Developmental Cell
Previews

Evolving Genital Structures: A Deep Look at Network Co-option

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http://dx.doi.org/10.1016/j.devcel.2015.08.022

Novel body structures are often generated by the redeployment of ancestral components of the genome. In this issue of *Developmental Cell*, Glassford et al. (2015) present a thorough analysis of the co-option of a gene regulatory network in the origin of an evolutionary novelty.

The emergence of new morphologies is a fundamental evolutionary process. In many lineages, morphological novelties have facilitated adaptation to new ecological niches or driven major changes in lifestyle of species. Novelties can be defined as additions of new body parts or radical changes in pre-existing structures, rather than minor modifications of pre-existing structures such as small changes in shape or size. While some examples of morphological change may or may not be categorized as novelties (where to put the limit between minor and radical change is always subjective), there are plenty of instances that fall, unquestionably, in the class of morphological novelties. For example, eyespots on butterfly wings (Monteiro, 2015) and limbs in terrestrial vertebrates (Schneider and Shubin, 2013), among many other examples (Held, 2014).

The origin of evolutionary novelties has puzzled evolutionary biologists since the 19th century (Wagner and Lynch, 2010). How do these new structures evolve? Is the generation of new genes and new gene regulatory networks a key part of the innovation process? Or, alternatively, do new structures arise from the re-use (co-option) of old genes and old regulatory networks? Over the past decades, evidence gathered from evo-devo studies has given a convincing answer to these questions: new structures are often generated by the redeployment of ancestral components of the genome (Shubin et al., 2009). In this issue of *Developmental Cell*, Glassford et al. (2015) present one of the first, to our knowledge, thorough analyses of the co-option of a gene regulatory network in the origin of an evolutionary novelty.

Glassford et al. (2015) aimed to elucidate the origin of the posterior lobe, a male-specific genital structure that emerged in the *melanogaster* clade (*Drosophila melanogaster* and closely related species; Figure 1). The posterior lobe is important for the interaction between male and female flies during copulation. Proper development of the posterior lobe requires the activity of the paired-domain transcription factor *Pox neuro* (*Poxn*). Glassford et al. (2015) showed that *Poxn* expression in the posterior lobe primordium is unique to species that develop the novel genital structure. This *Poxn* expression in the developing posterior lobe is driven by a transcriptional enhancer located downstream of the transcription start site (Boll and Noll, 2002; Figure 1).

Using a clever strategy, the authors exploited the *Poxn* posterior lobe enhancer as a tool to track the origin of the gene regulatory network upstream of *Poxn*. Two observations suggested that this enhancer is used in a different developmental context and implied that the genes that instruct the making of the posterior lobe belong to an ancestral gene regulatory network. First, this enhancer is present in *Drosophila* species that do not have posterior lobes. Second, the enhancers from non-lobed *Drosophila* species are able to drive expression in the posterior lobe of *Drosophila melanogaster* in reporter-gene assays. Hence, the next crucial step was to look for the ancient *Drosophila* structure from where the *Poxn* gene regulatory network was co-opted. Again, the posterior lobe enhancer of *Poxn* was a key part of the story. The authors examined the activity of the posterior lobe enhancer in many developmental stages and found that it drives *Poxn* expression in an embryonic structure called the posterior spiracle (Figure 1). The posterior spiracle is a larval breathing structure present in all *Drosophila* species that also requires expression of *Poxn* for its proper development. At this point, working with a model species proved to be a real advantage for the authors. In *D. melanogaster*, the components of the posterior spiracle gene regulatory network in which *Poxn* is embedded were known from previous genetic studies. Thus, Glassford et al. (2015) set out to search for the similarities and differences between the spiracle and lobe networks by performing a large number of functional and expression assays. These experiments revealed that most of the genes that are deployed in posterior spiracle development are also active players in the formation of the posterior lobe. Moreover, these experiments showed that the position that the shared genes occupy in the network is conserved between the two structures. In other words, both the components themselves and the topology of those components in the networks are almost completely conserved. This suggested that the posterior spiracle network was fully co-opted at the origin of the posterior lobe and subsequently diverged in the *melanogaster* clade lineage.

At this point in the study, the evidence for co-option was solid and the comparison of the networks had a high level of detail. But the authors did not stop there. They searched for additional elements in the network genes that would confirm

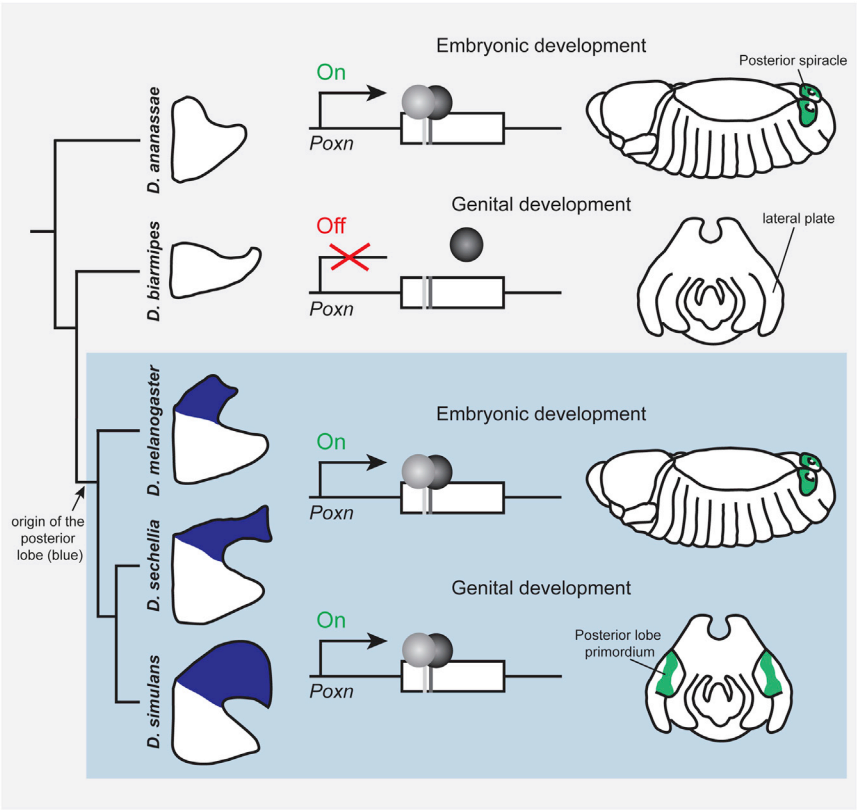

Figure 1. Co-option of the *Poxn* Gene Regulatory Network in the Evolution of the Posterior Lobe

The arrow in the phylogeny of *Drosophila* species indicates the lineage in which the posterior lobe originated. The posterior lobe (highlighted in blue) is an outgrowth of the lateral plate of the adult (schematized for each species). Expression of *Poxn* (green) in the embryo is necessary for the formation of the posterior spiracle (ancestral function). In species of the *melanogaster* clade, *Poxn* expression is also present in the posterior lobe primordium, a region of the developing lateral plate. This expression is necessary for the formation of the posterior lobe. The same enhancer of *Poxn* (white rectangle) drives expression in both the posterior lobe and the posterior spiracle. The binding sites (vertical gray lines) for Abd-B (dark gray circle) and STAT (light gray circle) are required in the two developmental contexts to generate *Poxn* expression.

the homology between the lobe and spiracle networks. Remarkably, they discovered more transcriptional enhancers that are used in the formation of both the spiracle and the lobe. Moreover, they found that transcription factor binding sites for STAT and Abdominal-B (two main transcription factors in the network) within the *Poxn* enhancer are used in both developmental contexts as well (Figure 1).

In general, co-option investigations are based on comparative gene-expression analyses and a few functional assays, often leaving relevant aspects of the co-option process unexplored. The study by Glassford et al. (2015) provides unprecedented evidence of co-option and an extraordinary level of detail in terms of the analysis of the components and connections within the network that was co-opted. However, there is still one loose end: the gene that activates the co-opted network in the posterior lobe remains unknown. One appealing candidate is the gene *unpaired* (a ligand for the JAK/STAT pathway), since its expression has been gained in the developing genitalia of lobed species. Unfortunately, the involvement of *unpaired* could not be corroborated in this study. Nevertheless, this work from Glassford et al. (2015) provides fertile ground to address still-unanswered questions. How is an ancestral network integrated in a new context? How is it that similar networks generate two dissimilar structures? The quick and obvious answer to the latter question would be that the function of unshared network components is what makes the difference. However, the existence of unshared components might not have functional consequences (i.e., "developmental system drift"; True and Haag, 2001). As an alternative, it can be hypothesized that differences in protein levels and cellular contexts in which proteins interact are the key events in the divergence of body parts built with similar networks. Certainly, further work will shed light on these exciting issues.

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