Supplemental Material

CBE—Life Sciences Education

Couch ***et al.***

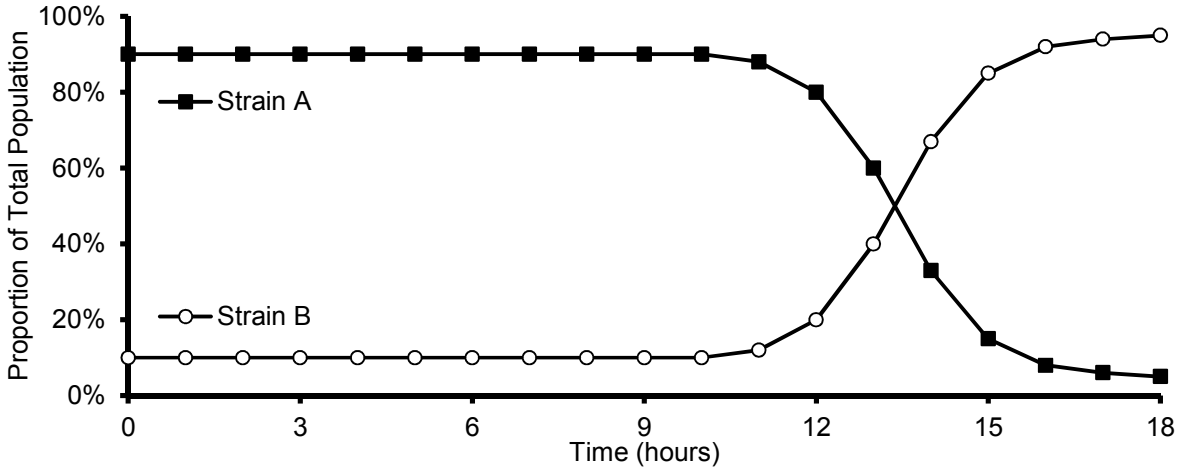
Supplemental Material 1. The Molecular Biology Capstone Assessment (MBCA)

The number to the left of each T/F statement represents statement difficulty (the fraction of students selecting the correct T/F response for that statement). An answer key is available upon request.

1. Originally a marine species, stickleback fish now live in both marine and freshwater environments. Marine sticklebacks have pelvic spines that protect them from marine predators. Freshwater sticklebacks do not have pelvic spines, which reduces their likelihood of being caught by freshwater predators that can grasp onto these spines. The lack of spines in freshwater sticklebacks results from a heritable mutation that disrupts the function of the *Pitx1* gene. This mutation could have first occurred:

0.57 a) in freshwater sticklebacks to intentionally avoid predation.
0.52 b) in a skin cell that is part of the pelvic spine.
0.68 c) prior to the establishment of a freshwater population.
0.69 d) independently of the environmental change.

2. Bacteria can multiply in liquid media containing nutrients and energy sources. You place 9,000 Strain A bacteria and 1,000 Strain B bacteria into media containing glucose and lactose, and you allow this culture to grow without further additions. At different time points, you remove small samples and determine the proportions of Strain A and Strain B bacteria in the population, resulting in the observed growth curves shown below.



These data could result from Strain B bacteria:

0.58 a) multiplying more slowly than Strain A bacteria during the first 10 hours.
0.69 b) multiplying at the same rate as Strain A bacteria where the two curves cross.
0.85 c) multiplying more rapidly than Strain A bacteria as nutrients become depleted.
0.88 d) utilizing lactose once the preferred glucose energy source becomes depleted.

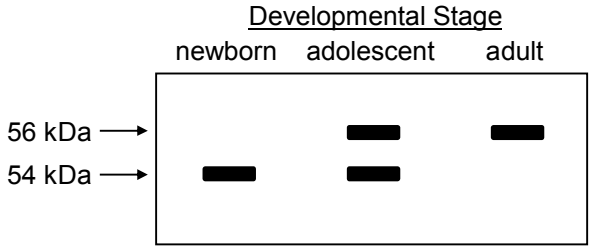
3. A population of squirrels lives in the flatlands. A small portion of that population moves to an adjacent mountain range where there are no other squirrels and becomes isolated from the parent population. After many generations, the number and frequency of alleles that determine coat color are different between the two populations. This could be because:

0.53 a) the group that moved to the mountains had different allele frequencies than the parent population.
0.45 b) inbreeding among the mountain squirrels caused new alleles to occur within this population.
0.84 c) mountain predators were more successful at catching squirrels with certain alleles.
0.59 d) different new alleles arose in the two populations after their initial separation.

4. The *NeuN* gene is normally transcribed in neurons, but not in liver cells. This difference could be because:

0.70 a) the sequence of DNA bases within the *NeuN* promoter is different in neurons and liver cells.
0.62 b) DNA bases within the *NeuN* promoter are chemically modified in liver cells, but not in neurons.
0.97 c) a transcription factor that activates *NeuN* expression in neurons is absent in liver cells.
0.56 d) a protein present in both cell types activates *NeuN* transcription in neurons, but not in liver cells.

5. Tau is a microtubule-associated protein that plays a key role in the development of certain brain disorders. To analyze normal Tau expression, mouse brain extracts are made at different developmental stages, separated on an SDS-PAGE gel, and analyzed by Western blot with antibodies recognizing only mouse Tau protein, as shown below.



The two different size protein products observed at different developmental stages could result from:

0.92 a) transcription of *Tau* DNA in different directions.
0.70 b) different RNA-binding proteins present at different stages.
0.67 c) translation of *Tau* mRNAs of different sizes.
0.78 d) different chemical modifications of Tau protein.

6. During the process of normal development, a frog cell undergoes mitosis to produce two non-dividing daughter cells. After migrating to separate locations within the embryo, each cell independently encounters the same signaling molecule, but one cell responds while the other cell does not respond. The two cells could have responded differently because:

0.63 a) they have different DNA content as a result of recombination between sister chromatids.
0.43 b) the dividing cell that gave rise to them had an uneven distribution of mRNAs.
0.52 c) the dividing cell that gave rise to them had an uneven distribution of proteins.
0.75 d) they were exposed to different signaling molecules during migration.

7. Many strains of *E. coli* bacteria are found in the human gut. These bacteria:

0.69 a) make mRNA and protein in different cellular compartments.
0.60 b) lack mitochondria.
0.71 c) have cell walls.
0.83 d) normally cause illness in the host.

8. The sequence below represents the coding strand, also called the non-template strand, of a gene from a rapidly mutating virus, starting with the sequence that encodes the translation initiation codon.

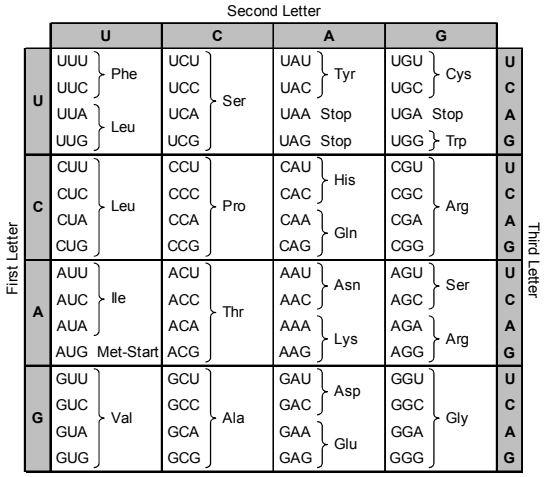
5'….ATGGCGACTATCGTTAAGTA….3'

You have isolated a mutant version of this gene that contains three base pair changes, underlined below.

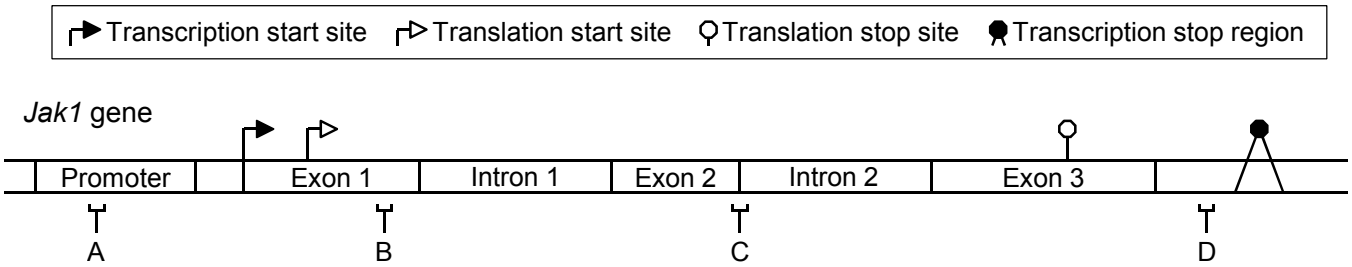
5'….ATGGCCACTATAGTTTAGTA ….3'

This combination of mutations will result in:

0.54 a) substitution of only one amino acid in the polypeptide product.
0.72 b) substitution of multiple amino acids in the polypeptide product.
0.57 c) a shorter mRNA transcript.
0.68 d) a shorter polypeptide product.



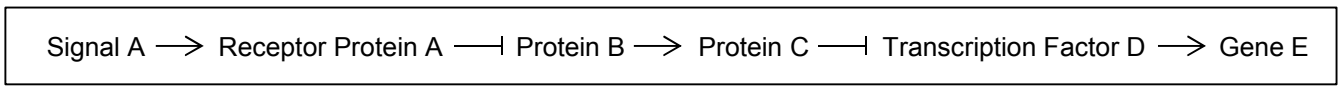
9. The *Jak1* gene encodes a transcription factor that binds to the promoter region of another gene. The *Jak1* gene diagramed below has only one promoter and is located in a region of the genome containing no other genes.



You discover a mutation in the *Jak1* gene that results in a protein with altered binding affinity. This mutation could be located:

0.68 a) A, in the promoter region.
0.84 b) B, within the first exon.
0.67 c) C, at a splice site.
0.84 d) D, downstream of the last exon.

10. The diagram below shows how a series of components in a signaling pathway normally interact. The pathway is initiated by the presence of Signal A at the cell surface. Pointed arrows (→) indicate that one component activates another component that is otherwise inactive, and blunt arrows (—|) indicate that one component inhibits another component that is otherwise active.



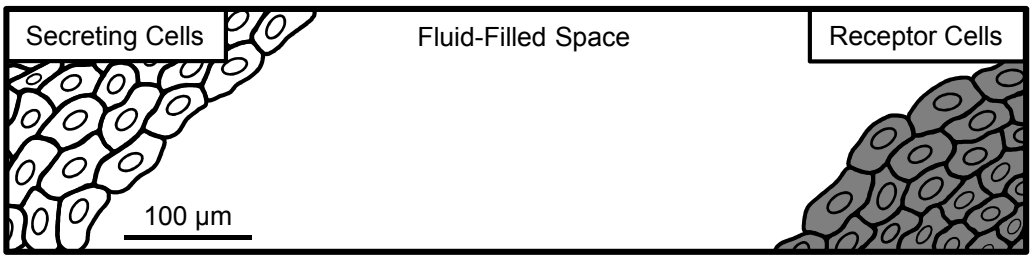
Assuming Signal A is present, this pathway will promote Gene E expression when:

0.61 a) there are no mutations in any genes encoding pathway components.
0.75 b) Receptor Protein A is non-functional.
0.58 c) Protein B is non-functional.
0.47 d) Receptor Protein A and Protein B are both non-functional.

11. A mouse embryo is produced with only one chromosome 16. Assuming normal sperm contribution, a chromosome separation error could have occurred in:

0.65 a) meiosis I, giving rise to an egg with no chromosome 16.
0.71 b) meiosis II, giving rise to an egg with no chromosome 16.
0.66 c) meiosis I, giving rise to an egg with one chromosome 16.
0.61 d) meiosis II, giving rise to an egg with one chromosome 16.

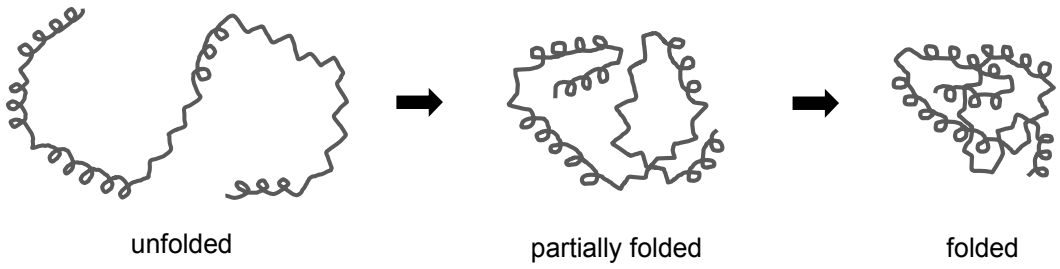
12. Two populations of cells are separated by a fluid-filled cavity, as shown below. The white cells secrete signaling ligands that bind to receptors on the surfaces of the gray cells.



A signaling ligand can travel across this 1 mm distance to a receptor by the following processes:

0.78 a) the receptor senses the ligand and draws the ligand across this space.
0.32 b) charged regions on the ligand and receptor attract each other across this distance.
0.34 c) a motor protein actively transports the ligand across this space.
0.74 d) the ligand moves across this distance sometimes towards and sometimes away from the receptor.

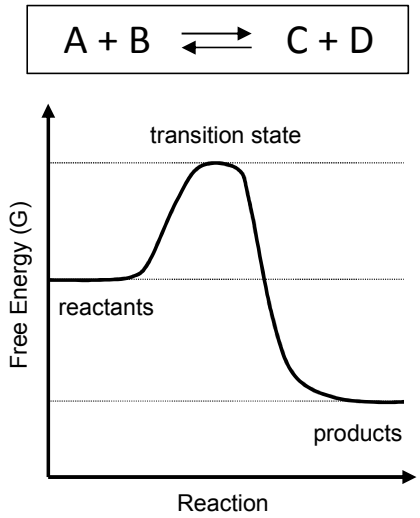
13. The gray lines below represent the amino acid backbone of a water-soluble protein. This protein is comprised of polar and nonpolar residues and folds under physiological conditions, as shown below.



The following statements describe the folding of this protein in an aqueous environment:

0.79 a) nonpolar side chains will tend to be located on the outer surface of the folded conformation.
0.76 b) the free energy of the protein and its surroundings is lower when the protein is folded than when it is unfolded.
0.70 c) the entropy of the protein is lower when the protein is folded than when it is unfolded.
0.51 d) the entropy of the surrounding water molecules is lower when the protein is folded than when it is unfolded.

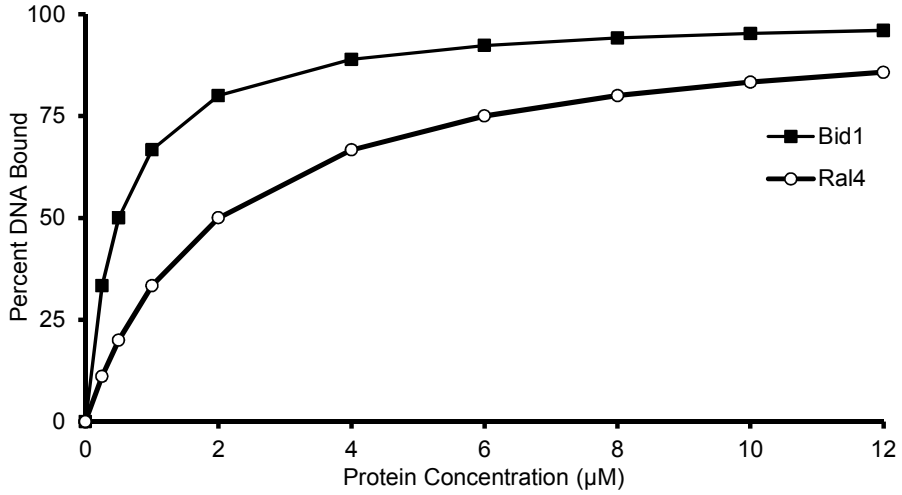
14. An energy diagram is shown below for a simple uncatalyzed biochemical reaction, showing the changes in free energy of the molecular components as the reaction proceeds from reactants through the transition state to products.



When the reactants are mixed at room temperature, no product formation is detected. If the solution is heated, the reaction proceeds slowly to equilibrium. If an enzyme is added to the solution at room temperature, the reaction proceeds rapidly to equilibrium. These different reactions can be characterized as follows:

0.73 a) at room temperature, the uncatalyzed reaction proceeds at an extremely slow rate.
0.91 b) when the solution is heated, the free energy of some reactant molecules reaches the level of the transition state.
0.49 c) binding to the enzyme raises the free energy of the reactant molecules to the level of the transition state.
0.62 d) when the enzyme-catalyzed reaction reaches equilibrium, the forward reaction rate exceeds the reverse reaction rate.

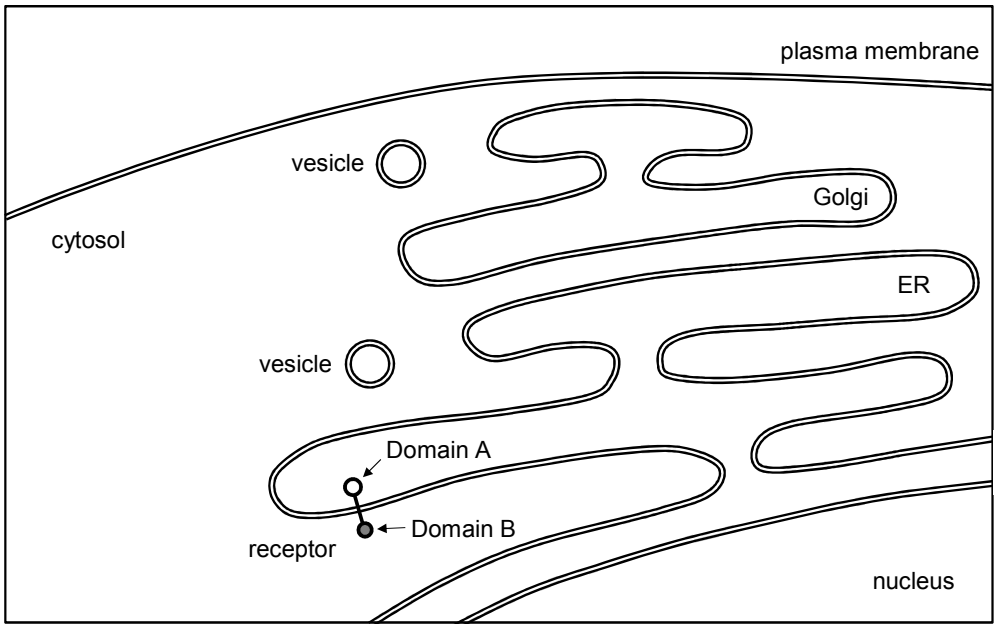
15. Bid1 and Ral4 are transcription factors that bind to the same DNA sequence. To investigate this binding, you purify Bid1 and Ral4, make separate solutions of these proteins at increasing concentrations, and add the same small amount of the DNA sequence to each solution. After allowing the solutions to equilibrate, you measure and plot the percent of the total DNA bound at each different protein concentration, as shown below.



Based on these results, you can conclude that:

0.96 a) Bid1 binds with higher affinity to the DNA sequence than Ral4.
0.47 b) Ral4 binds with higher affinity to the DNA sequence at 12 μM concentration than it does at 2 μM concentration.
0.92 c) if the two proteins were present together at 2 μM each, the percent DNA bound by each protein would be equal.
0.73 d) individual Bid1 and Ral4 proteins are unable to dissociate once they bind to DNA.

16. The transmembrane receptor protein shown below in the ER membrane is trafficked through the Golgi apparatus to the plasma membrane.



During this process, Domain A will be accessible to the cytosol when the receptor is embedded in:

0.71 a) the ER membrane.
0.62 b) the membrane of a transport vesicle.
0.74 c) the Golgi membrane.
0.60 d) the plasma membrane.

17. Tamoxifen is a drug used to treat cancers characterized by high levels of estrogen receptor. Tamoxifen works by binding to the estrogen receptor, blocking estrogen receptor signaling, and preventing estrogen-induced cell proliferation. Some people with these cancers respond well to tamoxifen, while others receive little benefit from tamoxifen treatment.

To investigate this problem, a genomic study is conducted with cancer patients undergoing tamoxifen treatment. DNA samples taken from non-tumor cells are sequenced at different sites spread across the genome where there are known single-nucleotide polymorphisms, called SNPs, in the population. The resulting data show that a statistically significant number of responding patients differ from non-responding patients at one particular nucleotide site. This nucleotide difference could be located:

0.84 a) within the estrogen receptor gene in a region that influences its expression or activity.
0.30 b) in a region genetically linked to the estrogen receptor gene, but not influencing its expression or activity.
0.66 c) in the coding sequence of an enzyme responsible for tamoxifen metabolism.
0.62 d) in the coding sequence of a signaling protein that functions downstream of the estrogen receptor.

18. In human female embryos, one copy of the X chromosome is randomly inactivated in each cell between the 4-cell and 32-cell stages. A woman is homozygous for a recessive X-linked mutation that results in her having all unpigmented skin. If she has children with a man who has all normally pigmented skin, their offspring could include:

0.66 a) a son with all normally pigmented skin.
0.82 b) a son with patches of pigmented and unpigmented skin.
0.81 c) a son with all unpigmented skin.
0.68 d) a daughter with patches of pigmented and unpigmented skin.

Supplemental Material 2. Detailed information required to answer each MBCA question.

1. Organisms do not induce targeted mutations to promote their own survival; mutations must be in germ cells to be heritable; specific mutations occur independently of environmental conditions, often arising prior to an environmental change that allows them to be beneficial.

2. The proportions of two groups in a population are determined by the multiplication rates for each group; two groups multiplying at the same rate will maintain constant relative proportions; two groups multiplying at different rates will exhibit changes in their relative proportions; bacteria growing in a semi-closed system will deplete certain resources over time; some bacterial strains can use alternative carbon energy sources (e.g. lactose), once a preferred energy source (e.g., glucose) becomes depleted.

3. Multiple processes can lead to the genetic divergence of a newly isolated population and a parent population; a small newly isolated population can have different allele frequencies than a parent population at initial separation (founder effect); different environments can cause different alleles to have reproductive advantages in the two populations (natural selection); different new alleles can arise by mutation in the two populations; while inbreeding increases the likelihood that rare recessive alleles will become homozygous, it does not serve as a source of new mutations.

4. The sequence of DNA present in different cell types is nearly identical; while there can be slight DNA sequence differences between cells due to somatic mutation, these differences are not thought to provide a mechanism for normal cell-type-specific gene regulation (except in certain cells of the immune system); chemical modification (methylation) of DNA bases in the promoter of a gene can affect rates of transcription initiation; different transcription factors are present in different cell types, and the presence of certain transcription factors will impact the transcription of a given gene; a single transcription factor can act differently in different cell types if it is differentially modified (e.g., phosphorylated) or acts in combination with other transcription factors (combinatorial control).

5. Genes are only transcribed in one direction; alternative splicing is mediated by binding of proteins to the RNA transcript and results in the production of mRNA templates of different lengths and compositions; proteins can be modified post-translationally (e.g., phosphorylation).

6. Daughter cells are normally identical in DNA content; while mitotic recombination can produce daughter cells with different DNA contents, this recombination takes place between non-sister chromatids; daughter cells can become phenotypically different from each other through differential segregation of mRNAs or proteins during initial cell division or exposure of daughter cells to different signaling molecules.

7. Since bacteria lack a membrane-bound nucleus, transcription and translation occur in the same compartment; bacteria do not have mitochondria (respiration occurs at the cytoplasmic membrane); many bacteria, including *E. coli*, have peptidoglycan-based cells walls; the human gut flora includes numerous strains of *E. coli* and other bacteria that do not cause illness under normal conditions.

8. Transcription produces an RNA sequence that is the same as the DNA coding strand, except that thymine is replaced by uracil; translation proceeds along the transcript from 5' to 3', canonically starting with the AUG translation initiation codon; some mutations are "silent" and do not alter the resulting amino acid sequence; a mutation resulting in a premature stop codon (i.e., a nonsense mutation) does not alter transcript structure, but does lead to a shorter polypeptide product; nonsense-mediated decay (NMD) requires a pioneer round of translation, and thus produces a polypeptide product.

9. Binding affinity is an intrinsic property of a protein and does not depend on protein expression levels; a mutation causing an alteration in binding affinity must alter the protein's amino acid sequence; mutations within the promoter or downstream of the final exon will not alter the resulting amino acid sequence; mutations within an exon or splice site could alter the resulting amino acid sequence.

10. Inactivated or non-functional proteins do not regulate the activity of the next pathway component in their normal manner; if two proteins in a signaling pathway are non-functional, the ultimate consequence is the same as if only the downstream protein were non-functional.

11. For an embryo to be produced with only one chromosome for a given homologous chromosome pair, one of the contributing germ cells must have been lacking that chromosome; homologous chromosomes separate in meiosis I; sister chromatids separate in meiosis II; a nondisjunction event in either meiotic stage can lead to a gamete lacking a given chromosome.

12. A signaling ligand encounters its cognate receptor by random diffusion; while electrostatic attraction between two molecules can have significant consequences at very short distances, this attraction is inconsequential at longer distances because of the high dielectric constant of water and the numerous other charges present in physiological environments; motor proteins do not transport materials outside of cells.

13. Spontaneous processes proceed towards states of lower free energy; the free energy of a system is inversely proportional to its entropy (disorder); water molecules in contact with nonpolar substances are more ordered; nonpolar substances, such as non-polar amino acid side chains, tend to aggregate in aqueous environments, reducing their water-exposed surface area; while a protein becomes more ordered during the folding process, this entropic cost is outweighed by the increased disorder (entropic gain) of the surrounding water molecules.

14. All reactions proceed towards chemical equilibrium, the point of lowest free energy for a given system; the rate at which a set of reactants is converted into products of lower free energy is directly proportional to the number of reactant molecules that can achieve the transition-state energy level; increasing the temperature of a reaction increases the free energy of the reactant molecules and allows a greater proportion of them to achieve the transition-state energy level; adding a specific enzyme lowers the free energy of the transition state for the reaction and allows a greater number of reactant molecules to achieve the transition-state energy level; binding to an enzyme does not raise the free energy of the reactant molecules; equilibrium is achieved when the rates of the forward and reverse reactions are equal.

15. The binding affinity of an interaction dictates the proportion of bound molecules at different concentrations; binding partners with higher affinity will exhibit a larger bound proportion than binding partners with lower affinity at the same concentrations; binding affinity is an intrinsic property of a protein and its binding partner, which does not change with the concentration of either species (except in cases of cooperative binding); for irreversible binding events, the target will be saturated (or nearly saturated) whenever the protein concentration exceeds the concentration of the binding partner.

16. When a vesicle buds from a membranous organelle, the inner contents of the vesicle are drawn from the inner contents of the organelle; when a vesicle fuses with a membranous organelle, the inner contents of the vesicle are released to the inside of the organelle; when a vesicle fuses with the plasma membrane, the inner contents of the vesicle are released into the extracellular space; a transmembrane protein originating in the ER membrane will be trafficked such that its cytosolic domain will remain oriented towards the cytosol throughout the entire process.

17. A single nucleotide change (SNP) can be associated (co-inherited at high frequency) with an observable phenotype; this nucleotide may be located within any number of different genes encoding protein products that function in a given signaling pathway or metabolic process; this nucleotide may also be located adjacent to any of these genes, serving as a marker of that genomic region.

18. Human females have two X chromosomes, males have one X chromosome and one Y chromosome; mating of a woman homozygous for a recessive X-linked allele (X^aX^a) and a man with a normal allele (X^AY) will result in hemizygous recessive sons (X^aY) and heterozygous daughters (X^AX^a); a hemizygous recessive son will exhibit the recessive phenotype; a heterozygous daughter will exhibit a mosaic phenotype in which some cells express the recessive allele and other cells express the normal allele due to random X-inactivation.
