

CHEM 527 Introductory Biochemistry Mutational Analysis

1. The α -chain of human hemoglobin is normally 141 amino acid residues long. Three unusual variants have been discovered in which the α -chain is longer than normal. Hb-Constant Spring and Hb-Icaria both have α -chains 172 residues long and differ from each other only by one amino acid at position 142. The first 141 residues are identical with the entire normal α -chain. The α -chain of Hb-Wayne is 146 residues long. The last 8 residues differ from both normal and Hb-Constant Spring α -chains. The amino acid sequences from residue 138 to 148 are presented below. Each of these inherited defects can be explained by single base mutational events.

Residue No.	138	139	140	141	142	143	144	145	146	147	148...
Normal	Ser	Lys	Tyr	Arg	-						
Constant Spring	Ser	Lys	Tyr	Arg	Gln	Ala	Gly	Ala	Ser	Val	Ala
Icaria	Ser	Lys	Tyr	Arg	Lys	Ala	Gly	Ala	Ser	Val	Ala
Wayne	Ser	Asn	Thr	Val	Lys	Leu	Glu	Pro	Arg	-	

- a. What is the termination codon for normal α -chain? Be specific as possible and explain your deduction.
 - b. From the above sequences it is possible to unambiguously deduce a 26-base sequence of normal α -chain mRNA. Write out 15 bases of this sequence extending 6 bases on either side of the normal termination codon.
 - c. Write out the base sequence of the sense strand of DNA coding for the mRNA in part b.
 - d. Specifically what are the base changes that can explain each of the three hemoglobin variants? Can this be ascertained unambiguously?
2. Baker's yeast, *Saccharomyces cerevisiae*, is a facultative anaerobe.
- a. What might be the phenotypic characteristics of a yeast defective in the gene for cytochrome c? (i.e. consider examples of carbon sources it could or could not grow on, the effect if any of O₂, and end products of metabolism likely to be found in the medium). Give examples of other mutants would have similar phenotypes.
 - b. The N-terminal amino acid sequence of cytochrome c from yeast is:

Thr-Glu-Phe-Lys-Ala-Gly-

The absence of what feature suggests that the protein is modified subsequent

to its synthesis on the ribosome?

- c. A series of mutants lacking cytochrome c activity were isolated. Most produced defective proteins which would cross react with antibodies to cytochrome c. Of the following classes of mutants, which would produce cross reacting material (CRM), which would not, and which may or may not. (Assume a mutant protein greater than 60% like the wild type protein will cross react).
- i. deletion of cytochrome c gene
 - ii. point mutation resulting in a single amino acid substitution
 - iii. mutation in the termination codon
 - iv. mutation in the initiation codon
 - v. amber mutation
 - vi. frameshift mutation
- d. Mutants lacking CRM were screened for revertants having a functional cytochrome c. Most of the revertants had the N-terminal sequence of the wild type cytochrome c. There were, however, several revertants with altered N-terminal sequences.

Revertant Sequence	Frequency	N-terminal
Wild type	21	Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..
A	4	Ala-Gly-Ser-Ala-Lys..
B	13	Met-Ile-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..
C	4	Met-Leu-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..
D	2	Met-Arg-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..
E	1 (10%)	Met-Val-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..
	(90%)	Val-Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys..

What is the nature of the original mutant lacking CRM and revertant mutation in each case? (Refer to the genetic code)

- e. With reference to Question 2d, what can be deduced about the specificity of the enzyme which modifies wild type cytochrome c?