Good genetics take home quiz essay example

Sociology, Community

- If K65R and D113E are conserved mutations, what type of mutations are Q151M and Q151N. They are non-conserved is not the correct answer.

K65R and D113E being conserved mutations implies that even after undergoing mutations they retain the original properties. There functionality remains almost the same. On the other hand, Q151M and Q151N, mutations will result in a change of their initial properties or additional functions may be gained in the process of mutation.

- The K65A RT mutant had a reduction in RT activity. Explain the reasoning behind this phenotype

In the cell, the presence of nucleoside triphosphates and nucleoside diphosphates contribute to the pyrophosphorolysis. The nucleoside triphosphates and nucleoside diphosphates act as a source for the pyrophosphate. However, the presence of ATP binds to the RT mutants.

- Harris et al. tested the pyrophosphorolytic activity of the RT mutants. To do this they added pyrophosphate to the reaction. In the cell the pyrophosphate is cleaved by pyrophosphotase to drive the polymerization reactions, so there is no pyrophosphate available to drive the reverse reaction. So, how do you think this reaction could potentially take place in the cell? Think carefully about this situation, the answer is not in the paper, but asking you to come up with an answer based on what you know about RT.

RTs are found in the body’s RNA and DNA and play a role in replication of genetics. The reverse pyrophosphorolytic activity would take place through polymerization reaction whereby a correctly positioned PPi group generates dNTP used in generating primer-end producing a base shorter than its original length. On the other hand, using a mer reverse substrate

RAG*1316/1109 that eliminates pyrophorolysis substrate creates a reverse pyrophosphorolytic activity.

- How did Harris et al. show that the K65A in HIV-1 RT mutation had lower mutation frequency compared to the wild type.

- The Y183 RT mutant is less active on RNA templates than DNA templates. What is the potential reasoning for this?

The nature of the dNTP binding pocket differs depending on RNA and DNA templates. Additionally, the polymerase activity on the RNA templates is lower compared to the DNA template.

- In the Harris paper, they did several experiments in which Mn2+ was the co-factor used. Why did the researchers want try the polymerization and fidelity experiments using Mn2+? Give an example.

Polymerases require the presence of divalent metal action for catalysis. Manganese has been observed to affect the polymerase activity, processing and fidelity if DNA synthesis largely. Manganese was used as a substitute for magnesium since it has a more intense effect. To examine the effect of the manganese, the researchers used fidelity mutants such as Y183F and Q151N and compared this with the effects of using magnesium. it was found out that these high fidelity mutants were unable to catalyze mis-insertion in the presence of magnesium. In presence of Manganese, the mutants are able to extend the primer.

- Explain what the mutations Q258C, G262C and W266C represent in the Huang et al. paper

Q258C, G262C and W266C represent the potential mutation sites in the HIV-1 RT.

- Huang uses the term primer grip. What is meant by the " Primer Grip" and why is this region important?

The primer grip is a region where both strands, that is the template strand and primer strand interact with the residues in helices H and I. the primer grip region has residues that are important in maintain the primer terminus orientation that is necessary for a nucleophilic attack.

- Huang et al. showed several interesting characteristics of HIV-1 RT, but the most pronounced affect was what? Explain your answer

HIV-1 RT has a high binding specificity. The DNA polymerase domain has four sub domains, which include fingers, palm, thumb and connection. Fingers domain, thumb and palm conjointly sub-domains form a cleft that is used as a template primer. The palm contains significant residues that are necessary for the polymerase catalytic activity.

- In the Huang et al paper they states " In our model, the guanidinium group of Arg72, which lies flat against the dNTP base, donates hydrogen bonds to the -phosphate, and the -amino group of Lys65 donates hydrogen bonds to the -phosphate." What do they mean by the -amino group of Lys65?

The -amino group of Lys65 contains a higher percentage of polypeptides and is very reactive thus used in reactions where enzymes are involved.

- The present structure allowed Huang et al. to deduce two primary conclusions as to how resistant phenotypes develop. Describe these two conclusions.

In the first conclusion, Huang indicates that the point mutations necessary for resistance such as Q151M and M1841/V reside near the incoming

nucleotide. Factors that contribute to the major changes are located further away from the site in question. In a very likely scenario, the primary mutation will most likely cause changes in position, reactivity or stability of the primer terminus or templating base. The second conclusion relates to the site of mutations. Huang notes that the sites of mutations resistance to the dideoxy class of inhibitors will likely interrupt the dNTP from the front whereas the sites of resistance to AZT will interrupt the 3’ pocket from the rear. In conclusion, the major mutations are localized based on the chemistry of the inhibitor, thus the likelihood of cross-resistance and resensitization.

- Explain the how the closed complex was deduced

During the binding of the template significant conformational changes takes place. The fingertips of the fingers domain shift closer to the palm. The closure of these fingertips relates to the bending of fingertips. This causes some of the residues to encounter incoming nucleoside triphosphate. As such, the large gaps that were there earlier are closed making t a closed complex.

Describe the importance of the different conformations the DNA takes within the active site of the RT

It is important since substrates may be having different shapes. Thus, to ensure efficiency they have to conform to different shapes of substrates. The DNA polymerases are required to ensure that there is accurate synthesis via the selection of the correct dNTP and prevent the incorporation of unwanted or incorrect substrates. As such, conformational alterations of the DNA are significant to maintain DNA polymerase fidelity.