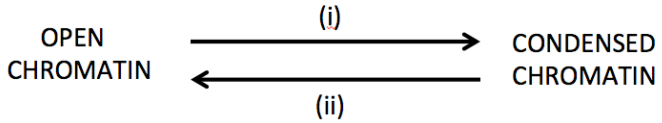
Genetics and Genomics in Medicine Chapter 6

Questions & Answers

Multiple Choice Questions

Question 6.1

With respect to the interconversion between open and condensed chromatin shown below:



Which of the directions (i) or (ii) would you anticipate would be the consequence of the following types of chromatin modification?

a) Histone acetylation.
b) DNA methylation.
c) Histone methylation.
d) Histone deacetylation.
e) DNA demethylation.

Answer 6.1

a) (ii)
b) (i)
c) (i) or (ii). It depends on the specific modification. Thus: (i) in the case of trimethylation of histone H3K9 or H4K20, but (ii) in the case of H3K4 methylation
d) (i)
e) (ii)

Question 6.2

With respect to microRNAs, which, if any, of the following statements, is false?

a) MicroRNA is a generic term that covers all tiny RNAs, ones that are less than 35 nucleotides long when mature.
b) MicroRNAs usually work as transcription factors.
c) MicroRNAs regulate target genes by binding to complementary sequences on one DNA strand of the target gene.
d) MicroRNAs normally regulate the expression of just a single target gene.

Answer 6.2

a) MicroRNA is a generic term that covers all tiny RNAs, ones that are less than 35 nucleotides long when mature.
b) MicroRNAs usually work as transcription factors.
c) MicroRNAs regulate target genes by binding to complementary sequences on one DNA strand of the target gene.
d) MicroRNAs normally regulate the expression of just a single target gene.

Explanation 6.2

All are false. There are other types of tiny RNA, including piRNAs and endogenous siRNAs. MicroRNAs regulate gene expression at the level of translation, not transcription. They bind to RNA sequences, notably in the 5' or 3' untranslated regions of mRNAs. They normally regulate multiple target genes.

Question 6.3

With respect to microRNAs, which, if any, of the following statements, is true?

a) MicroRNA genes often occur in gene clusters within introns of protein-coding genes and are usually transcribed by RNA polymerase II.
b) Like an mRNA a miRNA is initially produced as a larger precursor RNA that, like the great majority of mRNAs, usually has a 5' cap and a poly(A) tail.
c) MicroRNAs are produced by germ-line cells only.
d) An miRNA is initially produced as a duplex RNA but in order to work it needs to be converted into a single-strand RNA.

Answer 6.3

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d) An miRNA is initially produced as a duplex RNA but in order to work it needs to be converted into a single-strand RNA.

Explanation 6.3

Unlike piRNAs which are produced by germ-line cells, miRNAs are expressed in a wide range of somatic tissues.

Question 6.4

With respect to microRNAs, which, if any, of the following statements, is false?

a) A microRNA normally works by binding to perfectly complementary sequences within an RNA transcript, usually an mRNA.
b) Like the great majority of mRNAs an miRNA is usually produced as a larger precursor RNA that is capped and has a 3' poly(A) tail.
c) The precursor miRNA undergoes different types of post-transcriptional cleavage by endoribonucleases that are specific for double-stranded target sequences.
d) A nuclear endoribonuclease called dicer cleaves the miRNA precursor so that it forms a stem-loop RNA.

Answer 6.4

a) A microRNA normally works by binding to perfectly complementary sequences within an RNA transcript, usually an mRNA.
d) A nuclear endoribonuclease called dicer cleaves the miRNA precursor so that it forms a stem-loop RNA.

Explanation 6.4

MicroRNAs often bind to complementary target sequences that have one or more mismatches. Dicer is a cytoplasmic enzyme that coverts a single-stranded stem-loop miRNA into a duplex RNA with two strands held together by base pairing

Question 6.5

With respect to how miRNAs work, which, if any, of the following statements, is false?

a) An miRNA is initially composed of two RNA strands, a passenger strand that will be destroyed and a complementary RNA, the guide strand, that is required for it to work.
b) an active miRNA regulates target protein-coding genes by binding to complementary sequences in the mRNA
c) A single miRNA normally binds to transcripts from just one target gene
d) A single type of mRNA can be regulated by multiple different miRNAs.

Answer 6.5

c) A single miRNA normally binds to transcripts from just one target gene

Explanation 6.5

Individual miRNAs normally bind to transcripts from multiple target genes.

Question 6.6

MicroRNAs are important gene regulators, but the miRNAs are also regulated in turn by other RNAs. Which, if any, of the following classes of RNA are known to contain RNAs that regulate miRNAs?

a) Pseudogene RNA
b) Ribosomal RNA
c) Long noncoding RNA
d) Circular RNA

Answer 6.6

a) Pseudogene RNA
c) Long noncoding RNA
d) Circular RNA

Question 6.7

Which, if any, of the following is not regularly an epigenetic phenomenon that depends on DNA methylation or chromatin modification?

a) X-chromosome inactivation.
b) A position effect in which a gene is silenced by an inversion where both breakpoints occur within a euchromatic environment.
c) Establishment of heterochromatin at a centromere.
d) Imprinting

Answer 6.7

b) A position effect in which a gene is silenced by an inversion where both breakpoints occur within a euchromatic environment.

Explanation 6.7

This type of position effect can silence a gene simply on the basis that it severs the gene or separates the gene from crucial *cis*-acting regulatory sequences that need to be closely located to the gene they regulate.

Question 6.8

With respect to histone modifications, which, if any, of the following statements, is true?

a) histone acetylation always means adding an acetyl group to the side chain of a lysine residue.
b) in histone acetylation each lysine of the histone is acetylated.
c) in histone phosphorylation a phosphate group is transferred to the side chain of a serine .
d) in histone methylation it is the DNA that coils around a nucleosome that is methylated, not the histone itself.

Answer 6.8

a) histone acetylation always means adding an acetyl group to the side chain of a lysine residue.

Explanation 6.8

Variable lysine residues of histone proteins are acetylated. Histone phosphorylation often occurs at serine residues but not always. Histone methylation involves adding a methyl group to certain lysine or arginine residues in histones.

Question 6.9

Some DNA sequences in our cells have high frequencies of methylated cytosines (hypermethylation); some others have a low frequency of methylated cytosines (hypomethylation). Which of the two methylation states best describes the kind of sequences listed in a) to d)?

a) satellite DNA in pericentromeric heterochromatin.
b) promoters.
c) dispersed transposon repeats.
d) CpG islands
e) enhancers.

Answer 6.9

a) hypermethylated
b) hypomethylated
c) hypermethylated
d) hypomethylated
e) hypomethylated

Question 6.10

With respect to the DNA methylation mechanism in mammalian cells, which of the following statements, if any, is true?

a) The principal role of the DNMT1 DNA methyltransferase is in *de novo* methylation.
b) The DNMT3A and DNMT3B DNA methyltransferases require hemi-methylated DNA as a substrate and are responsible for methylating nascent DNA strands that are complementary to methylated parental DNA strands
c) Active DNA demethylation means removal of methyl groups from a hemi-methylated DNA double helix.
d) DNA methylation is not essential in mammalian development.

Answer 6.10
None.

Explanation 6.10
All are false.

Question 6.11

With respect to CpG islands in our genomic DNA, which, if any, of the following descriptions do not apply?

a) frequently occurring (there are about 30,000 in the human genome).
b) long DNA sequences (typically from 10 kb to 100 kb in length).
c) low CG dinucleotide frequency.
d) frequently associated with transcriptional start sites.

Answer 6.11

b) long DNA sequences (typically from 10 kb to 100 kb in length).
c) low CG dinucleotide frequency.

Explanation 6.11
CpG islands are short (often 1 kb or less) and have the normal CG frequency (but are hypomethylated because DNA methylation in these regions is suppressed)

Question 6.12

With respect to X-chromosome inactivation, which, if any, of the following statements are not correct ?

a) X-chromosome inactivation in mammals begins in the pre-implantation embryo
b) In humans all diploid cells that carry two normal X-chromosomes are subject to a random pattern of X-inactivation.
c) Once a decision has been made to inactivate an X chromosome (either the paternal or maternal X), all descendant cells show the same pattern of X-inactivation.
d) The inactivated X chromosome becomes a highly condensed Barr body in which genes are silenced across the length of the chromosome.

Answer 6.12

b) In humans all diploid cells that carry two normal X-chromosomes are subject to a random pattern of X-inactivation.
d) The inactivated X chromosome becomes a highly condensed Barr body in which genes are silenced across the length of the chromosome.

Explanation 6.12
In cells of the placenta the paternal X chromosome is always selected to be inactivated. About 15% of genes on the inactivated human X chromosome escape inactivation and are not silenced.

Question 6.13

With respect to noncoding RNA (ncRNA) , which, if any, of the following statements, is false?

a) Many long noncoding RNAs work in epigenetic regulation of gene expression.
b) Most regulatory long ncRNAs work as *trans*-acting regulators.
c) HOTAIR RNA is produced by a gene in the *HOXC* homeobox gene cluster at 12q13 but can regulate multiple genes within the HOXD gene cluster on chromosome 2.
d) HOTAIR RNA works as a scaffold that binds specific protein regulators at its two ends.

Answer 6.13

b) Most regulatory long ncRNAs work as *trans*-acting regulators.

Explanation 6.13
Most regulatory long ncRNAs work as *trans*-acting regulators

Question 6.14

With respect to epimutations, which, if any, of the following statements, is false, from a practical viewpoint?

a) The term *epimutation* means an unexpected change in chromatin conformation, causing a gene to be expressed in an abnormal way that is not related to its base sequence.
b) A primary epimutation is a change in chromatin confirmation that is not related directly to any change in the base sequence.
c) A secondary epimutation arises from a standard mutation that results in a profound change of expression in a gene that regulates chromatin conformation.
d) A “chromatin disease” is a disorder that is consistently caused by a primary epimutation.

Answer 6.14

d) A “chromatin disease” is a disorder that is consistently caused by a primary epimutation.

Explanation 6.14
A “chromatin disease” means a disease that results from a mutation in a gene that regulates chromatin structure/chromatin modification.

Question 6.15

Which, if any, of the following descriptions is false?

Uniparental disomy

a) means that in a diploid cell two copies of the same chromosome are inherited from one parent.
b) is very rare.
c) can be the outcome of a trisomic zygote that is unstable and ejects a chromosome from one parent, but keeps two copies of the same chromosome from the other parent.
d) can occur when a sperm fertilizes an egg that lacks one chromosome, and the resulting unstable zygote is able to recover by duplicating the single chromosome.

Answer 6.15
None.

Explanation 6.15
All are true.

Question 6.16

Which, if any, of the following descriptions is false?

a) Genomic imprinting in mammals really means that a very few genes are expressed from one allele only, according to the sex of the parent.
b) Imprinted genes are often found in clusters of genes, many of which are imprinted.
c) means that in a diploid cell two copies of the same chromosome are inherited from one parent
d) Within an imprinted gene cluster, all genes on one of the two parental chromosomes are silenced, but the equivalent genes on the other parental chromosome are not subject to silencing.

Answer 6.16

d) Within an imprinted gene cluster, all genes on one of the two parental chromosomes are silenced, but the equivalent genes on the other parental chromosome are not subject to silencing.

Question 6.17

Which, if any, of the following statements is incorrect?

a) In X-chromosome inactivation the inactivated X chromosome is epigenetically silenced by a transcript, the XIST RNA, that is produced from the active X chromosome.
b) The XIST RNA works by coating most of the X chromosome that is to be inactivated and then recruiting Polycomb proteins to condense the chromosome.
c) The inactivated X chromosome carries the kinds of histone modification that are typical of heterochromatin.

d) The pattern of X-chromosome inactivation is made randomly but once it has been established the same pattern of X-inactivation is propagated through all mitotic and meiotic cell divisions.

Answer 6.17

a) In X-chromosome inactivation the inactivated X chromosome is epigenetically silenced by a transcript, the XIST RNA, that is produced from the active X chromosome.
d) The pattern of X-chromosome inactivation is made randomly but once it has been established the same pattern of X-inactivation is propagated through all mitotic and meiotic cell divisions.

Explanation 6.17

X-inactivation works in *cis* and the XIST RNA is transcribed from a gene on the inactive X chromosome. Once made, the pattern of X-inactivation is propagated though mitotic cell divisions, but not through meiosis: a woman's maternal X can equally well have been the active or inactive one in her mother, and has the same chance as her paternal X of being inactivated in her own cells.

Question 6.18

With reference to imprinting disorders, which, if any, of the following statements is false?

a) About one quarter of individuals with Angelman syndrome lack a paternal chromosome 15.
b) With the exception of abnormal chromosome segregation, imprinting disorders always result from a deletion or inactivating mutation within, or spanning the imprinted gene cluster.
c) In some imprinting disorders, disease results from inappropriate biallelic expression of a gene.
d) Angelman and Prader-Willi syndrome are very different disorders but can be caused by precisely the same large deletion at 15q11-q13.

Answer 6.18

b) With the exception of abnormal chromosome segregation, imprinting disorders always result from a deletion or inactivating mutation within, or spanning the imprinted gene cluster.

Explanation 6.18

In more than half of cases with Beckwith Wiedemann syndrome the disorder results from inappropriate DNA methylation.

Question 6.19

a) With respect to imprinting control regions, which if any, of the following statements is true.
b) An imprinting control region is differentially methylated on paternal and maternal chromosomes
c) In some individuals with a disorder of imprinting, the disease occurs because an imprinted control region is inappropriately demethylated, and as a result a neighboring gene that it directly regulates is inappropriately inactivated.
d) In some individuals with a disorder of imprinting, the disease occurs because an imprinted control region is inappropriately demethylated, and as a result a neighboring gene is inappropriately activated.
e) In some individuals with a disorder of imprinting, the disease occurs because an imprinted control region is inappropriately methylated, and as a result a neighboring gene is inappropriately activated.

Answer 6.19
All are true.

Explanation 6.19

b) occurs in about 35-50% of cases of Silver-Russell syndrome when loss of methyl groups from the ICR1 imprinting control region on paternal 11p15 leads to silencing of *IGF2* (insulin-like growth factor 2) on the paternal chromosome 11, as well as the maternal chromosome 11 (where it is normally hypomethylated.
c) occurs in about 50% of cases of Beckwith-Wiedemann syndrome when loss of methyl groups from the ICR2 imprinting control region on maternal chromosome 11 leads to expression of the *KCNQ10T1* gene (as naturally occurs on paternal chromosome 11), and then production of a suppressor RNA that inhibits a growth-restricting gene, *CDKN1C.*
d) occurs in about 5% of Beckwith Wiedemann syndrome cases when the ICR1 imprinting control region is inappropriately methylated on maternal chromosome 11 so that both chromosome 11s express the *IGF2* (insulin-like growth factor 2) gene, instead of just the paternal chromosome 11.

See Table 6.7 and Figure 6.22 on page 184.

Question 6.20

With regard to the molecular pathogenesis of facioscapulohumeral dystrophy, which, if any, of the following can be implicated in the pathogenesis?

a) a variable number of tandem DNA repeats.
b) increased heterochromatin.
c) activation of a retrogene.
d) a regulator of methylation.

Answer 6.20

b) increased heterochromatin.

Explanation 6.20

The pathogenesis involves a *reduction* in the degree of heterochromatinization of the tandem D4Z4 repeats that leads to activation of the *DUX4* retrogene.

Question 6.21

Which, if any, of the following descriptions is inaccurate?
The epigenome

a) means the totality of epigenetic settings in a cell.
b) is variable between tissues.
c) does not vary between cells of the same type
d) can change in response to changes in the environment.

Answer 6.21

c) does not vary between cells of the same type

Explanation 6.21

Epigenomes are not as stable as genomes and are sensitive to small changes in the local environment.

Essay and List Questions

Question 6.22

Circular RNAs are very common in human cells. A human fibroblast, for example, has about 25,000 different circular RNAs. What is the role of these RNAs?

Answer 6.22

These RNAs serve a role in regulating genes that have homologous transcripts. The circular RNAs are often formed from transcripts that overlap protein-coding genes (they become circular by splicing the 5' end to the 3' end; the circular RNA is then protected from cellular exonucleases).

A circular RNA can then share sequences with a mRNA produced from an overlapping RNA transcript, such as sites that are recognition sequences for miRNAs, for example. By sharing miRNA-binding sites present in the untranslated regions of an mRNA, the circular RNA can compete with the mRNA for binding by miRNAs and so modulate the expression of the protein-coding gene.

Question 6.23

The nuclear genome in our cells makes four types of RNA polymerase, a simple RNA polymerase that is imported into mitochondria and is dedicated to transcribing mitochondrial DNA plus three types of multi-subunit RNA polymerase that transcribe nuclear DNA sequences. What types of DNA sequence are transcribed by the different nuclear RNA polymerases.

Answer 6.23

- *RNA polymerase I.* A nucleolar RNA polymerase, it is dedicated to transcribing three of the four types of ribosomal RNAs. Roughly 30-50 tandem DNA repeats containing successive sequences specifying the 18S, 5.8 S and 28 S ribosomal RNAs are present on each of the short arms of the five acrocentric chromosomes (chromosomes 13, 14, 15, 21 and 22). Each repeat is transcribed to make a large precursor RNA that is then cleaved to generate, the 18S, 5.8S and 28S rRNAs.
- *RNA polymerase II.* This polymerase transcribes all protein-coding genes and most of the genes that make snRNAs and miRNAs (which are often located within introns of protein-coding genes).
- *RNA polymerase III.* Transcribes all the genes that specify cytoplasmic (non-mitochondrial) tRNAs plus 5S ribosomal RNA, and also some genes that specify some other types of short RNA, including U6 snRNA, a few miRNAs, 7SL RNA, 7SK RNA, vault RNAs, Y RNAs, and the ribozymes RNase P and RNase MRP.

Question 6.24

List two examples of DNA-binding motifs commonly found in protein transcription factors. How do they bind to DNA?

Answer 6.24

1) *Zinc finger motif.* A Zn^{2+} ion is bound by four conserved amino acids (often cysteines and histidines) so as to form a loop ("finger"). Secondary structure elements in a zinc finger usually include an alpha helix and a beta sheet (held together by co-ordination with the Zn^{2+} ion), or two alpha helices, and in either case the primary contact is made by one alpha helix binding to the major groove in the DNA. Usually there are several consecutive zinc fingers in a protein and individual zinc fingers recognize specific trinucleotide sequences.
2) *Leucine zipper.* The leucine zipper is a helical stretch of amino acids rich in hydrophobic leucine residues, aligned on one side of the helix. These hydrophobic patches allow two individual alpha-helical monomers to join together over a short distance to form a coiled coil. Beyond this region, the two alpha-helices separate, so that the overall dimer is a Y-shaped structure. The dimer is thought to grip the double helix much like a clothes peg grips a clothes line. Leucine zipper proteins normally form homodimers but can occasionally form heterodimers (the latter provide an important combinatorial control mechanism in gene regulation).

Question 6.25

Splice junction sequences show a certain degree of sequence conservation. Give consensus sequences for the splice donor and splice acceptor sequences.

Answer 6.25

Splice donor consensus sequence. This is a purine-rich octanucleotide sequence that includes the invariant GU dinucleotide at the start of the intron (shown by bold and underlined font). Capital letters in blue font refer to nucleotides in the exon.

AG **gu**(a/g)agu

Splice acceptor consensus sequence. The invariant AG dinucleotide at the end of an intron (shown by bold and underlined font) is followed by a purine, but is preceded by a long sequence dominated by pyrimidines. Capital letters in blue font refer to nucleotides in the exon; n = any nucleotide.

(c/u)(c/u)(c/u)(c/u)(c/u)(c/u)(c/u)(c/u)(c/u)(c/u)(c/u) n (c/u) **ag** G

Question 6.26

How common is RNA editing in human cells and what types of RNA editing are seen?

Answer 6.26

The extent of RNA editing is believed to be limited. The most common types are:
A → I (adenosine to inosine) editing; C → U editing; and U → C editing.
A → I editing. Mostly occurs within the adenine of the CAG (glutamine) codon. Inosine base pairs with cytosine, and so effectively the CAG (glutamine) codon is replaced by CGG (arginine), and so this type of RNA editing is often referred to as Q/R editing (Q = glutamine; R = arginine, in the one letter amino acid code). This type of RNA editing is quite common during the maturation of mRNAs that will specify transmitter receptors or ion channels.
The other forms of RNA editing are quite rare but, for example, C → U editing occurs during processing of the precursor to the apolipoprotein B mRNA; and U → C editing occurs during maturation of the Wilms tumor *WT1* mRNA.

Question 6.27

The pseudogene *PTENP1* is an example of a functional pseudogene that has an important role in gene regulation. Explain what its role is.

Answer 6.27

PTENP1 is a processed pseudogene located at 9p13 and is very closely related to the parent *PTEN* gene that resides at 10q23. *PTEN* encodes an important protein dephosphatase that acts as a tumor suppressor. *PTENP1* originated when a *PTEN* mRNA was copied by a cellular reverse transcriptase to give a cDNA that integrated into a new chromosome location in the germ-line. Following its evolutionary origin by reverse transcriptase-mediated gene duplication, *PTENP1* acquired multiple inactivating mutations in its coding DNA sequence and does not make a protein.
Although it cannot make a protein, *PTENP1* is highly conserved and produces a ncRNA that regulates the expression of the *PTEN* gene (which needs to be very tightly controlled in cells). It does that by acting as an "miRNA sponge". That is, the *PTENP1* RNA transcript (which is very closely related in sequence to the *PTEN* mRNA) has shared binding sites for different miRNAs (see Figure 6.8B for known binding sites for miRNAs in the 3' untranslated region of *PTEN* mRNA). When *PTENP1* is strongly expressed it can flood the cell with transcripts that act as decoys for miRNA binding, thereby reducing miRNA regulation of *PTEN* to a minimum; or when *PTENP1* is weakly expressed, miRNA regulation of *PTEN* is maximized.

Question 6.28

List four types of epigenetic phenomena that involve DNA or chromatin modification

Answer 6.28

1) X-chromosome inactivation
2) Imprinting to produce monoallelic expression
3) A position effect in which a normally active gene is silenced after being moved within or close to a heterochromatin environment
4) Establishment of centromeric heterochromatin

Question 6.29

Match individual variant histones i) to iv) to one or more of the possible functions listed in a) to g).

Varient histones

i) CENP-A
ii) H2AX
iii) H2A.Z
iv) H3.3

Functions

a) DNA replication
b) As a barrier to stop heterochromatin spreading
c) DNA repair
d) Transcriptional activation
e) Kinetochore assembly
f) Recombination
g) Genome maintenance

Answer 6.29

i) e)
ii) c) and f)
iii) b), d) and g)
iv) d)

Question 6.30

DNA methylation is one epigenetic mechanism where it is easy to appreciate how the pattern of epigenetic settings is stably inherited from one cell generation to the next. What are the features of the DNA methylation mechanism that suggest this?

Answer 6.30

There are two key features. One is that the target for DNA methylation, the CG dinucleotide, is a palindromic sequence (one whose sequence from 5' to 3' is the same on both DNA strands). The second key feature is that the DNMT1 DNA methylase specifically works on hemi-methylated DNA.

Many CG dinucleotides in genomic DNA will not have a methyl group on the cytosine, but because of the above two key features of the DNA methylation mechanism those CG dinucleotides that do have a methylated cytosine can perpetuate the same pattern of DNA methylation in daughter cells. That happens because the DNMT1 methylase identifies any CG dinucleotide on the growing new DNA strand that is paired with a methylated CG on a parent strand, and then methylates the opposing cytosine on the new DNA strand.

Question 6.31

How does uniparental diploidy occur in humans, and what are the consequences?

Answer 6.31

Uniparental diploidy can occur so that a zygote has two male pronuclei (androgenetic embryo) or two female pronuclei (parthenogenetic or gynogenetic embryo). An androgenetic embryo is formed when an abnormal diploid sperm fertilizes an abnormal egg that lacks chromosomes. A parthenogenetic embryo is formed when the haploid set of chromosomes in an egg duplicates to produce an abnormal diploid egg cell.

An androgenetic embryo gives rise to a hydatidiform mole, an abnormal conceptus in which there is widespread overgrowth of the trophoblast but no fetal parts. A parthenogenetic embryo gives rise to an ovarian teratoma that is composed of disorganized embryonic tissues without the vital extra-embryonic membranes needed to support the pregnancy.

Question 6.32

According to the manner in which they work, three main classes of proteins that modify chromatin are recognized. What are these three classes and explain, with examples, what distinguishes the individual classes.

Answer 6.32

- *Writers*. These are proteins that add chemical groups to DNA or histones. They include DNA methyltransferases, histone methyltransferases, histone acetyltransferases, histone kinases and so on.
- *Erasers*. They remove the same types of chemical groups from DNA or histones that the writers can add. They include DNA demethylases, histone demethylases, histone deacetylases, histone phosphates and so on.
- *Readers*. They bind to specific chemical groups on DNA or histones to interpret defined epigenetic marks. They include proteins such as the meCG-binding protein (which specifically binds to methylated cytosines within the CG dinucleotide) and chromatin remodelers.

Explanation 6.32
See Table 6.6 on page 178 for some examples.

Question 6.33
What are the principal functions of DNA methylation in mammalian cells?

Answer 6.33
The principal general function of DNA methylation in mammalian cells is to stabilize, or lock in, patterns of transcriptional silencing so that transcription is suppressed in highly methylated regions of chromatin. Highly repetitive DNA sequences, such as satellite repeats in pericentromeric heterochromatin and dispersed transposons, are extensively methylated, but there is also significant—though more sporadic—methylation in the main body (exons and introns) of genes and in intergenic regions.
DNA methylation-based transcriptional silencing allows different forms of regulatory control. One of these is the regulation of gene expression, and it is the extent of DNA methylation in the key *cis*-acting regulatory elements that distinguishes actively transcribing genes from silenced genes. Patterns of gene expression that defines the identity of cells or that allow just one of the two alleles to be normally expressed can be locked in by DNA methylation.

Question 6.34
Give four examples of different ways in which the active allele at an imprinted locus is known to be inactivated or not inherited in an imprinting disorder.

Answer 6.34
1) Chromosome microdeletion (for example of the maternal 15q11-13 region in Angelman syndrome and the paternal 15q11-13 region in Prader-Willi syndrome)
2) Uniparental disomy. The normally expressed allele is not inherited; instead, both copies of a chromosome with the silenced allele come from one parent. Examples include: maternal disomy 15 in the case of Prader Willi syndrome; paternal disomy 15 in the case of Angelman syndrome; and maternal disomy 11 or maternal disomy 17 in the case of Silver-Russell syndrome.
3) Hypomethylation of imprinting control region (ICR). At the imprinted gene cluster at 11p15, the ICR1 control region is methylated on paternal chromosome 15, and that allows distant enhancer sequences to activate expression of the *IGF2* gene. Sometimes, however, there is a reduction in methylation at ICR1 on the paternal chromosome, and it is now bound by the CTCF protein which activates the assembly of an insulator region that prevents the remote enhancers from activating IGF2.
4) Inactivating point mutations in the normally active allele

Explanation 6.34
See Table 6.7 and Figure 6.22 on page 184 for fuller details

Question 6.35
Outline the major global changes in DNA methylation that occur during mammalian gametogenesis and early embryonic development.

Answer 6.35

- *Gametogenesis*. The primordial germ cells that will give rise ultimately to gametes are initially heavily methylated. As they enter the genital ridge, their genomes are progressively demethylated, erasing the vast majority of epigenetic settings. Thereafter, *de novo* methylation allows epigenetic marks to be reset; the sperm DNA becomes particularly heavily methylated; the egg DNA is also strongly methylated but less extensively than in sperm DNA.
- *Early embryogenesis*. Once a sperm has fertilized an egg, the introduced sperm genome (now within the male pronucleus) begins to undergo active DNA demethylation; after the male and female pronuclei fuse, global demethylation of the zygote begins and continues until the early blastula stage in the preimplantation embryo. Then a wave of genome re-methylation occurs, coincident with initial differentiation steps that give rise to different cell lineages. Genome methylation is extensive in somatic cell lineages but moderate in trophoblast-derived lineages (which will give rise to placenta, yolk sac, and so on). A subset of cells will develop into primordial germ cells with a progressive, but small, decline in levels of DNA methylation.

Explanation 6.35
See Figure 6.14 for more information

Question 6.36
Angelman and Prader-Willi syndromes are very different disorders that can be caused by the precisely the same deletion on chromosome 15. How is that possible?

Answer 6.36
The deletion in question spans 15q11-q13. The quite different phenotypes in Angelman and Prader-Willi syndromes arise because the deleted DNA segment spans an imprinted gene cluster, with some genes expressed only if they were inherited on the paternal chromosome 15, and other genes that are expressed only if they are inherited on the maternal chromosome 15. As a result, the same deletion can have different effects, according to whether it occurred on a maternal or paternally inherited chromosome 15.

In the case of Angelman syndrome, the pathogenic 15q11-q13 deletion is maternally inherited: pathogenesis seems to arise because of failure to express the maternal *UBE3A* allele (the paternal allele is silenced by imprinting).
In Prader-Willi syndrome, the pathogenic 15q11-q13 deletion is paternally inherited: pathogenesis seems to arise because of deficient expression of certain genes, notably the paternal *NDN* allele and certain paternal snoRNA alleles in this region.

Question 6.37

Rett syndrome is a classic chromatin disease. What is meant by a chromatin disease and what are the characteristics of Rett disease?

Answer 6.37

A chromatin disease is a disorder that arises from mutation in a chromatin modifier gene, that is, a gene that makes a product that regulates some aspect of chromatin modification, whether is be DNA methylation, histone modification, nucleosome spacing and so on. Because mutation in a chromatin modifier gene can affect basic patterns of chromatin modification across the genome (and so can affect the expression of multiple genes), chromatin disorders are often severe dominant disorders where affected individuals do not reproduce.
Rett syndrome is caused by mutations in the X-lined *MECP2* gene that encodes a chromatin modifier "reader" protein whose job is to recognize and bind 5-methylcytosine in 5-meCG dinucleotides. It is a progressive X-linked neurodevelopmental disorder that affects girls almost exclusively. Growth retardation results in microcephaly. Mental retardation can be severe, seizures are common, and autistic features are often seen.
Failure to produce any functional MECP2 protein was initially expected to be lethal and would explain why affected males are so rarely seen. Affected males can occasionally occur, however, often as a result of a post-zygotic inactivating mutation.

Question 6.38

In some imprinting disorders the normal allele of an imprinted gene locus is inactivated (so that both alleles are silenced). Illustrate how this happens in Angelman and Prader-Willi syndromes.

Answer 6.38

At an imprinted gene locus, either the paternal or maternal allele is consistently epigenetically silenced in at least some cells of the body of normal individuals. In disorders such as Angelman, and Prader-Willi syndromes, however, the normal allele at imprinted gene loci is usually inactivated by large deletions or other inactivating mutations, or is not inherited because of uniparental disomy.
In Angelman and Prader-Willi syndrome the key imprinted genes are at 15q11, and in the case of Angelman syndrome the problem is the *UBE3A* gene which is preferentially expressed on

maternal chromosome 15 but which is frequently lost through a large-scale deletion on maternal chromosome 15. The same region also has multiple genes that in normal situations are exclusively or preferentially expressed on the paternal chromosome 15, including many ncRNA genes, notably various *SNORD116* (HBII-85) snoRNA genes. In Prader-Willi syndrome, these genes are lost, frequently through large-scale deletion on paternal chromosome 15 or by maternal disomy 15 (when both copies of chromosome 15 are inherited maternally).

Question 6.39

In Beckwith-Wiedemann syndrome an allele that is normally epigenetically silenced is somehow expressed, resulting in biallelic expression. Illustrate how the expression of relevant genes is altered to cause the disease.

Answer 6.39

In Beckwith-Wiedemann syndrome normal growth patterns are disturbed so that affected individuals develop macrosomia. That can happen when both alleles of the *IGF2* gene at 11p15 are active or when both alleles of the neighboring *KCNQ10T1* gene are active. Normally, the *IGF2* gene and the nearby *KCNQ10T1* gene are expressed on the paternal chromosome 11 but not on maternal chromosome 11.

The *IGF2* (insulin-like growth factor 2) gene has a role as a growth factor and *KCNQ10T1* is normally expressed to produce a suppressor ncRNA that inhibits expression of two neighboring genes, including *CDKN1C* (cyclin-dependent kinase inhibitor 1C) which functions in growth restriction; if *CDKN1C* expression is suppressed by the KCNQ10T1 suppressor RNA, the lack of growth restriction leads to overgrowth. Paternal disomy 11 leads therefore to two active IGF2 alleles and two active *KCNQ10T1* alleles (which suppress *CDKN1C*), both of which cause overgrowth.

Expression of *IGF2* and *KCNQ10T1* are also regulated by imprinting control regions, respectively called ICR1 and ICR2. These two regions are differentially methylated on paternal and maternal 11p15 as follows:

- On maternal 11p15 the ICR1 element is unmethylated and bound by a CTCF protein that allows an insulator to form, which blocks a remote enhancer from activating *IGF2.* But methylation of ICR1 on paternal 11p15 stops CTCF binding and allows expression of *IGF2* and in some individuals with Beckwith-Wiedemann syndrome there is a gain of methylation on maternal ICR1 so that the maternal *IGF2* allele is also expressed.
- The ICR2 element acts as a promoter for *KCNQ10T1* and is normally unmethylated on paternal 11p15, so that the KCNQ10T1 suppressor RNA is produced and down-regulates the growth-restricting gene *CDKN1C*. But it is methylated on maternal 11p15 so that KCNQ10T1 suppressor RNA is not produced and the CDKN1C protein is produced to check growth. In about half of cases with Beckwith-Wiedemann syndrome, however, maternal ICR2 is unmethylated, just like paternal ICR2 and down regulates the production of the growth-restricting *CDKN1C* gene.

Question 6.40

A classical position effect means that a gene can be partly or fully silenced simply if it is moved to a different chromosomal location. Explain how this happens.

Answer 6.40

It happens when a gene is moved close to a heterochromatin environment. Across our chromosomes there are alternating regions of euchromatin and heterochromatin that are demarcated by *barrier elements.* That is, a gene within a euchromatin region will be separated from the nearest heterochromatin by a barrier element. However, certain types of chromosomal rearrangements such as translocations and inversions can move genes to new chromosomal regions where they are located close to a region of heterochromatin and are not protected by an intervening barrier. Without a barrier element to oppose it, the neighboring heterochromatin can seed the formation of new heterochromatin in the adjacent region that contains the transposed gene (*heterochromatin spreading*).

Explanation 6.40

See Figure 6.20 on page 180