I've emphasized in the first lecture, you know, that there's a lot of stuff that happens just in your ordinary life. I saw two examples of this. Yesterday's Boston Globe, just on the front page there was a discovery about Ã¬Heart Cell Discovery Raises Treatment HopesÃ®. Scientists announced yesterday the discovery of cells in the heart that can create new muscle cells raising hopes that doctors may find dramatic new ways to treat heart disease. The team showed that the cells, which are similar to stem cells, can be expanded from just a few hundred in the laboratory dish up to more than a million. And these can be guiding into becoming the pulsing muscles that power the heart. So when we were talking about those yeast dividing and saying how one cell becomes two, this is a general principle throughout life that cells come from other cells and they divide. And we'll see the relationship to that with DNA replication as we go along. In the case of yeast, as I said, they're just always the same. Your progeny are always the same. But in something like our own cells we start out as a single fertilized cell but somewhere along the way the cells have to become specialized. So the very early ones are the embryonic stem cells. They have the potential to become any cell in the body. But at some point, at one of these cell divisions the cells are going to have to start to become more specialized. And, for example, this one might be a lineage that would lead to heart muscle or to becoming a nerve or something. And at that point it loses its ability to become any cell in the body. And in many cases by the time you get out ultimately to the final cell that's making up the muscle or the nerve or something it has no capacity to regenerate. So that's why, for example, spinal cord injuries are so damaging because nerves at this point cannot be regenerated. Or heart disease, you get a damaged heart we're stuck. This is why this result is exciting. Because there seem to be at least a few cells in the heart that have the capacity to regenerate more heart muscle. Now, this is early on. It hasn't been rigorously shown to be a stem cell. But there's an example from the front of yesterday's paper about something we were virtually alluding to in class. There was also an article about AIDS testing. Again, you know, we're talk more about the HIV-1 virus. And then today on the front page of the Boston Globe yet again is Ã¬Romney Draws Fire on Stem CellsÃ®. And you can look at this. But, you know, he's sort of trying to straddle, I guess, between being supportive of research on the one hand and the concerns of the conservatives and the religious right on the other hand, and he's drawing fire from both sides. But it's an issue that is in our society today. You're going to be expected to make decisions on it, to know about it and understand. I'm just trying to drive home that what we're talking about isn't taking place in a vacuum. Nobody emailed me an idea as to what happened here. I showed you this little movie. This is water that is cooled below the freezing point but hasn't formed ice crystals, but if we put a little bit of this pseudomonas syringae in it then somehow that super-cooled water turned into ice. And I told you it was a protein on the surface. Nobody had any ideas. So why don't you turn to whoever is close to you and you can talk about it for 30 seconds and see if anybody can come up with an idea as to why. All right? I won't look. You know, just go ahead. Talk to somebody and come up with an idea. OK. Well, let's see. Did we manage to get any ideas? Anybody got the courage to try and guess what that protein might be doing? Pardon? It's a nonpolar molecule. It's not disturbing the bonds. It's an interesting idea. Do you have an idea then, are you able to extend that as to why then the ice would start to form? I mean it's certainly true that nonpolar bonds sort of interfere with the water. That's something we've talked about. Let's see. Any other ideas? Yeah? That's a version of the same idea, I think, hydrophobic because you think it wants to repel the water and push it together. That's interesting. You're sort of getting closer on these. Yeah? There it is. If you were to design a protein that basically could bind water molecules in a lattice that mimicked what you found in ice then the water molecules coming up and binding to these little pockets in the protein would present then a little field of stable water molecules that looked to the next water molecule like it was part of an ice crystal. And that's indeed how that bacterium does that trick. It's called the ice nucleation protein. And they do things like take this bacterium and they put it into things like when you're doing snowmaking, you put this in and then you spray the super-cooled water, and this makes it go into ice crystals and then it helps you get nice snow for ski resorts and things. That's at least one of the areas where it's used. OK. So I'm just going to show you this movie again. These are just baker's yeast, saccharomyces cerevisiae, a kind of single-celled yeast that's used in baking bread or making beer. And here we're seeing cells divide. And this particular kind of yeast has a way of doing, it kind of buds the daughter off from the side. Some double and then split down the middle. But you can see what's going on. There's a lot of cell growth going on. And the issue that we're going to address now is where does the energy come that's needed to do that? You know from your own experience that to build things, to make things takes energy. You cannot put up a bridge, you cannot put up a building, you cannot build a computer chip without somehow putting energy in. You're taking a bunch of matter in the universe and ordering it in a very specific way making new contacts that didn't be there. It's an energy-requiring process. And I'm going to talk today about where that energy comes from. And then I want to tell you a little bit, just a very brief historical thing along the way, because a point I've emphasized here is biology is an experimental science. And many of the greatest discoveries weren't because somebody had the idea and then went out to prove it. Very often we didn't even understand how it worked. And somebody was investigating a phenomenon, found some peculiar things, and then began to get insights. And the insights were what then led to a fundamental increase in our understanding. And this little bit of history involves some names that you see on the MIT buildings around here. One is Lavoisier who is a French scientist. And he was studying what happened when grapes were converted into wine, a good topic for a French scientist to be studying. So, in essence, what he was studying was glucose being converted to two molecules, excuse me, of -- -- ethanol and two molecules of carbon dioxide. This transformation, there's C6H12O6. Remember, carbohydrates have that composition. And so he was studying that. He managed to figure out that's what happened to the sugar when you were making the wine. And at that point he got beheaded. That terminated that part of his investigation. But this problem was then picked up by Lois Pasteur who, again, his name is on one of the MIT buildings. He worked in France as well. There's a Pasteur Institute in Paris. There's a nice museum in Lille in Northern France that has a lot of this. But he grew up in Arbois which is a town in sort of

Eastern France that, as you can see from the little picture of the village, winemaking was a major industry. So he was interested in that probably from when he was a small, small kid, although probably not dressed like that. But anyway. So one of the issues that he took on, which was a real problem for the wine growers in his little town and in France in general was sometimes wines would go bad. They'd come out sour and couldn't be drunk and then you'd lose all the profit that would have come from that wine. So there was a lot of interest in trying to figure out how to prevent wines from going bad. And so Lois Pasteur started to study this. And he discovered that there was this conversion that had been figured out now of two ethanol and two carbon dioxide. So this was a conversion. And we now refer to it generally as Ã¬a fermentationÃ®. But what he discovered with this conversion occurred -- -- if yeast were present. That the rate of this conversion varied as the number of yeast, so it went faster if there were more yeast. And the yeast stopped growing -- -- when the sugar ran out. So what he discovered here was a correlation. He hadn't proven anything. He just saw that if you watch sugar go to ethanol there were yeast around, if you had more yeast it went faster, and when you ran out of sugar the yeast stopped growing. There was something connected here. So he came up with the idea that the yeast were responsible for this conversion that was happening when you made wine. And it was further helped out in this because he discovered an alternative -- -- conversion in which C6H12O6 went instead to give two molecules of CH3CHOH. This molecule which you know, galactic acid, it too has C6H12O6 on both sides of the equation but it's a different molecule. And what he found was that this is the lactic acid you know as what's in yogurt. It makes yogurt sour. Or if you exercise really hard and your muscles are sore that's because you accumulate lactic acid in your muscles, and I'll tell you why that is in the next lecture. But what the other thing that Pasteur realized was when you got this alternative conversion you didn't have yeast present, you had some other organism. And so that was a huge advance just of practical value to the winemakers because they knew they had to have yeast in there to get wine and there problems were coming when some other organism that wasn't yeast got in there and it did something different with the sugar and made it into lactic acid instead of making it into ethanol and carbon dioxide. So there was Pasteur working away on a practical problem and it was, you know, a really major advance to the winemaking industry for him to do this, but it also then sort of unexpectedly led to another issue. And that was why were the yeast doing this? Because one of the things that Lavoisier had noticed and Pasteur noticed was that you did this conversion. The two ethanol plus two carbon dioxide. But you could account for virtually all of the carbon and hydrogens and oxygens that started out as sugar and seemed like virtually of them showed up in the ethanol and the carbon dioxide. So why was the yeast doing this? And the idea began to develop out of that was that rather than being used to make biomass, in which case you would have expected to see a whole lot of mass in the yeast cells and no so much up here, that instead most of this sugar was being used to make energy and that somehow the cell was getting the energy necessary to all that synthetic work involved in cell division by carrying out this conversion. And there's a fundamental relationship then between chemical energy and whether a reaction can proceed. And I'll just take it through in sort of your typical introductory chemistry reaction, A plus B going to C plus D. You know, there are certain classes of reactions that will go almost to completion. Probably an overstatement to say it's to go to completion, but it's effectively over here. Those are termed irreversible reactions, and there are certainly some of them. If I have hydrogen and oxygen and I light a little match, you pretty much go all the way to making water with a great big boom and no hydrogen or not much hydrogen and oxygen left on the other side. However, most reactions that one finds in nature don't have that quality. Instead they are going forward at some rate and back at another. And they reach eventually an equilibrium that's characterized by what's known as an equilibrium constant which is the product of the concentrations of the products over the product of the concentration of the reactants. And that's a characteristic of every particular chemical reaction. And we really have to worry about this in biology because if everything was irreversible that would be fine, but in order to do all this synthetic work you have to deal with a lot of reactions that aren't going to go to completion. And nature has had to figure out a way of doing that, just the same way that bridges and buildings don't spontaneously assemble and engineers and others have had to work out ways of putting all of those things together. So at some level you see the same kind of problem. Now, there's a way of expressing this energy associated with a chemical reaction that can be used to directly calculate whether a reaction is going to go and how far it will go. And a person who did this work is another person who's on one of the MIT buildings. It was [Willard? Gibbs who was a faculty member chemist who worked at Yale in the 1980s, excuse me, 1800s, and he came up with an expression that's now known as Ã¬Gibbs free energyÃ®. And what's important about this way of talking about the energy change associated with the chemical reaction is it considers not only the internal energy of the system but also the change in disorder. Or another way of saying that, for those of you who've run into the laws of thermodynamics, it combines the first and second laws of thermodynamics. And you have to consider both of those if you're going to consider whether a reaction will go. And you cannot measure an absolute free energy but you can measure a change. And this is the equation. It's the change associated with a chemical reaction is equal to the change associated with the chemical reaction under some set of standard conditions times RT times the log of the concentration of the products multiplied together over the concentration of the reactants. So if we could just go to the same example we were just thinking about, the energy change with that reaction that we were considering would have been this. So this is the energy change -- -- associated with the concentrations -- -- the reactants and products that we're considering. This is the energy change under standard, or the term standard conditions where everything, each reactant, each product is present under one molar concentrations. So not something you'd ever find in most cases, but it's a frame of reference. And then this is the universal gas constant -- -- which is two times ten to the minus third kilocalories per mole per degree Calvin, the temperature in absolute. This is the temperature in degrees Calvin. And the temperature for most biology, most life is around 25 degrees Centigrade, so that's equal to 298 degrees Calvin, which is about equal to 300 degrees Calvin. So for most -- And since the range in which life can occur on an absolute temperature scale is really pretty small, it sort of fluctuates in only very minor ways around 25 degrees Centigrade, then for most of the biological reactions we'll be thinking about this RT number is about 0.6 kilocalories per mole.

Now, biochemists actually have a special form of free energy they use, which we put a delta G prime. And in this case the delta G prime is equal to delta G prime under a set of standard conditions plus RT natural log of C products over the reactants. But the assumption is made that the reaction is in water which, I mentioned the other day, is 55 molar. Yeah? This is the degree Celsius. I've just expressed it in degrees Calvin. Sorry. My mistake. Excuse me. Because I was wrong is why. OK. Thanks for catching that. All right. So water is very concentrated. And so under these conditions the other convention is then you can set the hydrogen ions and water molecules to one. And you don't have to think about them when we're doing this. This is a convention that biochemists do. Now, this free energy, the delta G that gives free energy is a thermodynamic -- -- property. And I'll just share with you the same visual image I've had since I was an undergrad, which I think is not a bad way of thinking about it trying to understand what happens, that if we have a plot of the free energy as a function of what happens as the reaction goes along so that we have A plus B here and C plus D down here. When you go from reaction to products, the way I've drawn it, some kind of energy is given off in this kind of reaction. And if you know that you will know then that the reaction will be able to go forward because it's able to give off energy just the same way hydrogen and oxygen give off a lot of heat and stuff, and you know that reaction really goes a long way to completion. So it's kind of as if you were out here on your spring break on your skis already to go down the black diamond hill, you know, you can sort of see what would happen. Now, because it's a thermodynamic property it doesn't matter what route you take to get from the reactions to the products. So if you go down the double diamond slope or you go down the bunny slope you still end up with the same amount of energy coming out of the reaction. And that's important because if that wasn't true you could make a perpetual motion machine and you'd be very rich. The second thing that's important is that the free energy will tell you what would happen if the reaction went but it will not tell you whether it can go. If I did a demo here and I brought some hydrogen and some oxygen and I mixed them together in a vessel in the front of the class we could all sit here waiting for it to explode. But the likelihood is we would sit here for a very, very long time and not see an explosion, right? And the reason is that in order to get that hydrogen and oxygen close enough together we had to give them some extra energy and push them so they overcome repulsion and stuff. So if you were out here on your skis again getting already to go, but in fact you got off at the wrong stop on the ski lift and you were there, even though there would be energy getting down from here it's not going to happen at any discernable rate given the sort of little bounce in energy you have in your normal lives. So what we're doing when we do hydrogen and oxygen is by putting a match into it or something we're giving it enough energy that actually a few of the molecules get up here, they drop down, then they give up so much energy and heat that all the rest of them get pushed up and the thing goes. But that's sort of not a bad way of thinking about it. And we're going to talk in a minute about what determines how fast reactions go, not whether they go or not. And then, of course, at that point we're going to have to worry about this issue. But before that what I want to show you is that there's a direct relationship between this Gibbs free energy and the equilibrium constant. So we have this, well, what we could do is you have the reaction over there. So let's consider that reaction has come to equilibrium. And that means there'll be no further energy change. So we'll just set the delta G to zero. And that would mean then that delta G prime zero is equal to minus RT concentration C over D over concentration of A over B. You'll recognize this. That's the equilibrium constant, right? I'm sorry. There's a natural log in here. I didn't get it in. OK? So which is equal to minus RT the natural log of the equilibrium constant or the natural log of the equilibrium constant is equal to minus delta G prime zero over RT. Or another way of saying that is the K equilibrium is equal E to the minus delta G prime zero over RT. So if you think back to consequences of an equilibrium constant, if the reaction is going to go almost all the way then there are going to be mostly products, very few reactions, so the K equilibrium will be large. So if a reaction is going to go a long way then the equilibrium constant will be large. And in order for an equilibrium constant to be large then this delta G is going to have to have a large negative sign. So if the reaction -- -- is favorable then K equilibrium will be large and the delta G prime zero will have, at least within the scale of an activation energy, a large negative value. And let me give you a couple of examples. When we talked about carbohydrates, I briefly told you sucrose was what we call a disaccharide, two sugars joined together. What do we do when we join two things together pretty much usually in nature? You split out a molecule of water. So we take a molecule of glucose, a molecule of fructose, both carbohydrates, stick them together and we get table sugar. If we want to reverse that reaction we have to put in a molecule of water and we can run it the other way. We get glucose plus fructose. The K equilibrium for that reaction is 140,000. The delta G prime zero is minus seven kilocalories per mole. So that's an example of what I was just telling you, a fairly large negative value. If we think about a reaction that's not favorable, here's acidic acid. That's what makes vinegar sour. And the hydrogen ion can come off here to give you a hydrogen ion and the negative ion of acidic acid or acetate ion. The equilibrium constant for that one is, what is it, I think two times ten to the minus five. So only a little tiny bit of the acidic acid actually ionizes. And the K equilibrium constant then, excuse me, the delta G prime zero is plus 6.3 kilocalories per mole. So buried in this example is not showing you that a reaction that's unfavorable will have a positive free energy associated with it, whereas one that's favorable will have a negative free energy. This is also sort of telling you why you don't die when you put salad dressing on your salad, because if acidic acid ionized as thoroughly as sulfuric acid and you put an equivalent amount of sulfuric acid on our salads none of us would be here. It's only a little tiny bit that's going, and so that's what's happening. So what this really sets us up for is this fundamental problem in biology, and that is that this reaction here, you can see what it would go, this one doesn't go, but most of the reactions that you have to carry out in biology demand an energy input because they just won't go. We could sort of force this a little bit. We could raise the concentration of the reactions and it would give us a little bit more product, but that's not a useful solution to all the things. So this was a really fundamental problem that had to be solved in evolution in order for life to ever exist. And I'll give you just an example. If we consider taking a couple of molecules of glutamate, which is one of the amino acids we talked about, a couple of molecules of amino and making it into a couple of molecules of glutamine. Now, this is an amino acid needed for making proteins. This is an amino acid needed for making

glutamine. Now, this is an amino acid needed for making proteins. This is an amino acid needed for making proteins. The cell has to have both of them. Glutamate has two methylene groups and then are carboxyl group that's one of the acid amino acids. And glutamine the side chain -- -- is now amid. The delta G from zero associated with this reaction is plus seven kilocalories per mole, so it's as unfavorable almost as that one we're looking at. In fact, it's worse than the one we're looking at over there. The reason that this is sort of pushing the thing uphill energetically is that the electrons here actually distribute themselves back and forth. So you can kind of think of the molecule as going back and forth between these two forms. And that makes it more stable. And when you stick on the amine group to make the amid it cannot do that, and so you're actually pushing everything energetically uphill. So how does a cell accomplish this? There's energy available. If we consider what happens with C6H12O6 going to two lactate the delta G prime zero associated with that is minus 50 kilocalories per mole. So the cell has got a lot of energy out of making even that simple conversation of a sugar molecule into two lactate. But it somehow has to figure out how to use that energy in order to drive these unfavorable reactions. And the solution, which is really one of the secrets to life, is to use coupled reactions -- -- with a common intermediate. And if you look outside a cell, as Lavoisier did or Pasteur did, this is what you'd see. But if you could look inside the cell and see what's happening when that conversion is being made you'd discover that the full reaction looks like this. It's the sugar molecule plus two molecules of ADP plus two molecules of inorganic phosphate are going to give two molecules of lactate plus two molecules of ATP. What's ATP? It's a ribonucleotide. That's ADP. And what happens when you make ATP is an extra phosphate gets added onto that end of the molecule. So by having yet another phosphate on here you've got a whole role of negative charges. This is a molecule in which the various parts are not happy to be together because all these negative charges would like to push apart so when you break the bond of ATP then energy is released. So using ATP is a way of sort of storing chemical energy so you can use it in some other kind of context. And so by burning it, by carrying out the reaction in this way a cell is able to not only make a molecule of sugar, glucose into two lactate, it's able to generate ATP along the way. And the delta G prime zero for this reaction is minus 34 kilocalories per mole. So even though it's taking out some of that energy and putting it in ATP, this is a reaction that goes very, very efficiently. Then instead of trying to carry out just that reaction, what the cell is actually doing is taking the two glutamate plus the two molecules of ammonia plus two ATP. And then this is converting it to two glutamine plus two water. I think I failed to put that in here so you can correct it back there. Plus two ADP plus two molecules of inorganic phosphate. And so the Pi very commonly used in biochemistry to denote just inorganic phosphate ion. So what's happen here then are these two reactions going on. This reaction now, because ATP is involved, is now favorable, and the delta G for this reaction is minus nine kilocalories per mole. So by having an ATP hydrolyzed as part of the reaction mechanism, this reaction that used to be unfavorable is now favorable. And then the kind of cute thing then is if you sum this all up, the ATPs and the ADPs are on both sides of the equation so they just drop out. And what you're left with is C6H12O6 plus the two glutamines plus two ammonias going to give two glutamines, excuse me, two lactate plus two glutamines plus the two waters. And the delta G prime zero for this is minus 43 kilocalories per mole. So this is not, you can think of it as using energy in the form of ATP like this a little the way we use money in our society. I do some work at MIT. I don't get given food to eat or TV to watch the Super Bowl. Instead I get given money, then I go to the store, I give them the money, I end up with the food or the stuff. And if you're watching it from the outside you see me do work at school and then food, TV or whatever shows up at home. But what's happening is the money is serving as a common intermediate in those transactions. And that's what basically ATP is in the cell. It's energy money. And in making ATP the cell has to take this ribose with an adenine on it, I think I didn't put the adenine on here I realize. The adenine is sitting on the ribose now. There are two phosphates, both of which have a negative charge on them. And to create that third bond it has to push it together. It's a very sort of an intrinsically unstable molecule. When you break the bond it will give you energy back. And that's one of the really amazing secretes to life, and that's the underlying principal of why it is that life can go forward. Now, the second issue that we need to quickly address here is -- -- not only can a reaction go, which is what thermodynamics tells us, but how can fast can it go. And this epitomizes the problem that all chemical reactions face because literally every chemical reaction that you carry out involves bringing a couple of entities together. And as they get closer and closer and closer they don't want to be there so you have to sort of push them together in some kind of way or make sure they have enough energy to get together. And that's what we see represented here. And that's a special term called the activation energy. It's given the term delta G with a double-dagger. And that is what -- It's the size of that activation energy that limits how fast chemical reactions can go. So the solution you use in chemistry, most of you, is you use a catalyst. And the catalyst doesn't change the outcome of the reaction. It just changes how fast you get there. So there are many reactions you've heard about in chemistry. Just stick the thing at 500 degrees centigrade, put in a piece of platinum, and now the reaction will go a whole lot faster. By heating it up molecules have more energy. So if they have more energy they can get closer together just from that. And then what the platinum surface would do is allow the molecules to both stick and that would bring them in proximately and also help them come together. Well, you cannot raise the temperature in a biological system, but still you have to overcome this. But the principal then, what you have to do when you carry out a catalyst, what any catalyst would do is that it lowers this activation energy. And if you lower the activation energy then enough of the molecules, just at whatever condition they're in will have enough energy to be able to go. It won't change the size of the drop. It just changes how fast you reach that final equilibrium. And there are two forms of biological -- Two molecules that are biological catalysts. One of the molecules you know is enzymes. Enzymes are made of a protein. We spent a bunch of time working at that. One of the things I showed you the very first day, this is a thing made by the anthrax bacterium, anthrax lethal factor. What it actually is, it's a protein and it's an enzyme that's able to catalyze the cleavage of certain peptide bonds in proteins in our body. And in particular it goes after molecules that are involved in signaling processes inside of cells. And if we don't have those then we die. More recently it was discovered that RNA can be a catalyst. And these are called, if you have an RNA that's a catalyst it's called a ribozyme. And these seemed pretty exotic for a little while they first discovered the idea that a piece of RNA could serve as a catalyst in a biological system.

for a little while they first discovered the idea that a piece of RNA could serve as a catalyst in a biological system, but it eventually turned out that the ribosome, which we'll talk about in some detail which is the protein synthesizing machinery that creates those peptide bonds between each of the amino acids to make the proteins. It's a big conglomeration of RNA shown in gray and a bunch of different proteins that are shown in yellow, but the actual formation of the peptide bond, the thing that makes all proteins is actually catalyzed by a piece of RNA. And so the ribosome is actually a ribozyme. And it's ironic that that sense that a piece of RNA is catalyzing the bond that makes proteins possible. So we'll finish this up and get in then to glycolysis which is the most evolutionary ancient of these energy-producing systems on Monday. OK?