ANSWERS to WS: GENE SUITE: Lesson A: Why do we need Vitamin C in our diet?

1. Rat sequence yields a chain of 17 amino acids:
YPVEVRFTRGDDILLSP (Tyr-Pro-Val-Glu-Val-Arg-Phe-Thr-Arg-Gly-Asp-Asp-Ile-Leu-Leu-Ser-Pro)
AA: Y P V E V R F T R G D D I L L S P

2. Human sequence yields a chain of only 14 amino acids because a deletion causes a frameshift which creates a stop codon:
YLVGVRFTWRMTSY (Tyr-Leu-Val-Gly-Val-Arg-Phe-Thr-Trp-Arg-Met-Thr-Ser-Tyr)
AA: Y L V G V R F T W R M T S Y

3. In comparing the two sequences, students should observe that 6 of the first 9 amino acids match. From codon #346 on, none match.

4. Seven differences in the first 9 codons were reflected in only three differences in the first 9 amino acids. The absence ("deletion") of one nucleotide in the human sequence, corresponding to rat codon number 346, was associated with a left-shift in the reading frame, resulting in total lack of matches for amino acids 11-15 and termination of the human chain. This deletion alone would result in the human chain being only 345 amino acids long instead of being 440 amino acids long.

5. Single-base substitutions produce fewer amino acid changes than deletions do. Even if this were the only difference between the two gene sequences, which it is not, this one change alone would be expected to have a potentially dramatic effect on the ability of the enzyme to carry out its function in synthesis of Vitamin C.

6. We can hypothesize that a change such as this occurred in our ancestors, that they were not harmed by lack of the enzyme because Vitamin C was present in their fruit-rich diet, and that over generations, perhaps solely by chance, the mutated gene (allele) became common and the normal gene (allele) was finally lost completely.

7. How Can a Gene Become Permanently Inactivated?
Mutations may occur whenever chromosomes break or are copied. What kinds of mutations could prevent protein synthesis or result in the production of an amino acid sequence so altered that it cannot perform its expected function? Mutations in the **promoter** region of a gene can prevent its even being transcribed. To imagine how mutations in the coding region of a gene could affect the resulting protein, recall that enzymes must "match up with" their respective "substrates" in a manner similar to how a key must fit a lock, so any mutation that dramatically shortens a protein or causes a major change in shape is likely to prevent normal functioning.

The two types of mutations likely to produce dramatic changes are a **single-base substitution** that produces a **stop codon** (a **nonsense mutation**) or a **deletion** of one or more nucleotides that "shifts" the reading frame (a **frameshift mutation**).

References (sources of sequences):

Nishikimi, M.; Fukuyama, R.; Minoshima, S.; Shimizu, N.; Yagi, K.: Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J. Biol. Chem*. **269**: 13685-13688, 1994. PubMed ID : 8175804

Nishikimi, M; Kawai, T; Yagi, K.: Guinea pigs possess a highly mutated gene for L-gulono-gamma-lactone oxidase, the key enzyme for L-ascorbic acid biosynthesis missing in this species. *J Biol Chem* **267**:21967-72, 1992. PubMed ID: 1400507

Ohta, Y; Nishikimi, M.: Random nucleotide substitutions in primate nonfunctional gene forL-gulono-gamma-lactone oxidase, the missing enzyme in L-ascorbic acid biosynthesis. *Biochim Biophys Acta* **1472**:408-11, 1999. PubMed ID: 10572964