

# Chapter Eleven: Chromosome Structure and Transposable Elements

## COMPREHENSION QUESTIONS

### Section 11.1

- \*1. How does supercoiling arise? What is the difference between positive and negative supercoiling?  
*Supercoiling arises from overwinding (positive supercoiling) or underwinding (negative supercoiling) the DNA double helix. Supercoiling may occur:*  
*(1) when the DNA molecule does not have free ends, as in circular DNA molecules,*  
*or*  
*(2) when the ends of the DNA molecule are bound to proteins that prevent them from rotating about each other.*
2. What functions does supercoiling serve for the cell?  
*Supercoiling compacts the DNA. Negative supercoiling helps to unwind the DNA duplex for replication and transcription.*

### Section 11.3

- \*3. Describe the composition and structure of the nucleosome. How do core particles differ from chromatosomes?  
*The nucleosome core particle contains two molecules each of histones H2A, H2B, H3, and H4, which form a protein core with 145–147 bp of DNA wound around the core. Chromatosomes contain the nucleosome core with a molecule of histone H1.*
4. Describe in steps how the double helix of DNA, which is 2 nm in width, gives rise to a chromosome that is 700 nm in width.  
*DNA is first packaged into nucleosomes; the nucleosomes are packed to form a 30-nm fiber. The 30-nm fiber forms a series of loops that pack to form a 250 nm fiber, which in turn coils to form a 700-nm chromatid.*
5. What are polytene chromosomes and chromosomal puffs?  
*Polytene chromosomes are giant chromosomes formed by repeated rounds of DNA replication without nuclear division, found in the larval salivary glands of *Drosophila* and a few other species of flies. Certain regions of polytene chromosomes can become less condensed, resulting in localized swelling, or chromosomal puffs, because of intense transcriptional activity at the site.*
- \*6. Describe the function and molecular structure of the centromere.  
*Centromeres are the points of attachment for mitotic and meiotic spindle fibers and are required for the movement of chromatids to the poles in anaphase. Centromeres have distinct centromeric DNA sequences where the kinetochore proteins bind. For some species, such as yeast, the centromere is compact, consisting of only 125 bp. For other species, including *Drosophila* and mammals, the centromere is larger, ranging from several thousands to hundreds of thousands of basepairs of DNA sequence.*

- \*7. Describe the function and molecular structure of a telomere.  
*Telomeres are the ends of the linear chromosomes in eukaryotes. They cap and stabilize the ends of the chromosomes to prevent degradation by exonucleases or joining of the ends. Telomeres also enable replication of the ends of the chromosome. Telomeric DNA sequences consist of repeats of a simple sequence, usually in the form of  $5' C_n(A/T)_m$ .*
8. What is the difference between euchromatin and heterochromatin?  
*Euchromatin undergoes regular cycles of condensation during mitosis and decondensation during interphase, whereas heterochromatin remains highly condensed throughout the cell cycle, except transiently during replication. Nearly all transcription takes place in euchromatic regions, with little or no transcription of heterochromatin.*

#### Section 11.4

9. What is the C value of an organism?  
*The C value is the amount of DNA per cell of an organism.*
- \*10. Describe the different types of DNA sequences that exist in eukaryotes.  
*Unique-sequence DNA, present in only one or a few copies per haploid genome, represents most of the protein coding sequences, plus a great deal of sequences with unknown function.*  
*Moderately repetitive sequences, a few hundred to a few thousand base pairs long, are present in up to several thousand copies per haploid genome. Some moderately repetitive DNA consists of functional genes that code for rRNAs and tRNAs, but most is made up of transposable elements and remnants of transposable elements.*  
*Highly repetitive DNA, or satellite DNA, consists of clusters of tandem repeats of short (often less than 10 base pairs) sequences present in hundreds of thousands to millions of copies per haploid genome.*

#### Section 11.5

- \*11. What general characteristics are found in many transposable elements? Describe the differences between replicative and nonreplicative transposition.  
*Most transposable elements have terminal inverted repeats and are flanked by short direct repeats. Replicative transposons use a copy-and-paste mechanism in which the transposon is replicated and inserted in a new location, leaving the original transposon in place. Nonreplicative transposons use a cut-and-paste mechanism in which the original transposon is excised and moved to a new location.*
- \*12. What is a retrotransposon and how does it move?  
*A retrotransposon is a transposable element that relocates through an RNA intermediate. First, it is transcribed into RNA. A reverse transcriptase encoded by the*

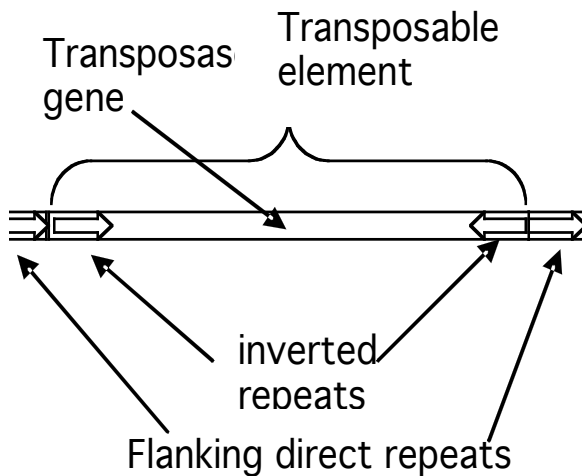
*retrotransposon then reverse transcribes the RNA template into a DNA copy of the transposon, which then integrates into a new location in the host genome.*

- \*13. Describe the process of replicative transposition through DNA intermediates. What enzymes are involved?

*First, a transposase makes single-stranded nicks on either side of the transposon and on either side of the target sequence. Second, the free ends of the transposon are joined by a DNA ligase to the free ends of the DNA at the target site. Third, the free 3' ends of DNA on either side of the transposon are used to replicate the transposon sequence, forming the cointegrate. The enzymes normally required for DNA replication are required for this step, including DNA polymerase. The cointegrate has two copies of the transposon and the target site sequence on one side of each copy. Fourth, the cointegrate undergoes resolution, which involves a crossing over within the transposon, by resolvase enzymes such as those used in homologous recombination.*

### Section 11.6

- \*14. Draw the structure of a typical insertion sequence and identify its parts.



15. Draw the structure of a typical composite transposon in bacteria and identify its parts.



### Section 11.7

- \*20. Briefly summarize three hypotheses for the widespread occurrence of transposable elements.

*The cellular function hypothesis proposes that transposable elements have a function in the cell or organism, such as regulation of gene expression.*

*The genetic variation hypothesis suggests that transposable elements serve to generate genomic variation. A larger pool of genomic variants would accelerate evolution by natural selection.*

*The selfish DNA hypothesis suggests that transposable elements are simply parasites, serving only to replicate and spread themselves.*

## APPLICATION QUESTIONS AND PROBLEMS

### Section 11.3

- \*21. Compare and contrast prokaryotic and eukaryotic chromosomes. How are they alike and how do they differ?

*Prokaryotic chromosomes are usually circular, whereas eukaryotic chromosomes are linear. Prokaryotic chromosomes generally contain the entire genome, whereas each eukaryotic chromosome has only a portion of the genome: the eukaryotic genome is divided into multiple chromosomes. Prokaryotic chromosomes are generally smaller and have only a single origin of DNA replication. Eukaryotic chromosomes are often many times larger than prokaryotic chromosomes and contain multiple origins of DNA replication. Prokaryotic chromosomes are typically condensed into nucleoids, which have loops of DNA compacted into a dense body. Eukaryotic chromosomes contain DNA packaged into nucleosomes, which are further coiled and packaged into successively higher-order structures. The condensation state of eukaryotic chromosomes varies with the cell cycle.*

22. a. In a typical eukaryotic cell, would you expect to find more molecules of the H1 histone or more molecules of the H2A histone? Explain your reasoning.  
*Because each nucleosome contains two molecules of histone H2A and only one molecule of histone H1, eukaryotic cells will have more H2A than H1.*
- b. Would you expect to find more molecules of H2A or more molecules of H3? Explain your reasoning.  
*Because each nucleosome contains two molecules of H2A and two molecules of H3, eukaryotic cells should have equal amounts of these two histones.*
23. Suppose you examined polytene chromosomes from the salivary glands of fruit fly larvae and counted the number of chromosomal puffs observed in different regions of DNA.
- a. Would you expect to observe more puffs from euchromatin or from heterochromatin? Explain your answer.  
*Euchromatin is less condensed and capable of being transcribed, whereas heterochromatin is highly condensed and rarely transcribed. Because chromosomal puffs are sites of active transcription, they should occur primarily in euchromatin.*

- b. Would you expect to observe more puffs in unique-sequence DNA, moderately repetitive DNA, or repetitive DNA? Why?  
*Highly repetitive DNA consists of simple tandem repeats usually found in heterochromatic regions and are rarely transcribed. Moderately repetitive DNA comprises transposons and remnants of transposons. Again, with the exception of the rDNA cluster, these sequences are rarely transcribed or transcribed at low levels. The most actively transcribed genes occur as single-copy sequences, or as small gene families. Therefore, more chromosomal puffs would be observed in unique-sequence DNA than in moderately or highly repetitive DNA.*
- \*24. A diploid human cell contains approximately 6.4 billion base pairs of DNA.
- a. How many nucleosomes are present in such a cell? (Assume that the linker DNA encompasses 40 bp.)  
*Given that each nucleosome contains about 140 bp of DNA tightly associated with the core histone octamer, another 20 bp associated with histone H1, and 40 bp in the linker region, then one nucleosome occurs for every 200 bp of DNA.*  

$$6.4 \times 10^9 \text{ bp divided by } 2 \times 10^2 \text{ bp/nucleosome} = 3.2 \times 10^7 \text{ nucleosomes (32 million).}$$
- b. How many histone proteins are complexed to this DNA?  
*Each nucleosome contains two of each of the following histones: H2A, H2B, H3, and H4. A nucleosome plus one molecule of histone H1 constitute the chromatosome. Therefore, nine histone protein molecules occur for every nucleosome.*  

$$3.2 \times 10^7 \text{ nucleosomes} \times 9 \text{ histones} = 2.9 \times 10^8 \text{ molecules of histones are complexed to 6.4 billion bp of DNA.}$$
- \*25. Would you expect to see more or less acetylation in regions of DNA that are sensitive to digestion by DNase I? Why?  
*More acetylation. Regions of DNase I sensitivity are less condensed than DNA that is not sensitive to DNase I, the sensitive DNA is less tightly associated with nucleosomes, and it is in a more open state. Such a state is associated with acetylation of lysine residues in the N-terminal histone tails. Acetylation eliminates the positive charge of the lysine residue and reduces the affinity of the histone for the negatively charged phosphates of the DNA backbone.*
26. Gunter Korge examined several proteins that are secreted from the salivary glands of *Drosophila melanogaster* during larval development (G. Korge. 1975. *Proceedings of the National Academy of Sciences of the United States of America* 72:4550–4554). One protein, called protein fraction 4, was encoded by a gene found by deletion mapping to be located on the X chromosome at position 3C. Korge observed that, about 5 hours before the first synthesis of protein fraction 4, an expanded and puffed-out region formed on the X chromosome at position 3C. This chromosome puff disappeared before the end of the third larval instar stage, when the synthesis of protein fraction 4 ceased. He observed that there was no puff at position 3C in a special strain of flies that lacked secretion of protein fraction 4. Explain these results. What is the chromosome puff at region 3, and why does its appearance and disappearance roughly coincide with the secretion of protein fraction 4?

*Chromosomal puffs correspond to relaxation of chromatin structure and transcriptional activity at the locus. The puff at region 3C indicates active transcription of that region, including the gene for protein fraction 4.*

27. Suppose a chemist develops a new drug that neutralizes the positive charges on the tails of histone proteins. What would be the most likely effect of this new drug on chromatin structure? Would you predict that this drug would have any effect on gene expression? Explain your answers.
- Such a drug would disrupt the ionic interactions between the histone tails and the phosphate backbone of DNA and thereby cause a loosening of the DNA from the nucleosome. The drug may mimic the effects of histone acetylation, which neutralizes the positively charged lysine residues. Changes in chromatin structure would result from the altered nucleosome-DNA packing and possible changes in interaction with other chromatin modifying enzymes and proteins. Changes in transcription would result because DNA may be more accessible to transcription factors.*

### Section 11.4

- \*28. Which of the following two molecules of DNA has the lower melting temperature? Why?

AGTTACTAAAGCAATACATC  
TCAATGATTCGTTATGTAG

AGGCGGGTAGGCACCCTTA  
TCCGCCCATCCGTGGGAAT

*The molecule on the left, with a higher percentage of A–T base pairs, will have a lower melting temperature than the molecule on the right, which has mostly G–C base pairs. A–T base pairs have two hydrogen bonds, and thus less stability, than G–C base pairs, which have three hydrogen bonds.*

29. In a DNA hybridization study, DNA was isolated from a particular species, labeled with  $^{32}\text{P}$ , and sheared into small fragments (S. K. Dutta et al. 1967. *Genetics* 57:719–727). Hybridizations between these labeled fragments and denatured DNA from different species were then compared. The following table gives the percentages of labeled wheat DNA that hybridized to DNA molecules of wheat, corn, radish, and cabbage.

Species	Percentage of bound wheat DNA hybridized relative to wheat
Wheat	100
Cabbage	23
Corn	63
Radish	30

What do these results indicate about the evolutionary differences among these organisms?

*The extent of DNA hybridization correlates with DNA sequence similarity. Corn DNA is more similar to wheat than radish DNA. Cabbage DNA is least similar. Therefore, corn is most closely related to wheat, followed by radish; and cabbage is the most evolutionarily distant from wheat.*

## Section 11.5

- \*30. A particular transposable element generates flanking direct repeats that are 4 bp long. Give the sequence that will be found on both sides of the transposable element if this transposable element inserts at the position indicated on each of the following sequences:
- 5'—ATTCGAACT**GAC**(transposable element)**TGAC**CGATCA—3'
  - 5'—ATTC**GAA**(transposable element)**CGAA**CTGACCGATCA—3'
- For (a) and (b) the target site duplication is indicated in bold.*
- \*31. White eyes in *Drosophila melanogaster* result from an X-linked recessive mutation. Occasionally, white-eye mutants give rise to offspring that possess white eyes with small red spots. The number, distribution, and size of the red spots are variable. Explain how a transposable element could be responsible for this spotting phenomenon.  
*Such a fly may carry an allele of the white-eye locus that contains a transposon insertion. The eye cells in these flies cannot make red pigment. During eye development, the transposon may spontaneously transpose out of the white-eye locus, restoring function to this gene so the cell and its mitotic progeny can make red pigment. Depending on how early during eye development the transposition occurs, the number and size of red spots in the eyes will be variable.*
- \*32. What factor do you think determines the length of the flanking direct repeats that are produced in transposition?  
*The length of the flanking direct repeats that are generated depends on the number of base pairs between the staggered single-stranded nicks made at the target site by the transposase.*

## Section 11.6

33. Which of the following pairs of sequences might be found at the ends of an insertion sequence?
- 5'—GGGCCAATT—3' and 5'—CCCGGTAA—3'.
  - 5'—AAACCCTTT—3' and 5'—AAAGGGTTT—3'.
  - 5'—TTTCGAC—3' and 5'—CAGCTTT—3'.
  - 5'—ACGTACG—3' and 5'—CGTACGT—3'.
  - 5'—GCCCCAT—3' and 5'—GCCCAT—3'.
- The pairs of sequences in (b) and (d) are inverted repeats because they are both reversed and complementary and might be found at the ends of insertion sequences. Sequences in (a), (c), and (e) would not be expected at the ends of an insertion sequence. The sequences in (a) are complementary, but not inverted. The sequences in (c) are reversed, but not complementary. The sequences in (e) are imperfect direct repeats.*

34. Two different strains of *Drosophila melanogaster* are mated in reciprocal crosses. When strain A males are crossed with strain B females, the progeny are normal. However, when strain A females are crossed with strain B males, many mutations and chromosome rearrangements occur in the gametes of the F<sub>1</sub> progeny and they are effectively sterile. Explain these results.  
*These results could be explained by hybrid dysgenesis, with strain B harboring P elements and strain A having no P elements. When sperm from strain A males fertilize eggs with P elements from strain B females, the progeny are normal because the strain B egg cytoplasm contains a repressor of P element transposition. However, when P<sup>+</sup> sperm cells from strain B fertilize P<sup>-</sup> eggs from strain A, the P elements undergo a burst of transposition in the embryo because the P egg cytoplasm lacks the repressor.*
- \*35. An insertion sequence contains a large deletion in its transposase gene. Under what circumstances would this insertion sequence be able to transpose?  
*Without a functional transposase gene of its own, the transposon would be able to transpose only if another transposon of the same type were in the cell and able to express a functional transposase enzyme. This transposase enzyme will recognize the inverted repeats and transpose its own element as well as other nonautonomous copies of the transposon with the same inverted repeats.*
36. A transposable element is found to encode a transposase enzyme. On the basis of this information, what conclusions can you make about the likely structure and method of transposition of this element?  
*This element probably has short inverted terminal repeats, and transposes through a DNA intermediate, using either a cut-and-paste nonreplicative mechanism or a copy-and-paste replicative mechanism. Because it does not encode a reverse transcriptase, it is not likely to be a retrotransposon.*
37. Zidovudine (AZT) is a drug used to treat patients with AIDS. AZT works by blocking the reverse transcriptase enzyme used by human immunodeficiency virus (HIV), the causative agent of AIDS. Do you expect that AZT would have any effect on transposable elements? If so, what type of transposable elements would be affected and what would be the most likely effect?  
*AZT should affect retrotransposons because they transpose through an RNA intermediate that is reverse transcribed to DNA by reverse transcriptase. If endogenous reverse transcriptases in human cells have similar sensitivity to AZT as HIV reverse transcriptase, then AZT should inhibit retrotransposons.*
38. A transposable element is found to encode a reverse transcriptase enzyme. On the basis of this information, what conclusions can you make about the likely structure and method of transposition of this element?  
*Like other retrotransposons, this element probably has long terminal direct repeats and transposes through an RNA intermediate that is reverse transcribed to DNA.*
39. A geneticist examines an ear of corn in which most kernels are yellow, but she finds a few kernels with purple spots, as shown here. Give a possible explanation for the

appearance of the purple spots in these otherwise yellow kernels, accounting for their different sizes. (Hint: See section on *Ac* and *Ds* elements in maize on pp. 304-305). *The appearance of purple spots of varying sizes in these few yellow corn kernels could be explained by transposition. The yellow kernels may be due to inactivation of a pigment gene by insertion of a Ds element in the plant bearing this ear. Because the Ds element cannot transpose on its own, the mutant allele is stable in the absence of Ac and the plant produces yellow kernels when fertilized by pollen from the same strain (lacking Ac). However, a few kernels may have been fertilized by pollen from a different strain with an active Ac element. The Ac element can then mobilize transposition of the Ds element out of the pigment gene, restoring pigment gene function. Excision of the Ds element earlier in kernel development will produce larger clones of cells producing purple pigment. Excision later in kernel development will produce smaller clones of purple cells.*

40. A geneticist studying the DNA of the Japanese bottle fly finds many copies of a particular sequence that appears similar to the *copia* transposable element in *Drosophila* (see Table 11.6). Using recombinant DNA techniques, the geneticist places an intron into a copy of this DNA sequence and inserts it into the genome of a Japanese bottle fly. If the sequence is a transposable element similar to *copia*, what prediction would you make concerning the fate of the introduced sequence in the genomes of offspring of the fly receiving it?  
*Because copia is a retrotransposon, the copia-like element probably transposes through an RNA intermediate. The recombinant transposon containing an intron will be transcribed into an RNA molecule containing the intron. However, the intron will be removed by splicing. Reverse transcription of the spliced RNA will generate copies of the transposon that lack the intron. Therefore, the daughter transposons will lack the intron sequence.*

## CHALLENGE QUESTIONS

### Section 11.3

41. An explorer discovers a strange new species of plant and sends some of the plant tissue to a geneticist to study. The geneticist isolates chromatin from the plant and examines it with the electron microscope. She observes what appear to be beads on a string. She then adds a small amount of nuclease, which cleaves the string into individual beads that each contain 280 bp of DNA. After digestion with more nuclease, she finds that a 120 bp fragment of DNA remains attached to a core of histone proteins. Analysis of the histone core reveals histones in the following proportions:

H1	12.5%
H2A	25%
H2B	25%
H3	0%
H4	25%
H7 (a new histone)	12.5%

On the basis of these observations, what conclusions could the geneticist make about the probable structure of the nucleosome in the chromatin of this plant?

*The 120 bp of DNA associated with the histone core is smaller than the 140 bp associated with typical nucleosomes. The new plant also is lacking histone H3. The new histone H7 apparently does not replace histone H3 in the nucleosome core because it is present in the same ratio as histone H1, or half of the ratios of nucleosomal core histones H2A, H2B, and H4. Finally, the 280 bp fragments with limited DNase digestion are larger than the 200 bp fragments seen with typical eukaryotic chromatin.*

*These observations suggest a model in which the nucleosome core consists of just six histones, two each of H2A, H2B, and H4, explaining the lack of H3 and the smaller amount of DNA. The longer DNA per nucleosome can be explained in part by a molecule of H7 either in the spacer between nucleosomes, or perhaps helping to cap nucleosomes in conjunction with H1.*

#### Section 11.4

42. Although highly repetitive DNA is common in eukaryotic chromosomes, it does not code for proteins; in fact, it is probably never transcribed into RNA. If highly repetitive DNA does not code for RNA or proteins, why is it present in eukaryotic genomes? Suggest some possible reasons for the widespread presence of highly repetitive DNA.

*Highly repetitive DNA may have important structural roles for eukaryotic chromosomes. Highly repetitive DNA is present in regions of the chromosome that are heterochromatic, near the centromere and near the telomeres. Highly repetitive DNA clusters may play important roles in facilitating chromatin condensation in mitotic or meiotic prophase. They may serve to insulate critical regions from chromatin decondensation or from transcription. Near the telomeres, they may serve as buffers between the ends of the chromosome and essential protein coding genes, to minimize deleterious effects from loss of telomeric DNA. Heterochromatic sequences may also regulate the expression of genes located in the heterochromatic region of the chromosome or near the heterochromatin/euchromatin border.*

43. In DNA hybridization experiments on six species of plants in the genus *Vicia*, DNA was isolated from each of the six species, denatured by heating, and sheared into small fragments (W. Y. Chooi. 1971. *Genetics* 68:213–230). In one experiment, DNA from each species and *E. coli* was allowed to renature. The adjoining graph shows the results of this renaturation experiment.
- Can you explain why the *E. coli* DNA renatures at a much faster rate than DNA from all of the *Vicia* species?  
*The E. coli genome is far smaller than the genomes of the plant species, and therefore has lower complexity. Given the same concentration of DNA, the E. coli DNA sequences are at higher copy number than the plant DNA sequences, and therefore renature faster.*
  - Notice that, for the *Vicia* species, the rate of renaturation is much faster in the first hour and then slows down. What might cause this initial rapid renaturation and the subsequent slowdown?  
*The most repetitive sequences in the Vicia genome will renature fastest. The single-copy sequences renature most slowly. The rapidly renaturing sequences are the highly repetitive sequences.*

**Section 11.6**

44. Marilyn Houck and Margaret Kidwell proposed that *P* elements were carried from *Drosophila willistoni* to *D. melanogaster* by mites that fed on fruit flies (M. A. Houck et al. 1991. *Science* 253:1125-1129). What evidence do you think would be required to demonstrate that *D. melanogaster* acquired *P* elements in this way? Propose a series of experiments to provide such evidence.

*This hypothesis requires not only that mites pick up P elements when they feed on P<sup>+</sup> fruit flies, but also that they can transmit the P elements to a host that does not have them.*

*Mites that have infected a laboratory colony of D. willistoni should be isolated and tested for the presence of P elements. If P elements are present in these mites, these mites should then be allowed to infect a colony of D. melanogaster that is free of P elements. After several generations the colony of D. melanogaster should be tested for the presence of P elements after they have been disinfected of the mites. One way to test for the presence of P elements would be to mate females from this test colony with males that are P<sup>+</sup>. Fertile progeny, testifying to a lack of hybrid dysgenesis, would indicate that these females are P<sup>+</sup>. These experiments would show whether mites are capable of transmitting P elements from one species to another.*