Biochemistry 401, Lecture 35. Today we're going to talk about replication. We're going to talk about replication in bacterial and eukaryotic systems and it's important to keep these two straight. We're going to discuss key enzymes and proteins that are involved in replication and also the regulation of this process. We're then going to talk about topoisomerase one and two specifically. So let's get started.! Hi. Today we're going to talk about replication. We're going to talk about replication in two model systems E.coli for prokaryotes and humans for eukaryotes. The replication is a little bit different in each of these systems so it's important to get this straight. So as with any system it's important to think about things in a big-picture perspective and for replication we want to concentrate on these things. First of all, what is happening? When does this happen? How does it begin? How is this beginning regulated? What enzymes are doing this? Are there any accessory proteins that help these enzymes to know where to start and be able to continue? What is the directionality of these enzymes – do they go five prime to three prime? Three prime to five prime? It's important to know this. So with this in mind, let's get started. Replication is DNA synthesis, in preparation for cell division. Replication is semiconservative. This means that the parent DNA separates and a daughter strand is made from each parental template so that the parent cell keeps a parental strand and a new daughter strand, and the daughter cell gets a parental strand and a new daughter strand as well. The process of replication in bacteria and in humans requires similar things. In each, replication requires a DNA template. So in this case, it's the parental strand and it requires ribonucleotides to make RNA primers, because first you must make RNA before you can make DNA. Deoxyribonucleotides for DNA synthesis are also needed, and finally polymerase enzymes, accessory enzymes, and proteins are also required. So those are the things that are similar, but what sorts of things are different? In bacteria there is generally one circular chromosome. In E. coli this is true and there are about 4.6 million base pairs on that chromosome. Each circular chromosome has one replication start site and the cell division is
based on cell size. This is the relative surface area to volume ratio. Each different bacterium has its own particular optimal size, and once that size is surpassed it's time to divide. In eukaryotes – for instance in humans –the chromosomes are linear. There are 46 linear chromosomes in humans and approximately 6 billion base pairs. That's a lot more DNA that must be copied during replication and as you'd expect there are multiple replication start sites to get this big job done quickly. Cell division is highly regulated and differs with respect to cell type, developmental state, intracellular signals, and extracellular signals. Let's talk about replication in E.coli first, because this is a little bit easier to understand. First of all, where does replication start on the chromosome, and what sort of enzymes are responsible for this beginning? In E.coli, the initiation of replication occurs at a place called the origin of replication. That makes sense, doesn't it? It's abbreviated ori and in E. coli it's oriC. The oriC is a DNA sequence in E.coli that consists of two main regions. The first is an AT-rich region. The AT repeats represent a site at which the DNA is easy to melt, because there are only two hydrogen bonds between AT base pairs and so it's relatively easy to melt. The second portion is the DnaA binding site. This is a site to which DnaA a binds. This DnaA recognizes the sequence and it binds in these specific spots and uses ATP hydrolysis to power the separation of the DNA strands at the ori locus. So now that the DNA is separated, it's important to make sure that this DNA does not reanneal or form higher-order structures like stem loops or things like that. We don't want our DNA to get bound up in knots, and so we're going to cover this DNA with something called single-strand

binding protein, SSB, that coats the DNA and prevents it from reannealing or forming higher-order structures. The next thing that happens is DNA a is going to help recruit DnaC and DnaB so it's important to know your ABCs of bacterial replication. DnaA binds first DnaB is loaded on to the DNA by DnaC. What does this mean? DnaB is a helicase that consists of six individual subunits that comprise a ring-like structure. DnaC opens this up and helps to place this DnaB on the chromosome at the start site of replication. So DnaA binds first, helps to separate the strands, DnaC binds and helps to load DnaB, the helicase, onto the DNA. So DnaB is associated with the primates. This primase is called DnaG in E.coli and this primase is going to synthesize RNA primers that help get replication going, because remember –DNA synthesis cannot happen without a primer first and in this case we're going to use an RNA primer. RNA first then DNA. So DnaG is bound to DnaB and DnaG is the primase and it synthesizes a short strand of RNA as a primer, and then DNA synthesis itself can get going. As we can see from this illustration, the DNA is going to be synthesized in two directions. There are actually two primosomes made up of DnaB and DnaG that are found on this chromosome. There's one going to the left and one going to the right, and so we're going to be replicating in two directions at once. Now – now the place at which this DNA is separated, okay, on either side is called a replication fork and it's called a replication fork because it looks like a fork in the road, right, and so we're going to have one to the left and one to the right, and right now we have the primosome bound – DnaB and DnaG. This is going to help to recruit the main replicative enzyme, which is Polymerase III and this enzyme is part of what's called a replisome. The replisome itself consists of three main parts: we have a beta-2 clamp that helps to hold the polymerase enzyme on the DNA strand, and we also have the polymerase itself of course, so Pol III core polymerase contains three portions an alpha, which is the actual catalytic portion, the theta (which frankly we really don't know what it does) and the epsilon. The epsilon actually is the exonuclease capability. So if this polymerase makes a mistake the epsilon can take off that nucleotide and give the polymerase another chance to get the right one there, but we have to have something that's going to open and close the beta-2 clamp to get it onto the DNA and to release it, and this is called the clamp-loader. Makes sense, huh? So each replisome is going to have a clamp-loader, beta-2 clamp and polymerase which is Pol III, and one's going to go to the left and one is going to go to the right, and each one of these replisomes is going to synthesize two strands of DNA at the same time. The top strand and the bottom strand. This brings up a problem. We know that DNA is synthesized 5 prime to 3 prime and the DNA template is read 3 prime to 5 prime but remember the convention – the top strand is 5 prime to 3 prime and the bottom strand is 3 prime to 5 prime. So if we separate these 2, one's going to be going in the right direction for it to be used as a template and read 3 prime to 5 prime and the other one's going to be going backwards. So we have two replication forks, one going this way one going that way. It's going to synthesize two strands of DNA. One is going in the right direction. It can be read 3 prime to 5 prime no problem and the other one can't. And how do we fix this? Well you have to keep the forks going in the proper direction. We're going to reverse one of the strands in each of these forks and synthesize them in little tiny bits called Okazaki fragments so that both strands can be copied at the same time. Let's take a closer look at that because it's a little bit complicated. So here in this slide we can see the replication fork that's progressing in the rightward direction and on the bottom we have the lagging strand and on the top we have the leading strand. Now the leading strand has already had its RNA primers synthesized and so it's just chugging

along no problem. The clamp has been loaded on to that strand – the beta-2 clamp and the core polymerase consisting of alpha, theta and epsilon are there. So here we have the top strand it has the beta-2 clamp on the DNA and the polymerase is attached, also, and it's going in the rightward direction, no problem. It's reading the DNA 3 prime to 5 prime. It's synthesizing the daughter strand DNA 5 prime to 3 prime. No problem for the leading strand. The lagging strand – that's the problem. It's turned backwards so that the polymerase can synthesize 5 prime to 3 prime reading the DNA strand 3 prime to 5 prime in short Okazaki fragments. Now for each fragment we have to synthesize RNA. So what does this mean? The DnaB is the helicase right and that's going along opening up the replication fork and the DnaG is attached and it starts to synthesize the RNA primer and then the polymerase can bind to the RNA to where that RNA is and can start synthesizing the short stretch of DNA. Once this DNA
gets to a specific length the beta-2 clamp will be released and therefore the polymerase will also be released and the clamp loader will load another beta-2 clamp a little further down on the DNA and another polymerase will bind to make another Okazaki fragment. We can't have that RNA stay there because we know the RNA is less stable than DNA we don't want to have RNA inside of our genome we want it all DNA all the way through and so there's another enzyme that's going to be employed and this is DNA Pol I now Pol I has the capability of taking nucleotides out going 5 prime to 3 prime so it acts almost like a snowplow to remove RNA in the Okazaki fragments and replace that RNA with DNA. So in E. coli DNA Pol I removes the RNA primer and synthesizes DNA to replace It but the thing is we still have going to have breaks in the DNA now where we have stopped synthesizing the DNA and where the next Okazaki fragment DNA is so we have to fix that and DNA ligase uses ATP to ligate that DNA and seal the break so we need the following enzymes for replication in E.coli first we need DNA a in order to separate the strands we need DnaC to recruit DnaB. DnaB is the helicase DnaG is the primase. The main polymerase is Pol III. It's going to synthesize the leading strand and the lagging strand. The lagging strand is synthesized in short fragments called Okazaki fragments. Once those are made Pol I comes along and removes the RNA from those Okazaki fragments and fills those in with DNA complementary to the template and then DNA ligase seals the break to make a continuous strand of DNA on the lagging strand. Finally, both sides of the strands are synthesized so we can have one parent strand and one daughter strand remain with the parent cell and one parent strand and daughter strand going to the daughter cell. So each bacterial cell will have its own genome consisting of one parental strand and one daughter strand. I hope that's clear. So what do you need to know? You need to know what DnaA, DnaC, DnaB, DnaG do. You need to know some of the things that comprise the replisome but only these – you need to know the beta – sliding clamp you need to know the core polymerase which consists of alpha, theta and epsilon, you need to know what alpha and epsilon do, you also need to know that there is the clamp loader but you don't need to know the subunits in the clamp loader. Pol I comes along and removes the RNA from those Okazaki fragments and fills those in with DNA and then DNA ligase seals the break. I hope this helps. This also might be helpful. There are two links for DNA synthesis that might be helpful to you. In eukaryotes it's a lot more complex. It has to do with the tissue type, It has to do the developmental stage, signals inside the cell and outside the cell that say it's time to divide. And only particular cells have the ability to divide. Those that don't have the ability divide have exited the cell cycle and are now at something that's called G0 this is the non-dividing stage. For a cell that's going to divide there