E1. The second possibility in which molecule A has +4 supercoils and molecule B has –1 supercoils fits these data. Molecule A would be more compacted because it has more supercoils. Also, molecule B would be more transcriptionally active since it is more negatively supercoiled. The first possibility does not fit the data because both molecules have the same level of supercoiling so molecule A and molecule B would have the same level of compaction. The third possibility does not fit the data because molecule B would be more compact.

E2. This type of experiment gives the relative proportions of highly repetitive, moderately repetitive, and unique DNA sequences within the genome. The highly repetitive sequences renature at a fast rate, the moderately repetitive sequences renature at an intermediate rate, and the unique sequences renature at a slow rate.

E3. It affects only the rate of renaturation. Denaturation occurs because the heat breaks the hydrogen bonds between the two strands. The rate of denaturation depends on the hydrogen bonding, not on the number of copies of a sequence. The rate of renaturation, however, depends on the two complementary strands "finding" each other. The rate at which two complementary strands find each other will be faster if the concentration of the two strands is higher. That is why highly repetitive sequences renature at a faster rate.

E4. The amphibian probably has fewer structural genes than the mammal. The extra DNA is due to highly repetitive DNA sequences, which do not encode genes.

E5. Supercoiled DNA would look all curled up into a relatively compact structure. You could add different purified topoisomerases and see how they affect the structure via microscopy. For example, gyrase relaxes positive supercoils while topoisomerase I relaxes negative supercoils. If we added topoisomerase I to a DNA preparation and it became less compacted, then the DNA was negatively supercoiled.

E6. 1. The repeating nucleosome structure was revealed from DNase I digestion studies.

2. Purification studies showed that the biochemical composition is an octamer of histones.

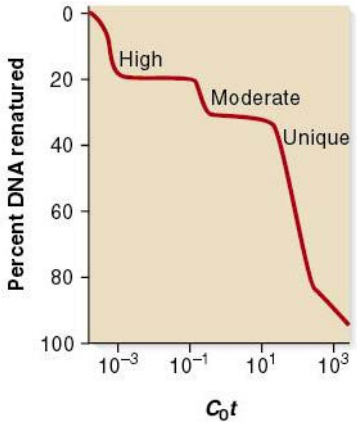
3. More recently, crystallography has shown the precise structure of the nucleosome.

4. Microscopy has revealed information about the 30 nm fiber and the attachment of chromatin to the nuclear matrix.

In general, it is easier to understand the molecular structure of something when it forms a regular repeating pattern. The eukaryotic chromosome has a repeating pattern of nucleosomes. The bacterial chromosome seems to be more irregular in its biochemical composition.

E7. Yes, if its base composition is similar to the main chromosomal DNA.

E8.



E9. A. One way is to do this by hand. You could make a series of solutions: 70% CsCl, 65% CsCl, 60% CsCl, 55% CsCl, 50% CsCl, 45% CsCl, 40% CsCl, 35% CsCl, and 30% CsCl. You could then add 1 ml of the 70% solution to the bottom, then gently layer 1 ml of the 65% solution on top, and then gently layer 1 ml of the 60% solution on top, and so on, until you finally add 1 ml of the 30% solution to the very top. This makes a step gradient; in this case, the gradient is found in 5% steps. Alternatively, some laboratories are equipped with a gradient maker. This is a machine that makes a continuous gradient. The experimenter would make a 70% CsCl solution and a 30% CsCl solution. The machine draws the 70% solution and 30% solution into a mixing chamber. After mixing, the solution is dripped into a centrifuge tube. At first, mostly the 70% solution is drawn into the mixing chamber, so the concentration of CsCl is greater near the bottom of the tube. Over time, more and more of the 30% solution (and less and less of the 70% solution) is drawn into the mixing chamber. Therefore, the solution dripped into the centrifuge tube gradually changes from a 70% solution to a 30% solution.

B. The gradient does not last forever, because diffusion eventually causes it to dissipate. Nevertheless, the gradient lasts long enough (at least many hours) so that the DNA sample can reach its equilibrium density.

C. Actually, you could add the sample anywhere because the DNA moves in the direction where the density of the DNA matches the density of the CsCl. It is usually easier to add it to the top. If you tried to add it in the middle, you might disrupt the CsCl gradient.

E10. You would expect all of the DNA in the sample to renature at a fast rate because it is a purified sample of highly repetitive DNA.

E11. With a moderate salt concentration, the nucleosome structure is still preserved so the same pattern of results would be observed. DNase-I would cut the linker region and produce fragments of DNA that would be in multiples of 200 bp. However, if a high salt concentration was used, the core histones would be lost, and DNase-I could cut anywhere. On the gel, you would see fragments of almost any size. Since there would be a continuum of fragments of many different sizes, the lane on the gel would probably look like a smear, rather than have a few prominent bands of DNA.

E12. You would get DNA fragments of about 446 to 496 bp (i.e., 146 bp plus 300 to 350).

E13. Lots of possibilities. You could digest it with DNase-I and see if it gives multiples of 200 bp or so. You could try to purify proteins from the sample and see if eukaryotic proteins or bacterial proteins are present.

E14. Histones are positively charged and DNA is negatively charged. They bind to each other by these ionic interactions. Salt is composed of positively charged ions and negatively charged ions. For example, when dissolved in water, NaCl becomes individual ions of Na^+ and Cl^-. When chromatin is exposed to a salt such as NaCl, the positively charged Na^+ ions could bind to the DNA and the negatively charged Cl^- ions could bind to the histones. This would prevent the histones and DNA from binding to each other.

E15. A. Since the *Alu* sequence is interspersed throughout all of the chromosomes, there would be many brightly colored spots along all chromosomes.

B. Only the centromeric region of the X chromosome would be brightly colored