Name_____ S.N.____ Date_____ Per____

MOLECULAR BIOLOGY AND PHYLOGENY

BACKGROUND: You have just completed an activity in which you made a cladogram showing the evolutionary relationships between seven organisms. The data used to draw that cladogram was based on shared characteristics that were inherited from their ancestors.

Biochemical characteristics, like similarities in nuclear DNA, mitochondrial DNA, or **protein structure**, can be used to produce cladograms also. If there is strong agreement between the patterns produced using anatomical similarities and those produced by using biochemical structures, it provides what we call "independent confirmation" of the cladogram. **Independent confirmation** is where two or more sources of evidence that are not dependent on each other produce the same pattern. The more independent confirmation that is available, the more confidence we have that the evolutionary relationships shown in the cladogram are correct.

In this activity, we will examine and compare the amino acid sequence of a protein. All seven of the organisms used in the previous activity produce this protein (it is a homologous protein). The complete sequence of amino acids for this protein has been determined for many organisms, including six of those seven animals. We assume that if we find fewer differences in the amino acid sequences, those animals are more closely related. (Be prepared to discuss why we can make this assumption.)

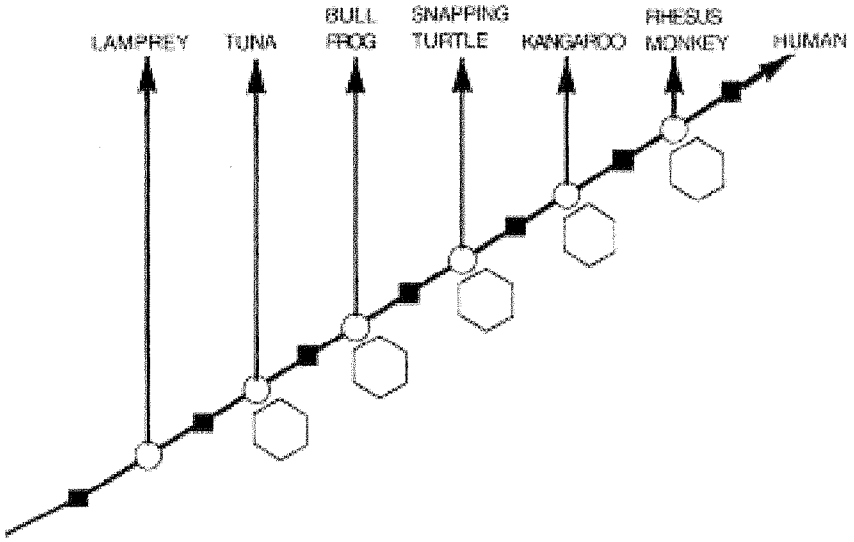
PROCEDURES: You have already done and discussed the activity entitled "Making Cladograms". The final cladogram produced in that activity (using anatomical similarities) is shown below. You may be asked to work in groups of four, and share the following task with your partners.

You will be provided with a chart (Fig.36.2) showing the amino acid sequence in a protein that is homologous for the 20 organisms shown, including six of the seven organisms already studied (data on this protein is not available here for the lamprey). The name of the protein is cytochrome-c. It is an enzyme that is important in the breakdown of food molecules by a cell. It helps release the energy in food molecules so that it can be used by the cell. Each amino acid is represented by a unique letter in the chart.

Compare the sequence of amino acids (letters) in **human** cytochrome-c to the sequences for each of the 5 remaining animals (shown in the diagram below) by counting the **number** of **differences**. HINT: Highlight or underline the organism being checked, then circle each amino acid which is **different** from the one above it in the human sequence (use pencil). It helps to use a ruler or other straight edge. Notice that the amino acids showing **no differences** in any of the organisms are surrounded with vertical boxes, so you can just ignore them when you scan the line of letters. When an organism has a "-" instead of an amino acid, that means there is no amino acid there. When comparing a "-" to an organism that has an amino acid at the position, it should be considered as a **difference**. When **both** organisms have a "-" at that spot, it is **not** considered as a difference.

Record the number of differences next to each animal's name. Compare your numbers with those of your team members. If there is a discrepancy, repeat the scan and count.

Record the confirmed number of amino acid differences in the appropriate hexagon on the cladogram below (below the vertical arrow pointing to that animal). Answer the Analysis questions (overpage).



ANALYSIS

1. Does the data from the amino acid sequence generally agree with the anatomical data that was used to make this cladogram? (i.e., do organisms with **fewer** shared anatomical traits also have **more** amino acid **differences**?)_____

2. Based on the molecular data, make a **general statement** about the "human-monkey" **relationship** as compared to the "duck-chicken" **relationship** (which shows **three** differences in their amino acids).

3. If the **molecular data,** the **structural similarities**, and the **fossil record** all support the same pattern of relationships, can we be fairly confident that the pattern is correct?_____ Why?

4. A) Using the **molecular** data, make a general statement which compares the "human-kangaroo" relationship ("H-K")to the "human-frog" ("H-F") relationship.
   B) Does the cladogram agree with this statement?_____ C) Explain your reasoning.
   A:

   C:

5. The chicken and the turkey are both birds and have the **same** sequence of amino acids in their cytochrome-c protein. Explain how two different species can have identical cytochrome-c and still be different species.

6. *Neurospora* and (bread mold) and *Saccharomycetes* (bakers yeast) are both fungi. Chickens and turkeys are both birds. A) What can you say about the **evolutionary relationships** between the two birds compared to the relationship between the two fungi? B) Explain your reasoning (use of simple diagrams might help).

7. In a short paragraph, summarize what important information can be **obtained** from cladograms (NOT the info that was used to make the cladograms).

AMINO ACID SEQUENCES IN CYTOCHROME-C PROTEINS FROM 20 DIFFERENT SPECIES

Amino Acid Number----->	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	20	1	2	3	4	5	6	7	8	9	30	1	2	3	4	5	6	7	8	9	40	1	2	3	4	5	6	7	8	9	50
Human	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	I	M	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	Y	S	Y	T	A	A
Rhesus monkey	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	I	M	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	Y	S	Y	T	A	A
Horse	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	T	Y	T	D	A
Pig, cow, sheep	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Dog	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	P	G	F	S	Y	T	D	A
Gray whale	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	V	G	F	S	Y	T	D	A
Rabbit	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	V	G	F	S	Y	T	D	A
Kangaroo	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	N	G	I	F	G	R	K	T	G	Q	A	P	G	F	T	Y	T	D	A
Chicken, Turkey	-	-	-	-	-	-	-	-	-	G	D	I	E	K	G	K	K	I	F	V	Q	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Penguin	-	-	-	-	-	-	-	-	-	G	D	I	E	K	G	K	K	I	F	V	Q	K	C	S	O	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	I	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Pekin duck	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	S	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	E	G	F	S	Y	T	D	A
Snapping turtle	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	V	E	K	G	G	K	H	K	T	G	P	N	L	N	G	L	I	G	R	K	T	G	Q	A	E	G	F	S	Y	T	E	A
Bullfrog	-	-	-	-	-	-	-	-	-	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	C	H	T	C	E	K	G	G	K	H	K	V	G	P	N	L	Y	G	L	I	G	R	K	T	G	Q	A	A	G	F	S	Y	T	D	A
Tuna	-	-	-	-	-	-	-	-	-	G	D	V	A	K	G	K	K	T	F	V	Q	K	C	A	Q	C	H	T	V	E	N	G	G	K	H	K	V	G	P	N	L	W	G	L	F	G	R	K	T	G	Q	A	E	G	Y	S	Y	T	D	A
Screwworm fly	-	-	-	-	G	V	P	A	G	D	V	E	K	G	K	K	I	F	V	Q	R	C	A	Q	C	H	T	V	E	A	G	G	K	H	K	V	G	P	N	L	H	G	L	F	G	R	K	T	G	Q	A	A	G	F	A	Y	T	N	A
Silkworm moth	-	-	-	-	G	V	P	A	G	N	A	E	N	G	K	K	I	F	V	Q	R	C	A	Q	C	H	T	V	E	A	G	G	K	H	K	V	G	P	N	L	H	G	F	Y	G	R	K	T	G	Q	A	P	G	F	S	Y	S	N	A
Wheat	A	S	F	S	E	A	P	P	G	N	P	D	A	G	A	K	I	F	K	T	K	C	A	Q	C	H	T	V	D	A	G	A	G	H	K	Q	G	P	N	L	H	G	L	F	G	R	Q	S	G	T	T	A	G	Y	S	Y	S	A	A
Fungus (*Neurospora*)	-	-	-	-	G	F	S	A	G	D	S	K	K	G	A	N	L	F	K	T	R	C	A	E	C	H	G	E	G	G	N	L	T	Q	K	I	G	P	A	L	H	G	L	F	G	R	K	T	G	S	V	D	G	Y	A	Y	T	D	A
Fungus (baker's yeast)	-	-	-	T	E	F	K	A	G	S	A	K	K	G	A	T	L	F	K	T	R	C	E	L	C	H	T	V	E	K	G	G	P	H	K	V	G	P	N	L	H	G	I	F	G	R	H	S	G	Q	A	Q	G	Y	S	Y	T	D	A
Fungus (*Candida*)	-	-	P	A	P	F	E	0	G	S	A	K	K	G	A	T	L	F	K	T	R	C	A	E	C	H	T	I	E	A	G	G	P	H	K	V	G	P	N	L	H	G	I	F	S	R	H	S	G	Q	A	Q	G	Y	S	Y	T	D	A

[CONTINUED FROM ABOVE]

Amino Acid Number----->	60	1	2	3	4	5	6	7	8	9	70	1	2	3	4	5	6	7	8	9	80	1	2	3	4	5	6	7	8	9	90	1	2	3	4	5	6	7	8	9	100	1	2	3	4	5	6	7	8	9	110	1	2
Human	N	K	N	K	G	I	I	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	V	G	I	K	K	K	E	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Rhesus monkey	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	V	G	I	K	K	K	E	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Horse	N	K	N	K	G	I	T	W	K	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	T	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Pig, cow, sheep	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	E	D	L	I	A	Y	L	K	K	A	T	N	E
Dog	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	T	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	K	E
Gray whale	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Rabbit	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	D	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Kangaroo	N	K	N	K	G	I	I	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	A	D	L	I	A	Y	L	K	K	A	T	N	E
Chicken, Turkey	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	V	D	L	I	A	Y	L	K	D	A	T	S	K
Penguin	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	A	D	L	I	A	Y	L	K	D	A	T	S	K
Pekin duck	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	S	E	R	A	D	L	I	A	Y	L	K	D	A	T	A	K
Snapping turtle	N	K	N	K	G	I	T	W	G	E	E	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	A	E	R	A	D	L	I	A	Y	L	K	D	A	T	S	K
Bullfrog	N	K	N	K	G	I	T	W	G	E	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	Q	D	L	I	A	Y	L	K	S	A	C	S	K
Tuna	N	K	S	K	G	I	V	W	N	N	D	T	L	M	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	I	K	K	K	G	E	R	Q	D	L	V	A	Y	L	K	S	A	T	S	-
Screwworm fly	N	K	A	K	G	I	T	W	Q	D	D	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	I	F	A	G	L	K	K	P	N	E	R	G	D	L	I	A	Y	L	K	S	A	T	K	-
Silkworm moth	N	K	A	K	G	I	T	W	G	D	D	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	V	F	A	G	L	K	K	A	N	E	R	A	D	L	I	A	Y	L	K	E	S	T	K	-
Wheat	N	K	N	K	A	V	E	W	E	E	N	T	L	Y	D	Y	L	L	N	P	K	K	Y	I	P	G	T	K	M	V	F	P	G	L	K	K	P	Q	D	R	A	D	L	I	A	Y	L	K	K	A	T	S	S
Fungus 1 (*Neurospora*)	N	K	Q	K	G	I	T	W	D	E	N	T	L	F	E	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	D	K	D	R	N	D	I	I	T	F	M	K	E	A	T	A	-
Fungus 2 (bkrs yeast)	N	I	K	K	N	V	L	W	D	E	N	N	M	S	E	Y	L	T	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	E	K	D	R	N	D	L	I	T	Y	L	K	K	A	C	E	-
Fungus 3 (*Candida*)	N	K	R	A	G	V	E	W	A	E	P	T	M	S	D	Y	L	E	N	P	K	K	Y	I	P	G	T	K	M	A	F	G	G	L	K	K	A	K	D	R	N	D	L	V	T	Y	M	L	E	A	S	K	-

AMINO ACID SYMBOLS
A = Alanine
C = Cysteine
D = Aspartic acid
E = Glutamic acid
F = Phenylalanine
G = Glycine
H = Histidine
I = Isoleucine
K = Lysine
L = Leucine
M = Methionine
N = Asparagine
P = Proline
Q = Glutamine
R = Arginine
S = Serine
T = Threonine
V = Valine
W = Tryptohpan
Y = Tyrosine

Symbols in light blue or gray represent amino acids which show NO differences in any organism on the list, so you can ignore them.

(adapted from Strahler, Arthur, *Science & Earth History*, 1987, p. 348)