Parallel embryonic transcriptional programs evolve under distinct constraints and may enable morphological conservation amidst adaptation

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ABSTRACT

Embryonic development evolves by balancing stringent morphological constraints with genetic and environmental variation. The design principle that allows developmental transcriptional programs to conserve embryonic morphology while adapting to environmental changes is still not fully understood. To address this fundamental challenge, we compare developmental transcriptomes of two sea urchin species, *Paracentrotus lividus* and *Strongylocentrotus purpuratus,* that shared a common ancestor about 40 million years ago and are geographically distant yet show similar morphology. We find that both developmental and housekeeping genes show highly dynamic and strongly conserved temporal expression patterns. The expression of other gene sets, including homeostasis and response genes, show divergent expression which could result from either evolutionary drift or adaptation to local environmental conditions. The interspecies correlations of developmental gene expressions are highest between morphologically similar developmental time points whereas the interspecies correlations of housekeeping gene expression are high between all the late zygotic time points. Relatedly, the position of the phylotypic stage varies between these two groups of genes: developmental gene expression shows highest conservation at mid-developmental stage, in agreement with the hourglass model while the conservation of housekeeping genes keeps increasing with developmental time. When all genes are combined, the relationship between conservation of gene expression and morphological similarity is partially masked by housekeeping genes and genes with diverged expression. Our study illustrates various transcriptional programs that coexist in the developing embryo and evolve under different constraints. Apparently, morphological constraints underlie the conservation of developmental gene expression while embryonic fitness requires the conservation of housekeeping gene expression and the species-specific adjustments of homeostasis gene expression. The distinct evolutionary forces acting on these transcriptional programs enable the conservation of similar body plans while allowing adaption.

1. Introduction

Developmental programs are capable of generating similar morphologies within the species, despite genetic variations and within broad environmental conditions (Garfield et al., 2013; Lasky et al., 2014; Levine et al., 2011; Nuzhdin et al., 2008; Runcie et al., 2012; Shaw et al., 2014). This flexibility of the developmental program is essential for keeping a wide genotypic pool adaptable in a changing environment and thus for the survival of the species. Understanding the relationship between the conservation of gene expression and morphological conservation as well as the association between expression divergence and adaptation, is key to understanding the evolution of embryogenesis through environmental changes (Carroll, 2008; Davidson, 2006; De Robertis, 2008; Shubin et al., 2009).

The relationship between the interspecies conservation of developmental gene expression and embryos morphological similarity is quite complex, as shown by recent comparative transcriptome studies (*e.g.,* (Hashimshony et al., 2015; Israel et al., 2016; Levin et al., 2016; Parikh et al., 2010; Xue et al., 2013)). Various comparative studies between different species, within a phylum, show that interspecies correlation in gene expression is higher between equivalent developmental stages that have similar morphology (Irie and Kuratani, 2011; Kalinka et al., 2010; Levin et al., 2012; Ninova et al., 2014; Piasecka et al., 2013; Yanai et al., 2011). The strength of the interspecies correlations of gene expression changes through developmental time, which resonates with existing models of morphological conservation. The early conservation model assumes the highest conservation (phylotypic stage) at early embryonic stages (Kalinka and Tomancak, 2012; Piasecka et al., 2013)

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whereas the hourglass model suggests that intermediate developmental stages are most resistant to evolutionary changes (Raff, 1996). The hourglass model is supported by the comparison of diverse vertebrate species (Irie and Kuratani, 2011) and by a comparative study of six closely related *Drosophila* species (Kalinka et al., 2010). However, the interspecies correlations between equivalent time points of two closely related *Xenopus* species increases with developmental progression, even after the morphological phylotypic stage (Yanai et al., 2011). Furthermore, a recent comparative study of 10 species representing 10 different phyla shows that the developmental time of highest interspecies correlations of gene expression within the phylum corresponds to the time of lowest interspecies correlations between the phyla (Levin et al., 2016). Thus, the link between morphological conservation and interspecies correlation of gene expression requires further investigation.

The complex relationship between the conservation of gene expression and morphological similarity might be explained by different constraints on different groups of genes. Many molecular processes within the developing embryo are not directly related to morphogenesis, *e.g.*, metabolic and physiological processes. If these non-morphogenetic processes evolve under different constraints than the morphogenetic processes, they could interfere with associating total gene expression with morphological similarity. Thus, to better understand the relationship between gene expression and morphological similarity, it is critical to study developmental transcriptomes in the light of molecular, cellular and physiological processes that occur in the developing embryo.

The sea urchin embryo presents an excellent system for comparative studies of developmental gene expression and its relation to developmental progression and embryo morphology (Garfield et al., 2013; Gildor and Ben-Tabou de-Leon, 2015; Israel et al., 2016). Sea urchin embryogenesis has been comprehensively studied for more than a century (Harvey, 1909; Hörstadius, 1939; Moore, 1929; Osborn, 1893). Gene regulatory networks models of sea urchin embryogenesis describe cell fate specification of most embryonic lineages up to gastrulation in unparalleled details (Ben-Tabou de-Leon et al., 2013; Li et al., 2014; Materna et al., 2013; Peter and Davidson, 2011; Saudemont et al., 2010). This extensive knowledge of embryogenesis and gene regulation is based on experimental studies in a few main species that occupy different habitats. Therefore, the sea urchin provides a good platform for studying the transcriptional programs that enable evolving embryos to conserve their morphologies despite environmental and genetic variation.

Two well-studied species are the purple sea urchin, *Strongylocentrotus purpuratus* (*S. purpuratus*) that inhabits the Pacific coasts of North America and *Paracentrotus lividus* (*P. lividus*) that inhabits the eastern Atlantic Ocean and the Mediterranean Sea. These species shared a common ancestor more than 40 million years ago (Smith et al., 2006), but despite their evolutionary distance and their geographically separate habitats they show great similarities in morphology and developmental gene regulation (Ben-Tabou de-Leon et al., 2013; Howard-Ashby et al., 2006a, 2006b; Lapraz et al., 2009; Li et al., 2012, 2013; Materna and Davidson, 2012; Materna et al., 2006; Nam et al., 2007; Range et al., 2007; Robert et al., 2014; Saudemont et al., 2010). We recently revealed a remarkable conservation of the expression kinetics of 25 developmental genes between these species (Gildor and Ben-Tabou de-Leon, 2015). We found a linear relationship between gene initiation times in the two species and used this ratio to scale the developmental rates of the two species, revealing strong conservation of gene expression dynamics. These findings suggest that the conservation of developmental gene expression might be essential for the conservation of embryo morphology; but will this conservation be seen in other sets of genes that do not control developmental processes?

Our goal here is to expand the comparison of expression kinetics to the entire transcriptomes of *P. lividus* and *S. purpuratus* and to illuminate the molecular basis of morphological similarity amidst genomic variation and environmental differences. To this end, we generated and analyzed *de-novo* developmental quantitative transcriptomes of *P. lividus* and compared them with the published developmental transcriptomes of *S. purpuratus* (Tu et al., 2014). We found that the expression profiles of both developmental and housekeeping genes are highly dynamic and strongly conserved. Among other gene sets, including some homeostasis and response to stress genes, we detected divergent expression. The interspecies correlations pattern of developmental genes is tightly related to morphological similarity between developmental time points and dramatically differs than that of housekeeping genes. Our study illustrates the various transcriptional programs that coexist in the developing embryo and evolve under different constraints.

2. Results

To study the transcriptional profiles of the Mediterranean sea urchin species, *P. lividus*, and compare them to those of the Pacific species, *S. purpuratus,* we collected *P. lividus* embryos at seven developmental stages matching to those studied in *S. purpuratus* (Tu et al., 2014, 2012), from fertilized egg to prism stage (Fig. 1A). Details on reference transcriptome assembly, quantification, annotations and identification of a set of 8139 putative homologous genes between *P. lividus* and *S. purpuratus* are provided in Section 4. Quantification and annotations of all the genes in *P. lividus* and in *S. purpuratus* are provided in Table S1. The analyses of this set of 8139 genes are described below.
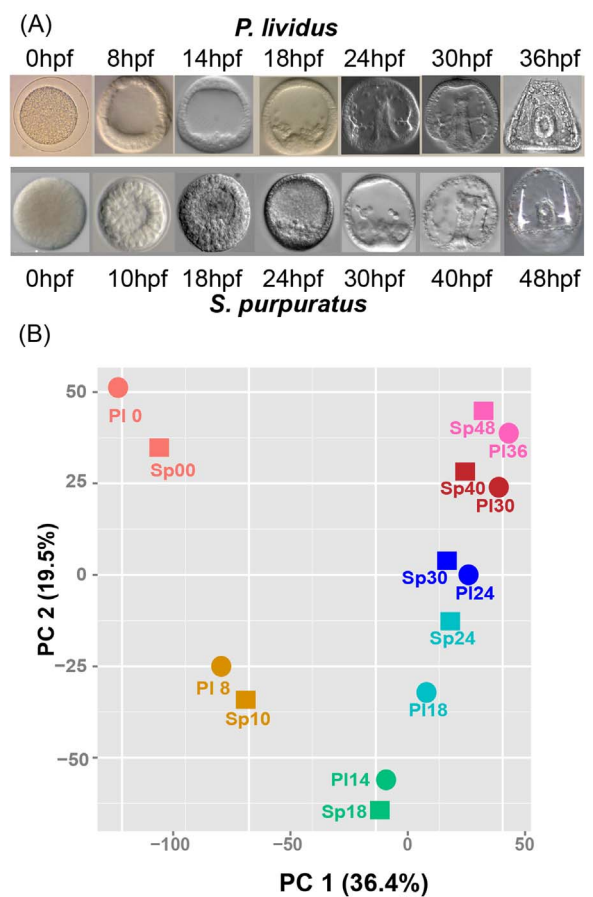
Fig. 1. Developmental time points studied and PCA analysis. (A) Images of embryos at the developmental time points studied in *P. lividus* and *S. purpuratus*. (B) First two principal components of expression variation between different developmental time points in *P. lividus* and *S. purpuratus*. Morphologically matching time points in the two species are marked in the same color.