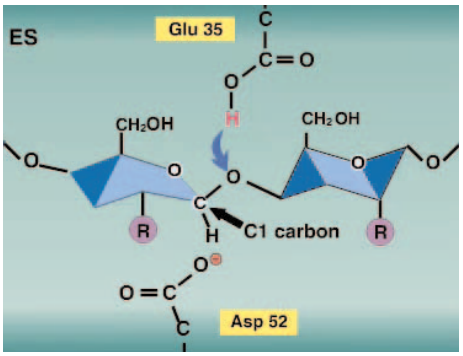
PDB ID number *: 1LSZ

Lysozyme is a small enzyme that binds to polysaccharide chains and breaks them apart by hydrolysis. It has two structural domains. One domain is composed mostly of α helices, while the other domain is composed mostly of β strands. The interface between the two domains forms a cleft in which the substrate binds. The structure shown here contains one of the products of the hydrolysis reaction.

Lysozyme acts as a catalyst by adding a molecule of water to the bond between two sugars, breaking the bond. This reaction is catalyzed by two strategically positioned amino acid side chains in the enzyme's active site: glutamate 35 and aspartate 52. The highlighted group on the reaction product shown here would have formed the bond cleaved in the reaction.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

3.8 Lysozyme II <TGGT>



The lysozyme enzyme cleaves polysaccharide chains. First, the enzyme and substrate associate, forming an enzyme-substrate complex. The enzyme catalyzes a hydrolysis reaction that cleaves the substrate into products, which are quickly released, allowing the enzyme to catalyze another reaction.

The cleft in the enzyme holds six sugar residues of a polysaccharide. The hydrolysis reaction occurs between residues.

Looking at the details of the reaction in solution, the sugar residues adopt their most stable three-dimensional conformation. However, after the polysaccharide enters into the enzyme–substrate complex, the enzyme forces the sugar shown on the left into a strained conformation that more closely resembles the transition state of the reaction and thereby helps to speed up hydrolysis.

Two amino acids within the enzyme facilitate the reaction. A glutamic acid donates a proton to sugar on the right and an aspartic acid attacks the C1 carbon atom of the sugar on the left. The attack on the C1 carbon results in a transient covalent bond between the sugar and the amino acid, and hydrolysis of the sugar-sugar bond.

The deprotonated glutamic acid then polarizes a water molecule, drawing a proton away from it. This allows the water oxygen to attack the C1 carbon, breaking the sugar-aspartate bond.

In this way, the two amino acids are returned to their original states, forming the enzyme-product complex. The enzyme and products dissociate.

Storyboard and Animation: Sumanas, Inc., (www.sumanasinc.com)

3.9 Oligomeric Proteins <GCCT>

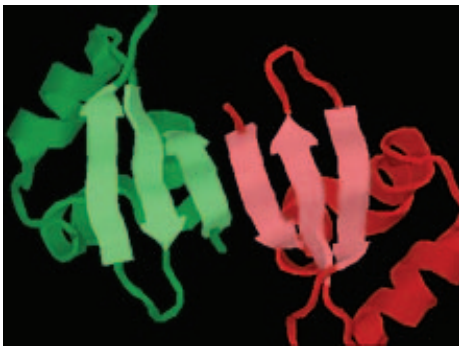
Many proteins are composed of multiple polypeptide chains, or subunits. The Cro repressor, for example, is a homodimer formed of two identical subunits. The subunits join in a head-to-head fashion as two small β sheets—one from each subunit—zipper up and form a larger β sheet.

The enzyme neuraminidase is composed of four identical subunits arranged in a square. Each pair of two subunits is held together in head-to-tail fashion, by repeated use of the same binding interaction. This becomes clear when the polypeptide chains are colored in a rainbow pattern, so that the same regions of each subunit have the same colors. All subunits adhere to each other through contacts between the orange and light-blue regions.

Hemoglobin is a tetrameric protein that transports oxygen. It is composed of two α subunits and two closely related β subunits. Oxygen binds to heme groups in the protein, which are shown in red. Each subunit can sense whether neighboring subunits contain bound oxygen. The protein subunits therefore communicate with one another through the interfaces that hold them together.

The tumor suppressor protein p53 is a tetramer of four identical subunits. Each p53 subunit contains a simple tetramerization domain composed of a single β strand connected to an α helix. The tetrameric form of p53 assembles as a dimer of dimers. Two copies of p53 interact via β strands, forming a two-stranded β sheet. Two such dimers interact via their α helices to form the tetrameric assembly.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: Cro repressor protein from bacteriophage lambda (5CRO); Neuraminidase of influenza virus (1NN2); Deoxy human hemoglobin (1A3N); p53 tetramerization domain (1C26)

3.10 Aspartate Transcarbamylase <CTAA>

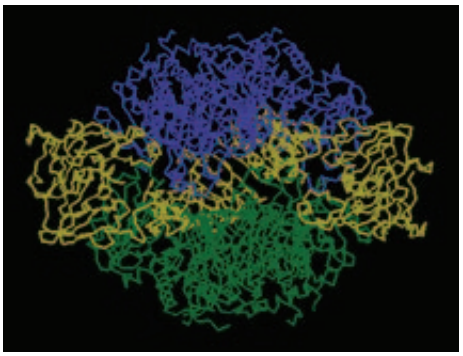
Aspartate transcarbamylase, or ATCase for short, is a well-studied example of allosteric regulation. ATCase catalyzes one of the early reactions in pyrimidine biosynthesis. This huge enzyme complex is composed of 12 subunits. Six are regulatory subunits that form a belt around the center of the complex. The remaining six subunits are arranged as two catalytic trimers, each positioned on one end of the enzyme.

ATCase alternates between two conformational states: an inactive tense or T state and a catalytically active relaxed or R state. ATCase is inactive when the inhibitor cytosine triphosphate is bound to its regulatory subunits. Binding of the two substrates, carbamylphosphate and aspartate, to the catalytic subunits switches the enzyme into the active R state.

The conformational change in ATCase from T state to R state involves a drastic change in the interactions between catalytic subunits. In the T state, glutamate 239 from a subunit of one catalytic trimer interacts with lysine 164 and tyrosine 165 from an adjacent subunit of the opposing catalytic trimer. With the transition to the R state, these subunit-subunit interactions are lost; the glutamate now interacts with the lysine and tyrosine from its own subunit. These atomic-level changes result in large movements of the subunits relative to one another.

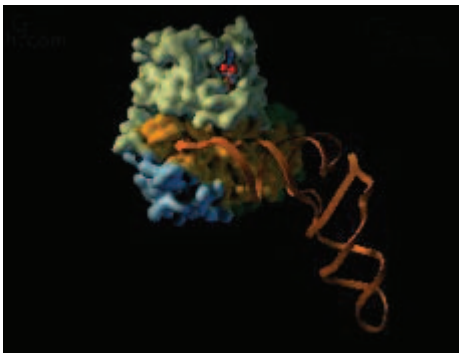
In each catalytic subunit of ATCase, the region of subunit–subunit interaction, our glutamate/lysine/tyrosine trio, is very close to the enzyme's active site. These conformational changes that affect the subunit–subunit interface in turn affect the active site residues. In the active R state, the active site side chains nestle up to the substrate to promote substrate binding and catalysis. In contrast, in the inactive T state, the active site side chains are dispersed.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number*: Aspartate transcarbamylase complex with CTP (5AT1); Compilation of aspartate transcarbamylase complex with CTP & aspartate transcarbamylase complex with PAM, MAL, and CTP (5AT1 & 8AT1)

3.11 EF-Tu <GTAA>



Elongation factor Tu has three domains, which are compactly arranged in its GTP-bound state. Here we show the surface of the protein with each of its domains in a different color.

Regions of all three protein domains contribute to the tRNA binding site.

An important dynamic element in the structure of elongation factor Tu is the switch helix.

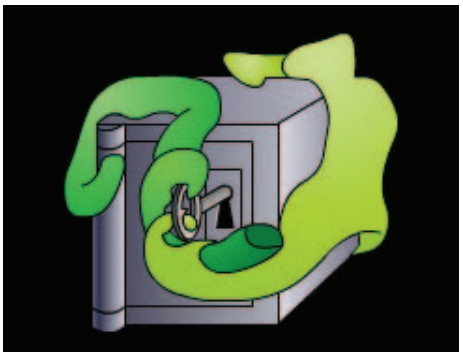
As GTP is hydrolyzed and the gamma phosphate is released, the switch helix rearranges.

This in turn leads to a major structural rearrangement of the three protein domains, which disrupts the tRNA binding site.

Thus upon GTP hydrolysis, the tRNA is released from elongation factor Tu.

Animation: Graham Johnson, Fivth Element (www.fivth.com)

3.12 The 'Safe Crackers' <ACTT>

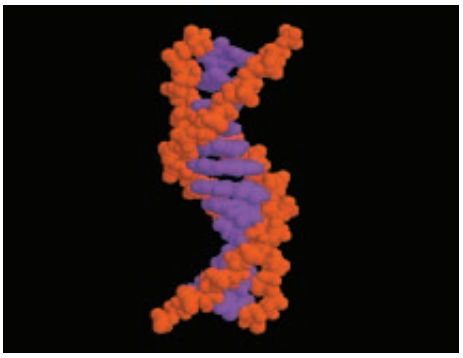


Individual proteins often collaborate as subunits of large protein assemblies, in which their individual activities may be coordinated.

Energetically favorable changes in substrates bound to one or more subunits, such as the hydrolysis of ATP, lead to orderly movements throughout the protein complex that accomplish a specific task.

Original illustrations and storyboard:
Nigel Orme and Christopher Thorpe

4.1 DNA Structure <CAGA>



PDB ID number *: 132D

Two DNA strands intertwine to form a double helix. Each strand has a backbone composed of phosphates and sugars to which the bases are attached. The bases form the core of the double helix, while the sugar–phosphate backbones are on the outside. The two grooves between the backbones are called the major and minor groove based on their sizes. Most protein–DNA contacts are made in the major grove, because the minor groove is too narrow.

The DNA backbone is assembled from repeating deoxyribose sugar units that are linked through phosphate groups. Each phosphate carries a negative charge, making the entire DNA backbone highly charged and polar.

A cyclic base is attached to each sugar. The bases are planar and extend out perpendicular to the path of the backbone. Pyrimidine bases are composed of one ring and purine bases of two rings. Adjacent bases are aligned so that their planar rings stack on top of one another. Base stacking contributes significantly to the stability of the double helix.

In a double helix, each base on one strand is paired to a base on the other strand that lies in the same plane. In these base pairing interactions, guanine always pairs with cytosine, and thymine with adenine.

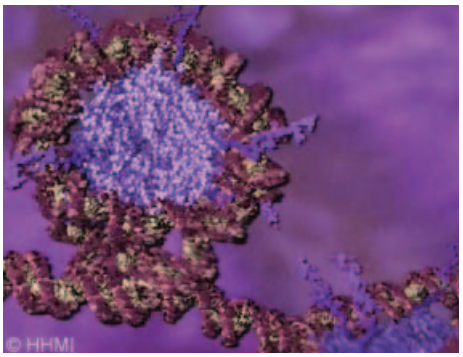
A GC pair is stabilized by three hydrogen bonds formed between amino and carbonyl groups that project from the bases.

In contrast, an AT pair is stabilized by two hydrogen bonds.

The specificity of base pairing ensures that the two strands are complementary.

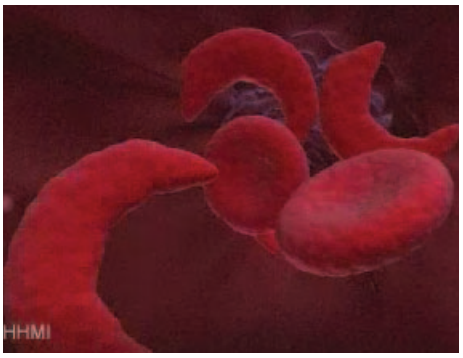
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

4.2 Chromosome Coiling <ACTA>



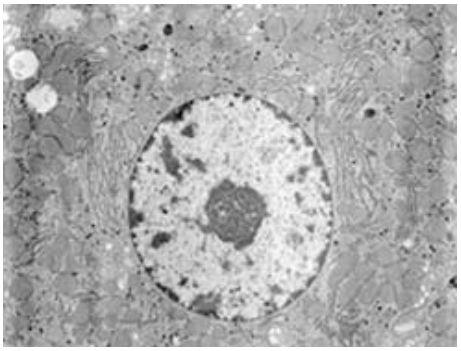
In this animation we'll see the way our DNA is tightly packed up to fit into the nucleus of every cell. The process starts with assembly of a nucleosome, which is formed when eight separate histone protein subunits attach to the DNA molecule. The combined tight loop of DNA and protein is the nucleosome. Six nucleosomes are coiled together and these then stack on top of each other. The end result is a fiber of packed nucleosomes known as chromatin.

4.3 Sickle Cell Anemia <TTTT>



Sickle cell anemia is a genetic disease that affects hemoglobin, the oxygen transport molecule in the blood. The disease gets its name from the shape of the red blood cells under low oxygen conditions. Some red blood cells become sickle-shaped and these elongated cells get stuck in small blood vessels so that parts of the body don't get the oxygen they need. Sickle cell anemia is caused by a single letter change in the DNA. This in turn alters one of the amino acids in the hemoglobin protein. Valine sits in a position where glutamic acid should be. The valine makes the hemoglobin molecules stick together when oxygen tension is low, forming long fibers that distort the shape of the red blood cells, and this brings on an attack.

4.4 Nuclear Structure: View 1 <GAGG>



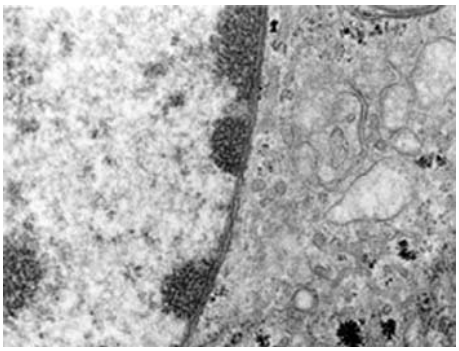
Find me:

- nucleus
- rough endoplasmic reticulum
- mitochondria
- nucleolus

Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

4.5 Nuclear Structure: View 2 <TTGC>



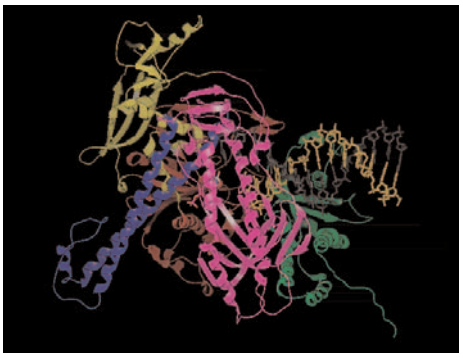
Find me:

- outer nuclear membrane
- inner nuclear membrane
- nuclear pore complex

Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

5.1 DNA Polymerase <GATT>



DNA polymerase faithfully replicates DNA by using the nucleotide sequence of the template strand, colored yellow, to select each new nucleotide to be added to the 3′ end of a growing strand, colored gray. In this animation, the different domains of DNA polymerase are colored differently.

Before a nucleotide can be incorporated into DNA at the 3′ end of the growing strand, the blue finger domain of the polymerase moves inward to correctly position the nucleoside triphosphate. A pyrophosphate group is released when each nucleotide is added.

In this view, the details of nucleotide selection at the active site are shown with the incoming nucleoside triphosphate and the template nucleotide in light blue. The growing strand is green, and the template strand is red. When the finger domain moves inward, the nucleoside triphosphate is tested for its ability to form a proper base pair with the template nucleotide.

When a base pair forms, the active site residues catalyze the covalent addition of the new nucleotide to the 3′ hydroxyl group on the growing strand, and the entire process repeats at speeds up to 500 nucleotides per second.

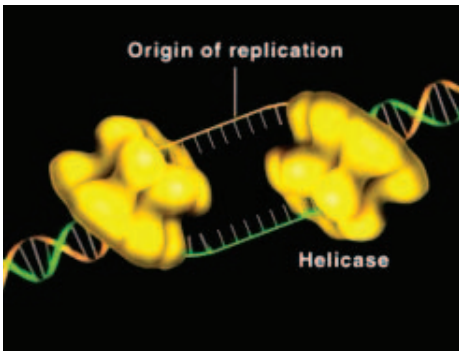
On rare occasions, approximately once every 10,000 nucleotide additions, the polymerase makes an error and incorporates a nucleotide that does not form a proper base pair onto the end of the growing strand. When this occurs, the polymerase changes conformation, and transfers the end of the growing strand to a second active site on the polymerase, where the erroneous, added nucleotide is removed. The polymerase then flips back to its original conformation, allowing polymerization to continue.

As a result, such a self-correcting DNA polymerase will make a mistake only about once every 10^7 to 10^8 nucleotide pairs.

Parts I & III:
Thomas A. Steitz, Howard Hughes Medical Institute, Yale University

Part II:
Lorena S. Beese, Duke University Medical Center

5.2 DNA Helicase <TGCC>



Helicases separate nucleic acid duplexes into their component strands using energy from ATP hydrolysis.

The crystal structure of this DNA helicase from bacteriophage T7, reveals an hexagonal arrangement of six identical subunits. Surprisingly, the ring is not six-fold symmetric, but is slightly squished.

A model for the mechanism of how the enzyme might work explains this structural asymmetry. Of the six potential ATP binding sites, two opposing ones bind ATP tightly, two are more likely to bind ADP and phosphate, and two are empty. These three states may interconvert in a coordinate fashion as ATP is hydrolyzed, creating a ripple effect that continuously runs around the ring.

Because of these conformational changes, the loops that extend into the center hole of the ring—that are proposed to bind DNA—oscillate up and down, as seen in this cross section. The oscillating loops might pull a DNA strand through the central hole, thus unwinding the double helix in the process.

A frontal view shows the full dynamics of this fascinating protein machine.

Dale B. Wigley and Martin R. Singleton
Imperial Cancer Research Fund

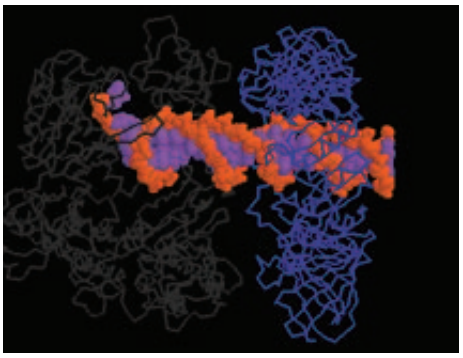
Tom Ellenberger
Harvard Medical School

Michael R. Sawaya
University of California, Los Angeles

5.3 Sliding Clamp <ACAT>

Sliding clamps allow DNA polymerases to remain attached to their DNA template. In this way, DNA polymerase can synthesize long stretches of DNA efficiently without falling off the template DNA. The multi-subunit clamp forms a ring that encircles the DNA helix; its structure thus relates to its function in a most intuitive way.

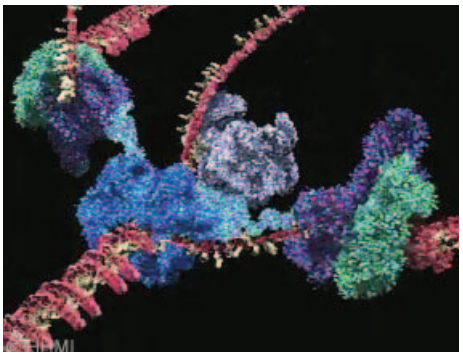
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PBD ID number *: 1QE4

5.4 Replication I <CCCG>

Using computer animation based on molecular research, we are able to picture how DNA is replicated in living cells. You are looking at an assembly line of amazing miniature biochemical machines that are pulling apart the DNA double helix and cranking out a copy of each strand. The DNA to be copied enters the production line from bottom left. The whirling blue molecular machine is called a helicase. It spins the DNA as fast as a jet engine as it unwinds the double helix into two strands. One strand is copied continuously and can be seen spooling off to the right. Things are not so simple for the other strand because it must be copied backwards. It is drawn out repeatedly in loops, and copied one section at a time. The end result is two new DNA molecules.



5.5 Replication II <AATA>

In a replication fork, two DNA polymerases collaborate to copy the leading-strand template and the lagging-strand template DNA.

In this picture, the DNA polymerase that produces the lagging strand has just finished an Okazaki fragment.

The clamp that keeps the lower DNA polymerase attached to the lagging strand dissociates, and the DNA polymerase temporarily releases the lagging-strand template DNA.

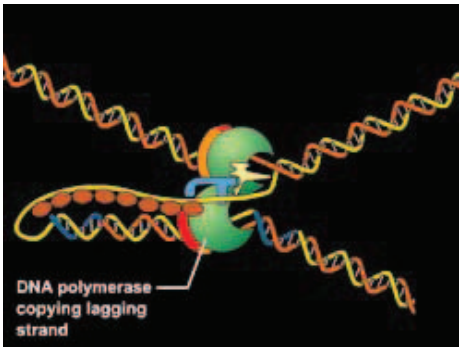
As the DNA helicase continues to unwind the parental DNA, the primase becomes activated and synthesizes a short RNA primer on the growing lagging strand.

The DNA polymerase binds to the DNA again and becomes locked in by the clamp.

The polymerase uses the RNA primer to begin a short copy of the lagging strand-template DNA.

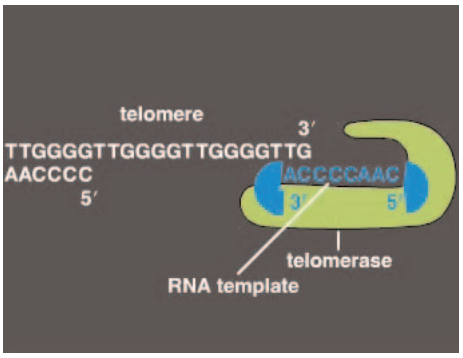
The polymerase stalls when it reaches the RNA primer of the preceding Okazaki fragment, and the entire cycle repeats.

Music: Christopher Thorpe



Original illustrations and storyboard: Nigel Orme and Christopher Thorpe

5.6 Telomere Replication <TCCT>



The ends of linear chromosomes pose unique problems during DNA replication. Because DNA polymerases can only elongate from a free 3′ hydroxyl group, the replication machinery builds the lagging strand by a backstitching mechanism. RNA primers provide 3′-hydroxyl groups at regular intervals along the lagging strand template.

Whereas the leading strand elongates continuously in the 5′-to-3′ direction all the way to the end of the template, the lagging strand stops short of the end.

Even if a final RNA primer were built at the very end of the chromosome, the lagging strand would not be complete.

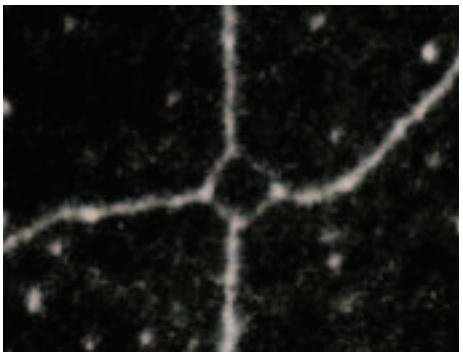
The final primer would provide a 3′-OH group to synthesize DNA, but the primers would later need to be removed. The 3′-hydroxyl groups on adjacent DNA fragments provide starting places for replacing the RNA with DNA. However, at the end of the chromosome there is no 3′-OH group available to prime DNA synthesis.

Because of this inability to replicate the ends, chromosomes would progressively shorten during each replication cycle. This "end-replication" problem is solved by the enzyme telomerase. The ends of chromosomes contain a G-rich series of repeats called a telomere. Telomerase recognizes the tip of an existing repeat sequence. Using an RNA template within the enzyme, telomerase elongates the parental strand in the 5′-to-3′ direction, and adds additional repeats as it moves down the parental strand.

The lagging strand is then completed by DNA polymerase alpha, which carries a DNA primase as one of its subunits. In this way, the original information at the ends of linear chromosomes is completely copied in the new DNA.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

5.7 Holliday Junction <CTAG>



The Holliday junction, an important intermediate structure in homologous DNA recombination, is formed when two homologous double-stranded DNA molecules reciprocally exchange DNA strands.

This junction can be visualized directly in the electron microscope.

In the cell, this junction is formed and stabilized by a specific group of helicase proteins, seen here in the background, which use ATP hydrolysis to move the junction up and down the DNA, as shown in this animation.

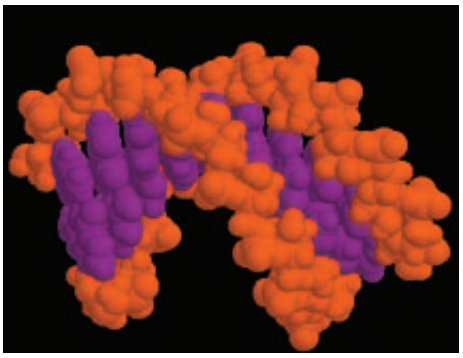
Electron microscopy:
David Dressler
University of Oxford

Huntington Potter
University of South Florida

Molecular animation:
David A. Waller
Astbury Centre for Structural Molecular Biology, University of Leeds

David Rice, Peter Artymiuk, John Rafferty, and David Hargreaves
Krebs Institute, University of Sheffield

6.1 RNA Structure <AATC>



PDB ID number *: RNA duplex containing a purine-rich strand (1RRR); RNA tetraloop (1AFX)

Like DNA, RNA strands also pair to form a double helix. The paired nucleotide bases are packed in the middle of an RNA helix, surrounded by the backbone. The RNA helix has a different geometry than a standard DNA helix. For example, the RNA helix has a significantly narrower and deeper major groove.

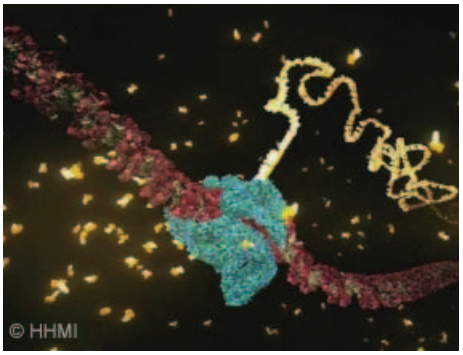
The backbone is composed of repeating ribose sugars and phosphate groups. Unlike the 2′ deoxyribose used in DNA, ribose has a hydroxyl group attached to the 2′ carbon. This 'extra' hydroxyl group influences the secondary structure. It is too bulky to allow RNA to fold like DNA, which is the primary reason why an RNA helix has a different structure.

Base pairing between strands is similar to that in DNA, except that adenine pairs with uracil instead of thymine. Thymine is exclusively used in DNA. The A-U pair also has two hydrogen bonds.

RNA strands often fold into complex structures. In this stem-loop structure, also called an RNA hairpin, a single-stranded RNA molecule folds back onto itself. The stem at the bottom is a classical RNA helix. Bases in the single-stranded loop, in contrast, are either exposed or engaged in nonstandard interactions.

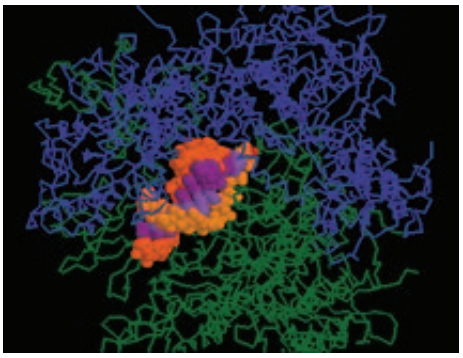
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

6.2 Transcription <CTAT>



Transcription is the process by which DNA is copied into RNA in the first step of gene expression. It begins with a bundle of factors assembling at the start of a gene, that is, a linear sequence of DNA instructions, here shown stretching away to the left. The assembled factors include an RNA polymerase, the blue molecule. Suddenly, RNA polymerase is let go, racing along the DNA to read the gene. As it unzips the double helix, it copies one of the two strands. The yellow chain snaking out of the top is the RNA, a copy of the genetic message. The nucleotide building blocks that are used to make the RNA enter through an intake hole in the polymerase. In the active site of the enzyme, they are then matched to the DNA, nucleotide by nucleotide, to copy the As, Cs, Ts and Gs of the gene. The only difference is that in the RNA copy, thymine is replaced with the closely related base uracil, commonly abbreviated "U." You are watching this process, called transcription, in real time.

6.3 RNA Polymerase II <GACT>



PDB ID number *: 1I6H

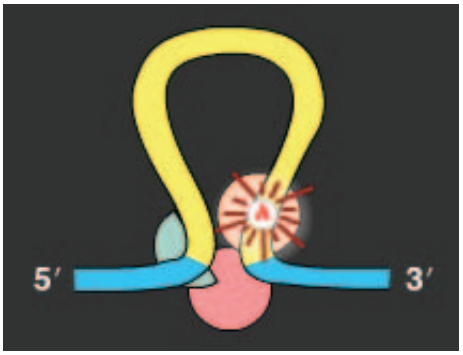
Eucaryotic RNA polymerase II transcribes all messenger RNA molecules in the cell. It is a huge complex of ten different protein subunits. The active site of the enzyme lies at the interface between the two largest subunits. In this structure a short stretch of a DNA-RNA hetero-duplex was co-crystallized. New nucleotides would be continually added to the 3′ hydroxyl group of the RNA shown in red.

In the active site of RNA polymerase II, a single-stranded DNA template is transcribed into a complementary RNA transcript. The initial product is a DNA:RNA hybrid from which the newly synthesized RNA strand is stripped off. It leaves the polymerase via an exit groove on the protein's surface.

A pore on the back side of RNA polymerase II extends from the protein surface all the way to the active site. The nucleotide triphosphates used to build the growing RNA transcript enter the polymerase through this pore.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

6.4 RNA Splicing <TCTT>



Eucaryotic genes typically contain introns, which have to be removed after transcription.

Before the RNA transcript leaves the nucleus, the cell splices out the intron sequences. A few short nucleotide sequences provide the cell with cues of what to remove. The elaborate molecular machine that carries out this task is called the spliceosome.

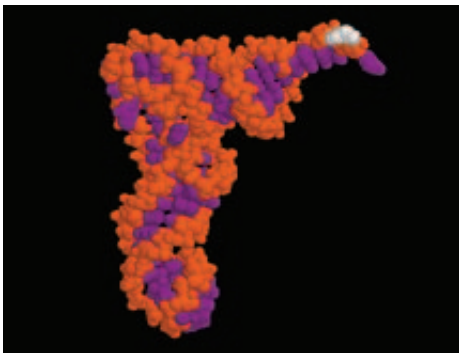
A branch-point binding protein (BBP) and a helper protein (U2AF) recognize the branch-point site within the intron, and an RNA and protein complex, called a snRNP, recognizes the 5′ splice site by forming base pairs within it. Next, another snRNP base pairs with the branch site, displaying the bound proteins. Additional snRNPs now come into play and several RNA rearrangements occur to break apart the U4/U6 base pairs and allow the U6 snRNP to displace U1 at the 5′ splice junction.

Now in position, a conserved adenine nucleotide in the intron attacks the 5′ splice site, cutting the sugar-phosphate backbone of the RNA. The end of the intron covalently bonds to the adenine nucleotide forming a lariat structure.

The spliceosome rearranges to bring together the exons, allowing the 3′ hydroxyl group of the first exon to react with the 5′ end of the other. After the two exons are joined into a continuous sequence, the lariat is released and degraded.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

6.5 tRNA <CGCA>



PDB ID number *: Phe-tRNA, elongation factor Ef-Tu: Gdpnp ternary complex (1TTT); Yeast aspartyl-tRNA synthetase (1ASY)

All tRNAs have a characteristic L-shape, with an amino acid attached to the 3′ end at the tip of the shorter arm. The anticodon loop is positioned at the opposing end of the molecule and contains the anticodon base triplet. Interactions between the equally conserved D and T loops are important in maintaining the tRNA's shape.

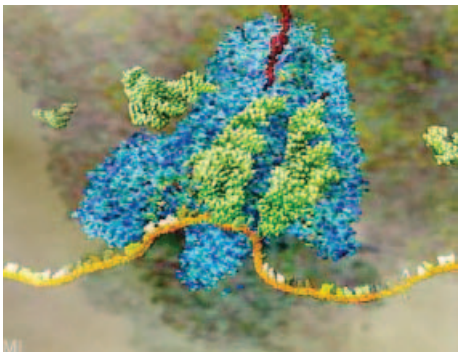
The amino acid, a phenylalanine in this case, is covalently attached to a conserved sequence, CCA, that is common to the 3′ terminus of all tRNAs.

The anticodon is comprised of three nucleotides complementary to the codon in the mRNA. The bases are exposed, and are thus freely accessible for basepairing during protein synthesis. In this example, the anticodon sequence is GAA, which would match a UUC codon on a messenger RNA, specifying phenylalanine.

The charging of tRNAs with the correct amino acids is carried out by aminoacyl-tRNA synthetases. As revealed in the cut-away view, the complex of phenylalanine-tRNA with its cognate synthetase shows an extensive contact surface that includes recognition sites for the anticodon base triplet. The tRNA's CCA end is deeply buried in the enzyme.

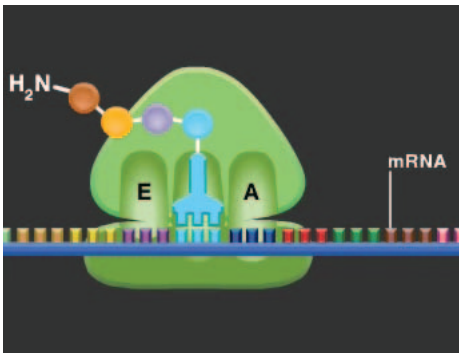
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

6.6 Translation I <CGCC>



When the mRNA is complete, it snakes out of the nucleus into the cytosol. Then in a dazzling display of choreography, all the components of a molecular machine lock together around the RNA to form a miniature factory called a ribosome. It translates the genetic information in the RNA into a string of amino acids that will become a protein. tRNA molecules, the green triangles, bring each amino acid to the ribosome. The amino acids are the small red tips attached to the tRNAs. There are different tRNAs for each of the twenty amino acids, each of them carrying a three-letter nucleotide code that is matched to the mRNA in the machine. Now we come to the heart of the process. Inside the ribosome, the mRNA is pulled through like a tape. The code for each amino acid is read off, three letters at a time, and matched to three corresponding letters on the tRNAs. When the right tRNA plugs in, the amino acid it carries is added to the growing protein chain. You are watching the process in real time. After a few seconds the assembled protein starts to emerge from the ribosome. Ribosomes can make any kind of protein. It just depends on what genetic message you feed in on the mRNA. In this case, the end product is hemoglobin. The cells in our bone marrow churn out a hundred trillion molecules of it per second! And as a result, our muscles, brain and all the vital organs in our body receive the oxygen they need.

6.7 Translation II <CACT>



To extend a growing polypeptide chain the ribosome must select the correct amino acids that are specified by the messenger RNA.

An aminoacyl-tRNA bound to elongation factor Tu, EF-Tu for short, enters the free A site on the ribosome. If the anticodon of the charged tRNA does not match the codon in the messenger RNA, the tRNA is rejected.

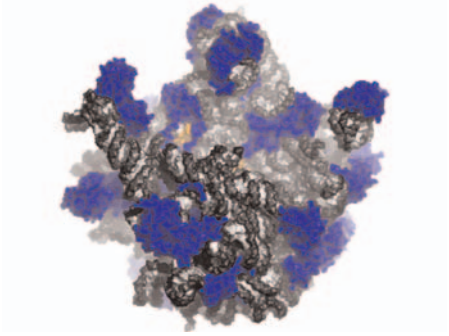
The process of trial and error repeats until the correct tRNA is identified.

Elongation factor Tu hydrolyzes its bound GTP and dissociates. If the tRNA is correctly matched and remains bound for a long enough time, it is committed to be used in protein synthesis.

The ribosome catalyzes the formation of the new peptide bond and undergoes a dramatic conformational change. Elongation factor G binds to the ribosome. Hydrolysis of GTP by elongation factor G switches the ribosome back to the state in which it can accept the next incoming tRNA.

Animation: Sumanas, Inc. (www.sumanasinc.com)

6.8 Ribosome Structure <AGGC>



T. Martin Schmeing
Thomas A. Steitz
Howard Hughes Medical Institute, Yale University

The crystal structure of the ribosome reveals many insights into the molecular mechanism of translation.

Zooming in on the large ribosomal subunit shows highly evolutionarily conserved RNA bases lining the active site of the peptidyltransferase center, which catalyzes polypeptide bond formation. There are no ribosomal proteins in the vicinity; peptide bond formation is catalyzed in an environment exclusively made of ribosomal RNA.

The 3′ end of a tRNA charged with an amino acid is bound in the active site. This amino acid represents the carboxy-terminal amino acid of a growing polypeptide chain on an actively translating ribosome with the peptidyl-tRNA bound to the P site on the ribosome. Conserved bases common to the 3′ end of all tRNAs base pair with the ribosomal RNA to position the amino acid precisely.

The incoming amino acid linked to its respective tRNA binds closely, again held precisely by base pairing interactions between a conserved base on the tRNA and ribosomal RNA. A network of hydrogen bonds positions the reactive groups with the precise geometry required to catalyze peptide bond formation.

The empty deacylated tRNA is released from the P-site.

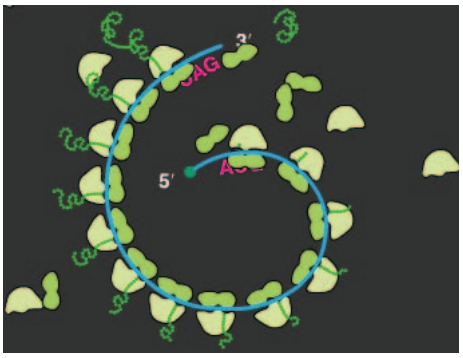
During the ‘translocation’ step of protein synthesis, the other tRNA, now containing the growing polypeptide chain, moves from the A- to the P-site, where it will be waiting for the next incoming amino acid to repeat the polymerization cycle.

The different states of the reaction cycle shown in this animation are based on actual crystal structures, in which large ribosomal subunits were crystallized with various aminoacyl-tRNA analogs bound to them, mimicking the discrete steps in the reaction cycle.

6.9 Polyribosome <GAAG>

As soon as a messenger RNA molecule is transported from the nucleus to the cytoplasm, ribosomes begin to translate the sequence into amino acids. Typically, many ribosomes translate the mRNA simultaneously. Each ribosome begins at the 5′ end of the mRNA and progresses steadily toward the 3′ end. New ribosomes attach to the 5′ end at the same rate as the previous ones move out of the way. These multiple initiations allow the cell to make much more protein from a single message than if one ribosome had to complete the task before another could begin. When a ribosome reaches a stop codon, the ribosome and the new protein dissociate from each other and from the mRNA. This electron micrograph depicts a membrane-bound polyribosome from a eucaryotic cell.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)



Electron Microscopy:
John Heuser
Washington University in St. Louis

6.10 Ribosome Ratchet <CGTT>

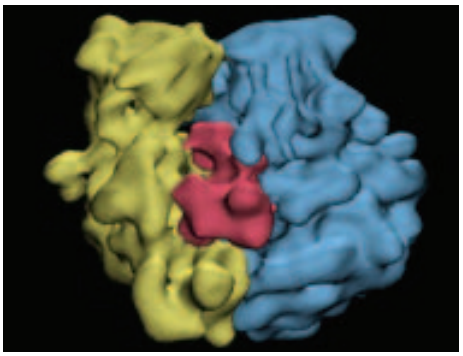
Comparison of two states of a bacterial ribosome, either with the initiator fMet-tRNA bound or with elongation factor EF-G bound, reveals the significant conformational changes that the ribosome is thought to undergo during each elongation cycle. The ratchet-like rearrangements at the interface between the two ribosomal subunits may help move the mRNA and tRNAs through the ribosome during protein synthesis.

The models shown here are a computer reconstruction made from many thousands of images of single ribosomes in vitreous ice that were observed with an electron microscope.

Animation: Amy Heagle Whiting, Howard Hughes Medical Institute, Health Research Incorporated at the Wadsworth Center, State University of New York at Albany

Final composition: Graham Johnson, Fivth Element (www.fivth.com)

Funded, in part, by NIGMS and NCRR, National Institutes of Health



Joachim Frank and Rajendra K. Agrawal
Howard Hughes Medical Institute
Health Research Incorporated at the Wadsworth Center, State University of New York at Albany

7.1 Homeodomain <ACGT>

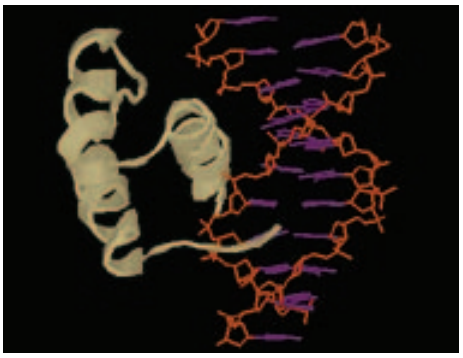
Homeodomains are found in many transcription regulatory proteins and mediate their binding to DNA. A single homeodomain consists of three overlapping α helices packed together by hydrophobic forces. Helix 2 and helix 3 comprise the DNA-binding element, a helix-turn-helix motif.

Amino acids in the recognition helix make important, sequence-specific contacts with bases in the DNA major groove.

Three side chains from the recognition helix form hydrogen bonds with bases in the DNA. A hydrogen bond is a strong, noncovalent interaction that forms when two neighboring electronegative atoms, like oxygen and nitrogen, share a single hydrogen.

In addition to the contacts between the recognition helix and the bases in the DNA major groove, an arginine residue from a flexible loop of the protein contacts bases in the minor groove.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PBD ID number *: 1APL

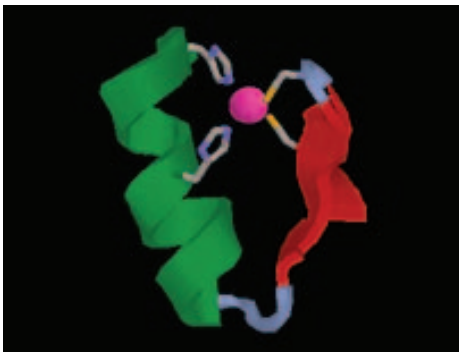
7.2 Zinc Finger Domain <ATCT>

Zinc finger domains are structural motifs used by a large class of DNA binding proteins. They use centrally coordinated zinc atoms as crucial structural elements.

A single zinc finger domain is only large enough to bind a few bases of DNA. As a result, zinc fingers are often found in tandem repeats as part of a larger DNA-binding region.

The helical region of each zinc finger rests in the major groove of the DNA helix. Basic side chains project out from the helix and contact bases in the DNA. The identities of these side chains determine the precise DNA sequence recognized by each zinc finger. Assembling different zinc finger motifs allows precise control over the sequence specificity of the protein. The specific contacts made between protein and DNA are hydrogen bonds.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: Zinc finger DNA-binding domain (1ZNF); Zif268-DNA complex (1ZAA)

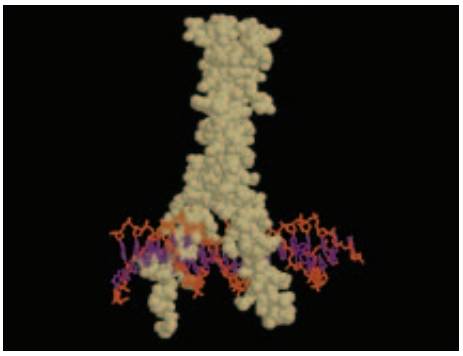
7.3 Leucine Zipper <TGTT>

A leucine zipper domain is comprised of two long, intertwined α helices. Hydrophobic side chains extend out from each helix into the space shared between them. Many of these hydrophobic side chains are leucines, giving this domain its name. A spacefilling view reveals the tight packing of side chains between the leucine zipper helices; this makes the domain especially stable.

Extensions of the two leucine zipper helices straddle the DNA major groove. Side chains from both helices extend into the groove to contact DNA bases.

The specific interactions between side chains and bases are hydrogen bonds. In this example, an arginine residue makes two contacts with a guanine base.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: 1YSA

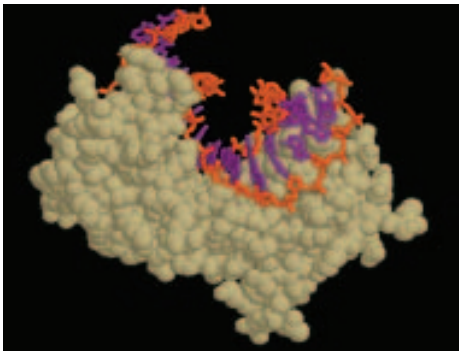
7.4 TATA-Binding Protein <TATA>

Eucaryotic transcription begins when RNA polymerase II binds to the promoter region of a gene. A crucial part of this initiation process is the recognition and binding of the TATA sequence, a short stretch of DNA rich in thymine and adenine nucleotides. The subunit of RNA polymerase II that binds to the TATA sequence is called the TATA-binding protein.

The TATA-binding protein binds to DNA using an eight-stranded beta sheet that rests atop the DNA helix like a saddle. Two protein loops drape down the sides of the DNA like stirrups.

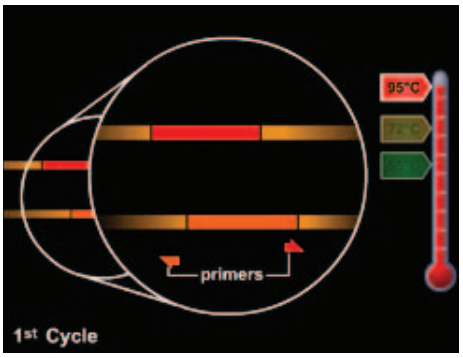
Binding of the TATA-binding protein introduces a severe kink in the DNA backbone. This kink dramatically bends the DNA helix by nearly ninety degrees and is thought to provide a signal to assemble the rest of the transcription complex at the initiation site.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: TATA element ternary complex (1VOL); Compilation of TATA element ternary complex & Gal4 complex with DNA (1VOL & 1D66)

8.1 Polymerase Chain Reaction <TACG>



Original illustrations, storyboard and music:
Christopher Thorpe

The polymerase chain reaction, or PCR, amplifies a specific DNA fragment from a complex mixture.

First, the mixture is heated to separate the DNA strands. Two different specific oligonucleotide primers are added that are complementary to short sequence stretches on either side of the desired fragment. After lowering the temperature, the primers hybridize to the DNA where they bind specifically to the ends of the desired target sequence. A heat stable DNA polymerase and nucleotide triphosphates are added. The polymerase extends the primers and synthesizes new complementary DNA strands. At the end of this first cycle, two double-stranded DNA molecules are produced that contain the target sequence.

This cycle of events is repeated. The mixture is again heated to melt the double-stranded DNA. The primers are hybridized and the DNA polymerase synthesizes new complementary strands.

At the end of the second cycle, four doubled-stranded DNA molecules are produced that contain the target sequence. In the third cycle, the mixture is heated, the primers are hybridized and DNA polymerase synthesizes new complementary strands. At the end of the third cycle, eight double-stranded DNA molecules are produced that contain the target sequence. Two of these molecules are precisely the length of the target sequence. In future cycles this population increases exponentially.

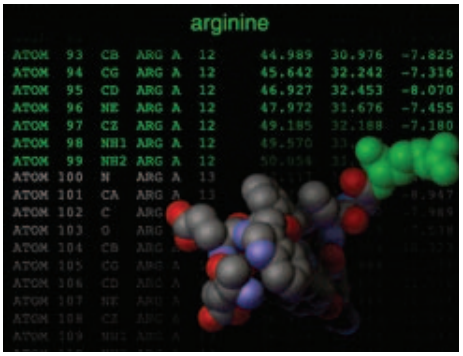
Cycle 4—heating, hybridization, DNA synthesis.

At the end of the fifth cycle there are 22 double-stranded DNA fragments of the correct length and 10 longer ones.

Cycle 6, 10, 15, 20 . . .

After 30 cycles there are over 1 billion fragments of the correct length but only 60 longer ones. The product therefore consists of essentially pure target sequence.

8.2 Anatomy of a PDB File <GGCC>



Graham Johnson
Fivth Element (www.fivth.com)

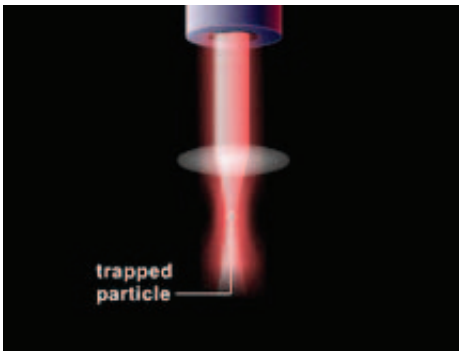
Three dimensional structures of macromolecules—that have been determined by NMR or x-ray crystallography—are archived in protein database files, or PDB files for short.

Each PDB file begins with a description of the molecule, credits to the authors who solved the structure and experimental details of the analysis. Next, the file lists the amino acid sequence of the protein.

The heart of the PDB file defines the precise position of each atom of the structure in three-dimensional space. Each atom is described in a separate line. The first few columns define the atom as part of a particular amino acid in the sequence. The later columns list a set of x, y, and z coordinates that precisely locate the atom in the structure. Programs such as Rasmol or Chime, used on this CD, directly read PDB files and use the coordinates to build three dimensional models on your computer screen. PDB files are stored in publicly accessible databases and can be readily downloaded from the Internet.

9.1 Laser Tweezers <CGCG>

The light of a laser beam that is focused into a cone through a microscope objective exerts small forces that can trap particles with high refractive indices near the focal point. This experimental set-up is called 'laser tweezers.' It can be used to move small particles, including cells and organelles.

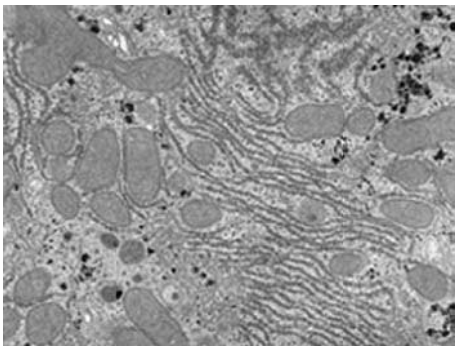


Christopher Thorpe

9.2 Liver Cell: View 1 <CACC>

Find me:

- rough endoplasmic reticulum
- smooth endoplasmic reticulum
- regions of continuities between rough and smooth endoplasmic reticulum
- mitochondria



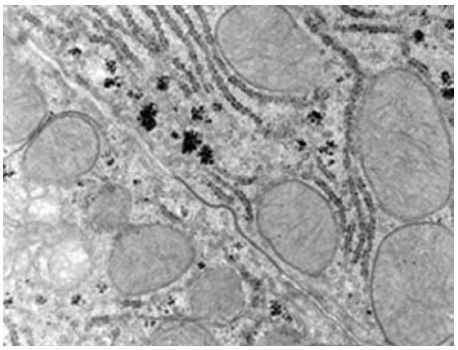
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

9.3 Liver Cell: View 2 <TACA>

Find me:

- plasma membranes
- tight junction
- rough endoplasmic reticulum
- glycogen granules



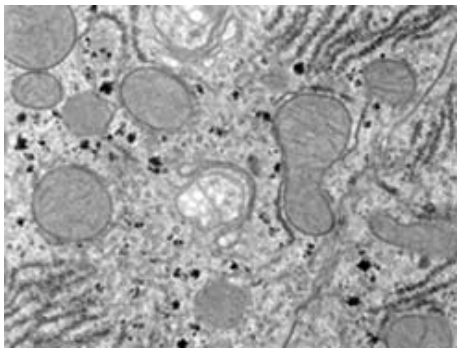
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

9.4 Liver Cell: View 3 <AATT>

Find me:

- Golgi apparatus
- plasma membranes
- lysosome
- mitochondria



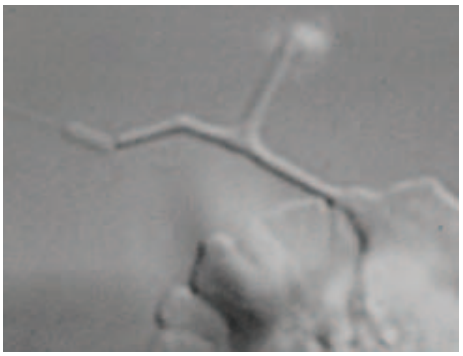
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

10.1 Fluidity of the Lipid Bilayer <CACA>

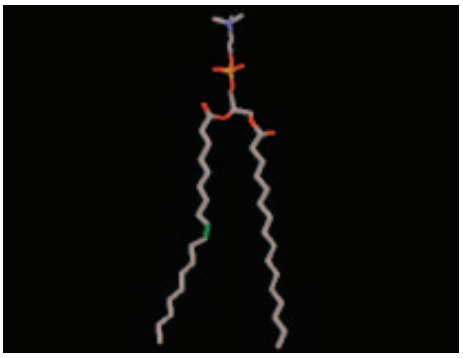
To demonstrate the fluidity of the lipid bilayer, a piece of the plasma membrane of this neuronal cell is pulled out with laser tweezers. Remarkably, moving this membrane tubule rapidly back and forth does not rupture the plasma membrane, which flows quickly to adapt to the mechanical distortion.

Music: Christopher Thorpe



Steven M. Block
Stanford University

10.2 Lipids and Lipid Bilayer <TAGC>



PDB ID source: Compact lipid molecule structure (Beckman Institute); Compilation of saturated and unsaturated fatty acids and cholesterol (Lipidat)

Phospholipids contain a head group, choline in this case, that is attached via a phosphate group to a 3-carbon glycerol backbone. Two fatty acid tails are attached to the remaining two carbons of the glycerol.

The head groups and the phosphate are polar, that is, they prefer to be in an aqueous environment.

In contrast the fatty acid tails are hydrophobic, that is, they are repelled from water. The fatty acid tails on phospholipids can be saturated, with no double bonds, or unsaturated, with one or more double bonds. The double bonds are usually in the *cis*-configuration, which introduces sharp kinks. When forming a bilayer, unsaturated fatty acid tails pack loosely, which allows the bilayer to remain fluid. If there were no double bonds, bilayers would solidify to a consistency resembling bacon grease.

Cholesterol is another lipid component of most cell membranes. It has a hydroxyl group, a tiny polar head group so to speak, attached to a rigid hydrophobic tail. Cholesterol can fill gaps between phospholipids and thus stabilizes the bilayer.

In a lipid bilayer, lipids arrange themselves so that their polar heads are exposed to water and their hydrophobic tails are sandwiched in the middle. In this model, water molecules are shown in red.

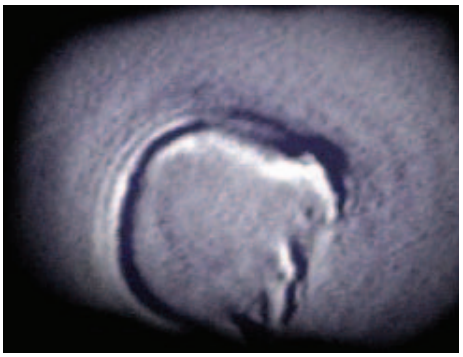
Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

Source: Beckman Institute, The Theoretical Biophysics Group University of Illinois Urbana-Champaign

H. Heller, M. Schaefer, K. Schulten. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid-crystal phases. *Journal of Physical Chemistry* 97:8343–8360, 1993.

Lipidat Database (www.lipidat.chemistry.ohio-state.edu/)

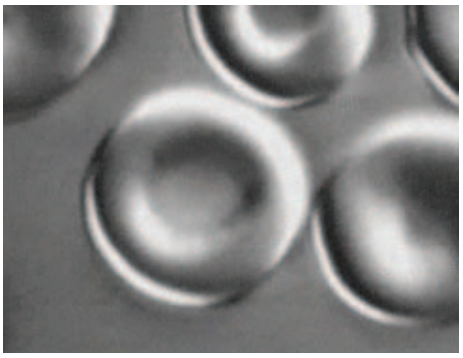
10.3 Membrane Disruption by Detergent <GCGA>



Steven M. Block
Stanford University

When detergent is added to this red blood cell, its membrane ruptures, and the cytosol spills out.

10.4 Membrane Effects in a Red Blood Cell <GTAC>



Red blood cells must deform when they squeeze through small blood vessels.

In this experiment a red blood cell is pushed and deformed with laser tweezers. It quickly springs back to its original shape because it has an extremely tough cytoskeleton to which the plasma membrane is anchored.

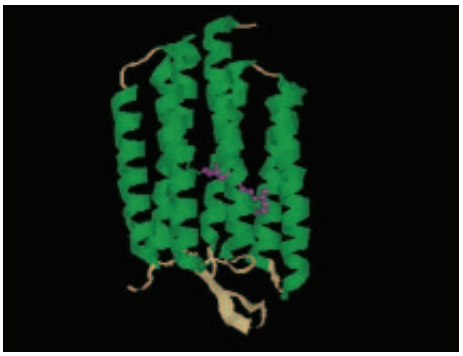
When the cell is placed in high-salt solution, however, the shape changes dramatically. Driven by the difference in osmotic pressure, water rushes out of the cell causing spikelike protrusions to form as the cell collapses.

Steven M. Block
Stanford University

Henry Bourne and John Sedat
University of California, San Francisco

Orion Weiner
Harvard Medical School

10.5 Bacteriorhodopsin <TTAA>



Bacteriorhodopsin is an abundant light-driven proton pump found in the membrane of *Halobacter halobium*, a purple archeon that lives in salt marshes in the San Francisco Bay Area. Bacteriorhodopsin is a multipass membrane protein that traverses the plasma membrane of the cell with seven long α helices. The helices surround a chromophore, retinal, that is covalently attached to the polypeptide chain and gives the protein and cells their characteristic purple color.

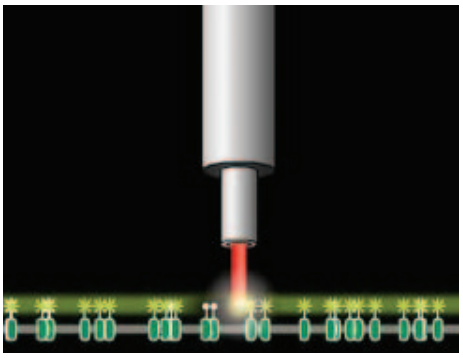
Retinal is a long, unsaturated hydrocarbon chain that is covalently attached to a lysine side chain of the protein. When retinal absorbs a photon of light, one of its double bonds isomerizes from a *trans* to a *cis* configuration, thus changing the shape of the molecule. The change in retinal's shape causes conformational rearrangements in the surrounding protein.

The light-induced isomerization of retinal is the key event in proton pumping. In the excited state, retinal is positioned so that it can transfer a proton to an aspartate side chain, aspartate 85, that is positioned towards the extracellular side of the protein. Aspartate 85 quickly hands off the proton to the extracellular space via a bucket brigade of water molecules. The now negatively-charged retinal takes up a proton from another aspartate, aspartate 96; this one is positioned towards the cytosolic face of the protein. Upon re-protonation, the retinal returns to the ground state. Aspartate 96 replenishes its lost proton from the cytosol, and the cycle can repeat. The net result: for each photon absorbed, one proton is pumped out of the cell.

PDB ID number *: Compilation of Bacteriorhodopsin Br state intermediate & Bacteriorhodopsin M state intermediate (1C8R & 1C8S)

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

10.6 FRAP <ATGT>



Jennifer Lippincott-Schwartz
NICHD, National Institutes of Health

The lateral mobility of membrane proteins can be measured in living cells by FRAP, which stands for fluorescence recovery after photobleaching.

For this purpose, membrane proteins are often expressed as fusion proteins with the green fluorescent protein GFP and observed with a fluorescence microscope.

A selected area of the cell is then bleached with a strong, computer controlled beam of laser light.

Those membrane proteins that are not anchored and therefore can diffuse in the plane of the membrane, quickly exchange places with their neighbors and fill back in the bleached area.

From the rate of this fluorescence recovery, the diffusion constant of the protein can be calculated.

Here, GFP is fused to a membrane protein that lies in the membrane network of the endoplasmic reticulum.

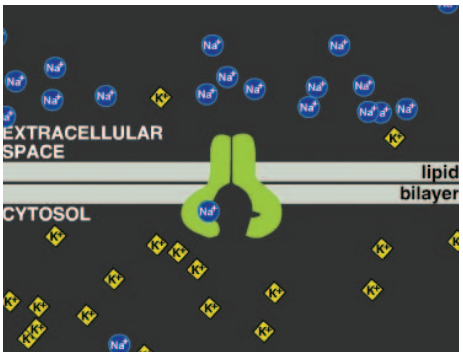
After bleaching, we observe quick recovery of the fluorescence, showing that the protein is very mobile in the plane of the membrane.

The same experiment can be repeated using a protein that is firmly anchored and not free to diffuse. Here, we observe GFP fused to a protein of the inner nuclear membrane that binds tightly to the meshwork of the nuclear lamina.

After photobleaching, no fluorescence recovery can be seen over the same time frame.

Final composition: Blink Studio Ltd. (www.blink.uk.com)
Video reproduced from: *The Journal of Cell Biology* 138:1193–1206, Figure 4B, 1997.

11.1 Na^+-K^+ Pump <GAGT>



Animal cells store energy in the form of ion gradients across the cell membrane. In the cytosol, the sodium ion concentration is kept low relative to the extracellular fluid, and conversely the potassium ion concentration in the cytosol is kept high.

Like water behind a dam, these gradients harbor potential energy that the cell taps to fuel cellular work.

Animal cells use a membrane pump, called the sodium–potassium pump, to maintain these ion gradients. To begin the pumping cycle, sodium ions enter binding sites on the cytosolic side of the pump. Although there are three sodium-binding sites on this pump, for simplicity only one is illustrated here.

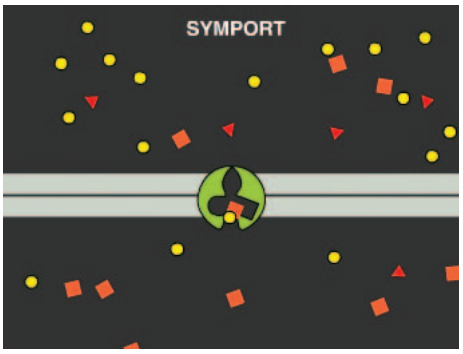
Pumping sodium against its concentration gradient requires energy, which is provided by cleaving ATP. ATP transfers a phosphate group to the pump in a high-energy linkage.

Phosphorylation causes a dramatic change in the pump's conformation, so that the sodium ions become exposed and released outside of the cell. This action also exposes binding sites for potassium ions in the pump. Although there are two potassium-binding sites, for simplicity only one is shown here.

Binding of the potassium ions triggers release of the phosphate group and the return of the pump to its initials conformation. The potassium is then released inside the cell, and the cycle repeats. A complete cycle takes about 10 milliseconds.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

11.2 Transport by Carrier Proteins <ACCC>



Cells possess a variety of membrane proteins to ferry solutes across the membrane. One type of transporter, called a uniport, carries only one type of solute, selectively bringing it from one side of the membrane to the other.

In contrast to uniports, coupled transporters carry two types of solutes. If both solutes are moving in the same direction across the membrane, the transporter is called a symport.

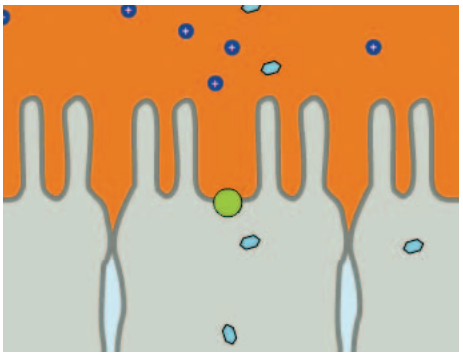
In this example, the solute represented by the circle is carried down its concentration gradient, from high concentration to low. The energy released by the movement of this solute drives the movement of the other solute, represented by the square, against its concentration gradient, from low to high concentration.

When the coupled transporter moves solutes in opposite directions across the membrane, it is called an antiport.

In this example, the solute represented by the circle is transported down its concentration gradient, fueling the transport of the other solute (represented by the triangle) against its concentration gradient, that is from low to high concentration.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

11.3 Glucose Uptake <GGAT>



One important task for cells lining the lumen of the gut is the uptake of glucose produced by digestion of food. Yet glucose is typically higher in concentration inside the cells than in the gut, and therefore transporting it into the cell requires energy. To this end, a glucose–sodium symport harvests the energy stored in the sodium gradient to pump glucose into the cell.

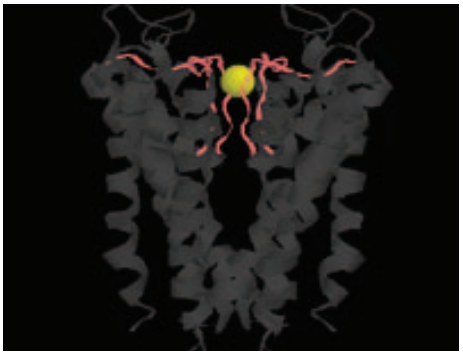
According to one model, sodium and glucose can both bind to the pump, but the binding of one makes the binding of the other more effective. When the binding sites of the symport are open to the lumen of the gut, the high sodium concentration makes sodium very likely to bind, and thus glucose will bind more efficiently.

Because the conformational change of the transporter will only occur when both sodium and glucose binding sites are filled, both solutes are transported across the membrane in strict unison and are released together into the cell.

On the cytosolic side of the membrane, the solutes could, in principle, also bind and thus be exported again by the same route that brought them into the cell. However, while there is plenty of glucose inside the cell, there is very little sodium. Therefore, the binding of both types of solutes only occurs very rarely, such that most of the glucose molecules that enter the cell will not leave by the same route. The import is therefore unidirectional.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

11.4 Potassium Channel <ATTA>



PDB ID number *: 1BL8

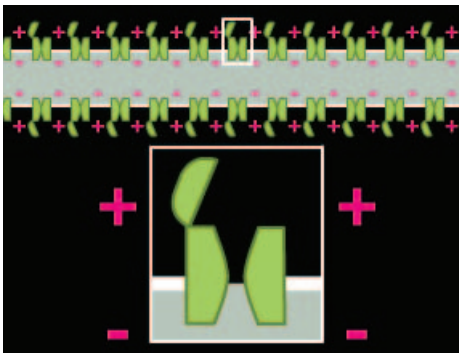
The bacterial potassium channel is a multipass transmembrane protein in the plasma membrane. It is built from four identical subunits that are arranged symmetrically. A pore in the center of the protein allows selective passage of potassium ions across the membrane.

Four rigid protein loops, one contributed by each subunit, form a selectivity filter at the narrowest part of the pore. This structure is responsible for the channel's high degree of selectivity for potassium ions over sodium ions.

In the selectivity filter, carbonyl groups line the walls of the pore. These carbonyl groups are spaced precisely to interact with an unsolvated potassium ion, balancing the energy required to remove its hydration shell. Passage of a sodium ion through the channel is energetically unfavorable because the sodium is too small for optimal interaction with the carbonyl groups.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

11.5 Action Potentials <CGAG>



The fundamental task of a nerve cell is to receive, conduct, and transmit signals. Neurons propagate signals in the form of action potentials, which can travel great distances along an axon without weakening.

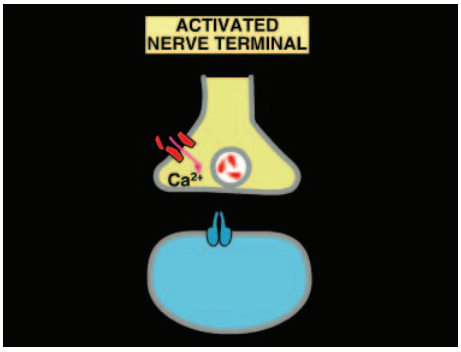
To transmit an action potential over such a distance without weakening requires that the signal is continuously reamplified along the way. The central molecular players in this process are the voltage-gated sodium channels, which undergo a cycle of finely choreographed conformational changes. When an action potential passes, sodium channels open in response to the membrane depolarization. Sodium ions rush into the axon, further depolarizing its membrane. Within a fraction of a thousandth of a second, however, the sodium channels switch to a new, inactivated state, in which they are closed but now also refractory to reopening. In this way, the membrane potential can recover quickly after an action potential has passed. The sodium channels then reconvert to the closed state, ready to be opened again when the next action potential is encountered.

Let's examine the changing state of the sodium channel during an action potential. When no stimulus is present, the sodium channels remain closed and the electrical potential measured across the membrane remains constant. However, if a depolarizing stimulus is applied by a brief pulse of electric current, the membrane will start to depolarize away from the resulting potential of about –80mV. Some of the sodium channels will open, permitting sodium ions to enter the axon along their concentration gradient. If the depolarization is sufficient, even more sodium channels open, and the membrane potential rapidly approaches the equilibrium potential for sodium (about +40 mV). At this point, the sodium channels close, adopting the inactive conformation, where the channel is unable to open again even though the membrane potential is still depolarized. The sodium channels will remain in this inactivated state until a few milliseconds after the membrane potential returns to its initial negative value.

The action potential is propagated along the length of the axon in only one direction. By examining the membrane potential and the state of the sodium channels along a length of the axon, we can see why this is so. As a depolarizing stimulus (represented in orange) reaches our section of the membrane, sodium channels open and current flows into the axon. This in turn depolarizes adjacent sections of the membrane (represented in blue), causing adjacent sodium channels to open, and the action potential is thus propagated along the axon. Sodium-channel inactivation prevents the depolarization from spreading backward along the axon.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

11.6 Synaptic Signaling <CTGA>



Electron Microscopy:
Cedric S. Raine
Albert Einstein College of Medicine

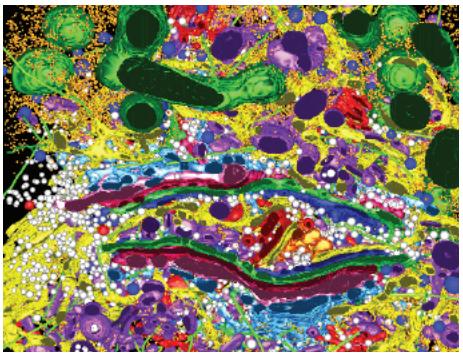
Neurons transmit chemical signals across synapses, like the ones shown in this electron micrograph. We can identify the dendrite of the receiving, or postsynaptic cell, as well as two presynaptic nerve terminals loaded with synaptic vesicles. Note the narrow cleft separating the pre- and postsynapitic cells.

The synapse converts the electrical signal of the action potential in the presynaptic cell into a chemical signal. When an action potential reaches a nerve terminal, it opens voltage-gated Ca^{2+} channels in the plasma membrane, allowing Ca^{2+} ions to flow into the terminal. The increased Ca^{2+} in the nerve terminal stimulates synaptic vesicles to fuse with the plasma membrane, releasing their neurotransmitter cargo into the synaptic cleft.

The released neurotransmitters diffuse across the synaptic cleft where they bind to and open the transmitter-gated ion channels in the plasma membrane of the postsynaptic cell. The resulting ion flows depolarize the plasma membrane of the postsynaptic cell, thereby converting the neurotransmitter's chemical signal back into an electrical one that can be propagated as a new action potential. The neurotransmitter is quickly removed from the synaptic cleft—either by enzymes that destroy it, or by reuptake into the nerve terminals or neighboring cells.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

12.1 Cell Compartments <ATCC>



Kathryn E. Howell
University of Colorado School of Medicine

Brad J. Marsh and J. Richard McIntosh
University of Colorado at Boulder

High voltage electron microscopy allows three-dimensionsional imaging of a segment of this insulin secreting pancreatic cell. Relatively thick slices of the cell are viewed in the microscope from different angles, which allows us to reconstruct a three-dimensional image.

Stepping through the image from the top reveals the complexity of cell structure.

Focusing on the Golgi apparatus, individual membranes can be traced, and we can appreciate the size and shape of various compartments.

Using these outlines, a computer can construct a three-dimensional model of the entire segment. Here we see the stacks of the Golgi apparatus, each traced in a different color. The *cis* Golgi, where proteins are first delivered to the organelle, is light blue and the *trans* Golgi network, where they exit, is light blue.

Shown in dark blue are the secretory vesicles into which insulin gets packaged after leaving the *trans* Golgi network.

Many little transport vesicles, shown in white, surround the Golgi apparatus. They transport cargo between the cisternae or back to the endoplasmic reticulum.

When all the other organelles are combined into a single image, we can see the incredible crowding of organelles in the cytosol. Here, mitochondria and microtubules are colored green. Endoplasmic reticulum and ribosomes are shown in yellow. The purple organelles are probably endosomes.

Given this apparent clutter, one cannot help but wonder how all these components work in synchrony to allow the cell to achieve its tasks.

12.2 Nuclear Import <AGTT>

Nuclear import and export can be directly visualized in living cells that express the green fluorescent protein GFP fused to the gene regulatory protein NF-AT.

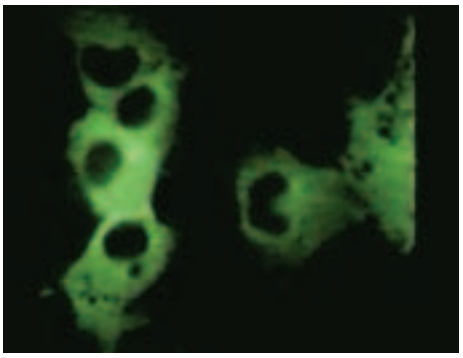
NF-AT is normally localized in the cytosol, and excluded from the nucleus. But when the cytosolic calcium concentration is raised, NF-AT migrates to the nucleus.

This is done here experimentally by adding an ionophore that allows calcium to enter the cells from the medium.

Upon removal of the ionophore, calcium levels return to normal and NF-AT is exported from the nucleus.

Readdition of the ionophore triggers reimport of NF-AT.

Final composition: Allison Bruce



Frank McKeon
Harvard Medical School

Futoshi Shibasaki
The Tokyo Metropolitan Institute of Medical Science

Roydon Price
Harvard University

Annie Yang
Harvard Medical School

12.3 Mitochondrial Protein Import <ACGG>

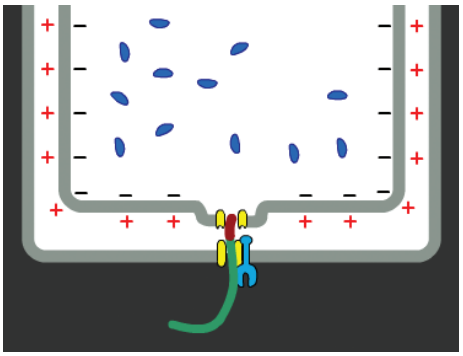
Mitochondria are organelles that have their own DNA and can make their own proteins. However, a vast majority of mitochondrial proteins are encoded in the nucleus and translated into protein in the cytosol. Proteins made in the cytosol must therefore be sorted and selectively delivered to their proper destinations, such as mitochondria, chloroplasts, peroxisomes, the ER, or the nucleus.

Precursor proteins destined for a mitochondrion have a short segment of amino acids, the signal sequence, that targets the proteins to this organelle. The signal sequence has affinity for a receptor on the mitochondrion's surface and delivers the precursor protein to a translocation apparatus for import.

At a contact site where the mitochondrion's two membranes are close together, the precursor protein snakes in an unfolded state through two sequential protein translocators, one in each of the mitochondrial membranes. Inside the mitochondrion, chaperone proteins are required to help pull the protein in. Chaperone proteins bind to the precursor protein as it appears on the inside of the mitochondrion, and thereby prevent the protein chain from backsliding through the translocation tunnel.

Once inside, an enzyme, called a signal peptidase, cleaves the signal sequence, which is no longer needed, from the precursor. The chaperone proteins are released as the protein chain folds into its three-dimensional structure.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)



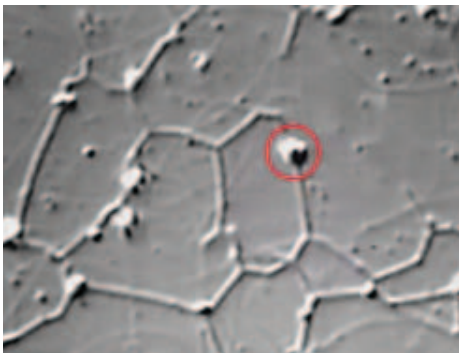
Electron Microscopy:
Daniel S. Friend

12.4 ER Tubules <TCGT>

The endoplasmic reticulum is a highly dynamic network of interconnected tubules that spans the cytosol of a eukaryotic cell—like a spider's web.

The network is continually reorganizing with some connections being broken while new ones are being formed.

Motor proteins moving along microtubules can pull out sections of endoplasmic reticulum membranes to form extended tubules that then fuse to form a network.



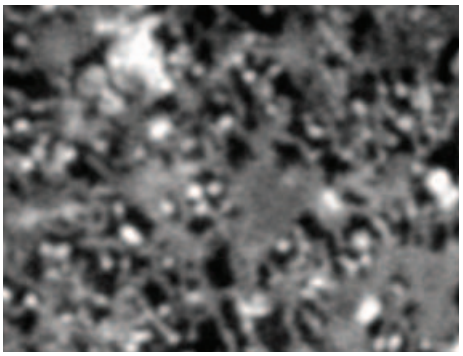
Part I:
Jennifer Lippincott-Schwartz
NICHD, National Institutes of Health

Part II:
Ron D. Vale
Howard Hughes Medical Institute
University of California, San Francisco

12.5 Nuclear Envelope Assembly <ATAA>

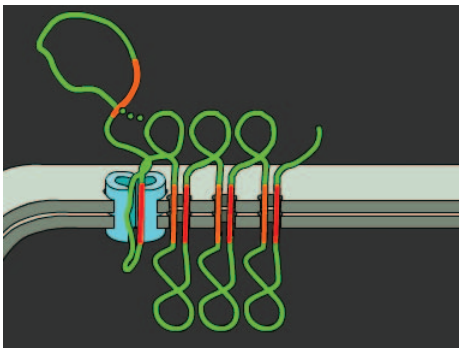
In this experiment, a network of fluorescently labeled ER membrane tubules is brought into contact with a layer of DNA on a glass slide. The tubules attach to the DNA and start to cover it, forming an increasingly dense network. Eventually, the membranes of adjacent tubules fuse to form continuous sheets that will completely cover the DNA.

In cells, this same process results in the construction of a new nuclear envelope after mitosis, when ER tubules attach to the DNA of decondensing chromosomes.



Martin W. Hetzer
Salk Institute of Biological Sciences

12.6 Protein Translocation <TTCC>



The endoplasmic reticulum (or ER) is the most extensive membrane system in eucaryotic cells. Proteins transported to the Golgi apparatus, endosomes, lysosomes, and the cell surface, all must first enter the ER from the cytosol.

As an mRNA molecule is translated into a protein, many ribosomes bind to it, forming a polyribosome. There are two separate populations of polyribosomes in the cytosol that share the same pool of ribosomal subunits.

Free ribosomes are unattached to any membrane. Membrane-bound ribosomes become riveted to the ER membrane and translate proteins that are translocated into the ER. These membrane-bound ribosomes coat the surface of the ER, creating regions called rough endoplasmic reticulum.

Two kinds of proteins are moved from the cytosol to the ER. Water-soluble proteins completely cross the ER membrane and are released into the lumen, while transmembrane proteins only partially cross the ER and become embedded in the membrane.

All these proteins are directed to the ER by a signal sequence of small hydrophobic amino acids. The signal sequence is guided to the ER membrane with a signal-recognition particle (or SRP) which binds the ER signal sequence in the new protein as it emerges from the ribosome. Protein synthesis then slows down until the SRP-ribosome complex binds to an SRP receptor in the ER membrane.

The SRP is then released, passing the ribosome to a protein translocation channel in the ER membrane. Thus the SRP and SRP-receptor function as molecular matchmakers, connecting ribosomes that are synthesizing proteins containing ER signal sequences to available ER translocation channels.

In addition to directing proteins to the ER, the signal sequence functions to open the translocation channel. The protein translocation channel then inserts the polypeptide chain into the membrane and starts to transfer it across the lipid bilayer. The signal peptide remains bound to the channel while the rest of the protein chain is threaded through the membrane as a large loop.

Once the protein has passed through the membrane it is released into the ER lumen. After the signal sequence has been cleaved off by a signal peptidase located on the luminal side of the ER membrane; the signal peptide is then released from the translocation channel into the membrane and rapidly degraded.

It is thought that a protein serving as a plug then binds from the ER lumen to close the inactive channel. But not all proteins that enter the ER are released into the ER lumen; some remain embedded in the ER membrane as transmembrane proteins.

For clarity's sake, the membrane-bound ribosome will be omitted to illustrate the translocation of transmembrane proteins into the ER membrane. In the simplest case, that of a transmembrane protein with a single membrane-spanning segment, the N-terminal signal sequence initiates translocation, just as for a soluble protein. But the transfer process is halted by an additional sequence of hydrophobic amino acids, a stop-transfer sequence, further in the polypeptide chain. The stop-transfer sequence is released laterally from the translocation channel and drifts into the plane of the lipid bilayer, where it forms a membrane-spanning segment that anchors the protein in the membrane.

As a result, the translocated protein ends up as a transmembrane protein inserted in the membrane with a defined orientation—the N-terminus on the luminal side of the ER membrane and the C-terminus on the cytosolic side. The transmembrane protein retains its orientation throughout all subsequent vesicle budding and fusion events.

In some transmembrane proteins, an internal signal sequence is used to start the protein transfer. In these cases hydrophobic signal sequences are thought to work in pairs: an internal start-transfer sequence serves to initiate translocation, which continues until a stop-transfer sequence is reached; the two hydrophobic sequences are then released into the bilayer, where they remain anchored.

In complex multipass proteins, in which many hydrophobic regions span the bilayer, additional pairs of stop and start sequences come into play: one sequence reinitiates translocation further down the polypeptide chain, and the other stops translocation and causes polypeptide release... and so on for subsequent starts and stops.

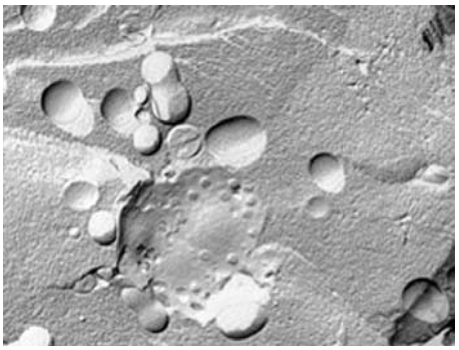
Thus, multipass membrane proteins are stitched into the lipid bilayer as they are being synthesized, by a mechanism resembling a sewing machine.

Storyboard and Animation: Thomas Dallman, Bioveo

12.7 Freeze Fracture of Yeast Cell <GATC>

Find me:

- outer nuclear membrane
- inner nuclear membrane
- nuclear pore complexes



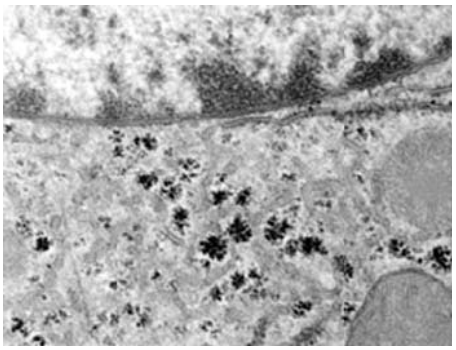
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

12.8 Liver Cell: View 4 <GGTC>

Find me:

- nuclear envelope
- nuclear pore complex
- ribosomes bound to outer nuclear membrane
- rough endoplasmic reticulum



Doug Bray
The University of Lethbridge, Canada

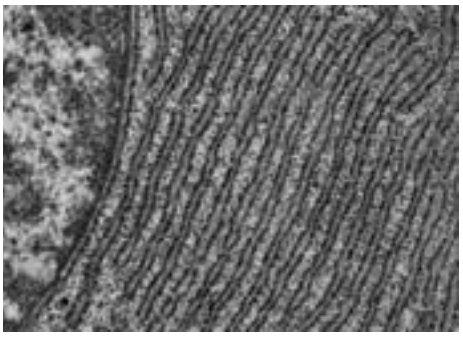
Brian Oates and Cyprien Lomas
The University of British Columbia

12.9 Pancreatic Secretory Cell <CGTA>

Find me:

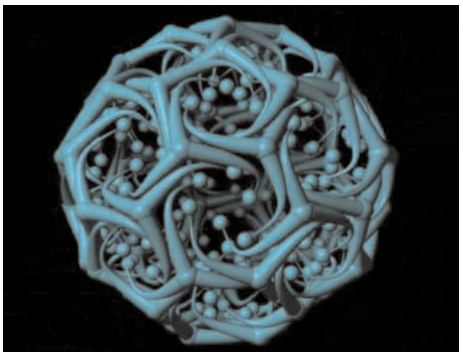
- nuclear lamina
- outer nuclear membrane
- nuclear pores
- ER lumen

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet. Springer-Verlag. New York, 1975.



Lelio Orci and Alain Perrelet

13.1 Clathrin <TATT>



Eucaryotic cells take in extracellular molecules through a process called endocytosis, in which the plasma membrane invaginates and pinches off cargo-filled vesicles. This movie shows a shows a series of electron micrographs that have been artificially morphed to show the process of endocytosis as it may occur.

The process involves a variety of molecules, including the cargo molecules that the cell takes in; the receptors that capture the cargo molecules; and molecules called adaptins that mediate contact between the receptors and the clathrin molecules that act to shape the vesicle forming at the plasma membrane.

Individual clathrin molecules can be seen in the electron microscope as three-legged structures, called triskelions. Each triskelion contains three heavy chains and three light chains.

When a clathrin coat forms on a membrane, the globular domains that make up the tips of the heavy chains bind to adaptins, which interact with cargo membrane proteins.

The assembly of a clathrin coat can occur spontaneously as numerous individual triskelions come together, interact through their leg domains, and ultimately form a closed cage.

Part I: Electron Micrograph, M.M. Perry and A.B. Gilbert
Part II: Electron Micrograph, Ernst Ungewickell, Hanover Medical School
Part III: Tomás Kirchhausen, Harvard Medical School

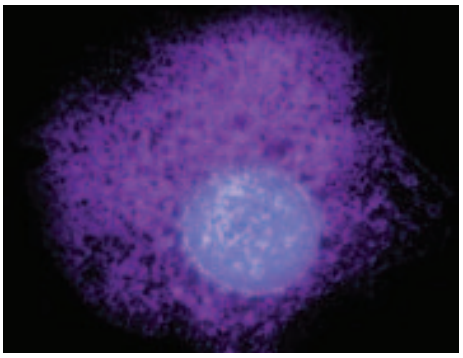
Electron Microscopy: Barbara M.F. Pearse, Medical Research Council, Laboratory of Molecular Biology

Animation:
Part I: Sumanas, Inc. (www.sumanasinc.com)
Part III: Alison Bruce

E. ter Haar, A. Musacchio, S.C. Harrison, and T. Kirchhausen. Atomic structure of clathrin: a β-propeller terminal domain joins an a-zig-zag linker. *Cell* 95:563–573, 1998.

A. Musacchio, C.J. Smith, A.M. Roseman, S.C. Harrison,T. Kirchhausen, B.M. Pearse. Functional organization of clathrin in coats: combining electron cryomicroscopy and x-ray crystallography. *Molecular Cell* 3:761–770, 1999.

13.2 Biosynthetic Secretory Pathway <GCTG>



Jennifer Lippincott-Schwartz
NICHD, National Institutes of Health

Fluorescently labeled membrane proteins start their journey to the plasma membrane after synthesis in the endoplasmic reticulum.

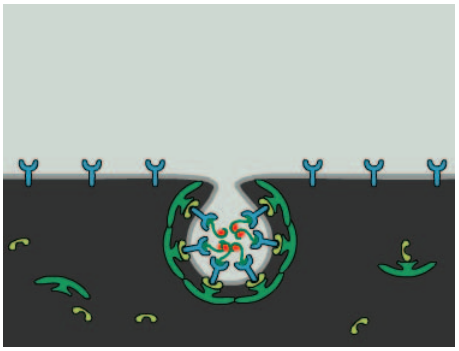
They are first dispersed throughout the extensive membrane network of the endoplasmic reticulum from where they move to exit sites that form in random locations in the membrane network. At each of these sites, the membrane proteins are concentrated and packaged into transport vesicles. Clusters of the transport vesicles fuse to form transport intermediates.

At the next stage, transport intermediates move along microtubule tracks to the Golgi apparatus near the center of the cell. The membrane proteins exit the Golgi apparatus. They move in transport vesicles that are now pulled outward on microtubules, which deliver them to the plasma membrane.

Each time a Golgi-derived vesicle fuses with the plasma membrane, its content proteins disperse.

Video reproduced from: *The Journal of Cell Biology* 143:1485–1503, Figure 1A, 1998.

13.3 Receptor-Mediated Endocytosis <GCTA>



Cholesterol circulates in the bloodstream and then enters cells by a process called receptor-mediated endocytosis. Instead of circulating freely, cholesterol molecules are derivatized and packed inside lowdensity lipoprotein particles, or LDLs. A protein and phospholipid layer surrounds the cholesterol molecules. The protein portion is recognized by LDL receptors on the surface of cells.

An adaptor molecule, called adaptin, binds to the tail of the LDL receptor that protrudes into the cytosol. Adaptin recruits clathrin molecules, which start coating the membrane. Assembly of the clathrin coat causes the membrane to bend and invaginate, forming a vesicle that buds off inside the cell, taking with it LDL receptors and the LDL particles bound to them.

Once inside the cell, the vesicle uncoats and fuses with the endosome, the intracellular compartment that first receives all endocytosed material. The endosome has a low internal pH, which causes the LDL receptors to release their cargo.

Empty LDL receptors are recycled to the plasma membrane in vesicles that bud off from the endosome. Each LDL receptor makes a round trip from the plasma membrane to the endosome and back every 10 minutes.

Meanwhile, the LDL particles need to be disassembled. The endosomal content is delivered to a lysosome, which contains hydrolytic enzymes that can digest the particles. Free cholesterol is liberated together with amino acids and small peptides generated by digestion of LDL proteins. The cholesterol is then released into the cytosol to be used in the synthesis of new membranes.

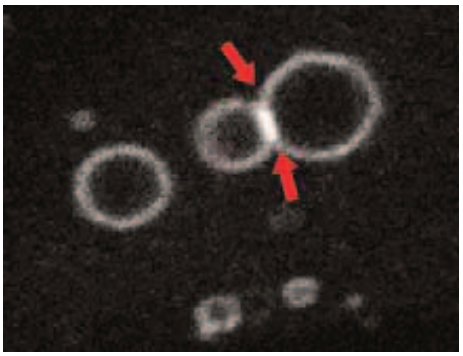
Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

13.4 Endosome Fusion <AAAA>

In these cells, fluorescent Rab5 protein has been overexpressed. Rab5 binds to endosomes and promotes their fusion with one another, thereby increasing the steady-state size of individual endosomal compartments.

Individual membrane fusion events can be observed at higher magnification.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



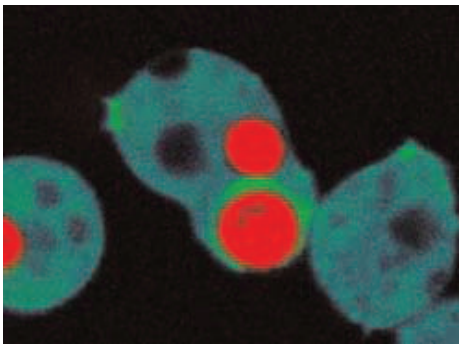
Philip D. Stahl, Alejandro Barbieri and Richard Roberts
Washington University School of Medicine in St. Louis

13.5 Phagocytosis <TCAT>

Phagocytosis allows cells to take up large particles, such as these yeast cells that are being engulfed by the slime mold *Dictostelium*.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

Video reproduced from: M. Maniak, R. Rauchenberger, R. Albrecht, J. Murphy, and G. Gerisch. Coronin involved in phagocytosis. *Cell* 83:91–924. © 1995, with permission from Elsevier Science.



Markus Maniak
University of Kassel, Germany

13.6 Exocytotic Transport <ACAG>

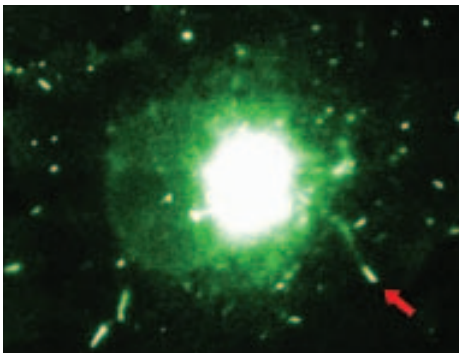
Passenger proteins exiting the Golgi apparatus on the way to the cell surface are often packaged into tubular transport vesicles of significant size.

Such tubular vesicles can branch and fragment before they fuse with the plasma membrane.

The transport vesicles move along microtubules which are stained here with a red fluorescent dye.

The green cell in the corner does not contain fluorescent microtubules.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



Jennifer Lippincott-Schwartz
NICHD, National Institutes of Health

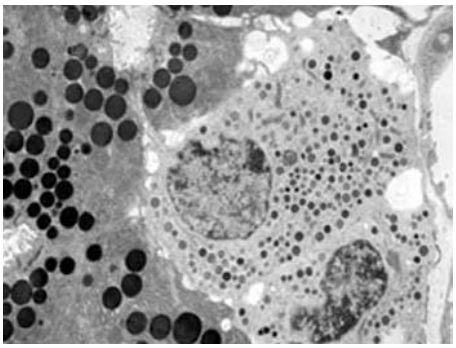
Patrick Keller and Kai Simons
European Molecular Biology Laboratory

13.7 Pancreas: View 1 <CAGC>

Find me:

- cell outlines
- nuclei
- secretory vesicles
- microvilli

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet. Springer-Verlag. New York, 1975.



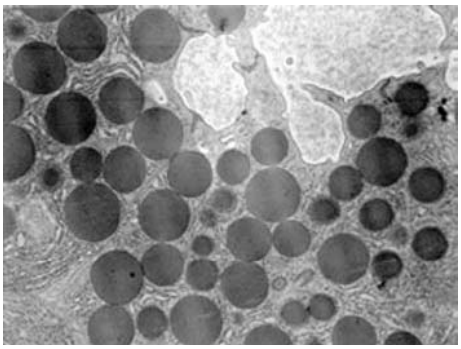
Lelio Orci and Alain Perrelet

13.8 Pancreas: View 2 <CCTA>

Find me:

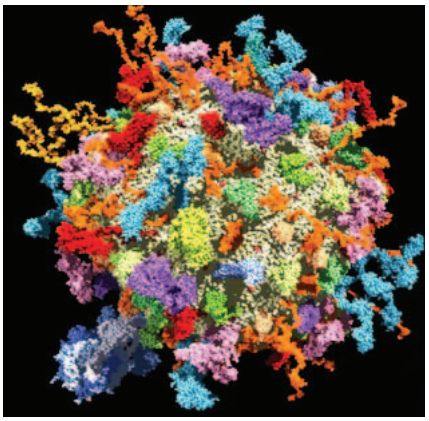
- secretory vesicles
- pancreatic duct
- tight junctions
- centrioles

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet. Springer-Verlag. New York, 1975.



Lelio Orci and Alain Perrelet

13.9 Synaptic Vesicle <ACCA>



Jürgen Haas
Helmut Grubmüller
Reinhard Jahn
Max-Planck-Institute for Biophysical Chemistry

This model represents a cutaway view of a synaptic vesicle, with the membrane lipids and proteins drawn to scale.

Each synaptic vesicle membrane contains approximately 7000 phospholipid molecules and 5700 cholesterol molecules. Each vesicle also contains close to 50 different integral membrane protein molecules, which vary widely in their relative abundance.

The most abundant protein is a SNARE protein, called v-SNARE synaptobrevin. This molecule participates in membrane fusion at the synaptic terminal, and there are about 70 copies of this protein per vesicle.

By contrast, the vesicle contains only one to two copies of V-ATPase. V-ATPase uses energy from ATP hydrolysis to pump H^+ into the vesicle lumen. The resulting electrochemical gradient provides the energy to import neurotransmitter molecules, such as glutamate, into the vesicle. Through a transporter in the membrane, protons flow down their concentration gradient to the outside of the vesicle, as glutamate enters by an antiport mechanism. In this way, a vesicle is loaded with thousands of glutamate molecules.

This model was created, in part, from an electron tomogram of a real synaptic vesicle. The tomogram combines visual slices of the vesicle at various angles to create a unified image. Other data, including structural data of the synaptic vesicle's proteins, have been combined with the data from the tomogram to create the three-dimensional model on the left.

Note, only 70% of the membrane proteins estimated to be present in the membrane are depicted in the model. A real vesicle would be covered by an even more dense forest of proteins.

14.1 Tomogram of Mitochondrion <CGAT>

Terrence G. Frey
San Diego State University

Guy Perkins
University of California, San Diego

A mitochondrion contained in a one-half micrometer thick section of chicken brain is viewed with a high voltage electron microscope. When the section is tilted in the microscope, it can be viewed from many different angles, and a large amount of three-dimensional detail becomes apparent. Images from such a series of tilted views can be used to calculate a three-dimensional reconstruction, or tomogram, of the mitochondrion.

The tomogram of the same tissue slice is shown here as a series of stacked images. The movie steps through the images one by one, from the bottom of the stack, to the top, and back. This allows us to trace individual membranes in three-dimensions.

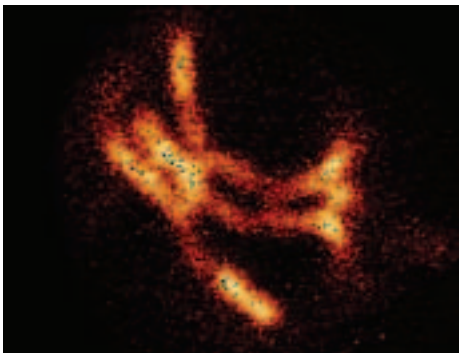
To create a three-dimensional model, membranes in an individual slice of the tomogram are traced. In this case the inner membrane is traced in light blue, where it parallels the outer membrane, and traced in yellow, where it folds into the cristae that protrude into the mitochondrial interior. The tracings from all sections are then modeled as three-dimensional surfaces, and displayed as a three-dimensional model by a computer program. Such a model can now be viewed from any angle.

In this view, only four cristae are shown and the others are omitted. The cristae are colored differently and show the variety of shapes and connections to the inner membrane in a single mitochondrion.

The model also shows the reconstitution of the outer mitochondrial membrane, represented in dark blue, as well as two fragments of endoplasmic reticulum. Regions of such close proximity between the two organelles are quite frequently seen in cells. Note that there is no continuity between the mitochondrial and endoplasmic reticulum membranes. Lipids are thought to be shuttled between the two organelles by special carrier proteins that operate in this gap.

Final composition: Graham Johnson, Fivth Element (www.fivth.com)

14.2 Mitochondrial Fission and Fusion <AGTA>



Gustavo Pesce
Howard Hughes Medical Institute, University of California, San Francisco

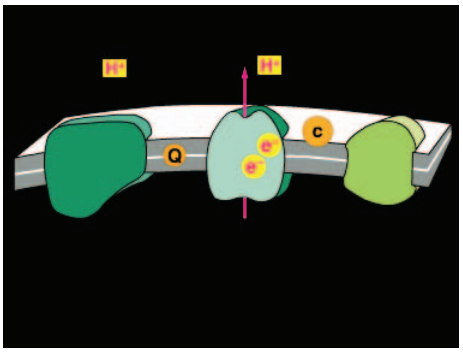
Peter Walter
Howard Hughes Medical Institute, University of California, San Francisco

Dynamic properties of the mitochondrial network can be seen in this living yeast cell which expresses the green fluorescent protein GFP fused to a mitochondrial signal sequence.

Membrane fusion and fission events constantly reshape the organelle.

Using a confocal microscope, an optical slice containing only the top focal plane of the cell is recorded in this movie; the remainder of the network is out of focus.

14.3 Electron-Transport Chain <TGGG>



The mitochondrion is the site of most of the cell's energy production. After food molecules are processed in the cytosol, they enter the mitochondrion, where they are further broken down. In the citric acid cycle, the molecules are stripped of high-energy electrons, which are donated to carrier molecules, such as NADH.

The carrier molecules transfer the high-energy electrons to a chain of proteins, called the electron transport chain, which is embedded in the inner mitochondrial membrane. The chain acts as a pump, using the energy of the electrons to move protons from one side of the membrane to the other. The pumping creates a proton gradient across the membrane, which the mitochondrion can tap to make the fuel molecule ATP.

The electron transfer begins at a multiprotein complex called the NADH dehydrogenase complex. This complex has a higher affinity for electrons than NADH, and easily strips away the high-energy electrons. As the electrons are transferred from one protein to another in the complex, energy is released and used to pump protons across the membrane.

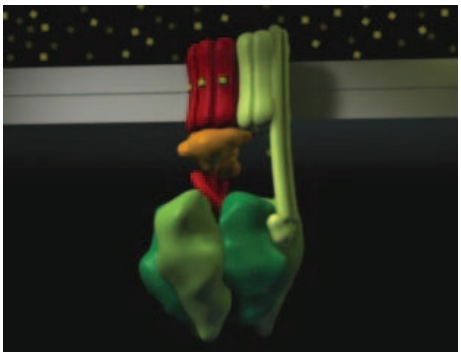
Electrons are then transferred to ubiquinone, a different carrier that shuttles them to the next way station, called the cytochrome *b*-c_1 complex, which again pumps protons as they flow through it. Because each complex in the chain has a higher affinity for the electrons than the previous one, the electrons keep moving through the chain unidirectionally.

Finally, cytochrome *c* delivers the electrons to the cytochrome oxidase complex, a third proton pump. The cycle repeats until the cytochrome oxidase complex has accumulated 4 electrons.

From there, they are handed over to molecular oxygen. Oxygen takes up the electrons as it combines with protons, forming water as product, thereby completing the step-wise path of the combustion of the food molecules.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

14.4 ATP Synthase—A Molecular Turbine <ATCG>



Video:
Masasuke Yoshida, Tokyo Institute of Technology

ATP synthase is a molecular machine that works like a turbine to convert the energy stored in a proton gradient into chemical energy stored in the bond energy of ATP.

The flow of protons down their electrochemical gradient drives a *rotor* that lies in the membrane. It is thought that protons flow through an entry open to one side of the membrane and bind to rotor subunits. Only protonated subunits can then rotate into the membrane, away from the static channel assembly. Once the protonated subunits have completed an almost full circle, and have returned to the static subunits, an exit channel allows them to leave to the other side of the membrane. In this way, the energy stored in the proton gradient is converted into mechanical, rotational energy.

The rotational energy is transmitted via a shaft attached to the rotor that penetrates deep into the center of the characteristic lollipop head, the F1 ATPase, which catalyzes the formation of ATP.

The F1 ATPase portion of ATP synthase has been crystallized.

Its molecular structure shows that the position of the central shaft influences the conformation and arrangement of the surrounding subunits. It is these changes that drive the synthesis of ATP from ADP. In this animated model, different conformational states are lined up as a temporal sequence as they would occur during rotation of the central shaft.

Like any enzyme, ATP synthase can work in either direction. If the concentration of ATP is high and the proton gradient low, ATP synthase will run in reverse, hydrolyzing ATP as it pumps protons across the membrane.

To show the rotation of the central shaft, a short fluorescent actin filament was experimentally attached to it. Single filaments attached to single F1 ATPases can be visualized in the microscope.

When ATP is added, the filament starts spinning, directly demonstrating the mechanical properties of this remarkable molecular machine.

Animation: Graham Johnson, Fivth Element (www.fivth.com)

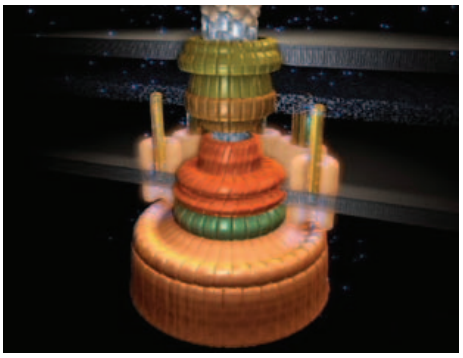
14.5 ATP Synthase—Disco <GAGA>

Subunits:
Center (gamma subunit): Toyoki Amano
Left (beta subunit 1): Hiroyuki Noji
Right (beta subunit 2): Satoshi P. Tsunoda
Back (beta subunit 3): Masaki Shibata

Dance direction:
Nagatsuta Bon-Odori

Camera work and production:
Hiroyuki Noji

14.6 Bacterial Flagellum <ACTA>



Video:
Howard C. Berg, Harvard University

3D Animation and Flagellar Structures:

Keiichi Namba, Protonic NanoMachine Project, ERATO, JST & Osaka University

Many species of bacteria propel themselves through their environment by spinning helical motorized flagella. *Rhodobacter* cells have one flagellum each, whereas *E. coli* cells have multiple flagella that rotate in bundles. Each flagellum consists of a helical filament that is 20 nanometers wide and up to 15 microns long and spins on the order of 100 times per second. These animations show a series of schematized and speculative models about how bacterial flagella might function and assemble.

Just outside of the cell wall, the filament is connected to a flexible rotating hook.

The filament, the hook, and a structure called the basal body (located below the cell's surface) make up the three parts of the flagellum. The basal body consists of a rod and a series of rings embedded in the inner membrane, the peptidoglycan layer, and the outer membrane.

Some of the rings make up the flagellar motor, which can be divided into two major parts: the stator, which is attached to the peptidoglycan layer and, as its name implies, remains stationary, and the rotor, which rotates.

The motor derives its power from a proton gradient across the membrane. In this example, a high concentration of protons exists outside and a low concentration exists inside the cell.

The protons flow through the interface between two types of proteins, called MotA and MotB that make up the stator.

Mutational studies suggest that a conserved aspartic acid in MotB functions in proton conductance. Each stator contains two MotB proteins and therefore also contains two of these important aspartic acids.

Although the molecular mechanism of rotation is not known, one possible model describes protons moving through the channels in the stators and binding to the aspartic acid in the Mot B proteins. This binding causes a conformational change in MotA proteins, resulting in the first power stroke that moves the rotor incrementally.

At the end of the first power stroke, the two protons are released into the cytoplasm. The proton loss causes a second conformational change that drives the second power stroke, once again engaging the rotor.

Although the mechanism for motor function is not yet certain, many details of flagellar assembly have been determined.

Flagella begin their assembly with structures in the inner membrane. 26 subunits of an integral membrane protein called FliF come together in the plasma membrane to form the MS ring. The FliG proteins assemble under the MS ring. FliG, along with FliM and FliN proteins, make up the rotor.

Flagellar proteins destined to be part of the extracellular portion of the flagellum are exported from the cell by a flagellum-specific export pathway and assembled at the center.

MotA and Mot B form the stationary part of the flagellar motor—the stator. Both are integral membrane proteins, but MotB is also anchored to the rigid peptidoglycan layer, keeping the stator proteins fixed in place.

The subunits of the rod portion of the rotor move up through the hollow cylinder in the assembly and, assisted by cap proteins, build up the rod in a proximal to distal fashion.

Another set of rings, called L and P rings, are found in gram negative bacteria, such as *E. coli.* They penetrate the outer membrane forming a bearing for the rod.

As the rod cap is exposed outside the L ring, it dissociates and is replaced by a hook cap that guides the assembly of the hook proteins.

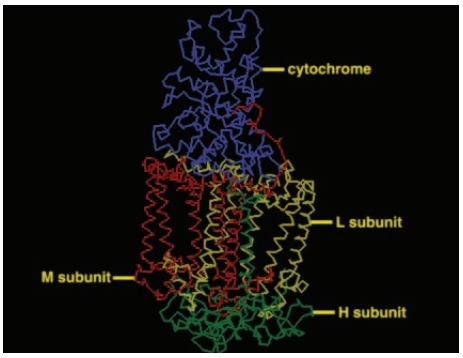
After the hook is assembled, the hook cap dissociates, and a series of junction proteins assemble between the hook and future filament.

Finally, yet another cap is built and filament proteins assemble. Like the rod and hook proteins, they travel through the hollow channel inside the filament to reach the distal end. The cap rotates which causes the subunits to build in a helical fashion. A complete filament can consist of 20,000 to 30,000 subunits.

14.7 Photosynthetic Reaction Center <ATCA>

The bacterial photosynthetic reaction center is a large complex of four protein subunits. Three subunits, called the H, L, and M subunits, contain hydrophobic α helices that span the membrane and anchor the complex. The fourth subunit is a cytochrome that is peripherally attached.

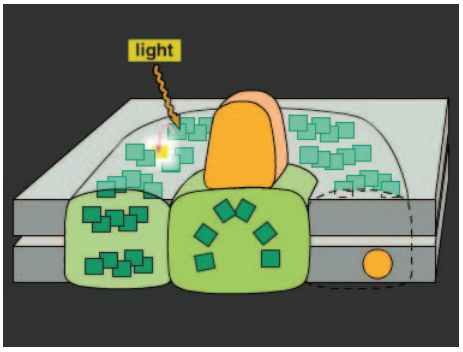
Energy transfer through the reaction center involves pigment molecules that are organized in the interior of the protein complex. Excited electrons generated after absorption of light move from centrally located chlorophylls to pheophytins. From there they move to a quinone, which is then released from the reaction center to feed the electrons into the electron transport chain. Electrons lost from the chlorophylls are replaced through a conduit of heme groups found in the cytochrome subunit.



PDB ID number *: 1DXR

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

14.8 Light Harvesting <GCAC>



In plant cells, chloroplasts carry out photosynthesis. These large, dedicated organelles contain a variety of membrane components that convert the energy in light into the energy carriers NADPH and ATP, which in turn fuel the production of sugars and other molecules required by the cell.

Chloroplasts have three distinct membrane systems: a two-membrane envelope akin to that surrounding mitochondria, and the internal thylakoid membrane system. Within the thylakoid membranes large antennae consisting of hundreds of light-absorbing chlorophyll molecules capture light energy. When a chlorophyll molecule absorbs light, the energy bumps from one chlorophyll molecule to another, until it passes to a special pair of chlorophyll molecules in the reaction center of photosystem II.

In the reaction center, the energy causes an electron in chlorophyll to jump to a higher energy level. This jump initiates a long series of electron transfers. First, a neighboring molecule accepts the high-energy electron. In the meantime, another neighboring molecule donates a low-energy electron to the deficient chlorophyll molecule. In turn, this donor molecule receives a low-energy electron from water. After this series of transfers occurs four times, two water molecules are split into one molecule of oxygen gas and four protons.

The photosystem shares the thylakoid membranes with an electron transport chain. When light bumps an electron out of the photosystem, the electron is removed by a small diffusible carrier molecule. As shown before, water replenishes lost electrons. The diffusible carrier molecule brings the electrons to the cytochrome b_6-f complex, which uses part of the electrons' energy to pump protons across the membrane. From there, the electrons travel to photosystem I.

Just like photosystem II, photosystem I absorbs light through its own antenna system and kicks electrons to an even higher energy level. After two such high-energy electrons have been produced and delivered to ferredoxin NADP reductase, they drive the reduction of $NADP^+$ to NADPH. To liberate a molecule of oxygen from two molecules of water, the cycle must occur twice more.

To make the system work, each member of the electron transport chain has to be finely tuned to have an appropriate tendency to receive or donate electrons, measured as a redox potential. When photosystem II is excited by light, it has a high tendency to donate electrons. The next component, having a lower redox potential, is more likely to receive electrons. The loss of an electron from photosystem II now makes it an excellent electron acceptor, receiving electrons from water. The next series of carriers in the chain make better and better acceptors, drawing the electron through the chain.

The released energy is used to generate a proton gradient that fuels ATP production. To produce another high-energy electron, photosystem I must also absorb a photon of light. This second energized electron has an even higher energy level than the first, and can pass to ferrodoxin NADP reductase. Two such electrons will produce a molecule of NADPH.

For every two water molecules split by photosystem II, 4 electrons are donated to produce NADPH.

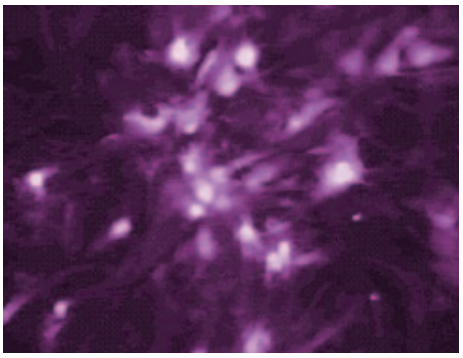
Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

15.1 Calcium Signaling <CGTC>

In this experiment, glial cells from the rat brain are grown in cell culture.

Calcium concentrations are visualized with a fluorescent dye that becomes brighter when calcium ions are present. In the presence of small amounts of a neurotransmitter, individual cells light up randomly as ion channels open up and allow calcium ions to enter the cell.

Occasionally, calcium waves are transmitted to adjacent cells through gap junctions at regions where the cells contact each other.



Ann H. Cornell-Bell
Viatech Imaging

Steven Finkbeiner
Gladstone Institute of Neurological Disease at the University of California, San Francisco

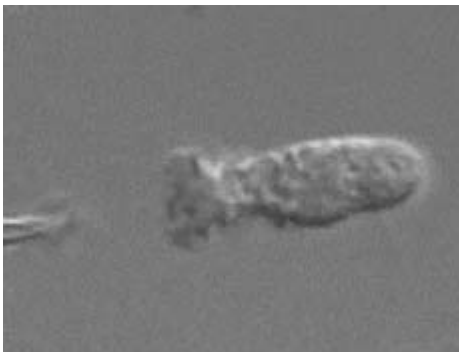
Mark S. Cooper
University of Washington

Stephen J. Smith
Stanford University School of Medicine

15.2 Chemotaxis of Neutrophils <GTCG>

These human neutrophils, taken from the blood of a graduate student, are mobile cells that will quickly migrate to sites of injury to help fight infection. They are attracted there by chemical signals that are released by other cells of the immune system or by invading microbes.

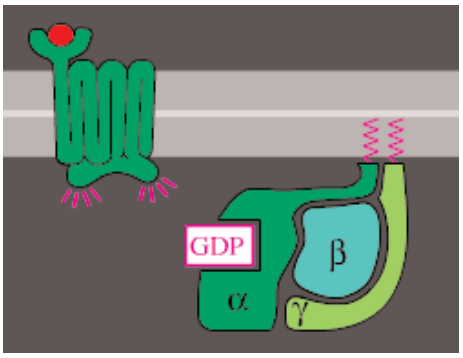
In this experiment tiny amounts of chemoattractant are released from a micropipette. When neutrophils sense these compounds they polarize and move towards the source. When the source of the chemoattractant is moved, the neutrophil immediately sends out a new protrusion, and its cell body reorients towards the new location.



Henry Bourne and John Sedat
University of California, San Francisco

Orion Weiner
Harvard Medical School

15.3 G-Protein Signaling <ATTC>



Many G-protein-coupled receptors have a large extracellular ligand-binding domain.

When an appropriate protein ligand binds to this domain, the receptor undergoes a conformational change that is transmitted to its cytosolic regions, which now activate a trimeric GTP-binding protein (or G protein for short).

As the name implies, a trimeric G protein is composed of three protein subunits called alpha, beta, and gamma. Both the alpha and gamma subunits have covalently attached lipid tails that help anchor the G protein in the plasma membrane.

In the absence of a signal, the alpha subunit has a GDP bound, and the G protein is inactive. In some cases, the inactive G protein is associated with the inactive receptor, while, in other cases, as shown here, it only binds after the receptor is activated. In either case, an activated receptor induces a conformational change in the alpha subunit, causing the GDP to dissociate.

GTP, which is abundant in the cytosol, can now readily bind in place of the GDP. GTP binding causes a further conformational change in the G protein, activating both the alpha subunit and beta-gamma complex. In some cases, as shown here, the activated alpha subunit dissociates from the activated beta–gamma complex, whereas in other cases the two activated components stay together.

In either case, both of the activated components can now regulate the activity of target proteins in the plasma membrane, as shown here for a GTP-bound alpha subunit. The activated target proteins then relay the signal to other components in the signaling cascade.

Eventually, the alpha subunit hydrolyses its bound GTP to GDP, which inactivates the subunit. This step is often accelerated by the binding of another protein, called a regulator of G-protein signaling (or RGS). The inactivated, GDP-bound alpha subunit now reforms an inactive G protein with a beta–gamma complex, turning off other downstream events.

As long as the signaling receptor remains stimulated, it can continue to activate G-proteins. Upon prolonged stimulation, however, the receptors eventually inactivate, even if their activating ligands remain bound.

In this case, a receptor kinase phosphorylates the cytosolic portions of the activated receptor. Once a receptor has been phosphorylated in this way, it binds with high affinity to an arrestin protein, which inactivates the receptor by preventing its interaction with G proteins.

Arrestins also act as adaptor proteins, and recruit the phosphorylated receptors to clathrin-coated pits, from where the receptors are endocytosed, and afterwards they can either be degraded in lysosomes or activate new signaling pathways.

Animation: Thomas Dallman

15.4 cAMP Signaling <AGAT>

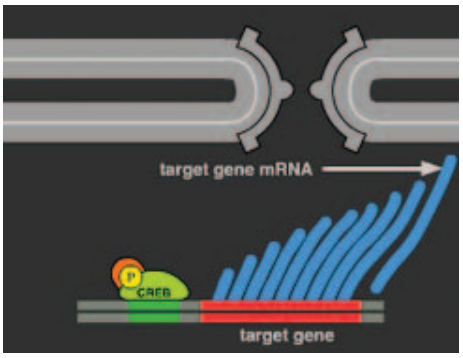
Adenylyl cyclase is a membrane-bound enzyme whose catalytic domain is activated by the GTP-bound form of the stimulatory G protein alpha subunit (or G-alpha-s for short).

Activated adenylyl cyclase converts ATP to cyclic AMP which then acts as a second messenger that relays the signal from the G-protein-coupled receptor to other components in the cell.

In most animal cells, cyclic AMP activates cyclic-AMP-dependent protein kinase (or PKA). In the inactive state PKA consists of a complex of two catalytic subunits and two regulatory subunits. The binding of cyclic AMP to the regulatory subunits alters their conformation and liberates the catalytic subunits which are now active and phosphorylate specific target proteins.

In some endocrine cells, for example, the activated PKA catalytic subunits enter the nucleus, where they phosphorylate a transcription factor called CREB. Phosphorylated CREB then recruits a CREB-binding protein. This complex activates transcription after binding to specific regulatory regions that are present in the promoters of appropriate target genes.

Animation: Thomas Dallman, Bioveo



Original illustrations:
Nigel Orme

15.5 Ras <GAAC>

The Ras protein is a representative example of the large family of GTPases that functions as molecular switches. The nucleotide-binding site of Ras is formed by several conserved protein loops that cluster at one end of the protein. In its inactive state, Ras is bound tightly to GDP.

As a molecular switch, Ras can toggle between two conformational states depending on whether GDP or GTP is bound. Two regions, called switch 1 and switch 2, change conformation dramatically. The change in conformational state allows other proteins to distinguish active Ras from inactive Ras. Active, GTP-bound Ras binds to, and activates, downstream target proteins in the cell signaling pathways.

A space-filling model shows that the conformational changes between the GDP and GTP bound forms of Ras spread over the whole surface of the protein. The two switch regions move the most.

Ras hydrolyzes GTP to switch off; that is, to convert from the GTP-bound state to the GDP-bound state. This hydrolysis reaction requires the action of a Ras GTPase activating protein, or Ras-GAP for short. Ras-GAP binds tightly to Ras burying the bound GTP. It inserts an arginine side chain directly into the active site. The arginine, together with threonine and glutamine side chains of Ras itself, promotes the hydrolysis of GTP.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

PDB ID number *: Compilation of c-H-Ras p21 protein catalytic domain complex with GDP & structure of p21-Ras complexed with GTP at 100K (4Q21 & 1QRA); Ras-Rasgap complex (1WQ1)

15.6 Calmodulin <CTTC>

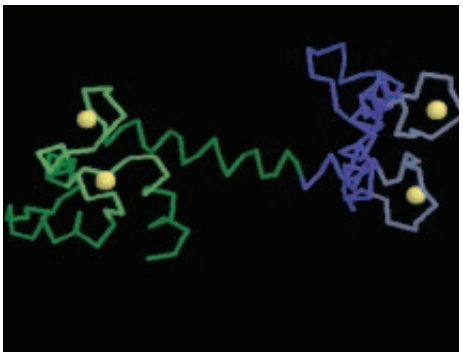
Calmodulin is a dumbbell-shaped protein formed by a single polypeptide chain. Its N-terminal and C-terminal globular domains are separated by an extended central helix. Each globular domain contains two high-affinity calcium-binding sites.

Binding of four calcium ions induces major allosteric changes in calmodulin. Most notably, the two globular domains rotate relative to each other. These conformational changes enable calmodulin to bind to target proteins and regulate their activity.

Reminiscent of a boa grabbing its prey, calcium-bound calmodulin captures helical peptides on target proteins by wrapping tightly around them. To make this possible, the central helix of calmodulin breaks into two helices now connected by a flexible loop. Although the calcium ions remain tightly bound during this remarkable reaction, they are not shown in the animated part of this movie.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

Source: Intermediate structures provided by Eric Martz (www.umass.edu/microbio/rasmol/) and calculated by the Yale University Morph Server, Mark Gerstein and Werner Krebs (bioinfo.mbb.yale.edu/)

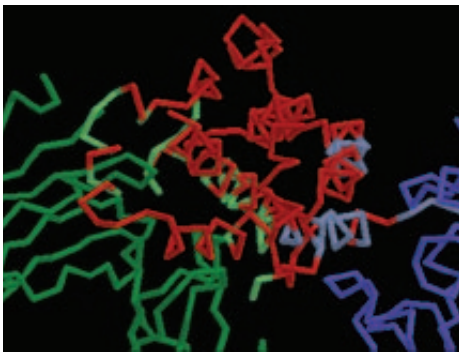


PDB ID number *: Calcium-free calmodulin (1CFD); Compilation of calcium-free calmodulin & calcium-bound calmodulin (1CFD & 1OSA); Compilation of calcium-bound calmodulin & calcium-bound calmodulin complexed with rabbit skeletal myosin light chain kinase (1OSA & 2BBM)

15.7 Growth Hormone Receptor <CGCT>

Human growth hormone receptor is a dimer of two identical subunits. Only the extracellular domains are shown. Its ligand, human growth hormone, binds in a cleft between the two subunits to activate the receptor. The lack of symmetry in this binding interaction is remarkable. While the receptor is a twofold symmetrical structure with two identical subunits, the growth hormone is a single chain asymmetric protein that binds as a monomer. Thus, the interfaces between each receptor subunit and the hormone are completely different.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

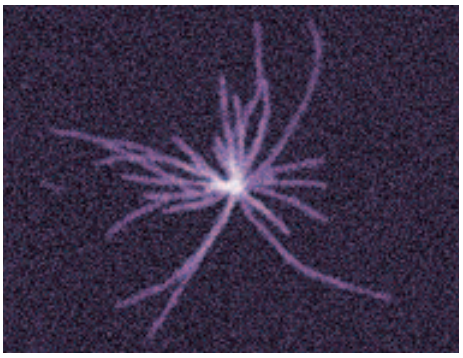


PDB ID number *: 3HHR

16.1 Dynamic Instability of Microtubules <CCCA>

Microtubules continually grow from this centrosome added to a cell extract. Quite suddenly however, some microtubules stop growing and then shrink back rapidly, a behavior called dynamic instability.

Music: Christopher Thorpe



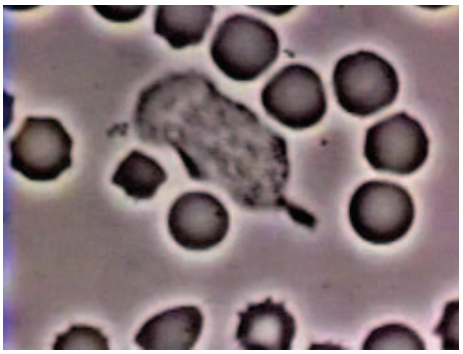
Timothy Mitchison
Harvard Medical School

16.2 Neutrophil Chase <TGTA>

Neutrophils are white blood cells that hunt and kill bacteria. In this spread a neutrophil is seen in the midst of red blood cells. *Staphylococcus aureus* bacteria have been added. The small clump of bacteria releases a chemoattractant that is sensed by the neutrophil. The neutrophil becomes polarized, and starts chasing the bacteria. The bacteria, bounced around by thermal energy, move in a random path, seeming to avoid their predator. Eventually, the neutrophil catches up with the bacteria and engulfs them by phagocytosis.

Digital capture: Tom Stossel, Brigham and Women's Hospital, Harvard Medical School

Music: Freudenhaus Audio Productions (www.fapsf.com)



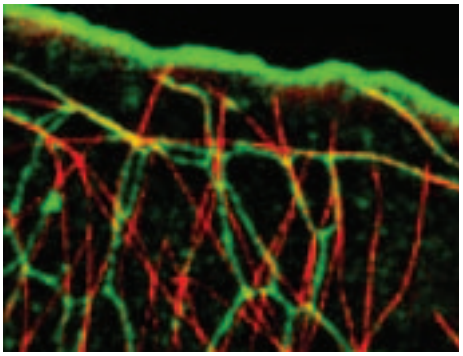
David Roger
Vanderbilt University

16.3 Microtubule and ER Dynamics <AAAT>

Governed by the principles of dynamic instability, microtubules constantly extend into the leading edge of a migrating cell and retract again.

Superimposed on the dynamic microtubule cytoskeleton (shown here in red), the membrane network of the endoplasmic reticulum (shown here in green) exhibits its own dynamic behavior as tubes are extended by motor proteins on the microtubule tracks.

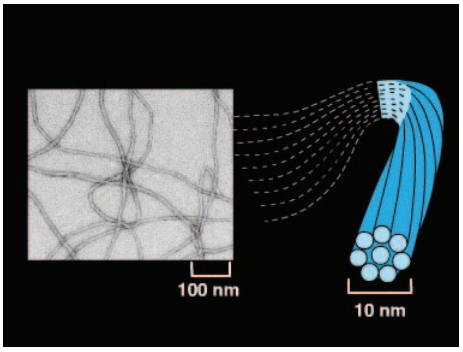
Video reproduced from: C.M. Waterman-Storer and E.D. Salmon. Endoplasmic reticulum tubes are distributed in living cells by three distinct microtubule dependent mechanisms. *Current Biology* 8:798–806. © 1998, with permission from Elsevier Science.



Clare M. Waterman-Storer
The Scripps Research Institute

Edward D. (Ted) Salmon
University of North Carolina at Chapel Hill

16.4 Intermediate Filaments <GCCA>



Electron Microscopy:
D.E. Kelly

Eucaryotic cells contain a complex network of filaments—intermediate filaments, microtubules, and actin filaments—that provide the cells with strength, structure, and movement. Although all eucaryotic cells contain microtubules and actin filaments, intermediate filaments are found only in vertebrates and a number of other soft-bodied animals.

Intermediate filaments are found in animal cells that require a lot of strength, such as the epithelial cells of the skin. Some of these filaments span the length of the cell, connecting cell–cell junctions called desmosomes.

These cables of intermediate filaments have a high tensile strength. Without these filaments, stretching or pressure on the epithelial sheet would cause it to rupture.

Each filament is ropelike, consisting of 8 thinner strands made of a precise hierarchical arrangement of protein subunits. At the lowest level, two monomers associate with each other to create a twisted dimer.

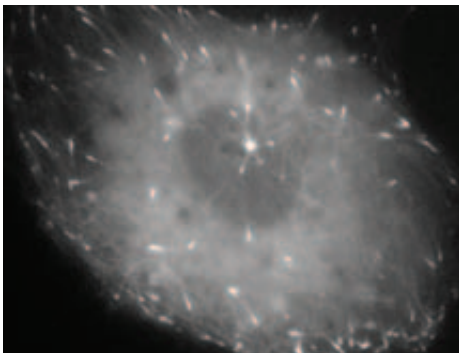
Two dimers then line up to form a staggered tetramer. Note that the two dimers are arranged in opposite orientations, with their amino terminal ends away from each other, so that the two ends of the tetramer are indistinguishable.

Tetramers then link end-to-end, thus building up one strand of an intermediate filament.

A total of eight strands stack together and twist around each other to create the intermediate filament. This stacking provides the extensive lateral contacts between the strands that give the filament its remarkable mechanical strength. An electron micrograph shows the appearance of intermediate filaments that have been assembled in a test tube.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

16.5 Microtubule Dynamics *in vivo* <TAAT>



Yuko Mimori-Kiyosue
KAN Research Institute

Shoichiro Tsukita
Faculty of Medicine, Kyoto University

EB1 is a protein that binds to the GTP-tubulin cap at the growing ends of microtubules.

Cells expressing an GFP-EB1 fusion protein reveal the spectacular dynamics of the microtubule cytoskeleton.

Note that many but not all microtubules in this cell grow from the centrosome.

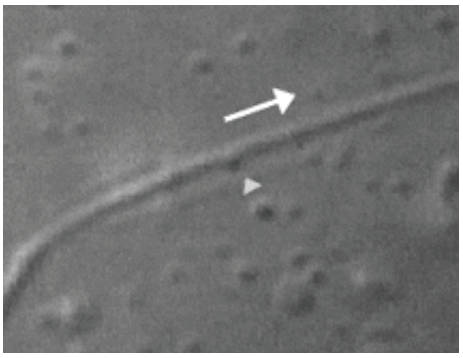
Only the ends of growing microtubules are visible in this experiment; those that are static or shrinking have lost their GTP-tubulin caps and do not bind EB-1.

In contrast, when all microtubules are labeled with GFP-tubulin, the true extent of the microtubule cytoskeleton emerges.

Both growing and shrinking microtubules can be observed.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

16.6 Organelle Movement on Microtubules <CAAT>



Nira Pollack
University of California, San Francisco

Ron D. Vale
Howard Hughes Medical Institute
University of California, San Francisco

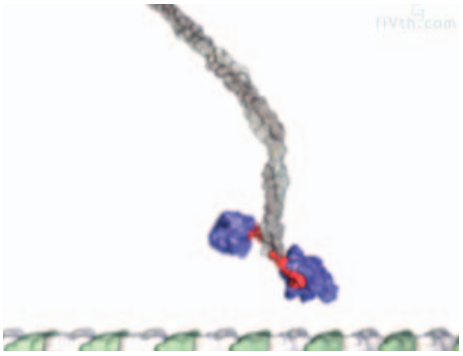
In this experiment a cell homogenate containing many different organelles is added to microtubules.

Motor proteins are normally attached to the organelles. When ATP is added as a fuel for the motor proteins, some organelles bind microtubules, and are moved along the tracks by their motors.

Most kinesin motors move towards the plus end of microtubules. Dynein motors always move in the opposite direction. Both motors are used to transport organelles, and occasionally a single organelle, which must have both types of motor attached, can be seen to switch directions.

The bi-directional traffic observed here is reminiscent of that in an intact cell.

16.7 Kinesin <GAAT>



Ron D. Vale
Howard Hughes Medical Institute
University of California, San Francisco

Ron Milligan
The Scripps Research Institute

The motor protein kinesin is a dimer with two identical motor heads. Each head consists of a catalytic core and a neck linker. In the cell, kinesins pull organelles along microtubule tracks. The organelle attaches to the other end of the long coiled-coil that holds the two motor heads together. The organelle is not shown here.

In solution, both kinesin heads contain tightly bound ADP, and move randomly, driven by Brownian motion. When one of the two kinesin heads encounters a microtubule, it binds tightly. Microtubule binding causes ADP to be released from the attached head. ATP then rapidly enters the empty nucleotide binding site.

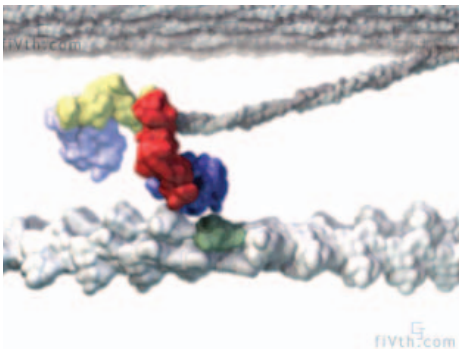
This nucleotide exchange triggers the neck linker to zipper onto the catalytic core. This action throws the second head forward, and brings it near the next binding site on the microtubule.

The attached trailing head hydrolyzes the ATP, and releases phosphate. As the neck linker unzippers from the trailing head, the leading head exchanges its nucleotide, and zippers its neck linker onto the catalytic core, and the cycle repeats.

In this way, kinesin dimers move processively, step-by-step, along the microtubule.

Animation: Graham Johnson, Fivth Element (www.fivth.com)

16.8 Myosin <ATAT>



Muscle myosin is a dimer with two identical motor heads that act independently. Each myosin head has a catalytic core and an attached lever arm. A coiled-coil rod ties the two heads together, and tethers them to the thick filament seen on top. The helical actin filament is shown at the bottom.

In the beginning of the movie, the myosin heads contain bound ADP and phosphate, and have weak affinity for actin.

Once one of the heads docks properly onto an actin subunit, phosphate is released. Phosphate release strengthens the binding of the myosin head to actin, and also triggers the force-generating power stroke that moves the actin filament. ADP then dissociates, and ATP binds to the empty nucleotide binding site, causing the myosin head to detach from the actin filament.

On the detached head, ATP is hydrolyzed, which re-cocks the lever arm back to its pre-stroke state. Thus, like a spring, the arm stores the energy released by ATP hydrolysis, and the cycle can repeat.

The actin filament does not slide back after being released by the motor head, because there are many other myosin molecules also attached to it, holding it under tension.

The swing of the lever arm can be directly observed on single myosin molecules, here visualized by high-speed atomic force microscopy.

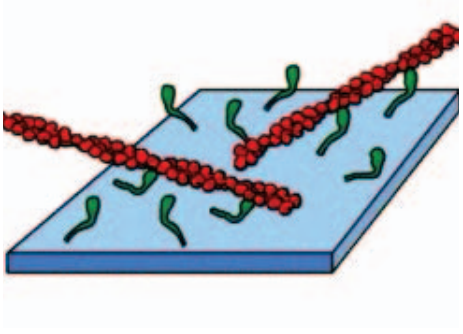
Animation: Graham Johnson, Fivth Element (www.fivth.com)

Part I:
Ron D. Vale
Howard Hughes Medical Institute
University of California, San Francisco

Ron Milligan
The Scripps Research Institute

Part II:
Toshio Ando
Kanazawa University, Japan

16.9 Crawling Actin <TTAT>

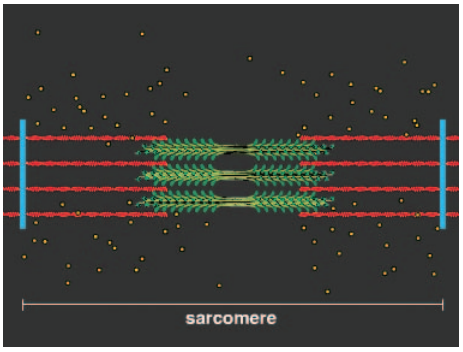


Myosin motors can be attached to the surface of a glass slide. Fluorescent actin filaments will bind to the motor domains of the attached myosins. When ATP is added, the myosin motors move the actin filaments.

This rapid movement can be observed in a fluorescence microscope as the actin filaments appear to crawl across the slide.

James Spudich
Stanford University School of Medicine

16.10 Muscle Contraction <CTGC>



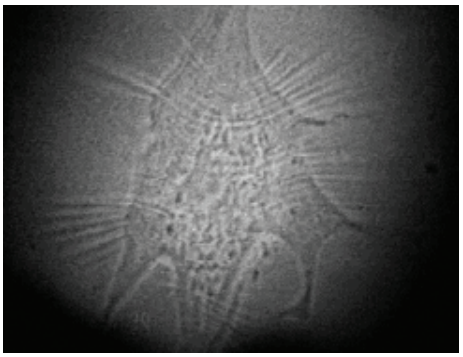
When a neuron stimulates a muscle cell, an action potential sweeps over the plasma membrane of the muscle cell. The action potential releases internal stores of calcium that flow through the muscle cell and trigger a contraction.

Muscle cells have an elaborate architecture that allows them to distribute calcium ions quickly throughout the cytosol. Deep tubular invaginations of the plasma membrane, called T-tubules, criss-cross the cell. When the cell is stimulated, a wave of depolarization—that is an action potential—spreads from the synapse over the plasma membrane and via the T tubules deep into the cell. A voltage-sensitive protein in these membranes opens a calcium-release channel in the adjacent sarcoplasmic reticulum, which is the major calcium store in muscle cells, thereby releasing a burst of calcium ions all throughout the cytosol of the cell.

Within a contractile bundle of a muscle cell, called a myofibril, the calcium interacts with protein filaments to trigger contraction. In each contracting unit, or sarcomere, thin actin and thick myosin filaments are juxtaposed but cannot interact in the absence of calcium. This is because myosin-binding sites on the actin filaments are all covered by a rodshaped protein called tropomyosin. A calcium-sensitive complex, called troponin, is attached to the end of each tropomyosin molecule. When calcium floods the cell, troponin binds to it, moving tropomyosin off the myosin-binding sites. Opening the myosin-binding site on the actin filaments allows the myosin motors to crawl along the actin, resulting in a contraction of the muscle fiber. Calcium is then quickly returned to the sarcoplasmic reticulum by the action of a calcium pump. Without calcium, myosin releases actin, and the filaments slide back to their original positions.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

16.11 Beating Heart Cell <AGGT>



Single heart muscle cells spontaneously contract when grown in cell culture. This cell is grown on a flexible rubber substratum. Each time the cell contracts, it pulls on the substratum which becomes wrinkled.

Although individual heart cells can beat with their own rhythms, they are coordinated in an intact heart so that all cells beat synchronously.

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Barbara Danowski
Union College

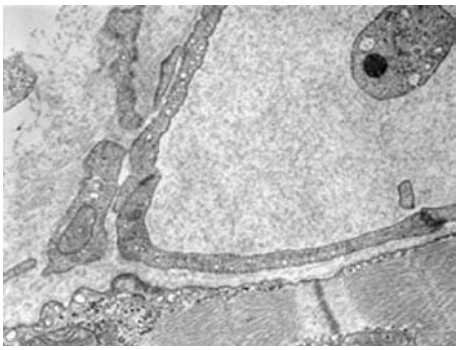
Kyoko Imanaka-Yoshida
Mie University

Jean Sanger and Joseph Sanger
University of Pennsylvania School of Medicine

16.12 Heart Tissue <TCAG>

Find me:

- endothelial cell surrounding blood vessel
- smooth muscle cell
- budding/fusing transcytotic vesicles
- junctions between endothelial cells
- white blood cell
- blood vessel (lumen)



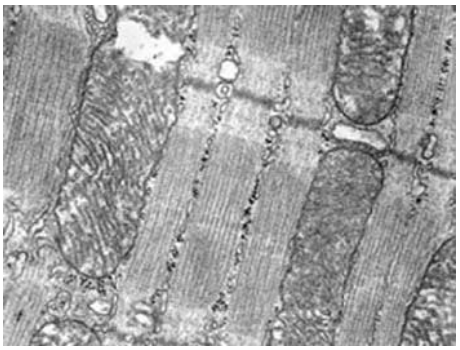
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

16.13 Heart Muscle Cell <GCAG>

Find me:

- mitochondria
- M-line
- thin filaments (actin)
- ribosomes
- Z-line
- thick filaments (myosin)



Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

17.1 Cdk2 <TAGA>

Cyclin-dependent kinases, or Cdks for short, are crucial regulatory proteins in the cell cycle. When activated, these kinases transfer phosphate groups from ATP to serine and threonine side chains on target proteins. When inactive, the active site of Cdks is sterically occluded by a loop, often referred to as the T loop.

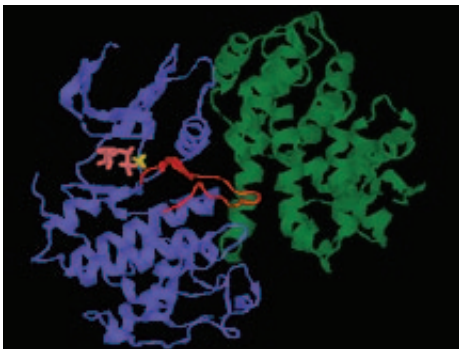
As their name suggests, cyclin-dependent kinases are activated by cyclins. Cyclin binding to Cdk pulls the T loop away from the active site and exposes the bound ATP, allowing it access to target proteins. Thus a Cdk can phosphorylate target proteins only when it is in a cyclin-Cdk complex.

A third protein called a Cdk-activating kinase is required for full activation of Cdk. This kinase adds a phosphate group to a crucial threonine in the T loop, thereby enabling Cdk to bind to and phosphorylate its target proteins.

Target peptides bind to the active site of the cyclin-Cdk complex so that the target serine or threonine side chains are precisely positioned with respect to the γ phosphate of the bound ATP.

Cdk inhibitor proteins, or CKIs, help regulate the rise and fall of cyclin-Cdk activity. Some inhibitors—like the one shown here—bind directly at the kinase active site and block kinase activity by interfering with ATP binding.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: Human Cyclin-dependent kinase 2 (1HCK); Cyclin A/Cyclin-dependent kinase 2 complex (1FIN); Phosphorylated cyclin-dependent kinase 2 bound to cyclin A (1JST); Phosphorylated Cdk2–cyclin A substrate peptide complex (1QMZ); p27/cyclin A/Cdk2 complex (1JSU)

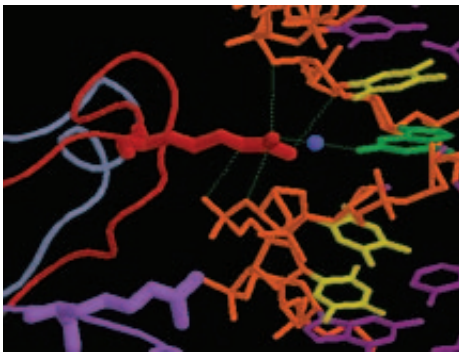
17.2 p53-DNA Complex <TGAA>

p53 is a tumor suppressor protein that prevents cells from dividing inappropriately. Loss of p53 function is associated with many forms of cancer. The DNA binding domain of p53 is folded as a β barrel. It exerts its function by binding to DNA as a negative transcriptional regulator.

The p53-DNA interface is complex. It involves several loops and a helix that extends from the β barrel core. Residues from one loop and the helix bind in the DNA major groove. Arginine 248 from another loop makes extensive contacts with the DNA backbone and, indirectly through water molecules, with bases in the minor groove. Mutations in arginine 248 are commonly found in tumor cells. Such mutations disrupt the ability of p53 to bind DNA.

Loop 2 does not bind to DNA directly but is essential for correctly positioning arginine 248 on the DNA. Three cysteines and a histidine from both loop 2 and loop 3 cooperate to sequester a zinc ion, forming the rigid heart of a zinc-finger motif. Mutations that disrupt interactions in this motif are also common in tumor cells.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: 1TSR

17.3 Plant Cell Division <TACT>

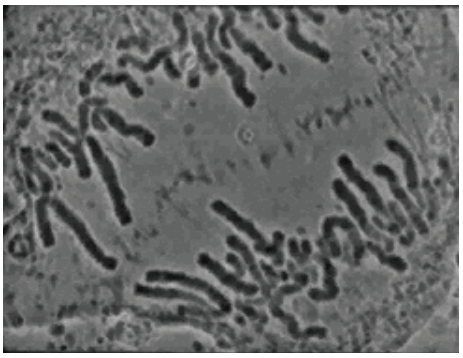
As this plant cell, taken from a lily, prepares for division, the chromosomes first condense. Next, the mitotic spindle lines them up in the center of the cell.

At the metaphase to anaphase transition, the sister chromatids of every chromosome pair separate suddenly, in striking synchrony. The chromosomes are pulled along the microtubules of the spindle to opposite ends of the cell.

After chromosome separation, membrane vesicles line up in the center and fuse with each other to form the new plasma membranes that separate the two daughter cells.

At telophase, the chromosomes decondense in the newly formed nuclei.

Final composition: Thomas Dallman



Andrew S. Bajer
Jadwiga A. Molè-Bajer
University of Oregon

17.4 Animal Cell Division <TCAA>

Differential interference contrast microscopy is used here to visualize mitotic events in a lung cell grown in tissue culture.

Individual chromosomes become visible as the replicated chromatin starts to condense.

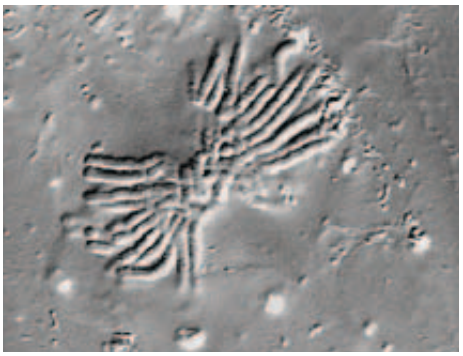
The two chromatids in each chromosome remain paired as the chromosomes become aligned on the metaphase plate.

The chromatids then separate and get pulled by the mitotic spindle into the two nascent daughter cells.

The chromatin decondenses as the two new nuclei form and cytokinesis continues to constrict the remaining cytoplasmic bridge until the two daughter cells become separated.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

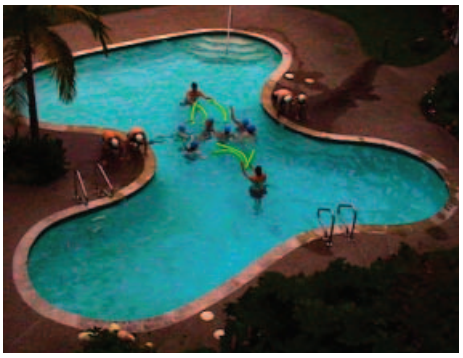
Video reproduced from: *The Journal of Cell Biology* 122:859–875, 1993. © The Rockefeller University Press



Edward D. (Ted) Salmon and Victoria Skeen
University of North Carolina at Chapel Hill

Robert Skibbens
Lehigh University

17.5 Interpretive Mitosis <CAAA>



Chromosomes: Mari Nishino, Han Li, Lisa Watson, Manisha Ray, Beatrice Wang, Sarah Foss

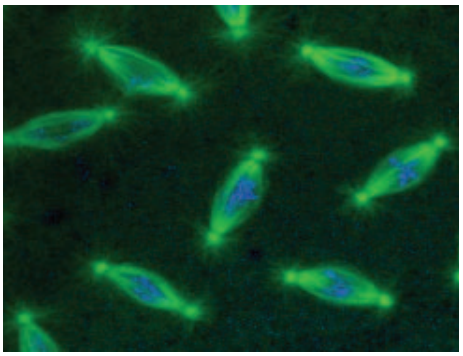
Cleavage Furrow: Ryan Joseph, Ahnika Kline, Chris Cain, Arthur Millius

Centrosomes: Ben Engel, Andrew Houk

Camera Work: Will Ludington

Directed & Edited: Ben Engel

17.6 Mitotic Spindles in a Fly Embryo <TTCT>



In an early *Drosophila* embryo, nuclei divide rapidly and in perfect synchrony. In this experiment, both DNA and tubulin are visualized with different fluorescent dyes.

After the mitotic spindle has assembled, the microtubules—shown in green—start pulling the blue chromosomes to either pole.

The chromosomes decondense and fill the newly formed round nuclei.

In preparation for the next round of mitosis, the centrosomes duplicate and migrate to opposite poles of each nucleus where they form new mitotic spindles and the process repeats.

The whole embryo rhythmically contracts with each division cycle.

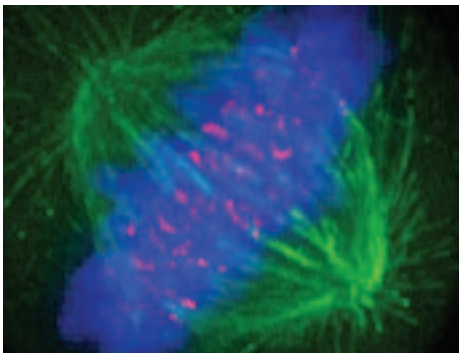
William Sullivan
University of California, Santa Cruz

Claudio E. Sunkel, Tatiana Moutinho-Santos, Paula Sampaio, Isabel Amorim and Madalena Costa
Institute of Biologia Molecular and Cell Biology, University of Porto, Portugal

17.7 Mitotic Spindle <GTTA>

The mitotic spindle of a dividing human cell is reconstructed here in its full beauty from multiple optical sections that were recorded with a fluorescent microscope. Microtubules are stained in green, DNA is stained in blue, and the kinetochores—where microtubules attach to the DNA—are stained in pink.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

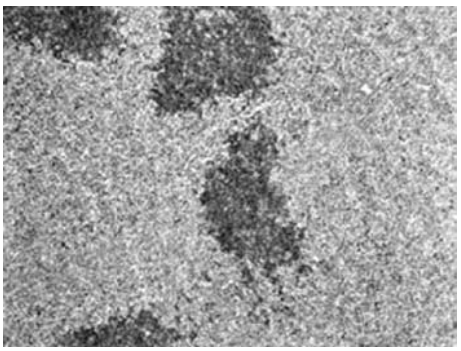


Kevin F. Sullivan
The Scripps Research Institute

17.8 Mitotic Chromosomes <CCAG>

Show me:

- microtubules
- kineticore
- chromosome



Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

18.1 Apoptosis <GCCC>

Apoptosis, a form of programmed cell death, has been induced in these cultured cells. Cell death is characterized by blebbing of the plasma membrane and fragmentation of the nuclei. Suddenly, cells weaken attachment to the substratum that they have been growing on and shrivel up without lysing.

In the following movies we observe the process at higher magnification.

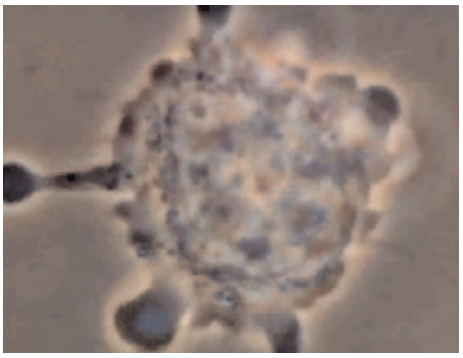
The mechanism of apoptosis involves many tightly controlled steps, three of which are demonstrated here by different visualization techniques.

One initial event is the sudden release of cytochrome *c* from mitochondria into the cytosol. This event has been visualized here using fluorescently labeled cytochrome *c*. Initially the greenish/yellow staining is restricted to a reticular pattern, which then suddenly disperses as the mitochondria release their content proteins into the cytosol.

At a later step, the lipid asymmetry of the plasma membrane breaks down. In normal cells, phosphatidyl serine is found only on the cytosolic side of the plasma membrane; but when cells undergo apoptosis, it becomes exposed on the outside of the cell. This event has been visualized here by adding a red fluorescent protein to the media which specifically binds phosphatidyl serine head groups as they become exposed. In an intact organism, exposure of phosphatidyl serine on the cell surface labels the dead cell and its remnants so that they are rapidly consumed by other cells, such as macrophages.

Finally—although apoptosing cells don't lyse—their plasma membranes do become permeable to small molecules. This event has been visualized here by adding a dye to the media that fluoresces blue when it can enter cells and bind to DNA.

All three of these events can be observed in the same group of cells.



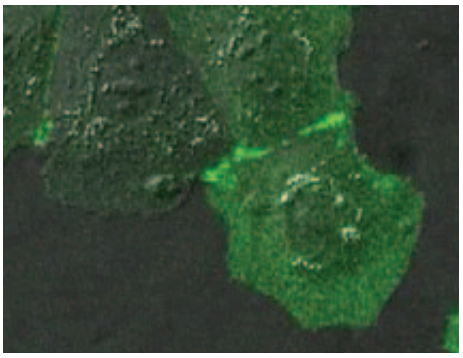
Part I:
Shigekazu Nagata, Kyoto University

Sakura Motion Picture Company, © 2007 Sakura Motion Picture Company

Part II:
Joshua C. Goldstein, The Genomics Institute of the Novartis Research Foundation

Douglas R. Green, St. Jude Children's Research Institute

19.1 Adhesion Junctions Between Cells <CGAA>



These epithelial cells express green fluorescent cadherin. They are grown at low density, so that isolated cells can be observed. Initially, labeled cadherin is diffusely distributed over the whole cell surface.

As cells crawl around and touch each other, cadherin becomes concentrated as it forms the adhesion junctions that link adjacent cells.

Eventually, as the cell density increases further, the cells become completely surrounded by neighbors and form a tightly packed sheet of epithelial cells.

Music: Christopher Thorpe

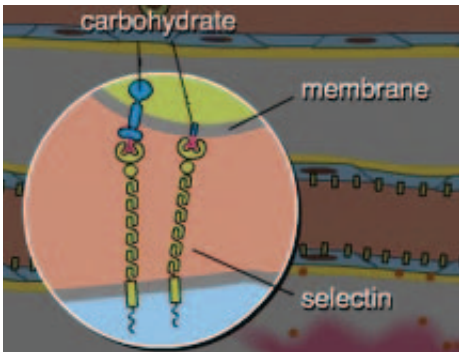
Stephen J. Smith
Stanford University School of Medicine

Cynthia Adams
Finch University of Health Sciences and Chicago Medical School

Yih-Tai Chen
Cellomics, Inc.

W. James Nelson
Stanford University School of Medicine

19.2 Rolling Leucocytes <CCCC>



Leucocytes are white blood cells that help fight infection. At sites of injury, infection, or inflammation, cytokines are released and stimulate endothelial cells that line adjacent blood vessels.

The endothelial cells then express surface proteins, called selectins. Selectins bind to carbohydrates displayed on the membrane of the leucocytes, causing them to stick to the walls of the blood vessels. This binding interaction is of sufficiently low affinity that the leucocytes can literally roll along the vessel walls in search for points to exit the vessel. There, they adhere tightly, and squeeze between endothelial cells—without disrupting the vessel walls—then crawl out of the blood vessel into the adjacent connective tissue.

Here, leucocyte rolling is observed directly in an anaesthetized mouse. The up and down movement of the frame is due to the mouse's breathing. Two blood vessels are shown: the upper one is an artery—with blood flowing from right to left . The lower one is a vein—with blood flowing from left to right. Leucocytes only adhere to the surface of veins; they do not crawl out of arteries.

Some leucocytes are firmly attached and are in the process of crawling through the vessel walls, whereas others have already left the vessel and are seen in the surrounding connective tissue.

When the blood flow is stopped temporarily by gently clamping the vessels, we can appreciate how densely both vessels are filled with red blood cells. Red blood cells do not interact with the vessel walls and move so fast under normal flow that we cannot see them. When the blood flow is restored, some of the leucocytes continue rolling, whereas all noninteracting cells are immediately washed away by the shear.

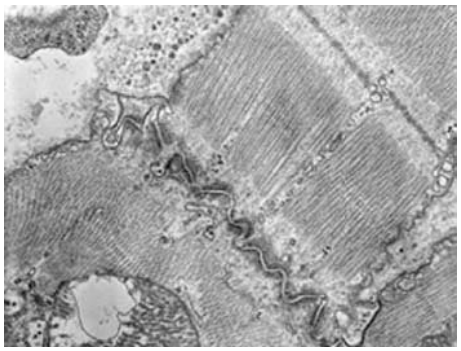
Animation: Blink Studio Ltd. (www.blink.uk.com)

Marko Salmi and Sami Tohka
MediCity Research Laboratory, University of Turku, Finland.

19.3 Junction Between Two Muscle Cells <AATG>

Find me:

- plasma membranes
- desmosomes
- Z disks
- transverse tubules and sarcoplasmic reticulum



Doug Bray
The University of Lethbridge, Canada

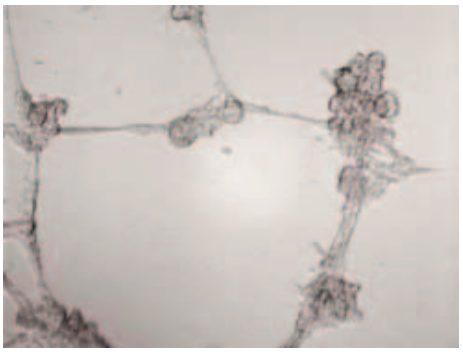
Brian Oates and Cyprien Lomas
The University of British Columbia

20.1 Breast Cancer Cells <CCGA>

Normal human breast epithelial cells can be grown in cell culture. They form structures that resemble the little sacs of cells from which the mammary gland is built. Cells assemble into a well organized, polarized epithelium that forms a closed sphere with an internal lumen. In the mammary gland, this space would be connected to ducts, and the cells would secrete milk into it.

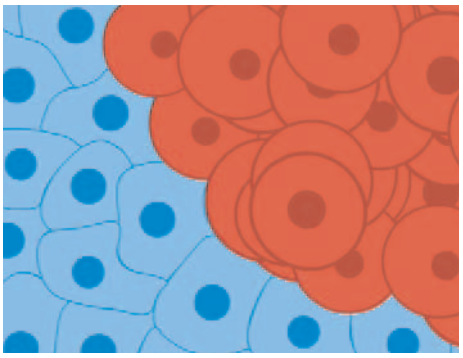
By contrast, these human breast cancer cells grown under the same conditions, divide aggressively and in an uncontrolled fashion. They are also more migratory and grow into disorganized clumps which would form tumors in the body.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



Mina J. Bissell, Karen Schmeichel, Hong Liu and Tony Hansen
Lawrence Berkeley Laboratories

20.2 Contact Inhibition <AGCC>



Sheryl Denker and Diane Barber
University of California, San Francisco

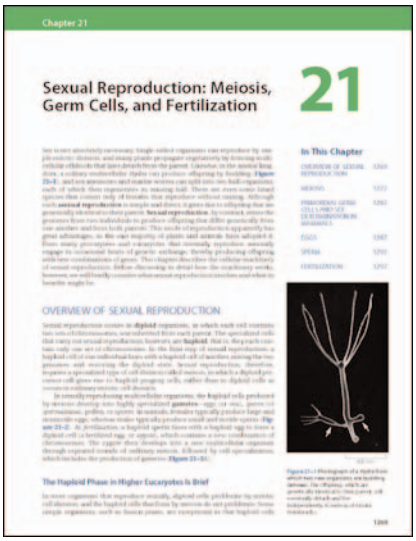
When normal cells are introduced into a Petri dish at low numbers, they begin to divide and proliferate. As the cells begin to touch one another, they slow their rate of division. This behavior is a consequence of the process called contact inhibition. Once the cells fill up the bottom of the dish, the rate of cell division slows further, and is balanced by the rate of call death such that the total cell number remains constant. This state is called confluence.

Contact inhibition ensures that the cells create a layer only one cell thick—a monolayer.

The behavior of cancer cells is quite different. If a cancer cell is seeded among normal cells, all of the cells will proliferate as before. However, once confluence is reached, the normal cells will regulate their growth while the cancer cells continue to divide in an unregulated manner, yielding a clump of cells, which is often called a focus.

Contact inhibition can be demonstrated *in vitro* by removing cells from a confluent monolayer. In this experiment, cells are removed by scratching the monolayer with a needle. The surviving cells at the edge of the wound now do two things. One, they begin to proliferate more rapidly, since they are no longer fully contact inhibited. And two, they migrate into the empty area of the wound, attempting to fill it up.

21.1 Chapter 21: Sexual Reproduction: <CAAG> Meiosis, Germ Cells and Fertilization


Chapter 21

Sexual Reproduction: Meiosis, Germ Cells, and Fertilization

21

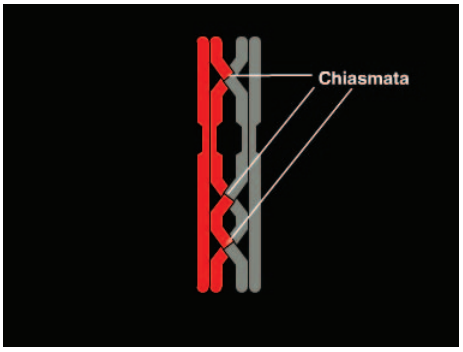
In This Chapter

OVERVIEW OF SEXUAL REPRODUCTION

The Haploid Phase in Higher Eucaryotes Is Brief

Sex is not absolutely necessary. Single-celled organisms can reproduce by simple mitotic division, and many plants propagate vegetatively by forming multicellular offshoots that later detach from the parent. Likewise, in the animal kingdom, a solitary multicellular *Hydra* can produce offspring by budding (**Figure 21–1**), and sea anemones and marine worms can split into two half-organisms, each of which then regenerates its missing half. There are even some lizard species that consist only of females that reproduce without mating. Although such **asexual reproduction** is simple and direct, it gives rise to offspring that are genetically identical to their parent. **Sexual reproduction**, by contrast, mixes the genomes from two individuals to produce offspring that differ genetically from one another and from both parents. This mode of reproduction apparently has great advantages, as the vast majority of plants and animals have adopted it. Even many procaryotes and eucaryotes that normally reproduce asexually engage in occasional bouts of genetic exchange, thereby producing offspring with new combinations of genes. This chapter describes the cellular machinery of sexual reproduction. Before discussing in detail how the machinery works...

21.2 Meiosis <AGTG>



Gametes, such as a sperm or an egg, are specialized cells used in sexual reproduction. In this micrograph of a clam egg, we can see a large number of sperm binding to its surface. Note the large difference in size between these male and female gametes.

Although the sperm are much smaller than the egg, a single sperm has the same number of chromosomes as the egg. When a single sperm and egg fuse during fertilization, each contribute a set of chromosomes to the resulting fertilized egg, called a zygote. The zygote will have the same number of chromosomes as the other cells in the body, since each parental gamete supplies a half-set of chromosomes.

Gametes are created through a special process of cell division called meiosis. During meiosis, a single germ-cell precursor with two sets of chromosomes must divide twice to create four gametes. Each of the four resulting gametes will have half the number of chromosomes as the germ-cell precursor, and each of the gametes will be genetically different from the other gametes

In order to understand why meiotic cell division results in 4 genetically dissimilar gametes, we need to look more closely at the key molecular events that occur during the meiotic cycle.

The germ-cell precursor begins with two complete sets of chromosomes, a maternal set and a paternal set. Through DNA replication, a complete copy of each set is made. The copies align with the original set of chromosomes, and then link tightly, forming twin sets of chromosomes, called sister chromatids.

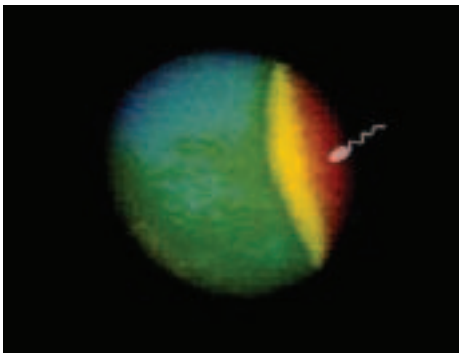
The maternal and paternal sister chromatids then align on the metaphase plate, where they form a set of four paired chromatids, called a bivalent. Crossover events occur between the nonsister chromatids, mixing chromosomal information at sites called chiasmata. This exchange of information, called recombination, is a major source of genetic variation.

After recombination, the reshuffled chromatids separate, and eventually the cells divide completely, ending Meitotic Division I.

Meitotic Division I is followed by a second stage of cell division, Mitotic Division II. Significantly, this second division occurs without DNA replication. Subsequently, the four resulting daughter cells, the gametes, will have one-half the number of chromosomes as the parent cells and, due to recombination, each gamete will be genetically different from the others.

Animation: Graphic Pulse, Inc. (www.graphicpulse.com)

21.3 Calcium Wave During Fertilization <AGGA>



When a sperm cell fuses with this sea urchin egg cell, calcium ions begin rushing into the cell at the site of fusion.

In these experiments, calcium concentrations are visualized and measured with a fluorescent dye that becomes increasingly brighter the more calcium is present.

Brightness is then translated into a color scale, and, in this three-dimensional display, into peak heights, where red and high peaks represent the highest calcium concentrations.

A second rise of the calcium concentration can be observed after fertilization. It occurs during the movements of the sperm and egg pronuclei to meet and fuse near the center of the egg.

Final composition: Allison Bruce

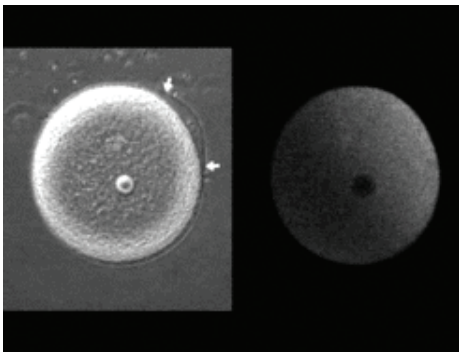
Part I:
Carolyn A. Larabell
Lawrence Berkeley National Laboratory

Jeff Hardin
University of Wisconsin, Madison

Part II:
Michael Whitaker
University of Newcastle Upon Tyne

Isabelle Gillot
University of Nice-Sophia Antipolis

21.4 Sea Urchin Fertilization <TGAC>



A sea urchin egg during fertilization is visualized here simultaneously by phase contrast microscopy and by fluorescence microscopy. The egg contains a fluorescent dye that becomes brighter in the presence of calcium ions.

When a sperm cell fuses with the egg, the fluorescence image shows a wave of calcium ions that sweeps through the cytosol, starting from the initial point of sperm–egg fusion. Following the path of the calcium wave, we see a membrane, called the fertilization envelope, rising from the cell surface. The fertilization envelope protects the fertilized egg from the outside environment, and prevents the entry of additional sperm. The rise in cytosolic calcium triggers an elevation of the fertilization envelope through the process of exocytosis.

Exocytosis releases hydrolytic enzymes stored in vesicles. Action of the released hydrolases causes a swelling of material surrounding the cell, which in turn elevates the fertilization envelope.

Exocytosis can be visualized directly in this system. For this purpose, the plasma membrane is labeled with a fluorescent dye, seen on the right. Each time a vesicle fuses, it leaves a depression in the plasma membrane which, in the optical sections shown, appears as a ring of increased fluorescent staining.

On the left, differential interference contrast microscopy is used to directly view the exocytic vesicles that underlie the plasma membrane. The vesicles are visible here, because they are densely packed with protein and consequently have a different refractive index from the surrounding material. Each time a vesicle exocytoses, it disperses its contents and disappears from the image. This effect is best seen when we step back and forth between adjacent frames of the movie.

Mark Terasaki
University of Connecticut Health Center

22.1 Chapter 22: Development of Multicellular Organisms <AGCT>


Chapter 22

Development of Multicellular Organisms

22

In This Chapter

UNIVERSAL MECHANISMS OF ANIMAL DEVELOPMENT

An animal or plant starts its life as a single cell—a fertilized egg. During development, this cell divides repeatedly to produce many different cells in a final pattern of spectacular complexity and precision. Ultimately, the genome determines the pattern, and the puzzle of developmental biology is to understand how it does so.

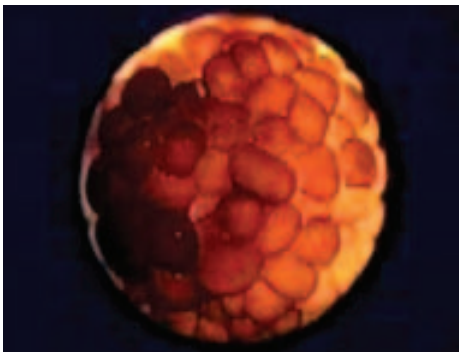
The genome is...

22.2 Developing Egg Cells <ATTT>

This frog egg cell has been fertilized and starts dividing. The first cell divisions occur very rapidly. Cells divide every thirty minutes.

This timing is very precise. Egg cells that have been fertilized at the same time divide and develop in almost perfect synchrony.

After a day or two, embryonic development is completed and tadpoles hatch from the eggs.



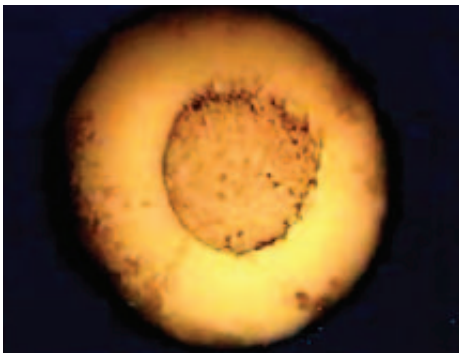
From "From Egg to Tadpole"
Jeremy Pickett-Heaps and Julianne Pickett-Heaps
Cytographics (www.cytographics.com)

22.3 Gastrulation <TCCC>

During gastrulation, cells of this developing frog embryo rearrange in a dramatic ballet of orchestrated cell movements. In a continuous motion, cells from the outer layer of the embryo sweep towards the vegetal pole and start invaginating, forming a deep cavity in the interior.

The paths of the cells and the topology of these rearrangements are best seen in this animation of an embryo that has been sliced open. The different cell layers that are formed in this way have very different fates.

Cells that line the newly formed cavity, called the endoderm, develop into the lining of the gut and many internal organs such as liver, pancreas, and lung. Cells in the middle layer, called the mesoderm, give rise to muscle and connective tissue. Cells remaining on the outside, called the ectoderm, go on to form the outer layer of the skin, as well as the nervous system.



From "From Egg to Tadpole"
Jeremy Pickett-Heaps and Julianne Pickett-Heaps
Cytographics (www.cytographics.com)

22.4 Spemann's Organizer <ATTG>

Hans Spemann and Hilde Mangold were pioneers of developmental biology. They showed how the pattern of the embryo is created by interactions between one group of cells and another. In 1924 they made a famous discovery. They found that a small piece of tissue called the Organiser, taken from a specific site in the early frog embryo and transplanted to another embryo, could control the behavior of neighboring cells and direct the formation of an entire body axis.

The key experiment is re-enacted here by a modern developmental biologist, using the frog *Xenopus Lavis*.

Two *Xenopus* embryos are maneuvered under the dissecting microscope. The embryos are beginning to gastrulate. The blastopore, where cells are tucking into the interior, is visible as a dark crescent in the embryo on the left. The dorsal lip of the blastopore contains the Organizer cells.

With a pair of forceps and a fine tungsten needle, a block of Organizer tissue is cut from the embryo on the left. Using a hair plucked from a human eyebrow, the block of tissue is gently pushed into a site on the ventral side of the other embryo.

An hour later, the graft has healed into the host embryo and the organizer cells have been integrated at an atopic site.

Two days later, the host embryo has developed into conjoined twins. The grafted Organizer has caused the host cells around the graft to form a second body axis, complete with central nervous system, eyes, somites, and other structures.

Experiment re-enacted by:
Edward M. De Robertis
Howard Hughes Medical Institute
University of California, Los Angeles

22.5 *Drosophila* Development <AACG>

During development, a *Drosophila* embryo undergoes many complex morphological changes. We first see migration of pole cells from the posterior end. These cells are destined to become the germ cells of the fly. A crest develops which separates a region that will develop into the head, mouth parts, and fore gut. At this stage, the future tail end of the body is folded over on the dorsal side. Body segments then become defined.

The first three segments will give rise to the head and mouth parts, the next three to the thorax, and the remaining ones to the abdomen. Eventually, the rear end of the embryo will retract back onto the ventral side and straighten out the embryo. Development to this stage takes about 10 hours.

We can appreciate the complexity of these events by morphing a series of individual scanning electron micrographs into a continuous temporal sequence: migration of pole cells; development of various surface indentations, including openings to the air ducts, or tracheal tubes; segmentation, and tail retraction.

A similar sequence viewed from the top—or the dorsal side. Pole cells migrate and then move into the interior as the hind gut invaginates. The rear end is temporarily folded over onto the dorsal side and eventually starts retracting to straighten out the embryo.

Early in development when seen from the bottom—or ventral side—a deep groove forms during gastrulation, as mesodermal cells migrate inward, where they become the precursor cells for many internal organs. The groove then seals off as the cells that remain exterior zipper up.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

Thomas C. Kaufman
Howard Hughes Medical Institute
Indiana University, Bloomington

SEM:
Rudi Turner
Indiana University, Bloomington

Morphing:
Michael Kaufmann, Jeffrey Giacoletti and Chris Macri
Indiana University, Bloomington

22.6 Early Zebrafish Development <GAGC>

The first divisions of a zebrafish egg occur synchronously about every 30 minutes and create a mass of cells sitting on top of a enormous yolk.

This blastoderm then begins to spread as a continuous sheath over the yolk.

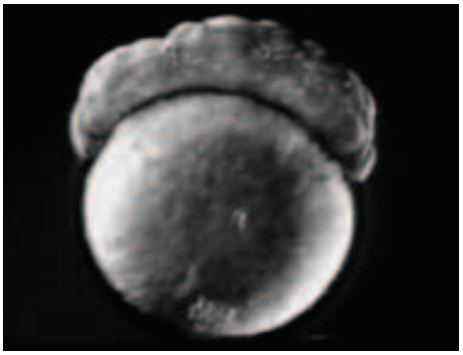
During this process, some cells from the external layer tug into the interior of the embryo. They will eventually form the lining of the gut, as well as the musculature, skeleton, and other internal tissues.

The first body segments, the head process and tail bud become visible.

The tail bud continues to extend, and we clearly see the eye develop.

17 hours into development, we can already see a recognizable vertebrate emerging, wrapped around the ball of yolk that will nourish it for the first few days of its existence.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



Rolf O. Karlstrom
University of Massachusetts at Amherst

Donald A. Kane
University of Rochester, New York

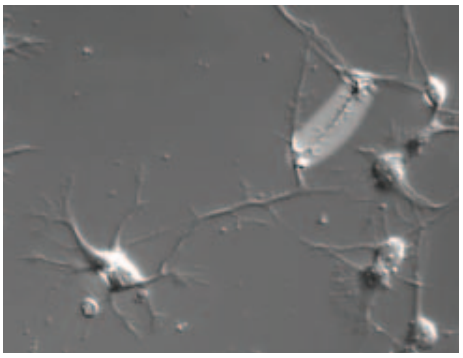
22.7 Neurite Outgrowth <AAGA>

Neuronal precursor cells, taken from the hippocampus of an embryonic rodent brain, differentiate in culture and send out long extensions, called neurites, that could later become dendrites or axons.

These neurites are pulled out of the cell body by growth cones that can crawl independently over the substratum. Occasionally, a growth cone releases from the substratum and the neurite retracts.

New growth cones can grow from the sides of existing neurites, forming branches.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



Frank B. Gertler and Lorene Lanier
Massachusetts Institute of Technology

22.8 Neuronal Pathfinding <TACC>

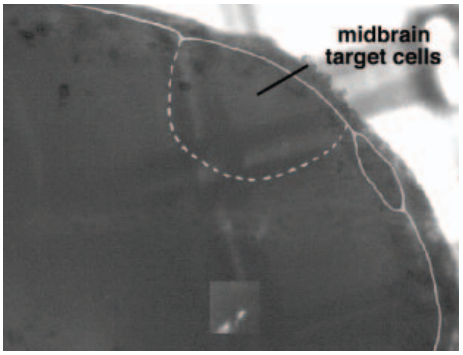
In the embryonic brain of the frog *Xenopus,* neurons extend axons from the eye to connect to appropriate target cells in the midbrain. Early in embryogenesis, these connections have to be made properly. Growth cones at the tips of the elongating axons guide cells in the right direction.

Growth cones elongate toward their targets by extending and retracting thin processes, called filopodia. In this way, the growth cones probe their environment for guidance. In this case, they cross paths as cues lead them on unerring courses toward their targets.

After entering the appropriate part of the midbrain, the optic tectum, the axons slow down and send out branches, which can sample numerous target neurons and establish synaptic connections.

These two axons took six hours to grow to their targets less than a millimeter away.

Final Composition: Allison Bruce



Sonia Witte and Christine E. Holt
University of Cambridge

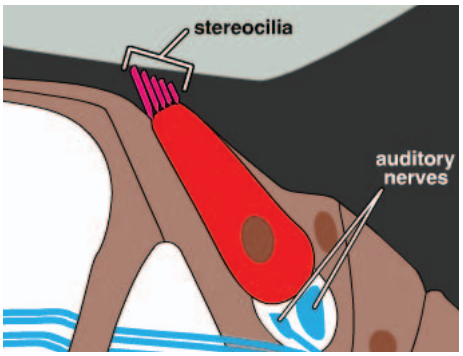
23.1 Chapter 23: Specialized Tissues, Stem Cells, and Tissue Renewal <GATA>


Specialized Tissues, Stem Cells, and Tissue Renewal

23

Cells evolved originally as free-living individuals, but the cells that matter most to us, as human beings, are specialized members of a multicellular community. They have lost features needed for independent survival and acquired peculiarities that serve the needs of the body as a whole. Although they share the same genome, they are spectacularly diverse: there are more than 200 different named cell types in the human body (see our web site for a list). These collaborate with one another to form many different tissues, arranged into organs performing widely varied functions. To understand them, it is not enough to analyze them in a culture dish: we need also to know how they live, work, and die in their natural habitat, the intact body...

23.2 Hair Cells I <TCCA>



The sound-sensitive cells within our ears are called hair cells. Each has a tuft of spiky extensions called stereocilia on its upper surface, and each sends signals to auditory nerve fibers through its basal surface.

The hair cells are embedded in a layer of supporting cells and are sandwiched between two sheets of extracellular matrix—the tectorial membrane and the basilar membrane. Sound vibrations cause the basilar membrane to vibrate, and this motion pushes the stereocilia against the tectorial membrane. The stereocilia tilt, triggering an electrical response in the hair cell. The activated hair cell, in turn, activates the auditory nerve cells.

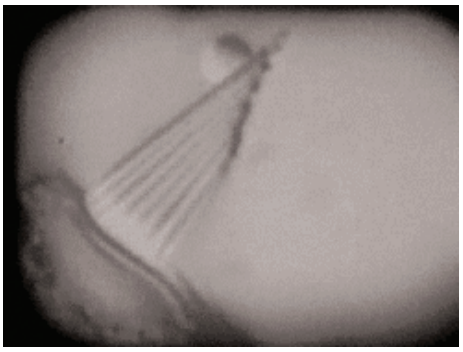
The hair cell membrane contains stretch-activated ion channels. These channels are closed when the stereocilia are not tilted. However, when they tilt, a linking filament from one stereocilium to a channel on the neighboring stereocilium pulls at the channel, opening it. Positively charged ions flow into the cell, depolarizing the membrane.

Storyboard and Animation: Sumanas, Inc. (www.sumanasinc.com)

23.3 Hair Cells II <CATA>

The stereocilia that project from hair cells vibrate in response to sound waves. Here the bundle of stereocilia projecting from a single hair cell is pushed with laser tweezers to simulate this movement. Movement opens stressactivated ion channels in the plasma membrane, leading to membrane depolarization. This is translated into the perception of sound.
Moving an individual stereocilium demonstrates the flexible attachment of these structures to the cell body.

Music: Christopher Thorpe



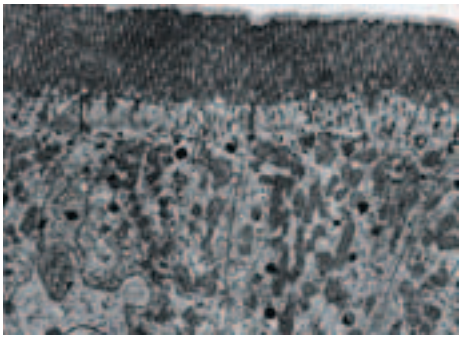
Steven M. Block
Stanford University

23.4 Gut Epithelium: View 1 <TATG>

Find me:

- tight junctions
- desmosomes
- carbohydrate layer
- adhesion belt
- microvilli

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet, Springer-Verlag. New York, 1975



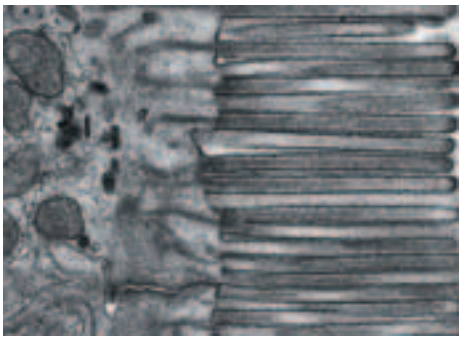
Lelio Orci and Alain Perrelet

23.5 Gut Epithelium: View 2 <GATG>

Find me:

- tight junction
- adherens junctions
- actin filaments
- adhesion belt

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet, Springer-Verlag. New York, 1975.



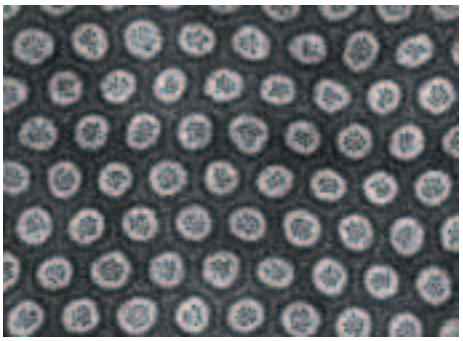
Lelio Orci and Alain Perrelet

23.6 Gut Epithelium: View 3 <CGGC>

Find me:

- actin filaments
- microvilli
- plasma membrane
- carbohydrate layer

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet, Springer-Verlag. New York, 1975



Lelio Orci and Alain Perrelet

23.7 Angiogenesis <GTTG>

As a normal part of growth and development, the body must generate new blood vessels to oxygenate the tissues. In a process called angiogenesis, new vessels sprout from existing ones. In this movie, we see endothelial cells sprouting to form new branches from the aorta of a zebrafish embryo. Each sprout is initially formed by one or a few endothelial cells.

The process begins when an endothelial cell of a small vessel is activated by an angiogenic stimulus, such as vascular endothelial growth factor (or VEGF). In response to the stimulus, the endothelial cell becomes motile and extends filopodia that guide the development of a capillary sprout.

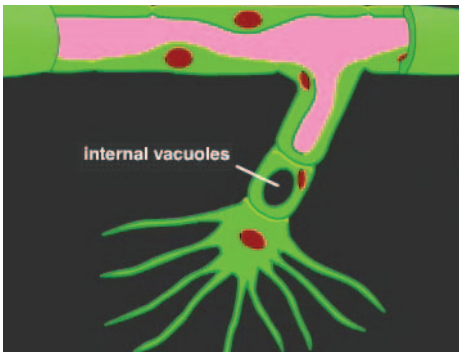
The leading or "tip cell" continues to move away from the capillary as cells behind it migrate in and divide, forming a stalk. The sprout begins to hollow out, forming a tube. In this process, pinocytic vesicles fuse with one another. The large vacuoles formed in this way then fuse with one another, creating a lumen that runs through the capillary sprout.

In culture, endothelial cells behave in a similar way—they spontaneously develop internal vacuoles that join up from cell to cell, creating a single lumen shared by many cells.

In the example shown here, the individual cells contain either a red or a green fluorophore. Note that the areas of green and red are distinct—even though cells share a lumen, they do not share cytoplasm and remain separate cells after the fusion events.

Angiogenesis is critical not only in normal development and wound healing, but also in the development of tumors. A tumor must stimulate blood vessel formation to grow more than a few millimeters in size. VEGF is a key activator of angiogenesis in both normal cells and tumors. When cells within a tumor become oxygen deficient, they begin to express VEGF. VEGF diffuses through the tissues, activating endothelial cells on nearby vessels. This results in capillary sprouting.

When the new vasculature provides enough oxygen to the growing tumor, the tumor cells stop producing VEGF, and capillary formation ends as well. Some new cancer therapies are targeted to block the action of VEGF, with varying clinical results.



Movie I:
Brant M. Weinstein
National Institutes of Health

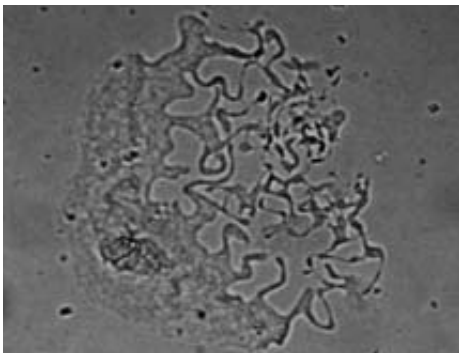
Movie II:
George E. Davis
University of Missouri School of Medicine

23.8 Megakaryocyte <GCAT>

Megakaryocytes are the precursor cells from which blood platelets derive. These gigantic cells undergo an elaborate fragmentation process that pinches off portions of the cell's cytoplasm. These fragments are the platelets, which are then swept away in the blood stream. Platelets are important for blood coagulation at sites of injury.

Final composition: Blink Studio Ltd. (www.blink.uk.com)

Video reproduced from: *The Journal of Cell Biology* 147:1299–1312, 1999. © The Rockefeller University Press.



Joseph E. Italiano, Jr.
Brigham and Women's Hospital and Harvard Medical School

23.9 Wound Healing <TGAT>

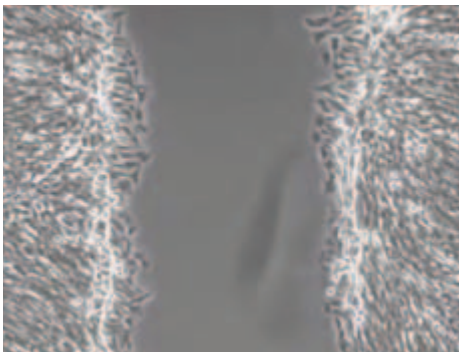
Fibroblasts grown in vitro in a culture dish form a confluent monolayer of cells. Cells in a monolayer are relatively static; contacting each other inhibits their migration.

Such cell layers can be wounded experimentally by scratching them with a needle.

In such an experiment, we can observe that the fibroblasts at the edge of the wound become migratory and quickly move to repair the gap.

Such cell migration is important for wound repair in an intact organism.

Animation: Blink Studio Ltd. (www.blink.uk.com)



Sheryl Denker and Diane Barber
University of California, San Francisco

23.10 Lymphocyte Homing <ACCG>

To visualize lymphocyte homing to a site of injury, a zebrafish larva was anaesthetized and its fin pierced with a needle to introduce a small wound.

A vein is seen at the bottom of the frame.

Because the fin is very thin and transparent, we can watch directly as lymphocytes crawl out of the blood vessel and migrate towards the wound.

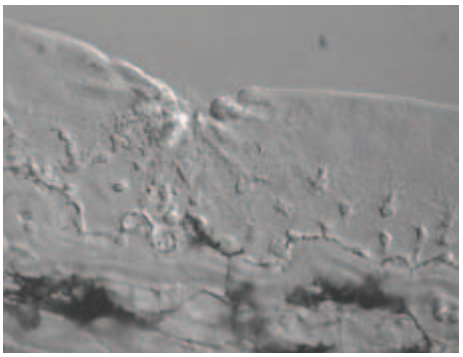
They are attracted there by chemicals released from damaged cells, invading bacteria, and other lymphocytes.

In a zoomed out view we can appreciate that lymphocyte invasion is restricted to the wounded area.

The static cells that are dispersed in the connective tissue are fibroblasts.

In these movies, 60 minutes of real time are compressed into 15 seconds.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



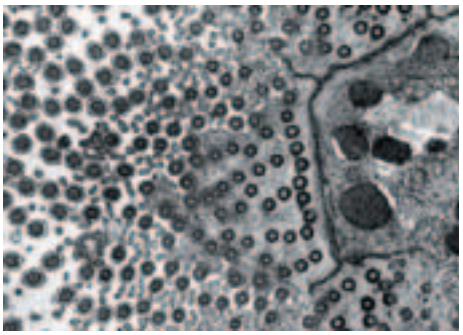
Michael Redd and Paul Martin
University College London

23.11 Tracheal Epithelium <ACGC>

Find me:

- cilia
- microtubules
- mucus secreting cell
- microvilli

Originally published in Freeze-Etch Histology: A Comparison between Thin Sections and Freeze-Etch Replicas by Lelio Orci and Alain Perrelet, Springer-Verlag. New York, 1975.

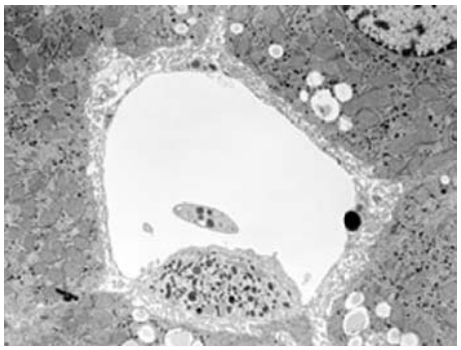


Lelio Orci and Alain Perrelet

23.12 Endothelial Cell in Liver <CCAA>

Find me:

- outline of endothelial cell
- outline of surrounding liver cells
- secretory vesicles with condensed content proteins



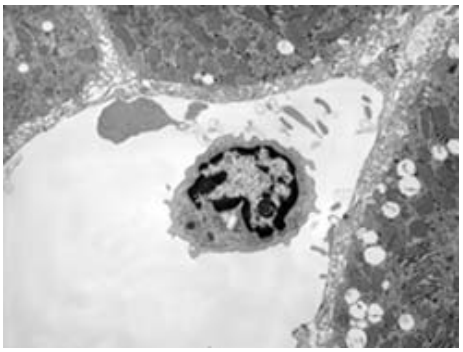
Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

23.13 Liver Cells: Sinusoid Space <CCGT>

Find me:

- microvilli of hepatocytes
- white blood cell (neutrophil)
- endothelial cells
- nucleus of neutrophil



Doug Bray
The University of Lethbridge, Canada

Brian Oates and Cyprien Lomas
The University of British Columbia

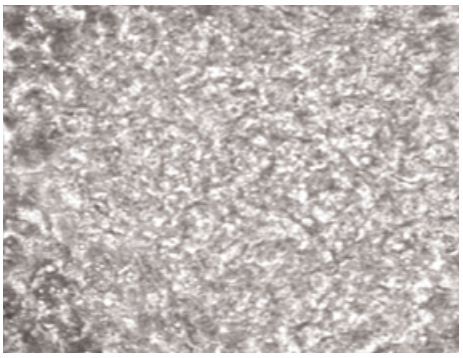
23.14 Embryonic Stem Cells <GGAA>

Embryonic stem cells, or ES cells, are able to differentiate into any cell type in the body.

ES cells derived from an embryo can be grown in culture. Upon exposure to an appropriate cocktail of signal molecules, the cells differentiate into specific cell types.

In this experiment, ES cells were exposed to signal molecules, inducing the differentiation program that specifies the development of heart muscle cells. After a few days in culture, the previously homogeneous, undifferentiated cells, organize into groups of highly specialized cells. Remarkably, the cells in these groups start contracting rhythmically, indicating they have formed a fully functional contractile apparatus, characteristic of muscle cells.

Examining GFP that has been expressed from a heart-muscle specific promoter, shows that the appropriate gene expression programs have been activated selectively in the beating cells.

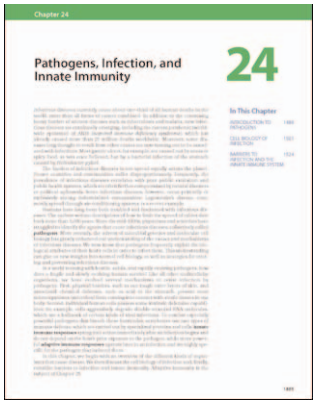


Bruce R. Conklin
Gladstone Institute of Cardiovascular Disease
University of California, San Francisco

24.1 Chapter 24: Pathogens, Infection, and Innate Immunity <AACC>

Infectious diseases currently cause about one-third of all human deaths in the world, more than all forms of cancer combined. In addition to the continuing heavy burden of ancient diseases such as tuberculosis and malaria, new infectious diseases are continually emerging, including the current pandemic (worldwide epidemic) of *AIDS (acquired immune deficiency syndrome)*, which has already caused more than 25 million deaths worldwide. Moreover, some diseases long thought to result from other causes are now turning out to be associated with infections. Most gastric ulcers, for example, are caused not by stress or spicy food, as was once believed, but by a bacterial infection of the stomach caused by *Helicobacter pylori*.

The burden of infectious diseases...



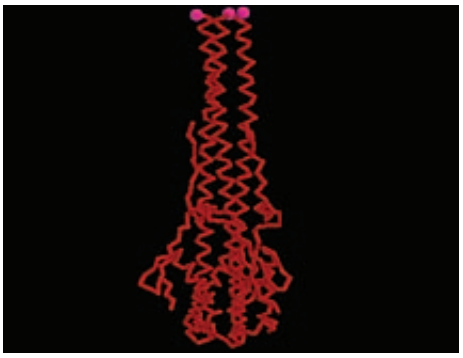
24.2 Hemagglutinin <ATAG>

Hemagglutinin is a membrane fusion protein expressed on the surface of influenza viruses. By mediating the fusion of viral and cellular membranes during infection, it allows the viral genome to enter the cell. During the fusion reaction, hemagglutinin inserts a hydrophobic fusion peptide into the host-cell membrane and thus transiently becomes an integral membrane protein in the two lipid bilayers. The transmembrane helix that anchors hemagglutinin in the viral membrane is omitted from this structure.

The fusion reaction is triggered by low pH which the virus encounters after up-take into endosomes of host cells. This change in pH leads to a massive structural change, including the formation of a long α helix in the core of the protein shown here. The fusion peptide, that was previously tucked away in the protein's stalk, is now displayed prominently at the tip of the helix, ready to slip into the host-cell membrane. The fusion peptide had to be removed from the protein to allow crystallization.

On the viral surface, hemagglutinin is a complex of three identical subunits. It is likely that the concerted action of a small number of hemagglutinin trimers is required to trigger a membrane fusion event.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: Hemagglutinin (1HGF); Compilation of hemagglutinin & structure of influenza hemagglutinin (1HGF & 1HTM)

24.3 *Listeria* Parasites <GTAT>

This mammalian cell has been infected with pathogenic *Listeria monocytogenes*. These bacteria move throughout the cytosol by recruiting host cell actin which polymerizes and pushes them forward, producing a comet's tail in their wake.

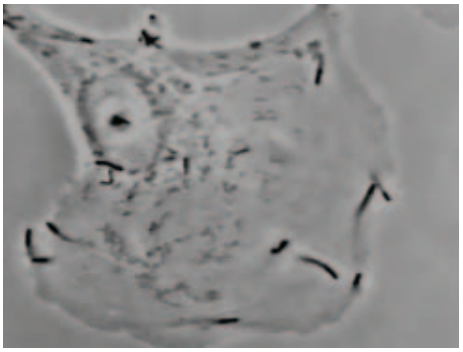
Whenever a bacterium is pushed into the plasma membrane, it creates a temporary protrusion and is then bounced back to continue its random path.

If we look closely, we can see a bacterium divide inside the host cell. Immediately after separation, the two daughter cells assemble their own actin tails and start moving about.

These bacteria can also form actin comet tails and move in cell extracts. Here, the bacteria are expressing the green fluorescent protein, and actin is labeled red with a fluorescent dye.

The dynamics of the actin tails, that propel the bacteria through the cytosol, can be modeled, based on known biochemical and physical properties of actin and actin filaments.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



Part I:
Julie A. Theriot
Stanford University School of Medicine

Daniel A. Portnoy
University of California, Berkeley

Part II:
Julie A. Theriot
Stanford University School of Medicine

Frederick S. Soo
Stanford University

Part III:
Jonathan B. Alberts
University of Washington, Seattle

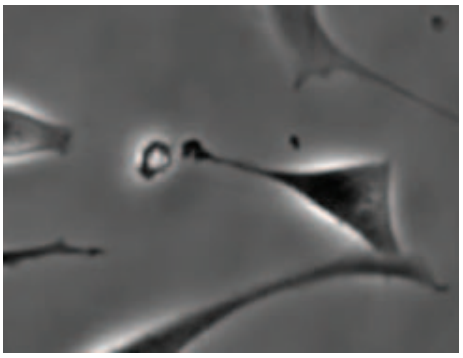
24.4 Killer T Cell <GTCA>

Cytotoxic lymphocytes, also called killer T cells, bind tightly to their target cells and then release toxic compounds by exocytosis into the cleft between the two cells.

Here, a killer T cell has attached to a fibroblast and proceeds to attack it. The fibroblast quickly retracts and rounds up.

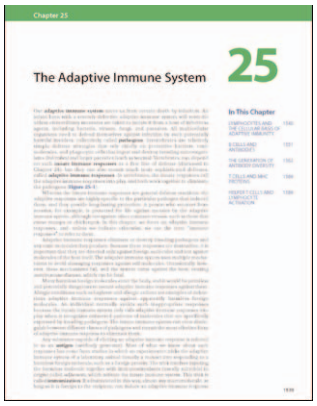
The movie is too short to tell whether it has actually been killed or will recover.

Final composition: Blink Studio Ltd. (www.blink.uk.com)



James Bear and Frank B. Gertler
Massachusetts Institute of Technology

25.1 The Adaptive Immune System <CTCT>



Our **adaptive immune system** saves us from certain death by infection. An infant born with a severely defective adaptive immune system will soon die unless extraordinary measures are taken to isolate it from a host of infectious agents, including bacteria, viruses, fungi, and parasites. All multicellular organisms need to defend themselves against infection by such potentially harmful invaders, collectively called **pathogens**. Invertebrates use relatively simple defense strategies that rely chiefly on protective barriers, toxic molecules, and phagocytic cells that ingest and destroy invading microorganisms *(microbes)* and larger parasites (such as worms). Vertebrates, too, depend on such **innate immune responses** as a first line of defense (discussed in Chapter 24), but they can also mount much more sophisticated defenses, called **adaptive immune responses**. In vertebrates, the innate responses call the adaptive immune responses into play, and both work together to eliminate the pathogens (**Figure 25–1**).

Whereas the innate immune responses...

25.2 Antibodies <GCCG>

PDB ID number *: Compilation of immunoglobin G1 & immunoglobin Fc and fragment B of Protein A complex (2IG2 & 1FC2); FabD1.3-lysozyme complex (1FDL)

Antibodies of the immunoglobulin G class are Y-shaped glycoproteins that circulate in the blood stream. They bind to and inactivate foreign molecules—the antigens—and mark them for destruction. Each IgG molecule consists of two light chains and two heavy chains. The heavy chains have carbohydrates attached. The regions of the antibody that bind to antigens are located at the very tips of the two arms.

Each arm of the antibody is composed of four domains. Two are called the variable domains, contributed by the heavy and light chains, and hence called V_H and V_L. The variable domains are attached to two constant domains, again one each from the heavy and light chains, and hence called C_H and C_L.

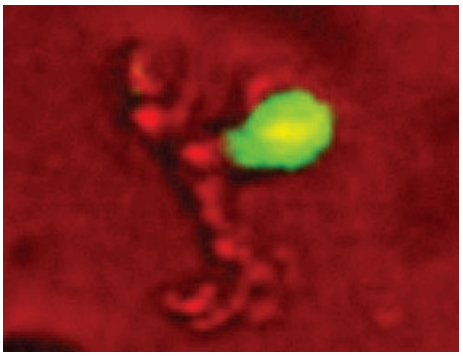
Variable and constant domains share a similar structure, called the Ig fold. Each domain consists of a pair of beta sheets, one with three strands and one with four. A single covalent disulfide bridge holds the two sheets together, which results in a rigid and very stable domain.

As their name implies, the variable domains vary in amino acid sequence from one antibody molecule to another, thus providing the vast diversity in structure required by the immune system. The antigen binding site in the variable domains is composed of hypervariable loops that are especially susceptible to sequence variations. Sequence variations in the hypervariable loops are responsible for the specificity of antibodies to particular antigens.

Antigens bind to the tip of each antibody arm, generally two molecules per antibody. Most antigens bind to an antibody via a large contact surface, providing a tight and highly specific association.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)

25.3 T Cell Activation <TTGA>



Matthias Gunzer and Peter Friedl
University of Muenster, Germany

In this video we can see a T cell that becomes activated when it interacts with a dendritic cell. The T cell is labeled with a dye that fluoresces when it binds calcium ions. At the moment the T cell is not activated. Its intracellular calcium concentrations are low, and so little green fluorescence is visible.

As the T cell contacts the surface of the dendritic cell, we can see it suddenly fluoresce bright green as it becomes activated. However, it still continues to move, crawling over the surface of the dendritic cell, perhaps to sample the cell's display of peptide:MHC complexes.

Eventually the T cell loses interest. While it is still contacting the dendritic cell you can see the fluoresence start to fade. The T cell will eventually migrates away from the dendritic cell.

25.4 MHC Class I <AAGT>

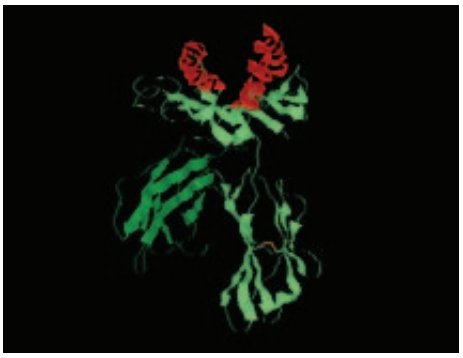
Class I Major Histocompatibility Complex proteins display short peptides, or antigens, derived from normal cell proteins. Peptide-loaded MHC proteins are located on the cell surface where they can be examined by passing T cells of the immune system. The MHC complex has two subunits. The smaller subunit, β_2 microglobulin, resembles an immunoglobulin domain. The larger α subunit also has an immunoglobulin-like domain which is linked to a head domain containing the antigen-binding groove.

The antigen-binding groove in the MHC head domain is built from two walls composed of long α helices that rest on a floor composed of an eight stranded β sheet. The peptide on display fits snugly between the helices in the groove.

The peptide backbone is bound at both ends by highly conserved regions of the MHC protein. Some peptide side chains extend downwards into specific binding pockets in the groove, while other peptide side chains project upwards where they can be recognized by T cells.

MHC class I proteins display their bound peptides on the cell surface for immune surveillance. Immune cells, called cytotoxic or killer T cells, for example, express T-cell receptors that bind to the MHC head domain and the bound peptide. If the cell expressing the MHC protein displays a peptide foreign to the immune system, the T cell is activated by this receptor-MHC interaction. The activated T cell then proceeds to destroy the abnormal cell. Cut-away views of this peptide-bound MHC protein complexed with a T-cell receptor reveal the exquisite precision with which the interacting surfaces fit together.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



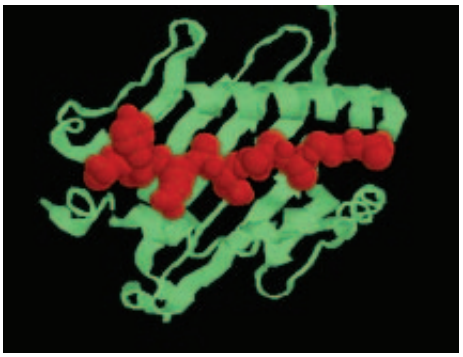
PDB ID number *: MHC class I molecule (1A1M); Human T-cell receptor, viral peptide and Hla-A 0201 complex (1AO7)

25.5 MHC Class II <GAAA>

MHC class II proteins have an overall similar structure to MHC class I proteins, although their subunit structure is distinct. MHC class II proteins are composed of two subunits that contribute to the structure of the head domain containing the antigen-binding groove.

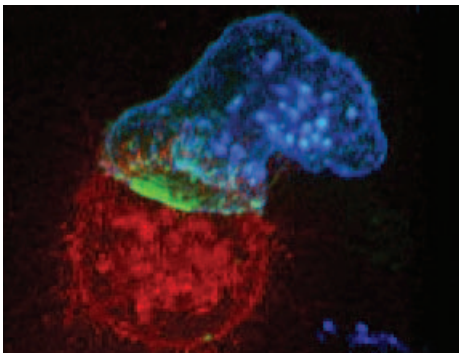
As a rule, MHC class II proteins bind longer peptides than MHC class I proteins. As seen in this comparison, the different shape of the antigen-binding groove allows the ends of the peptide to stick out. MHC class II proteins display peptides on the surfaces of specialized antigen-presenting cells and activate a different class of immune cells, called helper T cells.

Molecular modelling and animation: Timothy Driscoll, Molvisions (www.molvisions.com)



PDB ID number *: Compilation of MHC class I molecule & MHC class II/superantigen complex (1A1M & 2SEB)

25.6 The Immunological Synapse <ACGA>



In this 3D image, we are able to see the interaction between a T cell, colored blue, and an antigen presenting cell, in this case a B cell, colored red. When T cells are able to recognize their antigen on another cell, the zone of contact between the two cells, here colored green, forms an organized structure, called the immunological synapse.

In this second image, which shows a cytotoxic T cell recognizing its target, we can again see the synapse between the two cells. The cells are labeled with fluorescent molecules that show an adhesion molecule, the integrin alpha chain, CD11a, in green and a signaling molecule, lck, in red. The cytotoxic granules in the CTL are labeled blue.

If we look closely at the synapse, we can see its structure. The outer ring contains the adhesion molecule and the inner ring is divided into two parts: one containing the signaling molecules, and the other—dark in this image—is the secretory zone.

In this side view we can see that some of the cytotoxic granules, stained in blue, have moved close to the interface and are starting to fuse with the synapse. Other granules remain at the opposite end of the cell, perhaps where another synapse is starting to form.

Multiple synapses can be formed with the same antigen presenting cell, as we see here, where two CTL are trying to kill the same target. Each has formed a synapse organized into discrete signaling and secretory zones.

The signaling zones are indicated by the presence of the red stained signaling molecule, lck, while green labeled cytotoxic granules can be seen clustered just behind the synapse itself.

Part I:
Tomasz Zal
M. Anna Zal
Nicholas R.J. Gascoigne
The Scripps Research Institute

Part II:
Jane C. Stinchcombe
Giovanna Bossi
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