V(D)J gene Rearrangement and The Mechanisms Involved in Producing The Vast Antibody Repertoire.

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BACKGROUND

The genomic DNA in mammals has roughly six million nucleotides that are in a base pair set. Though high in number the amount of DNA that is actually coded for specific proteins is substantially less, estimated at nearly 30,000 bp. While various mammalian genomes are more closely understood since the introduction of better sequencing techniques. There are still a lot of questions that scientists are trying to understand better, one in particular is the mechanisms behind V(D)J recombination. This is responsible for yielding the antibody repertoire. There is complex regulation that occurs at specific loci of DNA and the story has been put together by immunology laboratories around the world. There are several regulatory sites and the RAG enzymes, RAG-1 and RAG-2, that play roles in recognizing binding sites (RSS) . This enzyme interacts with other proteins such as artemis 1, DNA-PK and ligases; RAG-1, creates a nick, then breaks the double-stranded DNA, Artemis opens the hairpin loops, then the ligation of the spliced ends of DNA is done by a ligase enzyme. The intervening DNA is excised when RAG-1 and RAG-2 make the DNA breaks, then the two coding regions are ligated. This sequence is signaled by the recombination signal sequence (RSS) and is the site of where the recombinase enzyme binds to. There are several other proteins that have specific roles in production of the antibody repertoire in pre-B cells. Histones are some of the proteins that are involved in V(D)J recombination. These enzymes and other protein structures will be elucidated later in this presentation.

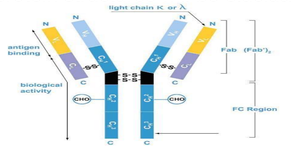


Fig 1: This is a diagram of the immunoglobulin receptor. The N-Terminus ends are where the antigen binding takes place. This protein structure is complex and held together by various bonds (hydrogen and disulfide bridges) and is in the tertiary conformation. The lambda and kappa (light chains) are also shown. The variable regions (V) are where a lot of the diversity takes place.

PURPOSE

The Feeney lab, located at the prestigious Scripps Research Institute, is working with cutting edge techniques to determine V(D)J gene recombination. This is a recombination that occurs in the genomic DNA on a particular locus that encodes the heavy and light chains of Immunoglobins. This spectacular event occurs to give rise to a repertoire that is vast and is known to be encoded in 10,000 + genes. The diversity is dependent on several factors such as; an enzyme complex called V(D)J recombinase and its recombination signal sequence. This recombinase interacts with several proteins that work together to cleave one of the sense strands, then another part of the complex bends the strand at the cleaved ends and creates a hairpin loop to facilitate the rearrangement. There are many factors involved such as histone modification and various transcription factors that play roles in rearrangement. Future studies are too include; Chromatin Immunoprecipitation (ChiP) assay ,which is used to quantify the fraction of a particular sequence that has DNA-binding protein bound. Real time PCR; which is a quantitative analysis that measures the saturation of DNA against the template in a logarithmic context

METHODS

1. Genotyping and breeding of mice includes maintaining various strains of mice, (Pax 5 +/-, RAG.GFP, EzH2, VaviCre) to list a few. Mating and weaning of mice according to what is needed by the post-doc researchers in the lab. Then keeping an up to date spreadsheet of complete count of mice, their genotype (what strain they are), keeping track of pregnant mice and new births.
2. Techniques used in the practice include, “tailing “ of the mice. This requires knocking out the mice briefly with a local anesthesia, place an ear tag, and then take a small specimen of mouse tail and place it into an eppendorf tube. These tails are then taken to another part of the lab to prepare the DNA from. This is a simple process that takes 100µl of a lysis buffer solution to lyse all of the non-genomic material like the cell membranes. Then it is placed on a heat block for ninety minutes to denature any proteins and cell membranes. Once this time is up then a neutralization solution is added to the tail sample. Then the samples are stored in the 4ºC cold room until the samples are needed for the Polymerase Chain Reaction (PCR), which is a reaction that takes a targeted section of DNA and amplifies the quantity exponentially. The parameters of each PCR reaction vary depending on the melting temperatures or each of the primer sets, sense or anti-sense and their required Taq polymerases.
3. Once a reaction mixture has been prepared, we aliquot PCR tubes either containing 20 µl or 25µl of sample per tube. Then placing it into a PCR machine and making sure the tubes are properly closed, the reaction times/temperatures and cycles are then selected. This process usually takes around two hours to complete.
4. Once the PCR reaction is complete, we now make an agarose gel for electrophoresis. This is a gel matrix that is used to verify DNA size fragments.
5. An example of a genotype that were are looking for is called hCre, this DNA is around 500 bp in length. Cre is a recombinase responsible for removing a gene between two flanked regions called loxP.

Fig 2 Steve Almos, is setting up a PCR reaction time, temperatures and cycles for reactions to occur. This will amplify a targeted region of DNA and make billions of copies of target DNA for genotyping.

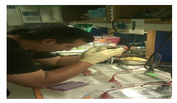
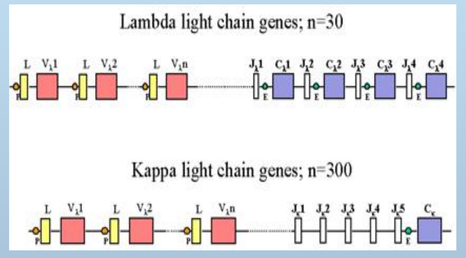
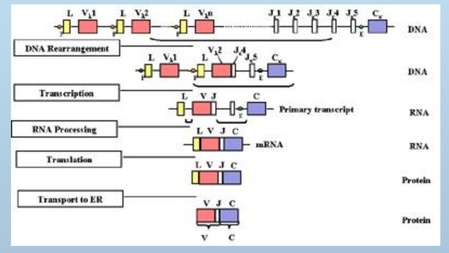
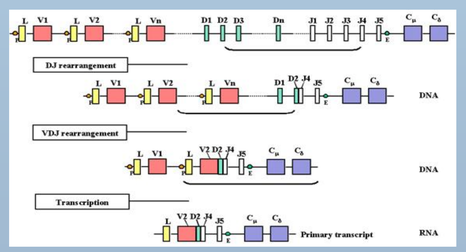


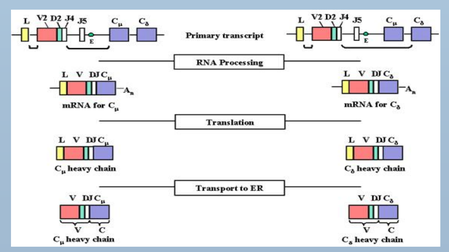
Fig 3: Steve Almos loading amplified DNA product into an agarose gel for electrophoresis.

Fig 4-7: These schematics show V(D)J recombination on the light and heavy chains, IgH and Igκ. In figure 4 please omit the lambda light chain, it is not the focus of our research.



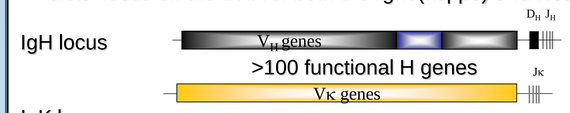




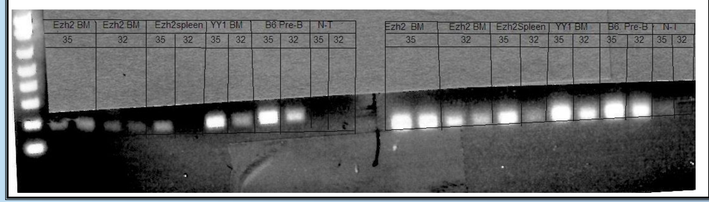


RESULTS

We are currently working on real-time PCRs to check proximal or distal locus on the DNA of both the light (kappa) and Heavy chains.



Below is a photograph of a 1.5% agarose gel, showing the rearrangement PCR of the vk21 and vk1 families. We are looking for rearrangement of proximal vs distal genes near the J gene region. This rearrangement is to find out how the contraction and looping of the locus to bring the distal V genes closer to the J genes. Each sample is a semi-quantitative look at how the rearrangement occurs on the locus of the light chain. This is because we measured the rearrangement from different strains of mice such as; Ezh2 BM,YY1 and B6. pre-B is designated as the positive control. This is to determine if there is a problem of rearrangement of the light chains.



CONCLUSION

I have learned a lot of information about V(D)J recombination in the seven weeks I have been in the Feeney Lab. I have performed a lot of regular PCR reactions and verified a lot of strains of mice by genotyping them and keeping track of them. I have been in charge of the vivarium where the many strains of mice are breeded,weaned and tailing them. I have also learned how to run real-time PCRs and rearrangement PCRs.

ACKNOWLEDGEMENTS

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