MOLECULAR CLOCKS: PROTEINS THAT EVOLVE AT DIFFERENT RATES

The longer two species have been evolving separately, the more amino acid differences accumulate in their proteins. Amino acid changes reflect mutations in the genes. The basic mutation rate is probably similar for all genes, but natural selection filters out those mutations that impair a protein's function. These functional constraints affect the rate at which amino acids are substituted in a given protein.

In this plate we look at four proteins that have changed at very different rates in the course of more than a billion years of evolutionary time. Histone structure is so rigidly defined for its DNA-binding function that in one billion years since plants and animals separated, only one amino acid difference exists between a pea and a cow. On the other hand, fibrinopeptides can take almost any amino acid change and still carry out their function in blood clotting, and therefore have a fast rate of change.

Begin by coloring the hourglasses in the upper right. Then color the molecules and the enlarged hourglasses, which compare present day human and horse amino acid differences. Color the histone first, then each protein as discussed.

The hourglasses represent time, and the sand grains represent each protein's amino acids. The "clocks" in the upper right were set at zero 90 million years ago, when the fossil record suggests that the major orders of placental mammals diverged from each other, as represented here by the horse and the human.

Notice that in the enlarged histone hourglass, none of the sand grains have dropped, showing that in the past 90 million years no amino acid substitutions have occurred in the histones of human and horse.

Histones interact with DNA in the chromosomes, providing structural support and regulating DNA activities such as replication and RNA synthesis. Their ability to bind to DNA depends upon a particular structure and shape. Virtually all mutations impair histone's function, so almost none get through the filter of natural selection. The 103 amino acids in this protein are identical for nearly all plants and animals.

In cytochrome c, there are 104 amino acids. The amino acids in this protein undergo mutations faster than histones do, but change slowly compared to hemoglobin and fibrinopeptides. The few fallen sand grains represent the 12 amino acid differences, or about 12% difference between horse and human cytochrome c. Cytochrome c is an enzyme necessary for the oxidation of food, the cell's main chemical reaction for producing energy. Cytochrome c is found in all aerobic (oxygen-using) cells, from yeast to multicellular animals. Its vital function limits the changes it can accept.

The beta chain of hemoglobin has 146 amino acids; 26 of them differ in horse and human, which is about 18%. Hemoglobin transports oxygen in the red blood cells from the lungs to other tissues throughout the body and so allows an efficient way to use energy. The exact sequence of amino acids is not so important in the hemoglobin molecule as long as it can bind and release oxygen. Because the amino acid substitutions do not interfere with the protein's function, natural selection allows more changes in hemoglobin than in the previous two protein molecules.

Fibrinopeptides are segments of the fibrinogen molecule and have about 20 amino acids. Human and horse amino acids differ in this protein by 86%. Fibrinopeptides are important in blood clotting. The segments simply act as spacers, keeping active sites of fibrinogen apart. When bleeding

occurs, the fibrinopeptides are cut out and discarded, leaving the sticky surfaces free to engage in forming clots. The actual sequence of amino acids is unimportant for this spacer function, so many amino acid substitutions have been tolerated.

Each protein, with its characteristic rate of change, pinpoints the timing of events in different evolutionary time frames. Histones time once-in-a-billion year events. Fibrinopeptides change rapidly, averaging one mutation per million years. Changes within the past five million years between closely related species can be timed with this clock. Biologist Russell Doolittle's fibrinopeptide sequences in 1970 pointed out the close relationship between chimpanzees and humans, prior to its confirmation in the 1980s.

Cytochrome c and hemoglobin have rates of change that are intermediate between histones and fibrinopeptides. Cytochrome c provided the first family tree of a sequenced protein, and hemoglobin was used as the first "molecular clock." Duplications in hemoglobin and the globin genes span a billion years and are intimately tied to changes in animal life. As animals became land dwelling and shifted their source of oxygen from the water to the air, changes in globin structure were vital. They could become larger in size because hemoglobin duplication enabled sufficient oxygen supply to their tissues.

Molecular clocks "tick" at different rates, and the same protein may change somewhat faster in one lineage, like rodents, than in another, like primates. To get the best time estimates of divergence between species, it is necessary to check the rate of molecular change against events in the fossil record, if there is one. Molecular clocks are not precise like digital clocks, so it is a good idea to use several different molecules if possible, just as the navigators a century ago carried several chronometers and took the average to get their longitude.

– adapted from *The Human Evolution Coloring Book*, 2d ed., by Adrienne L. Zihlman.
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