Recitation Section 22 Answer Key
May 2-3, 2005

# From Yeast to Humans—Essential Genes on the Evolutionary Continium.

## A. CBS protein in yeast and humans

Recall that earlier in the term we considered the human gene CBS and its yeast analog cys4. Recall that these genes each encode the protein cystathionine β-synthase that is responsible for converting homocysteine into cystathionine in the cellular pathway of creating cysteine.

1. What is cysteine? Is it important for organism's survival?

*Cysteine is an amino acid. It is used in production of proteins, and is thus, essential for organism's survival. However, external supplementation can sometimes alleviate the phenotype associated with inability to make own cysteine.*

2. What would you expect to be the result of complete absence of the protein product of the yeast *CYS4* gene to be? What about the same question for the human CBS protein?

*Yeast will not be able to make cysteine, resulting in cysteine auxotrophy. Humans lacking CBS protein should also be unable to make cysteine. Human phenotype may be more complex than just cysteine auxotrophy.*

3. Would you expect cells that contain no functional copy of CBS enzyme to accumulate some kind of a compound? If no, why not? If yes, what kind of a compound would you expect that compound to be?

*These cells should accumulate the compound that is the product of the previous step in the cysteine biosynthesis pathway. The compound is homocysteine, an intermediate in the cycteine biosynthesis pathway and one of the reactants in the step catalyzed by the CBS protein (the other reactant is serine).*

4. In the experiments we discussed earlier in the term, what was the phenotype of the *cys4* mutants on complete media?

*On complete media cys4 mutants had the same phenotype as the wild type yeast.*

5. As we told you a number of sessions ago, the deficiency in the human analog of *CYS4* gene, CBS, lead to a disease called cysteineurea, resulting in variety of serious conditions, including mental retardation, heart attack, and stroke. What accounts for such a big difference in phenotype between CBS protein deficient yeast and humans?

*The cause of the disease phenotype in humans is the accumulation on homocysteine, the product of the previous step in the cysteine biosynthesis pathway, and a reactant in the step catalyzed by CBS. Yeast cells apparently can either secrete excess homocysteine, or the excess of it within a cell does not interfere with the yeast cellular functions. Humans are a complex system, and the excess of homocysteine in the body leads to a variety of disourders. This is what is known as an emergent property—something we couldn't have predicted from observing the deficient phenotype in lower organisms.*

**B. Phylogenetic analysis**

Below is a figure from a research paper showing alignment of the amino acid sequences of human, rat, yeast, and *E. coli* CBS proteins.

Figure removed due to copyright reasons.

1. What do the dashes in the sequence represent?

*The dashes represent absence of amino acids in a particular protein sequence that would correspond to the amino acids in the sequence of another protein in the region of alignment.*

2. Are the DNA sequences encoding amino acids that are conserved across species above necessarily the same? Why or why not?

*No, the sequences are not necessarily the same. The genetic code is degenerate, i.e. most of the time several codons are available that encode the same amino acid. Thus, DNA sequence may change (due to a point mutation) without affecting the resulting protein sequence.*

3. What properties of the particular amino acids allow them to be grouped into the conservative groupings as described in the figure legend above?

*The amino acids grouped into conservative groupings have similar biochemical properties.*
*L, V, M, and I are all hydrophobic and roughly similar in size. F, W, and Y are all hydrophobic residues with large rings. A and G are the two smallest amino acids. S and T are similarly sized polar amino acids. R and K are positively charged amino acids with linear chains. D and E are negatively charged amino acids. The difference within the (D, N) and (E, Q) pairs is the acid vs. amino firm of each amino acid.*

4. Look at the genetic code table. Is there a relationship between the codons encoding amino acids in conservative groupings?

*Most of these groupings allow a one point mutation conversion between any member of a grouping and any other member of the same grouping. The two exceptions are the CGN encodings for arginine (R) that require two or three point mutations to be converted to an encoding for lysine (K), and the tryptophan (W) encoding that requires two point mutations to be converted to an encoding for either of the other members of its grouping.*

5. Is CBS a good candidate for creating a phylogenetic tree on the basis of its sequence? Why or why not?

*CBS is essential for cysteine biosynthesis, and, therefore, is likely to be relatively more conserved in evolution. As such, it is a fairly good candidate for phylogenetic tree construction. Even better candidates are genes that encode products essential for survival, such as rRNA subunits.*

6. Are human disease alleles of CBS likely to help with phylogenetic tree construction? Why or why not?

*These alleles are not likely to help because they represent versions of the gene that results in a protein with weakened or abolished functions. Such alleles are not likely to have been propagated through evolutionary lineage. They are likely the result of much more resent mutations that occurred after humans differentiated into a distinct species.*

7. If constructing a phylogenetic tree on the basis of CBS alignment, would it be more useful to work with the protein or cDNA sequences? cDNA sequences or DNA sequences? Why?

*It would be more useful to work with cDNA sequences than with protein sequences because with the cDNA sequences we can observe the silent as well as the missense mutations that occurred in each organism's lineage. Similarly, it is more useful to work with DNA than cDNA sequence because with DNA sequence we can observe mutations that occurred in the intron, as well as in the coding, regions of the gene.*

8. Do you expect the human wild type gene to complement yeast *CYS4* deficiency? Why or why not?

*There is a good chance that the product of the wild type human gene would complement yeast CYS4 deficiency. This is because amino acid metabolism is one of the basic cellular functions that is likely to be conserved across the evolutionary spectrum from yeast to humans. In fact, as can be seen in the figure above, predicted human and yeast protein sequences are 38% identical and 72% similar to each other.*

*The reason why essential genes tend to be conserved is that change in genetic material (that encodes a change in protein) occurs through mutation, a gradual, stepwise process. If a mutation in an essential gene results in a less viable phenotype (i.e. mutation is deleterious), then, even if just one more mutations would result in a vastly improved function of the protein in question, an individual carrying an original mutation is less likely to survive and procreate. If that individual does not have descendants, the mutation is question disappears from the gene pool. Thus, only small changes are likely to occur within essential genes. Those small changes accumulate over time to result in a larger degree of diversity between species farther away from each other on the evolutionary tree. But it is still true that the essential genes from species as different as yeast and humans are generally close enough to complement each other's deficiency.*

9. Is there a way to explore the relationship between human CBS protein and yeast *CYS4* protein?

*To test whether a human protein can complement yeast deficiency we can clone a human gene into a yeast vector, and check to see whether the deficiency is complemented. We have also described a human disease called homocysteinurea that results from deficiency in CBS. Thus, it would be interesting to see what the behavior of the human disease alleles in the yeast complementation system would be.*