

Amino Acid Sequences and Evolutionary Relationships Lab

Pre-Lab

HOMOLOGOUS STRUCTURES—those structures believed to have a common origin but not necessarily a common function—provide some of the most significant evidence supporting the theory of evolution. For example, the forelimbs of vertebrates often have different functions and outward appearances, yet the underlying similarity of the bones indicates a common origin. Although homologous structures can be used to demonstrate relationships between similar organisms, they are of little value in determining evolutionary relationships among those structures that are dissimilar.

Another technique used to determine evolutionary relationships is to study the **BIOCHEMICAL** similarity of organisms. Though molds, aardvarks, and humans appear to have little in common physically, a study of their proteins reveal certain similarities. Biologists have perfected techniques for determining the sequence of amino acids in proteins. By comparing the amino acid sequences in homologous proteins of similar organisms and of diverse organisms, evolutionary relationships that might otherwise go undetected can be determined. Biologists believe that the greater the similarity between the amino acid sequences of two organisms, the closer their relationship. Conversely, the greater the differences, the more distant the relationship. Further, biologists have found that such biochemical evidence compares favorably with other lines of evidence for evolutionary relationships.

Purpose: To compare amino acid sequences in proteins of several vertebrates.
To study amino acid differences and infer evolutionary relationships among some diverse organisms.

Materials: No special materials are needed.

Procedure

Part A: Comparing Amino Acid Sequences

- Examine Figure 1, which compares corresponding portions of hemoglobin molecules in humans and five other vertebrate animals. Hemoglobin, a protein composed of several long chains of amino acids, is the oxygen-carrying molecule in red blood cells. The sequence shown is only a portion of a chain made up of 146 amino acids. The numbers in Figure 1 indicate the position of a particular amino acid in the chain:

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101
Human	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Chimpanzee	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Gorilla	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Rhesus monkey	GLN	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Horse	ALA	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Kangaroo	LYS	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU

	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116
Human	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Chimpanzee	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Gorilla	ASN	PHE	LYS	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Rhesus monkey	ASN	PHE	LYS	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Horse	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	ALA	LEU	VAL	VAL	ALA	ARG
Kangaroo	ASN	PHE	LYS	LEU	LEU	GLY	ASN	ILE	ILE	VAL	ILE	CYS	LEU	ALA	GLU

Figure 1

Figure 1

- In Data Table 1, notice that the abbreviated names of the amino acids in human hemoglobin are printed.
- In the appropriate spaces in Data Table 1, place a "X" on all the amino acid in a chimpanzee

hemoglobin that is different from that in human hemoglobin. If there are no differences, leave the spaces blank.

4. For the remaining organisms, place a "X" on all the amino acids that do not correspond to those in human hemoglobin. Note: Always be sure that you compare the amino acid sequence of each organism with that of the human and not the organism on the line above.
5. Use Figure 1 to complete Data Table 2.

Part B: Inferring Evolutionary Relationships from Differences in Amino Acid Sequences

1. Another commonly studied protein is cytochrome c. This protein, consisting of 104 amino acids, is located in the mitochondria of cells. There it functions as a respiratory enzyme. Examine Figure 2. Using human cytochrome c as a standard, the amino acid differences between humans and a number of other organisms are shown.
2. Using Figure 2, construct a bar graph on Graph 1 to show the amino acid differences between humans and other organisms.
3. Now examine Figure 3. In this figure the cytochrome c of a fruit fly is used as a standard in comparing amino acid differences among several organisms. Construct a bar graph on Graph 2 to show these differences.

Species Pairings	Number of Differences
Human-chimpanzee.	0
Human-fruit fly	29
Human-horse	12
Human-pigeon	12
Human-rattlesnake	14
Human-red bread mold	48
Human-rhesus monkey	1
Human-screwworm fly	27
Human-snapping turtle	15
Human-tuna	21
Human-wheat	43

Figure 2

Figure 2

Species Pairings	Number of Differences
Fruit fly-dogfish shark	26
Fruit fly-pigeon	25
Fruit fly-screwworm fly	2
Fruit fly-silkworm moth	15
Fruit fly-tobacco hornworm moth	14
Fruit fly-wheat	47

Figure 3

Figure 3

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Use

Use figures 1 and 2. Use figures 2 and 3 to answer questions 5 through 9.

1. On the basis of the hemoglobin similarity, what organisms appear to be most closely related to humans? Explain your answer.
2. Among the organisms that you compared, which one appears to be least closely related to humans? Explain your answer.
3. On the basis of differences in their cytochrome c, which organisms appear to be most closely related to humans?
4. Which organisms appear to be least closely related to humans?
5. Which pair of organisms that appears to be most closely related to each other? Why?
a. snapping turtle-tuna b. snapping turtle-rattlesnake c. snapping turtle-pigeon
6. Agree or disagree with the following statement and give reasons to support your answer. "Fruit flies appear to be more closely related to silkworm moths than they are to screwworm flies."
7. Name the pair of organisms that appears to be equally related to humans on the basis of cytochrome c similarity.
8. Is it possible that the organisms in question 7 could be equally related to humans but not equally related to each other? Explain your answer.
9. Agree or disagree with the following statement. "Fruit flies and humans have about the same evolutionary relationship to wheat." Explain your answer.
10. There is a difference of only one amino acid in one chain of the hemoglobin of humans and gorillas. What might have caused this difference?
11. If the amino acid sequences in the proteins of two organisms are similar, why will their DNA also be similar?
12. How can biologists look at the organism's DNA to determine if two organisms are closely related or not?