S10 Biol 321 Extra Credit Option 10 pts total

Answers are due to CT by noon on Tuesday May 25.
No late submissions will be accepted

NOTE: The take-home quiz "rules" apply for completing this assignment. You are free to look up anything you want on the internet, but all the info that you need to work this problem is in the paper, your lecture notes or textbook.

Please word process your answers.

Read the attached paper <u>The Ubiquitin Pathway in Parkinson's Disease</u> and answer the following questions.

1. (2 pts all or nothing) Examine figure 1. The boxed sequences indicate the primers that were used to amplify the portion of the gene corresponding to the disease mutation. ***Write out the <u>first 5</u> bases of each of the primers.***

2. (1 pt.) Why is some of the sequence given in lowercase letters and some uppercase? ***One explicit sentence.***

3. (1 pt.) Ile93Met is shorthand for indicating that the disease causing mutation results in a substitution of Met for Ile. Based on side chain chemistry, is this a conservative or non-conservative amino acid substitution? ***One sentence explanation using proper terminology.***

4. (2 pts.) Two very different lines of evidence are presented that suggest that the Ile93Met is a disease-causing mutation. ***Summarize each line of evidence in one (or two at most) succinct sentence.***

5. (4 pt.) Examine Figures 1 and 2 carefully. Draw a set of ***simple*** diagrams that ***explains the different sized bands*** that are seen in Figure 2 as well as what you would predict for an individual that is homozygous for the mutant allele. Be sure to include a ***size scale (with appropriate units)*** and positions of BsmF1 site(s) in wildtype and mutant alleles. ***Include three panels: one panel for each of three possible genotypes.*** [Note, this particular restriction enzyme does not cut at its recognition site – but instead a few base pairs away – indicated by dark arrows in Figure 1] ***Your figure should be like Figure 2-20 in your textbook – but don't show the cross at the top.***

been found in the core of a mature cartilaginous tissue. Studies of the palaeohistology of vertebrates have assumed that cartilage can mineralize only on the surface. Any new descriptions of fossilized hard tissues, particularly from fishes, must take this discovery into account, and previous descriptions may need to be re-evaluated.

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The ubiquitin pathway in Parkinson's disease

Mutations of the α-synuclein gene[1,2] have been identified in some familial forms of Parkinson's disease, and α-synuclein protein has been shown to accumulate in the brains of patients with the disease[3]. These findings suggest that Parkinson's disease may be caused by the abnormal aggregation of α-synuclein protein. Here we have identified in a German family with Parkinson's disease a missense mutation in the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene. We show that this mutation, Ile93Met, causes a partial loss of the catalytic activity of this thiol protease, which could lead to aberrations in the proteolytic pathway and aggregation of proteins.

UCH-L1 is one of the most abundant proteins in the brain[4,5], comprising up to 2% of total brain protein. Immunoreactivity for this protein is found in Lewy bodies[6]. It belongs to a family of deubiquitinating enzymes, and is thought to cleave polymeric ubiquitin to monomers and to hydrolyse bonds between ubiquitin molecules and small adducts such as glutathione and cellular amines[7]. The abundance of UCH-L1 in human brain, its presence in Lewy bodies, and its involvement in the ubiquitin-dependent proteolytic pathway implicate it in the pathogenesis of Parkinson's disease.

We have sequenced the coding region of the UCH-L1 gene in probands from 72 families with Parkinson's disease, and identified a missense mutation in the fourth exon of the UCH-L1 gene that changes an isoleucine at position 93 to a methionine in one proband of a German pedigree. This Ile93Met change can be examined by a restriction endonuclease assay, because at the nucleotide level the C277G change introduces a new *Bsm*F1 site (Fig. 1). Mutation analysis of the affected brother of the proband showed that he too carries the Ile93Met mutation (Fig. 2).

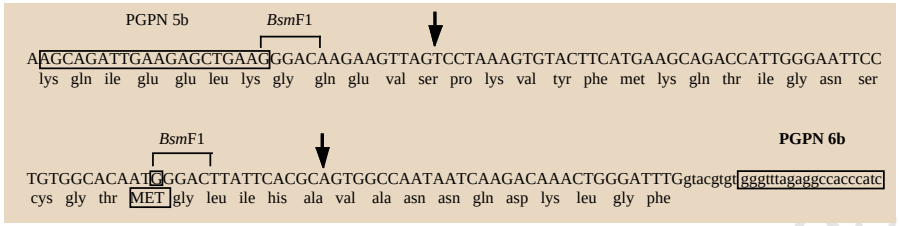

Figure 1 DNA sequence of a portion of exon 4 of the UCH-L1 gene. Boxes indicate polymerase chain reaction (PCR) primer sequences, and arrows indicate restriction sites for the *Bsm*F1 restriction endonuclease.

In both patients, the clinical syndrome is typical for Parkinson's disease. Symptoms began with resting tremor at age 51 for the proband and 49 for her affected brother, and progressed to rigidity, bradykinesia and postural instability. Both individuals showed a beneficial response to L-dopamine replacement therapy. A paternal uncle and the paternal grandmother were also affected, although, with the exception of the two siblings, all other individuals in the pedigree are deceased. The lack of phenotype in the father indicates that the mutation has incomplete penetrance in this family.

We analysed 500 chromosomes from control individuals of different ethnic backgrounds, 204 originating from German backgrounds. None of the 500 control chromosomes examined carried the Ile93Met change. Ile 93 is conserved in UCH-L1, UCH-L3 and the rat and mouse orthologues, as well as in the homologous genes of a yeast and the plant *Arabidopsis thaliana*. Thus, like mutations in α-synuclein, the Ile93Met mutation in the UCH-L1 gene is expected to contribute to the genetic aetiology of only a small number of patients with the familial form of the illness.

The mutant and wild-type proteins were expressed in *Escherichia coli* and assayed using two types of substrate. For the ubiquitin ethyl ester, rate measurements showed that the Ile93Met mutant protein cleaved the substrate (15 μM) at a rate of 2.41 U mg^{-1} compared with 4.08 U mg^{-1} for the wild-type protein. Because the K_m for this substrate is submicromolar, these rates represent V_{max} values and are consistent with previously determined rates[8]. With the substrate ubiquitin-7-amido-4-methycoumarin (Ub-AMC)[9], the V_{max} of the mutant enzyme was 0.20 U mg^{-1}, compared with 0.47 U mg^{-1} for the wild-type enzyme. Mutant and wild-type proteins exhibited similar K_m values, however, indicating that the mutant protein does not show decreased affinity for the substrate. Similarly, ubiquitin was an equally potent inhibitor of both the mutant and normal enzymes. The enzymatic activity values are consistent with Ile93Met UCH-L1 having a lower catalytic activity than the wild-type protein. Molecular modelling of the mutation suggests alteration in the geometry and fluctuation of the active site.

The roughly 50% reduction in catalytic activity of the Ile93Met protein should be interpreted with caution, however, as the natural substrate for the abundant UCH-L1 protein is not known. The reduced catalytic activity may affect the cleavage and turnover of the unknown substrate(s), leading to aggregation over time of the substrate(s), which can in turn act as a seed for other aggregation-prone abundant proteins. Alternatively, the Ile93Met substitution may render UCH-L1 prone to aggregation with the result that the protein accumulates. Finally, both models — reduced enzymatic activity and enhanced aggregation — may be in play at different stages of the illness.

The finding of mutations in the genes encoding α-synuclein and UCH-L1, and the identification of these proteins in Lewy

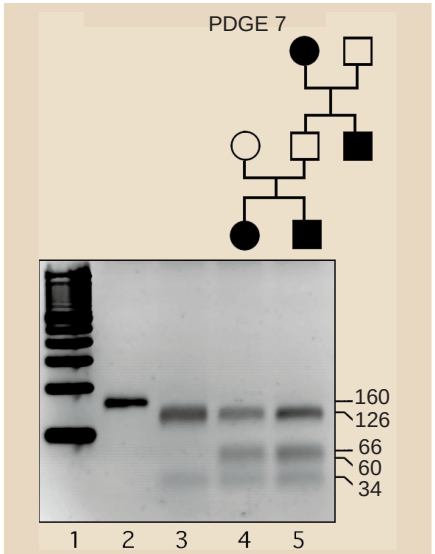

Figure 2 Mutation analysis for the Ile93Met mutation in kindred PDGE7. Lane 1 is a molecular marker; lane 2, undigested PCR product; lane 3, digested PCR product not carrying the mutation; lanes 4 and 5, PCR products from affected individuals carrying the Ile93Met mutation.

bodies in Parkinson's disease, indicate that aberrations in the folding, processing and degradation of proteins lead to neuronal degeneration.

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Whale ankles and evolutionary relationships

There are two main hypotheses for the relationships of the mammalian order Cetacea (comprising whales, dolphins and porpoises). The first hypothesis, mainly supported by DNA sequence data[1,2], is that one of the groups of artiodactyls (for example, the hippopotamids) is the closest extant relative of whales and that Artiodactyla are paraphyletic if Cetacea are excluded from it. The second hypothesis, mainly supported by palaeontological data[3,4], identifies mesonychians, a group of extinct archaic ungulates, as the sister group to whales. These two hypotheses are not mutually exclusive, because mesonychians and cetaceans could be sister groups, and this combined clade (Cete) could be the sister group to a group of artiodactyls.

The morphology of the ankle can be used to evaluate these hypotheses. Ankle specializations are universally used to characterize Artiodactyla, and would provide an excellent test for the inclusion of whales in that order. Unfortunately, the few cetacean ankle bones known are too incomplete or too reduced to allow meaningful comparison with other mammals.

We have recently recovered fragmentary Eocene astragali (ankle bones) from pakicetid and ambulocetid cetaceans[5] in Pakistan. We identified them as cetaceans because the deeply grooved trochlea resembles the partial astragalus of the holotype of *Ambulocetus natans*[5], and the large size of the astragali matches only a few mammals known from the associated freshwater and marine faunas, in particular perissodactyls, anthracobunids and sirenians. Astragali for known representatives or relatives of these mammals do not match the morphology of the new bones. The ambulocetid astragalus was found in marine sediments.

Three articular facets of artiodactyl ankles are highly specialized and are important for the relationship of whales. First, the astragalar head of artiodactyls is trochleated, meaning that it is wide, gently concave mediolaterally, and strongly convex dorsoplantarly, with the axis of this convexity perpendicular to the median plane. Second, the sustentacular facet is rectangular and covers the entire posterior aspect of the astragalus. Finally, the ectal (posterior calcaneo-astragalar) facet is reduced and placed on the lateral side of the bone. The combination of these features is found in all artiodactyls but not in any other mammal.

The cetacean astragalar head (Fig. 1) is wide and nearly flat both mediolaterally and dorsoplantarly. This is unlike the condyle of mesonychians, but is also unlike the convex trochleated head of artiodactyls. This important feature, often cited as the main defining character of artiodactyls[6], is inconsistent with the hypothesis that cetaceans should be included in the artiodactyls.

The cetacean sustentacular facet resembles that of artiodactyls in being long, but unlike that of artiodactyls it is narrow. In primitive mammals, including mesonychians, the sustentacular facet is short and rounded. The cetacean ectal facet is strongly reduced and placed laterally as in artiodactyls, not plantarly as in mesonychians and other primitive mammals. This position of the ectal facet is highly derived[7] and unique, occurring only in artiodactyls and these Eocene cetaceans. These features argue against close phylogenetic ties between cetaceans and mesonychians.

Our new ankle data do not unambiguously support either of the predominant hypotheses of cetacean relationships. Inclusion of Cetacea in Artiodactyla to the exclusion of mesonychians is consistent with the position of the ectal facet and the shape of the sustentacular facet. But the absence of a trochleated astragalar head argues against the inclusion of Cetacea in Artiodactyla, unless the flat head of the cetacean is interpreted as a secondary aquatic adaptation. Inclusion of Cetacea in Artiodactyla is also inconsistent with the derived similarities of the dentition and basicranium of cetaceans and mesonychians[8]. Sister-group relations between mesonychians and cetaceans are inconsistent with the derived similarities in the sustentacular and ectal facets between artiodactyls and cetaceans, both characters with little or no homoplasy in mammals. But, in any case, extensive convergence or reversals must have occurred in the dentition, basicranium and/or tarsus.

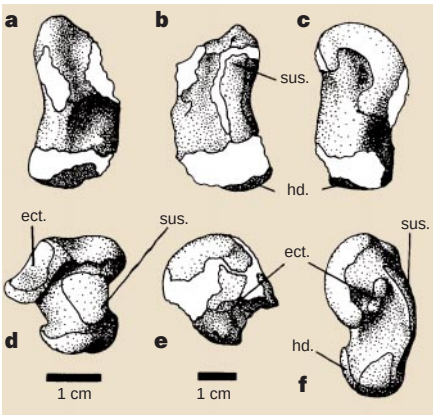

Figure 1 Astragali of: **a**–**c,** ?pakicetid cetacean shown in dorsal (**a**), plantar (**b**) and medial (**c**) view (H-GSP 97227, Locality 300); **d,** mesonychian (plantar view, *Disaccus europaeus*, MNHN Br 21 L); **e,** ?ambulocetid cetacean (lateral view, H-GSP 97113, Locality 9205, distal part missing); and **f,** artiodactyl (lateral view, *Sus scrofa*). Shown are ectal facet (ect.), sustentacular facet (sus.) and astragalar head (hd.). Left scale bar is for **a**–**d**, right scale bar is for **e**–**f**.

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