Cell cycle oscillations

Active Cdk1-Cyclin
Inactive Cdk1-Cyclin
Active APC

time
Many biological processes are regulated by controlling the stability and thus the abundance of crucial proteins. Here we use the cell cycle oscillator to introduce the most widespread form of protein degradation in eukaryotic cells, which is referred to as **ubiquitin-mediated proteolysis**. Its name comes from **ubiquitin**, a small (76 amino acid), strongly conserved protein found in all eukaryotes, which can become attached to other proteins, when the C terminal carboxyl group of ubiquitin forms an amide bond with the side chain amino group of a lysine on the protein destined for destruction. Once this has happened, further ubiquitins can be attached to lysine groups on the first ubiquitin creating a chain of ubiquitins, and once this chain reaches a certain length, it directs the ubiquitinated protein to a complex of proteases called the proteasome. This multi-protein complex uses the free energy of ATP hydrolysis to feed the ubiquitinated protein into the **proteasome**, the cellular equivalent of a garbage disposal, a cavity filled with active proteases that quickly chop the ubiquitinated protein into a series of short peptides and recycles the ubiquitin unscathed.
The **anaphase promoting complex** (APC) is the last enzyme in the sequence that puts ubiquitin on cyclin. The APC is another multi-protein complex and its activity is controlled by protein phosphorylation which is ultimately triggered by active Cdk1-cyclin complexes. If the steps from the activation of Cdk1-cyclin complexes to the activation of the APC are slow enough, Cdk1 will be active for a period, as the APC is getting activated, but once the APC is active, cyclin will be quickly ubiquitinated and then degraded, releasing naked and catalytically inactive Cdk1. Once the Cdk1 has been fully inactivated, the APC itself is turned off, and the oscillator is at the beginning the next cycle, with low cyclin levels, inactive and naked Cdk1, and a long cyclin half life.

Protein ubiquitination has several similarities to protein phosphorylation. It is effectively an irreversible reaction, placing a ubiquitin or a series of ubiquitins on a protein can alter its location, its activity, or target it for destruction, and just as there are many different protein kinases, each of which can phosphorylate a different set of proteins, so there are many different ubiquitinating enzymes each of which modifies a different set of proteins.
Similarities between phosphorylation and ubiquitination

<table>
<thead>
<tr>
<th></th>
<th>Protein Phosphorylation</th>
<th>Ubiquitination</th>
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<tbody>
<tr>
<td>Reversible reaction?</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Demodifying enzymes</td>
<td>Protein phosphatases</td>
<td>Deubiquitinases</td>
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<tr>
<td>Regulates</td>
<td>Protein activity, location, stability</td>
<td>Protein activity, location, stability</td>
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<tr>
<td>Number of human modifying enzymes</td>
<td>$\approx$500</td>
<td>$&gt;$500</td>
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Complications in the standard cell cycle

We also need to address another complication. The preceding description was couched in terms of a single cyclin, but most organisms contain several different types of cyclin that collectively control progress through the cell cycle. These are broadly grouped into four categories, mitotic cyclins (cyclin B), replication inducing cyclins (cyclin A and cyclin E), cyclins needed to progress through the G1 part of the cell cycle (cyclin D). Animal cells also contain several Cdk enzymes with each Cdk binding to a small subset of the cyclins. One of the reasons the cell cycle engine for the early embryonic cell cycle has been so revealing is that it can be driven entirely by the synthesis and destruction of cyclin B and the associated fluctuations in the protein kinase activity of the Cdk1-cyclin B complex.

Regulating progress through the cell cycle

So far we’ve discussed a simple and unregulated cell cycle. Once they are fertilized, frog eggs divide as fast as they can, without growing, and without being subjected to any of the checks and balances that control the cell cycles of our cells. In our cells, cell growth and cell proliferation must be precisely coordinated so that the average size of our cells stays the same over many cell cycles, and both processes must be regulated by signals from outside the cells that tell them when they need to proliferate and grow and when they need to remain quietly in G0. For example, when you skin your knee you need extra cell growth and proliferation to replace missing skin and produce new blood vessels, and the platelets that help your blood clot release factors that induce this growth and proliferation.
In animal cells the G1 cyclins appear to play a key role in controlling both cell growth and cell proliferation. These cyclins can’t be essential for the oscillation of the minimal cell cycle engine, since they are missing from early embryonic cell cycles. So what do the G1 cyclins actually do? The answer is that they remove a road block that has been placed in the G1 phase of the cell cycle, and is the most important difference that separates the early embryonic and standard cell cycles. It is the road block that allows signals from outside the cell to keep cells from progressing through the cell cycle. The road block means that the default decision of cells is to halt in G1 and then move to G0. They can only continue in the cell cycle if they receive specific signals from outside the cell that that send them into G1 and commit them to replicating their DNA and completing the cell cycle. In mammals, the signals that are needed to get past G1 are growth factors, small proteins that certain cells secrete to control the growth and proliferation of other cells. Biochemically, the road blocks are molecules that bind to and inhibit the Cdk-cyclin complexes that would otherwise drive cells into S phase, and the G1 cyclins overcome the roadblocks by targeting the inhibitors for ubiquitin-mediated proteolysis, a device that has echoes of how cells employ the proteolysis of mitotic cyclins to drive them out of mitosis. Almost every cancer that has been carefully investigated has defects in the machinery that regulates passage through the G1 roadblock, either because they express the G1 cyclins constitutively or because they have lost components of the roadblock.
3. CELL CYCLE CHECKPOINTS

Coordinating events in the cell cycle with each other

So far we have discussed the overall regulation of the cell cycle and the need to coordinate cell growth with the replication, mitosis, and division cycle. We also need to address how the events in this cycle are coordinated with each other. For example, cells have to make sure that they have completely replicated their chromosomes before they enter mitosis and try to segregate the sister chromosomes from each other. If there is a stretch of DNA that has not been replicated, it will be impossible to separate the sister chromosomes without breaking them, because in the unreplicated region the two sister chromosomes are represented by the two single strands of the DNA duplex which are wound around each other once every 10 base pairs. Once a chromosome has been broken, all sorts of bad things can happen, including losing all or part of a chromosome and linking the promoter of one gene to the protein coding part of another, exactly the type of mutation that might lead to constitutive expression of a G1 cyclin and uncontrolled cell growth and proliferation.
Cancer cells lack G1 roadblocks

Standard Cycle

NORMAL

Cdk Inhibitors Lost

CANCER

OR

Too much G1 cyclin

CANCER
Cell cycle checkpoints

a. Cell cycle arrests

b. Damage repair
How do cells solve these problems of making sure that one of the steps in the cell cycle is completed before the next one begins? Most cells rely on a class of regulatory circuits called **cell cycle checkpoints**.

A cell cycle checkpoint is a surveillance system that monitors whether one of the steps of the cell cycle has been completed. If it has not, the checkpoint sends two signals. One goes to the cell cycle engine, arresting its progress to allow the errant process more time, and the other starts repair mechanisms that help fix any defects the checkpoint has spotted. For example, treating a mammalian cell that has replicated its DNA with ultraviolet light or X-rays damages the cell’s DNA. If the damage were left unrepaired and the cell went through mitosis, the progeny cells would end up with mutations in their DNA. To prevent this, the DNA damage checkpoint detects the damaged DNA and sends two signals. The first inhibits the activation of Cdk1-cyclin B complexes thus keeping the cells from entering mitosis, and the second induces the transcription of enzymes that will help to repair the damaged DNA. Once the damage has been repaired, the signals from the checkpoint disappear and the cell can enter mitosis and divide to produce progeny in which the consequences of the initial insult have been made as small as possible.
Depending on the cell type and the type and extent of damage, checkpoints can also induce a third and more dramatic response, the cellular equivalent of suicide. This is a process known as apoptosis, or programmed cell death, in which cells induce the expression of genes that will kill them. The logic here is that of a completely altruistic cooperative. If a cell has been severely damaged, even with repair pathways, it is likely to produce daughters that contain mutations, and if these mutations could initiate the progression to cancer, the best thing for the cooperative to do is to kill the offending cell. And since the insulted cell is the best judge of how badly damaged it is, the logical conclusion is that this cell should kill itself. This exaggerated sense of propriety is one of our best defenses against cancer, and almost all cancers have mutations in the pathways that would normally lead to programmed cell death. One of the most commonly mutated genes in human tumors is named p53, and the normal version of this protein plays two crucial roles in the DNA damage response, making sure that the cell cycle arrests in cells whose DNA has been damaged, and inducing programmed cell death in cells with severe damage.

Programmed cell death is also tremendously important in normal development and the functioning of the immune system. An example from development is that our fingers and toes emerge out of flat paddles because carefully controlled cell death eliminates the tissue between our digits, and in the immune system, developing cells that would recognize and destroy our own bodies are detected and induced to kill each other in the thymus.
Apoptosis kills damaged or unwanted cells
Mitosis and the cytoskeleton

a. Phosphorylation controls cellular architecture

b. The cytoskeleton: roadways & scaffolding

c. Microtubules are dynamically unstable

d. Self assembly by exploration with selection:
   Chromosome capture by exploration with selection

We finish our consideration of the cell cycle by studying the details of mitosis, the process that separates the replicated chromosomes into two identical sets, and triggers the cell division that will lead to one set appearing in each of the two daughter cells. This topic will introduce two important ideas. The first is self assembly, the ability of simple set of rules for molecular interactions to give rise to large, complicated, structures that have properties, such as the ability to segregate chromosomes, that none of their individual components possess. The second is exploration with selection, the idea that selection acting on random variation can give rise to interesting and highly improbable structures. We believe this idea explains processes as diverse as protein folding, the assembly of the mitotic spindle, the development of the nervous system, and Darwinian evolution.
Before we can get to these ideas we need to describe the events of mitosis in more detail. The first sign that a cell is about to enter mitosis is a period called **prophase**. During prophase, the chromosomes condense; they become visibly distinct from each other as very thin threads that shorten and thicken until each chromosome can be resolved into a pair of sister chromosomes. Prophase ends when the nuclear envelope breaks down, abolishing the distinction between nucleus and cytoplasm. Dramatic changes also occur in the organization of **microtubules**, long fibers that radiate throughout the cell from a **microtubule organizing center** that is called the **centrosome** in animal cells. Cells are born with a single microtubule organizing center, which duplicates during interphase. As mitosis progresses, the microtubule network undergoes profound changes that lead to the formation of the **mitotic spindle**, an array of microtubules shaped like an American football with the centrosomes at its ends. (During mitosis, microtubule organizing centers are often called **spindle poles**.) Specialized regions of the chromosomes called **centromeres** attach them to microtubules (many cell biologists distinguish the **kinetochore**, the complex of proteins that attaches chromosomes to microtubules from the centromere, which they define as the piece of DNA on which this complex assembles. Here we define the centromere to include both objects). The two members of each pair of sister chromosomes attach to microtubules originating from opposite poles of the spindle and move to a position midway between the two spindle poles. When all the chromosomes are lined up like this, the cell is in **metaphase**.
The cell remains briefly in metaphase before dissolving the linkage between the sister chromosomes, a critical event that marks the beginning of **anaphase**. Because sister chromosomes are no longer held together, they separate from one another and move along the microtubules to opposite poles of the spindle. As the chromosomes near the poles, the physical process of cell division, called **cytokinesis**, begins. A contractile ring pinches the cell into two daughter cells, each containing a complete set of chromosomes and a spindle pole. As cytokinesis proceeds, the chromosomes decondense and acquire a nuclear envelope, re-forming an interphase nucleus, and the microtubule array returns to its interphase pattern, marking the beginning of the next cell cycle.

Cells produce genetically identical daughters because they replicate their chromosomes in S phase and the segregate the sister chromosomes from each other in mitosis. As Watson and Crick pointed out in their 1953 paper, the double helical structure of DNA and the rules of base pairing immediately suggested how one strand of DNA could serve as the template for assembling its partner. Mitosis lacks such an obvious template, since a roughly spherical interphase nucleus breaks down and its contents rearrange to form a football shaped spindle, with the two members of each sister chromosome pair attached to opposite ends of this structure. This is a spectacular example of self assembly. You start mitosis with a set of components that is homogenously distributed through space or **isotropic** and then the interactions of those components with each other gives rise to an elaborate structures whose properties are different in different directions and is thus called **anisotropic**.
Mitosis: the movies

Video Enhanced DIC Microscopy of Mitosis in Newt Lung Cells
(Tamiea granulosa)

Victoria Skeen,
Robert Skibbens, and
E. D. Salmon
University of North Carolina at Chapel Hill
(see Skeen et al., '92, J. Cell Biol., 122:4667-4676)
Frame Time: 100 MS/NSEC

Aaron Straight
To illustrate what we mean, consider the assembly of a mitotic spindle in the early embryonic cell cycle of a fertilized frog egg. A dramatic increase in the protein kinase activity of Cdk1-cyclin B complexes drives the cell into mitosis by initiating a series of reactions that completely rearrange the architecture of the cell. Before the spindle can be built, cells must do three other things: they have to dissolve the physical barrier between the microtubules, which are in the cytoplasm, and the chromosomes, which are in the nucleus, they have to compact their chromosomes so that they are small enough and strong enough to be dragged through the viscous solution of proteins that makes up the cell’s interior, and the behavior of the microtubules has to change to help them find the centromeres quickly.

In eukaryotes the chromosomes are kept separate from the cytoplasm by the nuclear envelope. This physical barrier is composed of two lipid bilayers that lie on the cytoplasmic side of a proteinaceous shell called the nuclear lamina, made up of thin filaments that are the result of polymerizing long thin proteins called nuclear lamins. The Cdk1-cyclin B complexes enter the nucleus and phosphorylate the nuclear lamins, altering their physical properties and causing them to dissociate from each other, thus depolymerizing the filaments and dissolving the nuclear lamina. The mechanisms that break the membrane that separates nucleus into cytoplasm into thousands of tiny vesicles are less clear, but they are also initiated by Cdk1-cyclin B complexes.
The second change affects the chromosomes themselves. They go from being loosely packed diffuse objects that we cannot distinguish from each other to highly compacted (or condensed) rods which are easily seen as distinct objects by light microscopy. Again this change is brought about by Cdk1/Cyclin B’s ability to phosphorylate a specific class of molecules, called condensins, which help to compact the DNA.
Monomers (entire proteins) are held together by non-covalent bonds

The last major change associated with mitosis affects the behavior of the microtubules, one of three classes of filament, which collectively make up the cytoskeleton, the physical framework of the cell. The three classes are microtubules, actin filaments, and intermediate filaments. The names of the filaments are partly based on their diameter and structure. Actin filaments were once known as microfilaments and are the thinnest filaments at 5 nm in diameter, and they are often found bundled together in more or less regular arrays. Intermediate filaments are 10 nm in diameter and microtubules are hollow tubes 25 nm in diameter.

All of the filaments are polymers and it is important to point out two ways in which they differ from proteins and nucleic acids the two sorts of polymers that much of this course has discussed. First, the subunits of the cytoskeletal polymers are not simple building blocks, like amino acids or nucleotides, but are themselves macromolecules, being intact, fully folded proteins composed of several hundred amino acids. Second, the linkages between the different subunits of the polymer are not covalent bonds, as they are between amino acids in proteins, or nucleotides in nucleic acids, but non-covalent interactions, involving all the different types of interactions (including hydrogen bonding, the hydrophobic effect, Van der Waals forces, and ionic bonds) that David mentioned when he discussed how small molecules bind to proteins. When the polymers assemble, new subunits associate with the ends of an existing polymer, and when they disassemble subunits dissociate from the ends of the polymer.
Actin filaments and microtubules have two roles, they define the physical shape of the cell by acting as stiff scaffolding and they are the roadways of the cell, which molecular motors can drag cargo along. For example in your muscles, it is the coordinated movement of millions of copies of the molecular motor myosin along a beautifully organized array of actin filaments that shortens the muscles and thus move one part of your body relative to another. In both their scaffolding and transport roles, the individual actin filaments and microtubules are dynamic, often lasting for as little as a minute, and this flexibility allows cells to change their shape and move from one place to another. In some, specialize cells, like muscle and nerve cells, the filaments are much, much more stable but this is the exception rather than the rule. In contrast, intermediate filaments, such as the nuclear lamins, appear to be only scaffolding since we know of no motors that move along them, and I will point out other differences between them and the other two types of filament as we go on and discuss the behavior of microtubules and their role in mitosis in more detail.
Microtubules are long rigid polymers of the protein tubulin, up to 50 um long. Each tubulin subunit is a dimer of made up of two protein molecules, one α tubulin and one β tubulin. An individual microtubule is a hollow tube, composed of 13 protofilaments, long head to tail polymers of tubulin molecules, whose sides stick to each other to form the tube. Because tubulin is an asymmetric protein, the microtubules made from it have a defined polarity and the three dimensional structure of the two ends (called plus and minus) of the polymer are different, and different motor proteins can move along the microtubule in opposite directions. Microtubules and actin filaments share two properties, which differentiate them from intermediate filaments. First, they are both polar because they are made of asymmetric subunits, giving the polymers a direction that motors can exploit to move along them, whereas intermediate filaments are made up of symmetric subunits so that both ends of an individual intermediate filament are chemically identical and the polymer has no polarity that motors can use to guide them. Second, actin and microtubules have the very unusual property that at the same time and in the same part of the same cell, some filaments are growing while others are shrinking.
Microtubules are polymers

How do microtubules form in the first place. This is an important question because the addition of one more subunit to a growing polymer is much easier than what is called nucleation, the initial meeting of several subunits that is needed to form the smallest possible filament. For a microtubule to nucleate spontaneously, 13 tubulin subunits must come together and form a ring of subunits. This is a highly improbably event, and while it can happen in a test tube it requires very high concentrations of tubulin and happens very slowly. Inside cells, microtubules are nucleated by specialized structures called microtubule organizing centers, and in animal cells these are the centrosomes. They contain rings of a special form of tubulin called gamma tubulin, which serves as the nucleus for forming a microtubule, essentially by acting as a template to which tubulin subunits can add one at a time.
**Microtubules are nucleated at organizing centers**

Microtubules are dynamically unstable

Inside the same cell, one microtubule can grow while another one shrinks. To understand how this is possible we need to examine the details of how the filaments grow and shrink. We concentrate on microtubules but the same basic ideas apply to actin filaments. The key is that inside cells microtubules and actin filaments are at steady state rather than being at equilibrium. From our discussion of steady states, you will realize that this implies that chemical pathway by which the filaments are assembled must be different from the one by which they are broken down.
Equilibrium polymers can grow OR shrink

\[ M_n + M \rightarrow M_{n+1} \]

Growing
(high [monomer])

\[ M_n + M \rightarrow M_{n+1} \]

Shrinking
(low [monomer])

We start by discussing the fate of an equilibrium polymer, like intermediate filaments. The addition of monomers to the filament is an equilibrium reaction. A monomer adds to a polymer containing \( n \) subunits to make a polymer containing \( n + 1 \) subunits. We cannot easily write down an equilibrium constant in the traditional form but we can say something about the monomer concentration at equilibrium, when monomers are being added to and lost from the ends of polymers at equal rates. The rate at which they are being added is given by

\[
\text{Rate of subunit addition} = k_{on} \times [M] \times [\text{ends}]\]

where \( k_{on} \) is the rate constant for adding subunits, \([M]\) is the concentration of free monomer and \([\text{ends}]\) is the concentration of polymer ends. The rate at which subunits are lost is given by

\[
\text{Rate of subunit loss} = k_{off} \times [\text{ends}]\]
Equilibrium polymers can grow OR shrink

\[ \text{Growing (high [monomer])} \]

\[ M_n + M \rightarrow M_{n+1} \]

\[ \text{Shrinking (low [monomer])} \]

\[ M_n + M \rightarrow M_{n+1} \]

where \( k_{\text{off}} \) is the rate constant for losing subunits. At equilibrium these two rates must be equal, and at the in this situation we call the monomer concentration the critical concentration \([M]_{CC}\) and we can find this as follows:

\[
\text{Rate of subunit addition} = \text{Rate of subunit loss} \\
k_{\text{on}} \times [M]_{CC} \times \text{[ends]} = k_{\text{off}} \times \text{[ends]} \\
k_{\text{on}} \times [M]_{CC} = k_{\text{off}} \\
[M]_{CC} = \frac{k_{\text{off}}}{k_{\text{on}}} 
\]

Above the critical concentration all the polymers in the cell must grow, since the monomer concentration is greater than the critical concentration, and below it they must all shrink so every polymer of this type in the cell must behave in the same way.
Non-equilibrium polymers can grow AND shrink

\[ M_n + M \rightleftharpoons M_{n+1} \]

In contrast, within the same cell, some filaments of a non-equilibrium polymer can grow, while others shrink. How can this be? The answer is that the monomer can exist in two different chemical forms. For form 1 (red in the diagram), there is a strong preference to bind to filaments that are in this form causing these filaments to grow, and for form 2 (black) there is a strong preference for monomers to be lost from filaments so that these filaments shrink. If free energy is used to convert one form into another and the opposite conversion has a different chemical path, this is a non equilibrium reaction and the relative proportion of the two forms is set by the rate of these two interconversions, which can be varied independently.
Microtubules can grow AND shrink
This is exactly what happens for tubulin. When it is not attached to a microtubule, the majority of the tubulin subunits in the cell contains GTP rather than GDP, and the bound GTP molecules hydrolyze very slowly to GDP. But when a GTP-tubulin subunit adds to the plus end of a microtubule, and the rate of GTP hydrolysis goes up dramatically and the GDP that is produced cannot escape from the tubulin subunit until the subunit is released by depolymerizing the microtubule. The hydrolysis of GTP, like that of ATP, releases energy and that this energy can be used to do work. In the case of tubulin, this work is to produce subtle changes in the three dimensional structure of the tubulin subunit, so that the structure of tubulin bound to GTP is different from that of tubulin bound to GDP. We refer to these two different structures as different conformations of tubulin, and because their structures are different, albeit subtly, the different conformations can show different chemical behavior.
Microtubules can grow AND shrink

The consequence of having different reaction rates for tubulin in solution and on microtubules is that a growing microtubule adds GTP-tubulin subunits and that these then rapidly hydrolyze their GTP to GDP. As long as the subunits add fast enough relative to hydrolysis, the plus end of the microtubule has a very short cap of GTP tubulin at its very end, but that underneath all the tubulin has hydrolyzed its GTP to GDP. This cap is crucial because GTP hydrolysis induces conformational changes in the tubulin molecules. In its GDP-bound conformation, tubulin reaches its minimum free energy by forming curved rather than straight protofilaments and as a result the protofilaments want to spring apart from each other destroying the microtubule. As long as the microtubule ends with a cap of GTP-bound tubulin subunits these forces can be resisted, but if the rate at which GTP tubulin get hydrolyzed at the end of a growing microtubule temporarily exceeds the rate at which new subunits are added, the GTP tubulin cap disappears, and the tendency of the protofilaments to splay apart can’t be resisted. Once they lose contact with each other, the protofilaments rapidly lose tubulin subunits from their end causing the whole microtubule to start to shrink rapidly. This dramatic transition is called catastrophe since the microtubule goes instantly from growing to shrinking just as stock prices did in October 1929. Once the microtubule is shrinking, it is hard to add new subunits, but occasionally, just by chance, the end of the shrinking microtubule adds enough GTP tubulin subunits to convert it from shrinking to growing, a process known as rescue.
Microtubules can grow AND shrink

We now examine the individual steps of this cycle in slightly more detail. We begin with the tubulin subunits that are free in the cytoplasm. They mostly are mostly bound to GTP because the equilibrium between the GTP bound and GDP bound forms of tubulin is strongly in favor of the GTP bound form at the relative concentrations of GTP and GDP that exist in the cell. Two factors favor the binding of GTP-tubulin to the growing plus end of a microtubule, there is more GTP-tubulin than GDP-tubulin, and the association constant for GTP-tubulin to the end of a microtubule is higher than that of GDP-tubulin.

Once GTP tubulin has bound to the end of a microtubule, the free energy of binding can be used to distort the structure of the bound tubulin. This distortion then lowers the activation energy for the hydrolysis of GTP, explaining why the binding of tubulin encourages it to hydrolyze its GTP. Here the free energy released when two molecules bind to each other is used to distort the structure of one of the two molecules and as a result a chemical reaction (GTP hydrolysis) which used to be kinetically unfavorable becomes favorable. Once the hydrolysis has occurred, the lowest free energy conformation of the tubulin subunit changes, and, as described above, this change makes the microtubule prone to depolymerization by making the preferred state of the protofilaments one in which they are curved instead of straight. Here a chemical change is altering the free energy landscape so that the tubulin subunits now prefer a different conformation to the one they had before hydrolysis.
Microtubules can grow AND shrink

The microtubule is held together by interactions between the GTP-bound tubulin that forms the GTP cap at its very tip. Even though the protofilaments would like to spring apart because they are made almost entirely of GDP-tubulin subunits, the interactions within the cap are strong enough to make a strong kinetic barrier to depolymerization. So what determines the fate of the microtubule is the state of the tubulin in its final layer. As long as the final layer is GTP-tubulin, the microtubule keeps growing, but if the proportion of GDP-tubulin subunits grows too high, the protofilaments splay apart and the microtubule undergoes catastrophe and starts to shrink rapidly.
To understand how the GTP cap can disappear, we need to understand the distinction between the average rates of reactions, when that average is computed over many molecules, and the fluctuations in the rates at which individual molecules are reacting. Inside a test tube, the average length of the microtubules may be increasing, but some individual microtubules will still suffer catastrophes and shrink. Similarly, the average length of the microtubules may be decreasing, but some individual microtubules can be rescued and start to grow. To see why, you have to appreciate when we talk about the reactions of individual molecules we need to think about the probability that something happens in a given time, in just the same way that we had to think about the probability of rolling six at least once after rolling a dice a certain number of times. For a typical growing microtubule in a mitotic cell, tubulin subunits are adding to the plus end at about 300 subunits a second. This means that the average interval between the binding of one subunit and the next is 3 milliseconds but sometimes this interval is longer and sometimes it is shorter. More specifically, the fastest 5% of the subunits add less than 0.2 milliseconds after their predecessors and the slowest 5% of the subunits add more than 9 milliseconds after their predecessors. It is exactly this sort of fluctuation that leads to the growth and shrinkage of the cap of GTP-bound tubulin subunits.
In a similar vein, once the tubulin subunits have been incorporated into the growing microtubule the GTP they carry is hydrolyzed on average within a few milliseconds after it has been incorporated, but once again some individual subunits hydrolyze their GTP much faster and some do so much more slowly. The consequence of the fluctuations in the time it takes to add tubulin subunits or hydrolyze the GTP on those that have recently bound is that the size of the GTP cap fluctuates. Periodically, these fluctuations make the cap disappear altogether, and when that happens there is nothing to resist the intrinsic tendency of the protofilaments containing GDP-tubulin to spring apart and a catastrophe occurs.

Thus, even in a population of microtubules where the average length of microtubules is not changing, most individual microtubules will be either growing or shrinking. This highlights an important distinction between equilibrium and steady state. If microtubules were polymers at equilibrium, this could not happen. Although individual microtubules might occasionally add a subunit or two while others where losing theirs, these discrepancies would be microscopic and all of the microtubules would either by growing or shrinking roughly in unison. But microtubules are steady state creatures growing and shrinking by different paths, since they grow by the addition of GTP-tubulin to the end of microtubules with GTP caps, which is an energetically favorable reaction, and shrink by the loss of GDP-tubulin from the ends of microtubules that lack a cap, which is also an energetically favorable reaction.
If both growing and shrinking reactions are energetically favorable aren’t we invoking some molecular equivalent of perpetual motion? The answer is no because every time a tubulin molecule binds to a microtubule it does so carrying GTP, every time it falls off it does so after hydrolyzing the GTP to GDP, and before it can bind to a microtubule again, it must exchange the GDP for GTP. Thus in every circuit it makes, each tubulin molecule consumes one high energy phosphate bond, and it is the hydrolysis of this bond that converts the structure of the tubulin subunit from a form that supports polymerization to one that encourages shrinkage. A simple experiment that supports this idea is replacing the GTP on tubulin with a derivative whose terminal phosphate bond can’t be hydrolyzed. These tubulin subunits polymerize into microtubules that never have catastrophes and simply keep growing until they exhaust all the tubulin subunits free in solution.
Microtubules are more dynamic in mitosis

It is this probing and the ability of the chromosomes to regulate the behavior of microtubules that explains how cells can form a spindle. When the nucleus breaks down, the centrosomes are nucleating microtubules in all directions with equal probability so that you can think of the centrosome as laying at the center of a sphere of growing and shrinking microtubules. As they grow and shrink, the ends of some of these microtubules encounter either the arms of chromosomes or their centromeres. These encounters reduce the catastrophe rate of the microtubules, modestly if the end gets close to a chromosome arm, and strongly if it contacts a centromere. These interactions have two effects, they attach the chromosomes to the spindle poles, and they lead to a much higher density of microtubules pointing from the centrosomes towards the chromosomes than pointing away from them and towards the periphery of the cell. This asymmetry occurs not because microtubules are preferentially nucleated towards the chromosomes but because the microtubules that encounter the chromosomes are stabilized, and the microtubules that do not meet chromosomes are not.

Astonishing as the assembly of the spindle from two centrosomes, microtubules, and a collection of chromosomes is, some cells do even better. For example, in the oocytes of vertebrates, there is no functional microtubule organizing center and it is the chromosomes themselves that nucleate the chromosomes, and the action of microtubule motors associated with the chromosomes and free in solution that produces the familiar spindle.
Again, we are witnessing a fundamental principle in biology, the establishment of order by selectively favoring a subset of what are initially random walks from some starting point. This idea is sometimes called **exploration with selection**, and is involved in many, many fundamental processes in biology, over a wide range of sizes and times. At the smallest and quickest level, it explains how proteins can fold enormously faster than they would if they had to explore every single arrangement of their polypeptide chain in space, and then pick the one with the lowest energy, a process that would take longer than the our estimates of the lifetime of the universe. Turning to larger and slower processes, we have just seen how the stabilization of some microtubules rather than others produces the football-shaped spindle. At the level of whole organisms, exploration with selection is a crucial feature of development. For example, far more nerve cells are created than will be needed to innervate all the tissues of the developing embryo, but only those cells that reach the desired targets are supplied with special factors that promote their survival. The ones that end up elsewhere induce programmed cell death. Finally, and most importantly this is the principle of evolution by mutation and selection that we have already encountered in thinking about AIDS and cancer. Even though most mutations are neutral or deleterious, a tiny fraction confer an advantage to the virus or cell that possesses them. Because their descendants contain the same mutation, these mutant viruses or cells increase in frequency with each generation and eventually take over the population. For AIDS and cancer this spells disaster for the patients, but when advantageous mutations occur in the germ line of an organism they are responsible for the evolution.
Exploration with selection is a general principle

<table>
<thead>
<tr>
<th>Process</th>
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<td>Protein Folding</td>
<td>0.1 - 10 nm</td>
<td>1 ms - 10 min</td>
</tr>
<tr>
<td>Spindle Assembly</td>
<td>10 μm</td>
<td>1 min - 1 hour</td>
</tr>
<tr>
<td>Nervous system development</td>
<td>1 μm - 1 m</td>
<td>1 day - 1 year</td>
</tr>
<tr>
<td>Evolution</td>
<td>0.1 nm - 100 m</td>
<td>1 month - 10⁹ yr</td>
</tr>
</tbody>
</table>
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