## Using Cytochrome c to Establish Phylogeny

Characteristics like similarities in nuclear DNA, mitochondrial DNA, or protein structure, can be used to establish evolutionary relationships. If there is strong agreement between the patterns produced using anatomical similarities and those produced by using molecular biology, it provides independent confirmation of a perceived relationship. Independent confirmation is where two or more sources of evidence that are not dependent on each other support the same conclusion. The more independent confirmation that is available, the more confidence one can have that suspected evolutionary relationships are actually correct.

A cladogram for seven animals constructed from anatomical similarities is shown in Figure 1. In order to confirm the relationships indicated in the cladogram, we will use the amino acid sequence for cytochrome c, a nearly ubiquitous protein in the electron transport chain.

1. a) Provide a reason that the amino acid sequence of cytochrome c would be different in different species.

   b) Write a hypothesis regarding the relationship between the amino acid sequences of cytochrome c in various species and how closely related those species are believed to be.

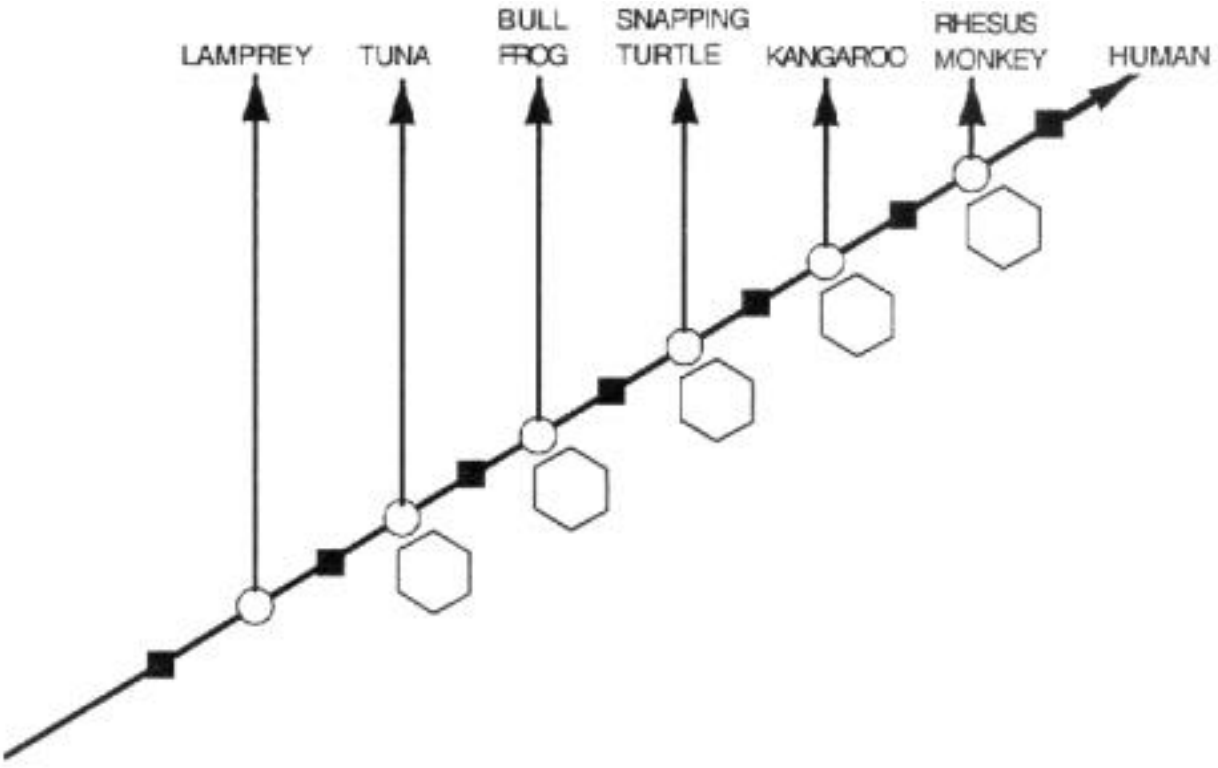


**Figure 1 Cladogram for seven species**

2. Compare the sequence of amino acids in human cytochrome c to the sequences for each of the five remaining animals in the cladogram in Figure 1 (the sequence for the lamprey is not given) by counting the number of differences. It might help to use a ruler to guide you and circle each amino acid which is different from the human sequence. Amino acids showing no differences in any of the organisms are in blue and can be ignored. When an organism has a '-' instead of an amino acid, it means there is no amino acid there and it should be considered as a difference. When both organisms

have a '-' at any given locus, it is not considered a difference. Record the number of differences in the appropriate hexagon on the cladogram.

3. State whether the amino acid sequence data generally agree with the anatomical data that was used to make the cladogram. Justify your response.
4. State whether the amino acid data agree with your hypothesis.
5. The chicken and the turkey are both birds and have the same sequence of amino acids in their cytochrome c. Explain how two different species can have identical cytochrome c and still be different species.
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7. Neurospora and baker's yeast are both fungi. Chickens and turkeys are both birds. Describe the evolutionary relationship between the two birds compared to that between the two fungi.
8. Complete Table 2 by counting the amino acid differences between each pair of organisms.

**Table 2 Number of amino differences between species**

| | horse | donkey | whale | chicken | penguin | snake | moth | yeast | wheat |
|---|---|---|---|---|---|---|---|---|---|
| horse | 0 | | | | | | | | |
| donkey | | 0 | | | | | | | |
| whale | | | 0 | | | | | | |
| chicken | | | | 0 | | | | | |
| penguin | | | | | 0 | | | | |
| snake | | | | | | 0 | | | |
| moth | | | | | | | 0 | | |
| yeast | | | | | | | | 0 | |
| wheat | | | | | | | | | 0 |

9. Construct a cladogram using the data in Table 2. The two most closely related species have the fewest differences in amino acid sequence. Start by placing the two most closely related species on the two shortest branches of the tree. Place the next two closest species on the next shortest branches. Place the species which is the next closest on the next longest branch. Continue until all the species have been placed.
10. a) Provide reasoning for the choice of using cytochrome c to establish phylogeny in this activity.

    b) Identify three other types of data that can be considered when establishing phylogeny.