Genome Biology

RESEARCH HIGHLIGHT

What is it about 'eye of newt'?

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Abstract

The newt transcriptome opens up many new possibilities in the study of regeneration, and the novel gene families identified shed light on lineage-specific mechanisms.

Introduction: the regeneration aim

Of all the areas of life science research, regenerative medicine and regenerative biology together arguably hold the greatest mystique for human societies. From Prometheus through to the yellow spandex-clad mutant Wolverine, our heroes and icons through history embody our deep desire to overcome the biological frailties that make us susceptible to physical, pathological and age-related damage. Those aspiring to Prometheus-like self-renewal now have cause for optimism, thanks to a nexus of ideas currently in circulation that promise to allow regenerative medicine to impact upon our lives in ways that were previously confined to science fiction. For example, we now understand some developmental fate signaling mechanisms well enough to generate specific structures *in vitro* from stem cells [1].

While the growth or engineering of new functional adult tissues is at the core of ongoing work, it is becoming clear that functional integration with systemic biological functions, such as the immune, circulatory and nervous systems, adds an extra level of complexity we have only limited insight into. For example, while we can produce optic cups from stem cells [1], integrating these structures into adult tissue is an entirely more complex problem. Perhaps the most obvious way to achieve a complete understanding of regeneration and functional integration is to study those animals, such as hydra, planarians, annelids, starfish and urodele amphibians, where robust regenerative abilities are *de rigueur*. The last of these groups, urodele amphibians, is the subject of an article published in this issue of *Genome Biology* [2] that sheds new light on the genomic basis of regeneration in this lineage.

Leveraging urodele regeneration: 'eye of newt' and the 'newt within'

Major model organisms, which we traditionally associate with progress in our understanding of fundamental cellular and developmental mechanisms, are conspicuously absent from the above list of animals capable of regeneration. Historically, animals that regenerate were broadly ignored by developmental biologists, as their life history traits afforded none of the features that allowed first classical and then modern molecular genetic approaches. In fact, among animal species, currently only the zebra fish offers the opportunity to study profound limb and organ regeneration with a fully established set of genomic and molecular genetic tools [3].

Among the list of regenerators, urodele amphibians, consisting of newts and salamanders (axolotls), are the closest group to mammals, and so arguably are of greatest interest to regenerative medicine. One of the key remaining questions in urodele research is how conserved molecular regenerative mechanisms, albeit latent in mammals with regard to profound regeneration, are combined with novel lineage-specific molecular components. These two distinct contributions (referred to by Professor Jeremy Brockes, of University College London, as the 'newt within' for conserved features and 'eye of newt' for features particular to urodele/regenerative lineages) imply different approaches to how we might achieve regeneration in humans. Progress on this issue has been slow, as a comparative genomic approach using urodeles has been impeded by a lack of well-defined genome-level data. Even now, with sequencing costs plummeting and throughput rocketing, the genome sizes of urodeles are prohibitively large for effective sequencing. The smallest reported urodele genome is that of the pygmy salamander, *Desmognathus wrighti*, at approximately 13 Gbp, with the lowest estimates for the species most often employed in regeneration studies, *Ambystoma mexicanum* and *Notophthalmus viridescens*, at over 20 Gbp and 30 Gbp, respectively [4]. When these genomes are eventually sequenced, the assembly and annotation problems will be on a scale that has not previously been

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tackled in the animal kingdom. In an article published in this issue of *Genome Biology*, however, Looso *et al*. [2] make a serious contribution to reinvigorating the study of urodele regeneration through taking an alternative approach to genome sequencing. They present a comprehensive transcriptome complemented with proteomic data, establish a basis for effective genome-wide expression studies in the newt *N. viridescens* and identify many urodele-specific genes. The study data bring newts to the forefront as a reference for comparative genomics with a view to elucidating the molecular mechanisms of regeneration.

A comprehensive newt transcriptome

Looso *et al.* combined a previous expressed sequence tag (EST)-based approach with new data from the 454/Roche and Illumina platforms. These different data sources allowed a cross-platform approach that took advantage of the strengths of some approaches to compensate for the weaknesses of others. Perhaps most importantly, Looso *et al.* looked at different tissues, incorporating ESTs from a regenerating heart time course from a previous study [5], and 454/Roche data from a normalized library of a range of developmental, regeneration and adult tissues. Finally, well over half a billion paired-end Illumina reads from ventral and dorsal regions of the regenerating lens provided impressive coverage.

Various assembly approaches resulted in a dataset of 120,922 transcripts; of these, just under one-third had their homology verified to existing protein coding genes and ESTs in NCBI databases by BLAST algorithms. Searching for Gene Ontology terms in UniProt databases enabled functional annotations to be assigned to just over one-quarter of transcripts. Together, the two annotation strategies enabled the annotation of about 40% of all transcripts. An analysis of gene discovery rate and coverage against known signaling pathways and conserved gene families confirmed that the study had yielded a high level of gene discovery, as well as very respectable coverage. Finally, the study also directly confirmed the coding potential of almost 12% of all transcripts using a high throughput mass spectrometry approach.

Distilling 'eye of newt'

In line with expectations, conserved signaling and developmental gene networks appeared to be well represented in the new dataset, and these will help greatly in ongoing studies of newt regeneration. In other words, the transcriptome study has provided researchers with plenty of very helpful data to assess the 'newt within' contribution to regeneration. But what does it teach us about novel genes and mechanisms for the 'eye of newt' contribution? Looso *et al.* addressed this question by focusing on those transcripts confirmed by mass spectrometry to be *bona fide* protein coding transcripts. Even with this conservative approach, which at this stage excluded most novel transcripts that are likely to be protein coding, over 826 sequences were found to be entirely unique or to only have homology within urodeles (Figure 1). Only 131 of these contained domains described in the Pfam database.

Iterative BLAST searches within the remaining transcripts identified several potential new protein families. Of note, the transcripts within one such family shared a common signal peptide sequence. The authors performed RT-PCR analysis to look at the expression of some of these urodele-specific genes and potential new gene families, forming the basis for future detailed analyses of potential roles in newt regeneration (Figure 1).

Previous work investigating the function and structure of Prod1 (a urodele-specific regulator of limb regeneration) demonstrates that lineage-specific genes can have central roles in key aspects of newt regeneration, and serves as a blueprint for studies investigating how these genes might interact with conserved signaling pathways [6,7]. The Prod1 findings underpin the hypothesis that taxon-specific machinery will play key roles in regeneration, by plugging into conserved molecular machinery. The dataset produced by Looso *et al.* is an important step in testing this hypothesis by allowing the identification of the usual suspects shared with mammals ('the newt within') and by clearly showing that there are plenty of evolutionary novelties in urodeles ('eye of newt'). Possession of a tool of the caliber of Looso *et al.*'s transcriptome will not only facilitate the description of newt-specific regeneration genes and conserved regeneration genes, but - crucially - will also help comparative work with other species. Such an approach could advance our comprehension of the evolution of regeneration and might help us answer questions about changes in regenerative ability. For example, why can the newt undergo lens regeneration throughout its life, whereas the axolotl, its close relative, can only do so at restricted developmental stages [8,9]? Quite literally, in this example, we might ask: What is so special about the eyes of newts?

Concluding remarks

Animal regeneration, as far as we understand it, involves many conserved genetic circuits. For example, axial regeneration in planarians can be explained in terms of highly conserved signals and transcription factors. However, unsurprisingly, the detailed study of regeneration has almost exclusively (with notable exceptions) dealt with conserved candidate genes, biasing data toward support of this idea. Pleiotropic redeployment of conserved gene networks, together with evolutionary changes in their activity in adult tissues, may underpin the evolution of increased or decreased regenerative ability in some lineages. If this is the case, there may also have

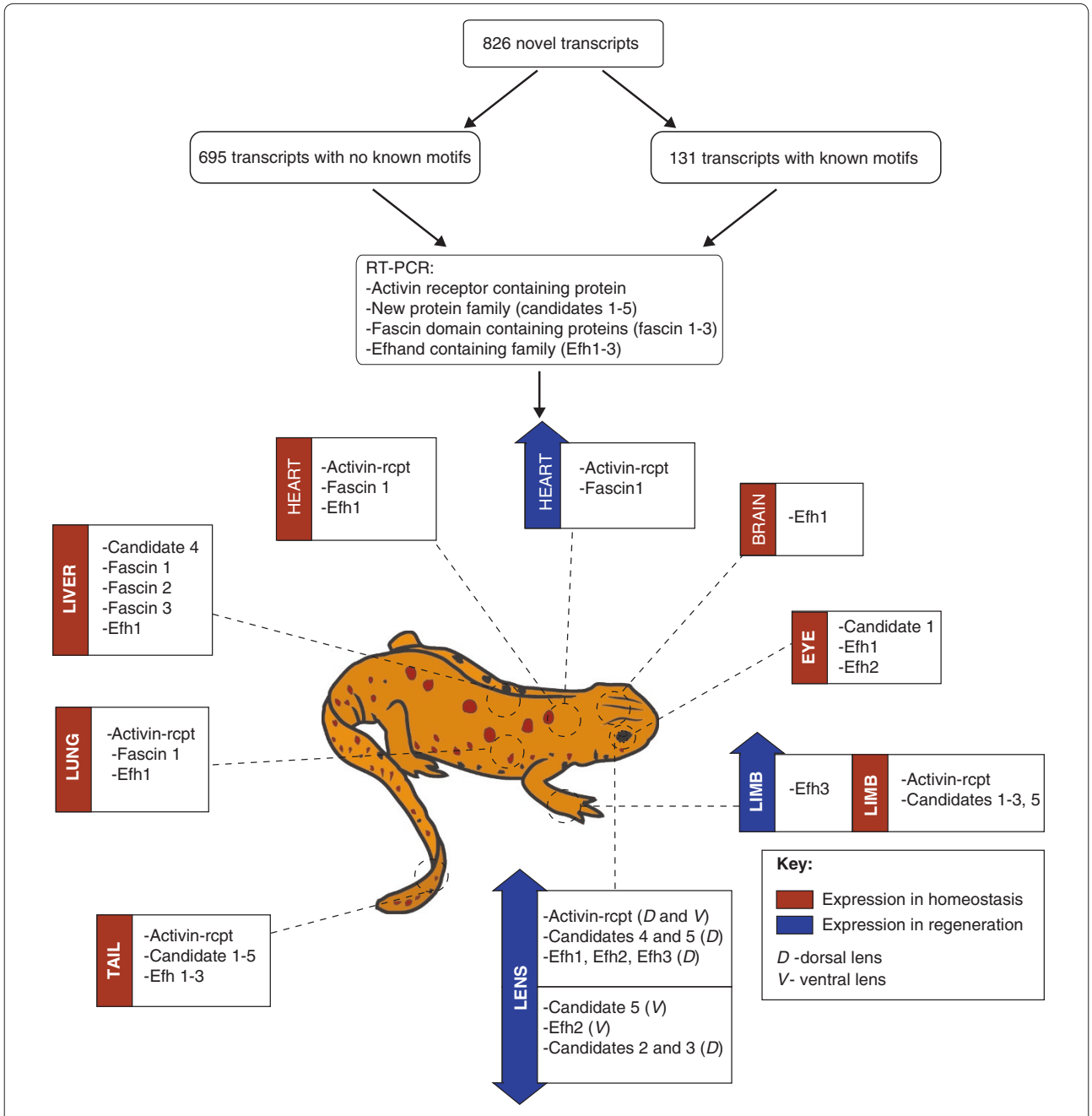

Figure 1. Distilling novel 'eye of newt' components of the newt transcriptome. Looso *et al.* [2] identified 826 novel protein-coding sequences with either no similarity to other species or similarity to urodeles only. Of those, 131 contained known motifs, while the rest did not. RT-PCR experiments on a select group of novel transcripts identified the tissues where these genes are expressed. These transcripts included those encoding a protein containing an activin receptor domain, a candidate family of new proteins with no known domains (five transcripts), three proteins containing the fascin domain and three proteins containing the EF-hand domain. Red boxes show expression of the tested transcripts in a homeostatic organ. Blue boxes demonstrate significant upregulation or downregulation (upward or downward arrow) of the tested transcripts in regenerating tissues. D and V stand for dorsal and ventral lens, respectively.

been ample opportunity for the incorporation of taxon-specific molecular novelties. Rather than just being a matter of evolutionary interest, the extent to which either of these possibilities is true will have a broad impact upon how we approach the prospect of enhancing regeneration in mammals. Happily, urodele regeneration researchers are now equipped with considerable ammunition with which to pursue these questions.

Abbreviations
EST, expressed sequence tag; Gbp, gigabase pairs; RT-PCR, reverse transcriptase polymerase chain reaction.

Competing interests
The authors declare that they have no competing interests.

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