Minireview

Ascidian gene-expression profiles

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Abstract

With the advent of gene-expression profiling, a large number of genes can now be investigated simultaneously during critical stages of development. This approach will be particularly informative in studies of ascidians, basal chordates whose genomes and embryology are uniquely suited for mapping developmental gene networks.

Molecular analysis of development has traditionally involved studies of one or a few genes at a time. This approach has revealed powerful regulatory genes, which have become the foundation for understanding pattern formation during metazoan development. But with one notable exception [1], the detailed genetic networks in which developmental genes function have remained elusive. Researchers working on the development of several model organisms are now breaking the single-gene tradition by using expressed sequence tag (EST) analysis to identify random cDNA clones from libraries derived from different stages and tissue types, and high-throughput *in situ* hybridization to categorize the corresponding mRNAs by their expression domains. In concert with antisense-mediated inhibition of gene expression and other molecular tools of developmental biology, EST analysis and other methods of gene-expression profiling can shed new light on the genetic circuitry underlying developmental processes. Here, I review recent gene expression-profile analysis in ascidians and the promise of this approach for studying developmental gene networks.

Ascidians

The ascidians are members of the tunicate (or urochordate) branch of the chordate tree and have been popular models in embryology and evolutionary biology for more than a century [2,3]. Their chordate features include a dorsal nervous system and a notochord in the larval phase of the life cycle, and pharyngeal gill slits in the adult phase (Figure 1). The favorable attributes of ascidians for traditional developmental biology include rapid embryogenesis, stereotypic cleavage divisions of the zygote and early embryo, well-documented cell lineages, low embryonic cell numbers, few larval tissue types, and a simplified larval body plan. Ascidian development starts with the localization of determinants in the egg; inductive signaling between different cells then takes place during the cleavage period, followed by simple morphogenetic movements that lead to the formation of a tailed (tadpole) larva; and the swimming tadpole is later radically reorganized into a sessile filter-feeding adult during metamorphosis (Figure 1).

The tadpole larva, with its distinct head and tail (Figure 1b), has attracted researchers to ascidian development because of its simplicity and its resemblance to the vertebrate body plan. The head contains endoderm cells, mesenchyme cells, and a central nervous system (CNS) equipped with two pigmented sensory organs. The tail contains a central notochord, flanked dorsally by a nerve cord (the posterior extension of the CNS), ventrally by a central strand of endoderm cells (the posterior extension of the head endoderm), and laterally by bands of striated muscle fibers, whose rhythmic contractions are responsible for larval swimming. The tadpole is covered by a single-layered epithelium of epidermal cells. During metamorphosis, the tail is retracted into the head, and head tissues become the progenitors of most adult tissues and organs.

Small genomes for rapid developers

A remarkable characteristic of tunicates is their small, compact genomes. The ascidian *Ciona intestinalis* has a

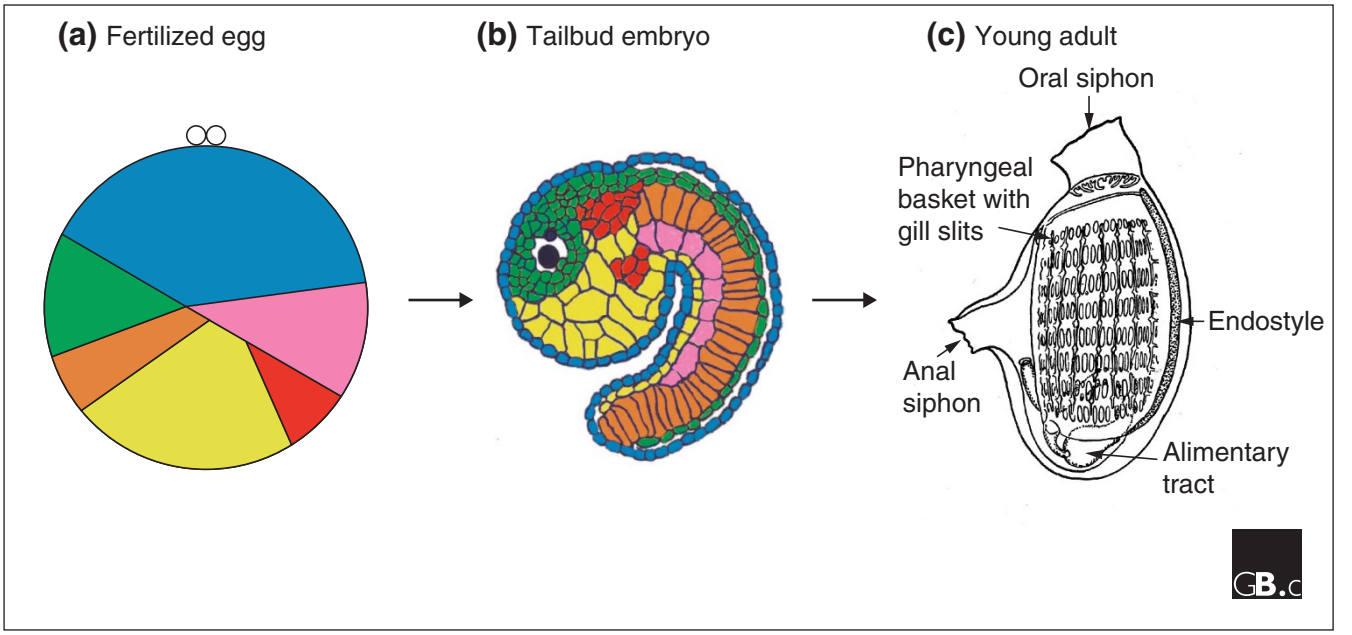

Figure 1
Ascidian development. **(a)** A fate map of the ascidian egg, showing the territories that generate epidermis (blue), muscle (pink), mesenchyme (red), endoderm (yellow), notochord (orange), and central nervous system and nerve cord (green). Most localized mRNAs are confined to the muscle territory, colored in pink [12]. **(b)** A diagram of a section through the anterior-posterior axis of a tailbud-stage embryo, showing the position of the larval tissues derived from the different territories in the fertilized egg. Colors are as in (a). **(c)** A young post-metamorphic adult.

haploid genome size of 160 megabases (Mb) [4], about the same size as that of *Drosophila melanogaster*. The tunicate *Oikopleura dioica* (which belongs to the larvacean subgroup) has a 50-65 Mb genome [5], by far the smallest of any known chordate. Both genomes contain about 15,500 genes, which are packed into a length of DNA equivalent to only about 5% of the human genome. One reason for the small size of tunicate genomes is a low frequency of duplicated genes. For example, in vertebrates there are two genes encoding troponins (muscle function regulators) and four members of the muscle transcription factor MyoD family, whereas these are each represented by a single gene in ascidians [6,7]. In other cases, such as the *hedgehog* gene family [8], gene duplication has not been as extensive as in vertebrates. In ascidians, alternative transcript splicing, rather than gene duplication, appears to be the norm for diversifying protein function [6,7]. Another factor responsible for the small genome is a relatively low ratio of non-coding to coding DNA sequence: overlapping or closely spaced genes, genes with very small introns, and intronless genes may be common in ascidians [9,10]. The low frequency of duplicated genes in the ascidian genome is in striking contrast to vertebrates, whose morphological complexity may stem in part from gene duplication and diversification [11].

The miniature tunicate genome may be an ancestral chordate feature, which persists in tunicates because of strong selective pressure for rapid development. Larger genomes may be a disadvantage as their correct replication would be difficult during the brief interphases in the cell division cycles of cleaving tunicate embryos. But whatever the reason for their origin and maintenance, the compact genomes of tunicates are invaluable for genomic studies because enhancers and other gene regulatory elements are compressed immediately upstream of the transcription start sites of the genes they regulate, simplifying their identification.

EST and *in situ* hybridization analysis

The analysis of ESTs and gene expression in two ascidian species, *Halocynthia roretzi* [12] and *C. intestinalis* [13], have revealed the expression patterns of a large number of cDNAs, which encode housekeeping proteins, signaling molecules, and transcription factors expressed at critical stages of development. The EST results tend to confirm earlier embryological studies. Classical experiments have shown that ascidian development is highly determinate, in other words that the territories destined for most cell fates are already specified in the fertilized egg (Figure 1a). The fact that a high proportion of the ESTs identified in fertilized eggs and early cleaving embryos represent localized maternal mRNAs corroborates this developmental autonomy [12,13]. The localized mRNAs tend to follow a few simple patterns [12], which may be sufficient to generate substantial cell-type diversity in the embryo. Later-stage EST analysis has been reported only for *C. intestinalis* [13]. At the 32-110

cell stage, which encompasses the period in which the fates of most embryonic cells are being restricted, about 17% of the ESTs are confined to a particular cell lineage, substantiating embryological studies [2]. Most embryonic tissues begin to differentiate during the tailbud stage (Figure 1b), and at this time about 37% of the ESTs represent mRNAs expressed in only one of the six larval tissues. Here, the ESTs provide a new insight: in contrast to vertebrates, neither of two ascidian *hedgehog* genes is expressed in the notochord; one is maternal and the other confined to the ventral nerve cord [8]. Thus, the ancestral chordate may not have used precisely the same Hedgehog signaling system as modern vertebrates to specify medial fates in the embryo. In the tadpole, which consists of fully differentiated cells and adult progenitors, 25% of the ESTs show tissue-specific expression. Finally, in young postmetamorphic adults (Figure 1c), about 31% of the ESTs show specific expression in various tissues and organs. Although the EST and expression-profiling analyses have yet to contribute to our understanding of developmental mechanisms, they provide useful markers for future experimental and comparative investigations and offer a large number of genes for mapping of regulatory networks.

Gene networks specifying muscle and notochord cells

To map gene networks, it is necessary to know the component genes, their *cis*-regulatory elements, and their positive and negative effects on cell-fate determination. This is a daunting prospect, particularly in vertebrates, largely because of gene duplication and possible redundancy. There are compelling reasons for choosing ascidians as a simplified system to map chordate gene networks. First, as described above, single-copy genes are the norm, and a large number of ESTs have been categorized into developmental expression domains that potentially indicate their presence in the same network. Second, gene functions and interactions can be studied by antisense inhibition [14,15] and ectopic expression through mRNA injection or electroporation-mediated transgenesis [16,17]. Moreover, the ability to transform thousands of embryos simultaneously by electroporation may permit genome-wide searches for *cis*-regulatory sequences [18]. Third, gene-regulatory elements are typically located only a short distance upstream of transcription start sites [19], thus simplifying their identification. Finally, ascidian gene networks may be 'shallow': key regulatory genes and their downstream structural gene targets are separated by a limited number of steps [20]. Although these attributes have yet to be combined to completely map an ascidian gene network, the pathways of larval muscle and notochord differentiation described below are ripe for analysis.

Most of the tail muscle cells are specified by maternal determinants [21]. The zinc-finger-protein gene *macho-1*, a member of the Zic family, appears to encode one of the muscle determinants: maternal *macho-1* transcripts are localized in the prospective muscle-forming region of the egg (Figure 1a), antisense inhibition of the gene blocks muscle-cell differentiation, and ectopic expression induces muscle cells [22]. The fact that zygotic muscle actin mRNAs begin to be transcribed at the 32-cell stage [20], when *macho-1* mRNA (and presumably protein) are still present in the embryo, suggests that there are only a few steps between muscle determinants and downstream structural genes. Other genes in the muscle network may be *Tb6* (a transcription factor that triggers muscle formation after ectopic expression [23]), *snail* (which represses expression of the notochord determinant *Brachyury* and thus represses the notochord cell fate (see below) in the muscle lineage [24]), and the single *MyoD* -family gene [7]. In addition, EST projects have identified a host of maternal and zygotic genes that could cooperate with or act downstream of *macho-1* to determine muscle cell fate [12,13]. The mapping of these genes into a network will help establish how maternal determinants interact with zygotic genes to specify embryonic cell fates by a cell-autonomous mechanism.

In contrast to the autonomous process of muscle determination, a conditional process specifies the notochord cells [25]. Homologs of fibroblast growth factors and bone morphogenetic proteins mediate the inductive events, sparked by triggering of a signaling cascade involving the small GTPase Ras and expression of the ascidian homolog of *Brachyury* (*Bra*) in the prospective notochord cells. The transcription factor Suppressor of hairless appears to activate *Bra* expression. The *Bra* gene is thought to play a central role in notochord differentiation, because *Bra* mRNA overexpression induces ectopic notochord formation [26]. The ascidian homolog of the winged-helix transcription factor HNF-3β (now called Fox5A), which is expressed in endoderm and notochord, is thought to act synergistically with Bra during notochord differentiation [27]. A gene network with great potential for mapping lies between *Bra*, *Fox5A*, and the downstream structural genes involved in notochord differentiation.

Antisense-inhibition studies indicate that two different cytoskeletal actin genes function downstream of *Fox5A* in notochord development [28]. In seminal studies, 39 genes downstream of Bra were identified in a subtractive hybridization screen carried out between wild-type embryos and embryos overexpressing *Bra* [29]. Many of these downstream genes, as well as other genes whose expression is restricted to notochord cells, were also identified in the EST analysis [13]. The products of these genes include enzymes, extracellular matrix proteins, and cytoskeletal proteins. At least one of the latter class (tropomyosin) appears to be a direct target of Bra [30], attesting to the simplicity of gene networking in ascidians. The mapping of these genes and ESTs into detailed networks will help to establish how signaling events control notochord-cell differentiation.

According to Nori Satoh [2], whose group has done many of the ascidian gene-expression studies described here, one of the goals of developmental biologists is to explain the entire process of embryogenesis in terms of molecular biology. Recent progress in gene-profile analysis suggests that this ambitious goal may indeed be attainable for ascidians.

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