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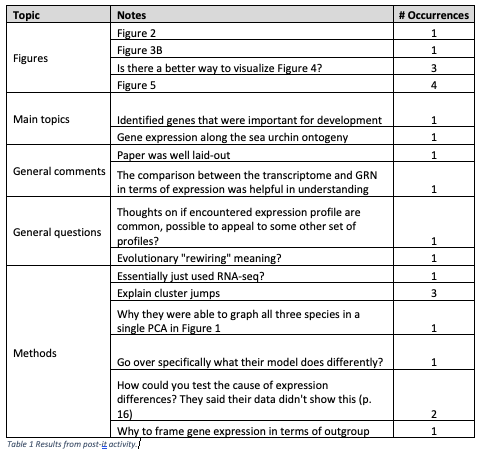
Dr. Prada

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Discussion Summary: Developmental Biology

For this week’s discussion on developmental biology participants read Israel et al.’s 2016 paper on comparative sea urchin development and Imam et al.’s 2014 paper on the transcriptional regulation network’s impact on photosynthesis in an anaerobic bacteria. Prior to the discussion, we reminded participants about some major themes in developmental biology that were reflected in the readings. We recalled 1) that phenotypic expression is more complex than we realize, involving the interaction of multiple genes, transcription factors, and enhancers, 2) that the original gene is never changed. It is instead duplicated and the copy is changed, and 3) that genetic regulation of necessary “core” functions is highly conserved. After a quick reminder of these main themes, we then introduced the party to an activity called a thought cloud. The purpose of this activity was to gather the group’s collective thoughts on the readings using sticky-notes for the purpose of visualizing gaps in knowledge and main takeaways from the paper.

Prior to discussing the Israel et al. 2016 paper, we requested that participants fill out three post-its each with questions and comments regarding the paper. We then organized the notes based on the following topics: figures, main topics, general questions and comments, and methodology. Table 1 below summarizes the results of this activity. Overall, there were many questions regarding the figures, especially figures 4 and 5. Specifically, there were many questions about the cluster jumps and their significance. Because there was so much uncertainty with the figures, we later reviewed meaning and methodology behind each figure.



After gathering the group’s collective thoughts and questions about the paper we asked participants to summarize the goals of the paper. In summary, Israel et al. examined the the evolution of lecithotrophy in sea urchins from the ancestral planktotrophic phenotype. They sought to determine if this evolution in life history pattern was primarily driven by expression changes in the transcriptome or by changes in the gene regulatory network (GRN). The paper accomplished this by comparing expression patterns in the whole genome and in the GRN of three species of urchin. Two of these urchins were within the same genus, however one was planktotrophic and the other was lecithotrophic. The third urchin species, another ancestral planktotroph, acted as an outgroup. Figure 1 showed this relationship between the urchins using a phylogeny of the urchins studied in this reading. Figure 1 also included an illustration of the morphological differences between lecithotrophic and planktotrophic urchins, as well as a principal components analysis (PCA) showing differences associated with life stage (PC 1) and between genuses (PC 2).  In the PCA we saw that differential expression between species decreased as urchin development progressed. We also saw differential expression between same-genus urchins and the outgroup species, thus reflecting their phylogenetic relationship.

After examining general trends in the PCA we discussed how the authors were able to graph all three species in a single PCA. To review, the authors mapped the transcriptome each species to their appropriate reference. They then normalized the number of reads within each species to the mean number of reads across all species. This allowed the authors to compile a transcriptome containing the normalized reads of all three species from which they could compute the PCA. After reviewing the methodology behind the first PCA we looked at Figure 2.

As our thought cloud revealed some uncertainty about the clustering technique used in figure 2 we went over the composition of this figure extensively. In 2A, a series of graphs depicted change in expression of different gene clusters at successive life stages in the outgroup species. 2B showed a similar series of graphs, however it was different in that genes from all three urchin species were grouped in clusters based on their expression patterns. Each graph displayed a distinct pattern of expression over successive life stages. Each of these patterns was deemed an ‘expression profile.’ The different expression profiles found in the paper included conservation, divergence, genus-level change, and cluster jumps. Finally, 2B visualized the prevalence of each expression profiles in relation to the others. We summarized that most of the differences were between species, and not between differences in life history.

We then discussed the similarities and differences between figures 1, 2, and 3. Like figure 1, figure 3 showed a pca, and like figure 2 it showed changes in expression of genes during larval development. However, it was different in that it highlighted two genes that were differentially expressed between species. 3A showed change in expression of these two genes in the three urchin species during their successive life stages, 3B showed a PCA of all comparable genes in all three species, and 3C and 3D showed the changes in timing of expression of genes during larval development.

Figure 4 illustrated the gene regulatory network of the sea urchin, which is a combination of three larger networks which either regulate the skeletogenic, endomesoderm, or the ectoderm. This figure uses prior knowledge of the urchin’s GRN to not only illustrate the intricacies of the GRN but also where changes occurred which lead to the arise of lecithotrophic urchins. In this figure, there is a number of changes which have caused cascading effects, which affected genes downstream. This is especially apparent for VEGFR, a gene that codes for lipids, which is shown to affect another gene in the ectoderm, although it was located in the skeletogenic. This was one of the larger changes, since the shift in VEGFR affected a number of other processes. This figure was also crucial because it helped to emphasize that the majority of the changes were small “jumps” and shifts, specifically located in the skeletogenic network. Throughout their work, they found that the majority of changes affected the adult body formation timeline (i.e. the adult body formed faster), the formation of a functional stomach as a larvae, and the formation of the skeleton (which was smaller and simpler in the lecithotroph).

The final figure we discussed for this paper was figure 5, which was: 1) comparing the two planktotrophic species of urchin to each other, and 2) comparing the lecithotrophic urchin to both the planktotrophic urchins. This figure is important because it shows that between the two comparisons there are clearly more genes that are coexpressed between the two planktotrophic urchins than there are between the planktotrophic and the lecitrophic. Indicating that the planktotrophic urchins are most alike, although one of the planktotrophic and the lecitrophic are sister species.

For the Imam et al. paper we asked participants to summarize the paper to the best of their knowledge before going over the figures. In summary, the purpose of this paper was to examine the role of five transcription factors, which have been previously associated with photosynthesis, in the photosynthetic bacteria, *R. sphaeroides*. They found that not all, but most of these previously recorded transcription factors were crucially involved with photosynthesis, as well as discovered several new ones. They also found examples of duplication, such as with FrnL and CrpK. While these two share several functions such as photosynthetic growth, CrpK cannot detect oxygen, leading them to believe CrpK is a duplicate of FrnL. We discussed the importance of duplication and that the creation of new phenotypes is not the result of new gene or transcription factors. Instead, they are the result of slight modifications in genes already present. After summarizing the paper, we quickly went over the figures. Given the fact that the figures in this paper were all similar we went over only a couple figures. In almost all the figures, there was: 1) a map of the binding sites across the chromosome, 2) a differential expression heat map, and 3) a position weight matrix logo.

The map of the chromosome explained where the binding sites were located. In the first figure this was very helpful since it didn’t provide any comparison like in figure 2, however, it was important to show that FnrL and CrpK were well distributed across the chromosome whereas Prra was located in a very small section. This expansion of FrnL and CrpK helps to ensure that at least the majority of the binding sites are still present in the future generations, indicating that these families are most likely crucial for photosynthesis function. The heat map usually compared the expressions in the “wild type” to the expressions in an altered gene (usually a deletion). This helped to indicate what operons were activated or repressed by the presence of FnrL, Prra, or CrpK. Figures 1 and 3 also had position weight matrix logos, which indicated the code for that particular transcription factor and any variation in said code. This is useful in comparing FrnL and CrpK in figure 3. They found that the binding sites specific to either FrnL or CrpK usually shared a similar “core” group of base pairs but had a few more bases added to one end or the other, differentiating them. The more generalize binding sites (those that can be activated by either FrnL or CrpK) usually only had that center region, allowing them to be activated by either FrnL or CrpK.