

Biodiversity - Amino Acid Sequences and Evolutionary Relationships



INSTRUCTOR:

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How do similarities in amino acid sequences of various species provide evidence for evolution?

An important technique used in determining evolutionary relationships is the biochemical comparison of organisms. Organisms that are not similar in physical appearance may show similarity in amino acid sequences for homologous proteins. Two proteins are commonly studied by scientists who are attempting to deduce evolutionary relationships from differences in amino acid sequences. One is hemoglobin, and the other is cytochrome c. Researchers believe that the greater the amino acid-sequence similarity of two organisms, the more closely related they are, in an evolutionary sense. Conversely, the greater the difference between their amino acid sequences, the more distant is their relationship. For the various organisms studied, biochemical evidence for evolution compares favorably with structural evidence for evolution. In this investigation, you will look at sections of the hemoglobin protein and the cytochrome c protein, compare the amino acid similarities and differences in a number of organisms, and deduce evolutionary relationships among them.

Materials: paper, pen or pencil, & highlighter

Part A: Cytochrome c

The greater the time that organisms have been diverging from a common ancestor, the greater the difference that can be expected in amino acid sequences for proteins found in their bodies.

1. Cytochrome c, a protein found in the mitochondria of many organisms, consists of a chain of 104 amino acids. Figure 1 shows the corresponding parts of discontinuous cytochrome c amino acid sequences of nine vertebrates.
2. Using the amino acid chart (figure 1 of answer sheet), mark the amino acids in each vertebrate's sequence that differ from the human sequence.
3. Now, for each vertebrate, count the amino acids in the sequence that differs from the human sequence (keep a record of the number of differences on a piece of scratch paper). In Table 1 of the answer sheet, list the eight vertebrate sequences in descending order according to their degree of evolutionary closeness to humans (the one at the top is the most closely related).
4. On your answer sheet, answer the following questions: *According to this line of evidence, which organism is most closely related to humans? Which is least closely related to humans?*

Part B: Hemoglobin

5. Look at the hemoglobin sequences for the five organisms shown in Figure 2 of the answer sheet. Hemoglobin is the oxygen-carrying molecule of the red blood cells.

Only a portion of the chain has been shown here, between amino acid numbers 87 and 116 in the sequence of 146 amino acids.

6. Using the amino acid chart (figure 2 of answer sheet), mark the amino acids in each vertebrate's sequence that differ from the human sequence.
7. For each organism, count the amino acids in the sequence that differ from the human sequence as you did for Part A and list them in Table 2 on the answer sheet. Be sure to list them in descending order according to their degree of evolutionary closeness to humans.
8. On your answer sheet, answer the following questions: *In the study of hemoglobin, which vertebrate is most closely related to humans? Least closely related to humans?*

Answer Sheet: Amino Acid Sequences and Evolutionary Relationships

The numbers along the side of the figure refer to the position of these sequences in the chain. The letters symbolize the specific amino acids in the chain.

Figure 1: Cytochrome c amino acid sequences

	H o r s e	C h i c k e n	T u n a	F r o g	H u m a n	S h a r k	T u r t l e	M o n k e y	R a b b i t
42	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln
43	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
44	Pro	Glu	Glu	Ala	Pro	Gln	Glu	Pro	Tyr
46	Phe	Phe	Tyr	Phe	Tyr	Phe	Phe	Tyr	Pro
47	Thr	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser
49	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr
50	Asp	Asp	Asp	Asp	Asp	Asp	Glu	Ala	Asp
53	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
54	Asn	Asn	Ser	Asn	Asn	Asn	Asn	Asn	Asn
55	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
56	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly
57	Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile
58	Thr	Thr	-	Thr	Ile	Thr	Thr	Ile	Thr
60	Lys	Gly	Asn	Gly	Gly	Gln	Gly	Gly	Gly
61	Glu	Glu	Asn	Glu	Glu	Gln	Glu	Glu	Glu
62	Glu	Asp	Asp	Asp	Asp	Glu	Glu	Asp	Asp
63	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr
64	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu
65	Met	Met	Met	Met	Met	Arg	Met	Met	Met
66	Glu	Glu	Glu	Glu	Glu	Ile	Glu	Glu	Glu

100	Lys	Asp	Ser	Ser	Lys	Lys	Asp	Lys	Lys
101	Ala	Ala	Ala	Ala	Ala	Ala	Thr	Ala	Ala
102	Thr	Thr	Thr	Gly	Thr	Ala	Thr	Ala	Thr
103	Asn	Ser	Ser	Ser	Asn	Ala	Ser	Asn	Asn
104	Glu	Lys	-	Lys	Glu	Ser	Lys	Glu	Glu

Table 1: Cytochrome c amino acid sequence similarity between humans and various animal species

SPECIES	# OF DIFFERENCES FROM HUMAN CYTOCHROME c

A. According to this line of evidence, which organism is most closely related to humans?

B. Which is least closely related to humans? _____

Figure 2: Hemoglobin amino acid sequences

	H u m a n	C h i m p a n z e e	G o r i l l a	M o n k e y	H o r s e
87	Thr	Thr	Thr	Gln	Thr
88	Leu	Leu	Leu	Leu	Leu
89	Ser	Ser	Ser	Ser	Ser
90	Glu	Glu	Glu	Glu	Glu
91	Leu	Leu	Leu	Leu	Leu
92	His	His	His	His	His
93	Cys	Cys	Cys	Cys	Cys
94	Asp	Asp	Asp	Asp	Asp
95	Lys	Lys	Lys	Lys	Lys

96	Leu	Leu	Leu	Leu	Leu
97	His	His	His	His	His
98	Val	Val	Val	Val	Val
99	Asp	Asp	Asp	Asp	Asp
100	Pro	Pro	Pro	Pro	Pro
101	Glu	Glu	Glu	Glu	Glu
102	Asn	Asn	Asn	Asn	Asn
103	Phe	Phe	Phe	Phe	Phe
104	Arg	Arg	Lys	Lys	Arg
105	Leu	Leu	Leu	Leu	Leu
106	Leu	Leu	Leu	Leu	Leu
107	Gly	Gly	Gly	Gly	Gly
108	Asn	Asn	Asn	Asn	Asn
109	Val	Val	Val	Val	Val
110	Leu	Leu	Leu	Leu	Leu
111	Val	Val	Val	Val	Ala
112	Cys	Cys	Cys	Cys	Leu
113	Val	Val	Val	Val	Val
114	Leu	Leu	Leu	Leu	Val
115	Ala	Ala	Ala	Ala	Ala
116	His	His	His	His	Arg

Table 2: Hemoglobin amino acid sequence similarity between humans and various animal species

SPECIES	# OF DIFFERENCES FROM HUMAN Hemoglobin

A. In the study of hemoglobin, which vertebrate is most closely related to humans?

B. Least closely related to humans? _____

Analysis Questions:

- What are some other methods used to determine evolutionary relationships?

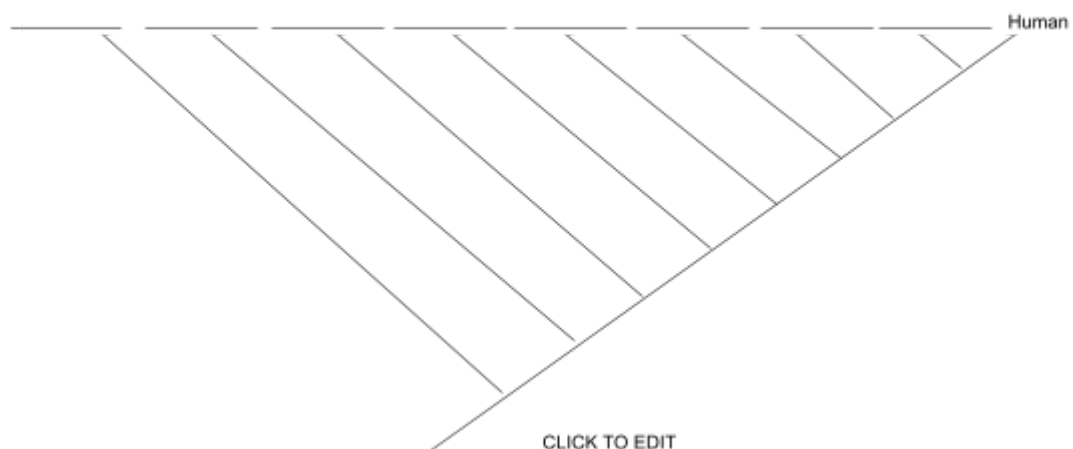
2. How is the biochemical method different from these methods?

3. Why can it be said that proteins behave like molecular clocks? (Remember, clocks tell time!)

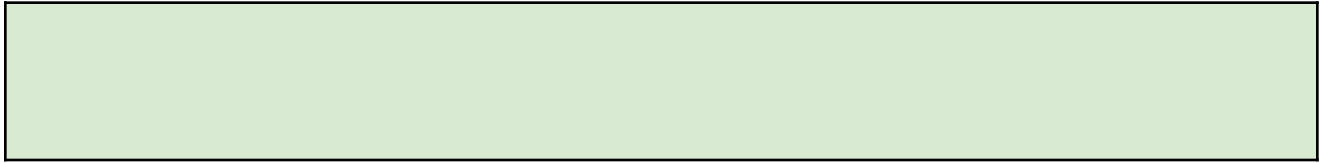
4. There is a difference of only one amino acid in one portion of hemoglobin of gorillas and humans. What could have been responsible for this change?

5. If the amino acid sequences are similar in gorillas and humans, why would you expect their DNA to also be similar?

Using the information from Table 1 of your answer sheet, complete the diagram below.



6. What do we call a diagram that shows the evolutionary history (and relatedness) of a group of organisms?



Penelope:

The poison dart frog (Dendrobatidae) is a group of small, brightly colored frogs that are native to Central and South America. One notable feature of these frogs is that they produce toxic secretions that can be used for defense or hunting. Here are some potential organisms that researchers might compare to the poison dart frog when analyzing DNA and amino acid sequences:

- Other **frogs**: The poison dart frog belongs to the Anura order, which includes all frogs and toads. Researchers might compare the DNA and amino acid sequences of the poison dart frog to those of other Anurans to investigate similarities and differences in their genomes.
- **Bacteria** and **fungi**: Many species of bacteria and fungi produce toxins that are similar in structure to those found in poison dart frog secretions. Researchers might compare the DNA and amino acid sequences of the poison dart frog to those of these microorganisms to investigate whether there are any shared genetic pathways involved in toxin production.
- **Mammals**: While the poison dart frog and mammals may seem like unlikely comparators, some researchers have suggested that the evolution of toxicity in the poison dart frog may be similar to the evolution of venom in some mammals (e.g., shrews, solenodons). Researchers might compare the DNA and amino acid

sequences of the poison dart frog to those of these mammals to investigate whether there are any shared genetic mechanisms involved in producing toxic substances.

Based on these potential comparators, researchers could investigate questions such as those I listed earlier. For example, they might ask:

- 7.** How similar are the DNA and amino acid sequences of the poison dart frog to those of other frogs, bacteria, and fungi that produce toxins? [LINK](#)
- 8.** Are there any unique genes in the poison dart frog that are not found in other toxin-producing organisms? What might these sequences be responsible for?
- 9.** Can we infer anything about the evolutionary relationships between the poison dart frog and other toxin-producing organisms based on the similarities and differences in their DNA and amino acid sequences? [LINK](#)