**Ian Bishop**

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**Developmental Biology Reading Response**

*Word Count: 763*

This week our focal topic was developmental biology and one important subtopic was gene regulatory networks (GRNs), how complex they can be, and the extent to which patterns of evolutionary change vary at the periphery and core of these networks. Imam et al. (IM) sought to characterize the regulons (a set of non-contiguous genes/operons whose expression are regulated by a particular transcription factor) of four transcription factors (TFs), of which two were previously known (FnrL, PrrA), and two only predicted (CrpK, MppG). All four are involved in bacterial photosynthesis in Rhodobacter sphaeroides. They used chromatin immunoprecipitation sequencing (ChIP-seq) to identify the binding sites of these TFs and compared their findings with a previous reconstruction of the photosynthesis TRN. ChIP-seq analyses corroborated the predicted network findings and previously ChIP-chip findings, but in general these two previous efforts underestimated the extent and complexity of this network, as all four TFs bound to many more sites than previously determined. IM paired their ChIP-seq analyses with a DNA microarray and found hundreds of differentially expressed genes (DEGs) present at binding sites for each of the examined TFs, where expression varied between WT and knockout strains for each TF. One last important finding was that binding sites were found to be highly redundant for 2/4 (FnrL and CrpK), and IM attempted to relate this redundancy to minor variations in binding motifs that would have different consequences in aerobic/anaerobic contexts.

It took a little longer than it should have to figure out what the actual point of this paper was, but this was more the result of a poorly structured narrative than that IM lacked an interesting research question. They should have from the beginning presented these findings as a validation of their previous reconstruction of the photosynthesis TRN in purple non-sulfur bacteria, kind of as a proof of concept of their reconstruction (though perhaps this wouldn't be novel enough for publication). Somewhere deep in the paper they say it explicitly that ~70% of operons predicted to be regulated by FnrL were validated as binding sites by ChIP-seq. To that end, I was more excited to see that the putative TFs found in that earlier work were experimentally determined to be important components of this TRN than the simple finding that "new knowledge was obtained", a phrase that they repeated often. However, this is just a matter of framing.

While IM sought to more generally *characterize* important components of the GRN via TF-binding site detection (via ChIP-seq), using the photosynthetic pathway’s importance as a rationale for the study, Israel et al. (2016) (IM) examined the sea urchin GRN from a more evolutionary standpoint, adopting a gene expression clustering approach and placing it into a phylogenetic context to seek? out the developmental differences between two developmental nutritional modes: planktotrophy and lecithotrophy. Early developmental expression data was collected for an outgroup organism with the ancestral feeding mode (planktotrophy) via RNA-seq, and genes were clustered into different groups depending on their temporal patterns of expression. Gene expression for in-group organisms that were either planktotrophic or lecithotrophic (a derived feeding mode) was similarly collected and assigned to the outgroup cluster groups. IR state that this is an advance in analytical technique because developmental expression must be looked at in a time-dependent manner. They found that more changes to developmental expression (movement of a gene's expression profile from one cluster to another) in the derived, evolved lecithotroph than in the ancestral planktotroph. This expression change was accentuated when only looking at the GRN as opposed to global expression, suggesting its prime importance in the evolution of this new nutritional mode.

Overall, the graphical presentation of this article was good, possibly with the exception of Fig4B. For this figure, I am still interested in discussing ways to visualize changes in particular genes within a network that do not clutter up the page and distract. How can jump expression profile change (via jump score change) be clarified for more central and more peripheral GRN nodes. Maybe this is a personal problem.

Both papers, despite their widely differing methods (comparative gene expression clustering vs. ChIP-seq) help identify important regulatory genes in the TRN/GRN of their organisms of interest. I do wish that IM would have considered some FISH localization, a method that IR subsequently applied to putatively important genes. The utility of FISH has been a common theme through many of the papers we've discussed in the past month, and is a nice way to concretize some of the genomic/transcriptomic findings into functional knowledge.

**References:**

Imam, S., Noguera, D. R., & Donohue, T. J. (2014). Global Analysis of Photosynthesis Transcriptional Regulatory Networks. *PLoS Genetics*, *10*(12), e1004837. <https://doi.org/10.1371/journal.pgen.1004837>

Israel, J. W., Martik, M. L., Byrne, M., Raff, E. C., Raff, R. A., McClay, D. R., & Wray, G. A. (2016). Comparative Developmental Transcriptomics Reveals Rewiring of a Highly Conserved Gene Regulatory Network during a Major Life History Switch in the Sea Urchin Genus Heliocidaris. *PLOS Biology*, *14*(3), e1002391. <https://doi.org/10.1371/journal.pbio.1002391>