The multidimensionality of cell behaviors underlying morphogenesis: a case study in ascidians

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**Summary**

Databases where different types of information from different sources can be integrated, cross-referenced and interactively accessed are necessary for building a quantitative understanding of the molecular and cell biology intrinsic to the morphogenesis of an embryo. Tassy and colleagues\(^{(1)}\) recently reported the development of software tailor-made to perform such a task, along with the generation and integration of three-dimensional anatomical models of embryos. They convincingly illustrated the utility of their approach by applying it to the early ascidian embryo. *BioEssays* 28:874–879, 2006. © 2006 Wiley Periodicals, Inc.

**Introduction**

Understanding how cell behaviors are individually executed and subsequently integrated to generate a final morphological structure represents a major challenge in developmental biology today. Bridging the gap between molecular and anatomical data is a requisite goal necessary to meet this challenge. If we are to construct accurate models of how information flows through genetic networks to control development, we must establish methods that project information onto a cell-based anatomical framework. Since embryonic development takes place in three dimensions over time, to fully understand the events required to build an embryo, we must observe morphogenetic processes in multiple dimensions in living systems. A key goal of such an approach is to integrate data of different types, making it available through a user-friendly interactive interface. Tassy and colleagues\(^{(1)}\) have admirably met this challenge with the early ascidian embryo (http://crfb.univ-mrs.fr/aniseed/).

The 3D Virtual Embryo software allows users to interactively analyze the dynamic changes in shape, volume, geometry, spatial arrangement and surfaces of contact occurring during early embryonic development of different cells within the ascidian embryo. It also permits the visualization, at any of the stages currently available, of the descendants of each cell and the tissue(s) that they will ultimately contribute to. While the integration of three-dimensional (3D) embryonic models with the arsenal of molecular tools and databases already available for ascidians discloses novel possibilities, such as the prediction in silico of the consequences of rearrangements and ablations of individual cells on the global embryonic landscape.

**The ascidian embryo: a simplified model of chordate development and evolution**

Ascidians (or Tunicates), commonly known as sea squirts, are marine invertebrate chordates usually found attached to submerged substrates in shallow waters in a wide variety of climates and geographic locations. The dual life cycle of an ascidian comprises two divergent extremes: a motile larval stage, exhibiting an elementary chordate organization, and a sessile adult form, in which chordate features such as notochord and dorsal neural tube are lost.

Ascidian zygotes are easily obtained through in vitro fertilization from hermaphroditic adults and in less than 24 hours develop into larvae. Early embryonic development takes place through stereotypical cleavages, which along with maternally inherited cytoplasmic determinants, account for the invariant cell lineage, which has been extensively analyzed.\(^{(2)}\) About 30 minutes after fertilization, most ascidian embryos undergo the first of a few symmetrical cell divisions, which give rise to blastulae. Gastrulation movements start around the 110-cell stage, and are followed by the formation of neural folds, which roll into a primordial neural tube,
resembling in a rudimentary form the neural tube of vertebrates. Approximately a day after fertilization, larvae hatch and begin to swim, before settling and metamorphosing. Notwithstanding their anatomical simplicity, ascidian embryos provide a valuable model for the basic mechanisms of vertebrate development, and recent phylogenetic studies suggest that tunicates might be more closely related to vertebrates than initially believed, and that they may represent the closest relatives of extant vertebrates.\(^{(3)}\)

Even though the rapid development, the relative ease of embryonic manipulation and the partial transparency make ascidian embryos an ideal model for detailed embryological studies on living embryos, there is still much to be learned about key topics, such as the significance of geometric constraints for cell behavior, the developmental role of asymmetric cell divisions and cell–cell interactions, and the effects of cell ablations on the propagation of short-range and long-range signals needed for tissue induction and specification. By applying recently developed technologies, such as high-resolution optical imaging and biometric interactive interfaces, to a classical model embryo, the software developed by Tassy and colleagues is beginning to fill some of these gaps.

### Molecular resources and online databases

The availability of a compact sequenced genome, extensive regulatory and gene expression data coupled with invariant cleavage, robust rapid development and ease of generating transgenics make the ascidian an attractive model system for investigating chordate development and genome organization.\(^{(4)}\)

A first draft of the *Ciona intestinalis* genome became available at the end of 2002\(^{(5)}\) along with an EST collection covering approximately 80% of the estimated genes,\(^{(6)}\) and has been recently joined by a draft of the genome of the sister species *Ciona savignyi*\(^{(7)}\) (http://www.broad.mit.edu/annotation/ciona/). In addition, EST data for a more distantly related ascidian species, *Halocynthia roretzi*, are also publicly available.\(^{(8)}\) Managing these large data sets requires the development of novel software that must perform a variety of tasks, including keeping track of biological information, ranging from molecular data to anatomical information on cell behaviors. Different databases also need to be accessible for visualization and navigation by the visitor. The ANISEED (Ascidian Network for In Situ Expression and Embryological Data) website developed by Tassy and colleagues does just that. While logical to navigate, it contains an essential introduction to the ascidian model system and a useful set of “Search tools”, reflecting the molecular resources that have been rapidly accumulating for different ascidians, in particular for the cosmopolitan model species *Ciona intestinalis*.

### Accessing gene expression data

The first set of search tools available from ANISEED, called “Expression queries” allows visitors to search in situ gene expression patterns in *Ciona intestinalis* and *Halocynthia roretzi* embryos at different stages of development, as well as in *Ciona* juveniles (Fig. 1). The majority of patterns and images come from the “Ghost” database, created by the Satoh laboratory (Kyoto University; http://ghost.zool.kyoto-u.ac.jp/indexr1.html), while the data for *Halocynthia* embryos come from the MAGEST consortium\(^{(9)}\) (http://www.genome.jp/magest). Expression patterns can be searched by selecting either the tissue(s) or the gene of interest. Such searches can also be performed on the Ghost and the MAGEST web sites, but the ANISEED site offers the advantage of linking data from the two species. The availability of another impressive set of expression data, obtained from cDNA libraries constructed in the Satoh laboratory from embryos at different developmental stages and from various adult tissues, has resulted in the creation of a new and interesting feature, the “Digital Differential Display”. This option permits the user to perform an in silico differential display, by selecting a pool of libraries for tissue(s) or stage(s) of interest and digitally subtracting it from a different pool.

The second set of tools, called “Embryological queries”, is built upon the 3D imaging data generated by the authors and includes a series of options that permit the visualization of the fate of different blastomeres at various developmental stages (Fig. 1). Finally, the “Molecular queries” ranges from traditional BLAST searches to searches of Gene Ontology databases. Under this menu, a novel tool that will likely become useful in the near future is the “Molecular weight search”, which allows a search for predicted proteins of a defined molecular weight through the provisional proteomes of *Ciona* and *Halocynthia*.

### Reconstruction and exploration of 3D anatomical models

There are now a number of technologies for imaging the 3D structure of an embryo, but optical microscopy is the most popular method capable of generating data at subcellular resolution. To generate accurate 3D models of embryos ranging from a zygote to 44 cells, Tassy and colleagues fixed consecutive staged *Ciona intestinalis* embryos, stained them with phalloidin which labels filamentous actin and then imaged them to generate z-stacks of xy images (Fig. 1). This xyz data was then computationally rendered to produce 3D models of embryos. These data are accessible through the “3D Virtual Embryo”, which is available as a separate downloadable module of the ANISEED database. The temporal window analyzed extensively covers the pre-gastrula stages of embryonic development. 19 interactive virtual embryos, covering 1-, 2-, 4-, 8-, 16-, 32-, 44-cell stages of *C. intestinalis* and a 32-cell stage embryo of *H. roretzi*, can be digitally...
searched, rotated, zoomed and sliced via a series of commands and options. For example, volumetric data on different blastomeres can be gathered using one of the many available geometry descriptors and were used by the authors to identify sister cells with different volumes, presumably deriving from unequal cleavages. This phenomenon had been previously reported and was attributed to the centrosome-attracting body, a structure found in vegetal blastomeres (B-lineage), promoting the asymmetric positioning of the mitotic spindle. It is conceivable that unequal cleavages lead to a differential segregation of cytoplasmic determinants, resulting in the generation of daughter cells with different developmental fates.

The numerous gene expression data included in the ANISEED database can be projected onto the 3D embryo scaffolds using the “From genes to structures” option. The reciprocal interface, “From structures to genes”, permits the identification of genes expressed in selected 3D structures. Finally, the complementary interfaces “From fates to structures” and “From structures to fates” offer the visualization of all cells that will form a given structure, and all the fates that cells of a selected cohort will adopt, respectively. Noticeably, the gene(s) of interest can be searched as a “stained molecule” in wild-type embryos and as more data become available as a “deregulated molecule”, in perturbed embryos.

**Predicting the consequences of cell ablation**

Over a century ago, the first cell ablation experiments were performed by Laurent Chabry and led to the discovery of the early determination of cell fates in ascidians. Cell ablations are currently performed using sharpened tungsten needles.
Areas of cell contact in the regulation of inductive interactions

Despite being frequently referred to as a mosaic, the ascidian embryo requires inductive interactions for tissue specification and patterning, in particular in the case of the neural tissue. For example, FGF9/16/20 has been described in Ciona as the inducer responsible for the formation of anterior neural tissue. Surprisingly, even though 16 blastomeres (competent cells) are in contact with the cells secreting FGF9/16/20 (inducing cells), only 4 out of these 16 blastomeres will respond by adopting a neural fate. The authors found an explanation to this puzzling result by calculating the surface area of contact between inducing cells and competent cells in the 3D virtual embryo.

In both Ciona and Halocynthia, the blastomeres responsive to FGF9/16/20 (a6.5 and b6.5) had the greatest surface area of contact with the inducing cells (A-line blastomeres), suggesting that a larger interface was necessary for promoting a neural fate. The authors proved this hypothesis by cutting 8-cell Ciona embryos, either from anterior to posterior (creating “lateral halves”) or from left to right (bisecting “anterior halves”), including a-line and A-line, from “posterior halves”, including b-line and B-line cells. In both cases, the surface area of contact between a-line competent cells and A-line inducing cells was increased. The embryo fragments were cultured to the 32-cell stage, then assayed for expression of Ci-Otx, a marker of anterior neural cells. In both the anterior and lateral halves, cells such as the a6.7 pair, which normally express Ci-Otx only sporadically, exhibited robust and consistent expression suggesting they had adopted a neural fate.

Adding seamless continuity by live imaging

While the information presented by Tassy and colleagues is built on a 3D anatomical scaffold obtained from a series of embryos fixed at successive time points, this approach can be taken a step further to establish 3D models with seamless temporal continuity by imaging living specimens. The acquisition of 3D time-lapse (i.e. 4D) data from living embryos requires that the embryo remains immobilized and healthy, and develops normally while being imaged. The development of reliable methods to mark cells is a key prerequisite to imaging live cell behaviors, and as Tassy and colleagues point out, can be achieved through the use of genetically encoded fluorescent proteins that highlight different subcellular compartments.

Plasma membrane localization of fluorescent proteins will provide information on cell morphology superceding data obtained with phalloidin staining. Fluorescent tagging of the plasma membrane can be achieved through lipid-modifications such as myristoylation, prenylation or glycosylphosphatidylinositol tags (Fig. 2A). Furthermore, data from imaging the plasma membrane can be complemented by multiplex labeling using spectrally distinct fluorescent proteins that localize to other cellular compartments, for example the nucleus, which can be tagged using histone fusions (Fig. 2B). Labeling the nucleus provides a descriptor of cell position, division and death. Furthermore nuclei do not overlap in 4D space, and in more complex embryos, at later developmental stages, nuclear labeling will likely aid in the construction and analysis of 3D embryos. Genetically encoded reporters would be used in conjunction with cis-acting regulatory elements that drive robust expression in all or a subset of cells in transgenic embryos.

3D time-lapse observations of developing embryos will likely extend the groundwork of Tassy and colleagues and provide data on the detailed temporal sequence of cell divisions, movements and interactions driving morphogenesis. For example, 4D recordings of early Ciona embryos labeled with a membrane-tagged fluorescent proteins reveal detailed information on the rapid, coordinated asynchronous wave of cell divisions in animal cells at the onset of gastrulation (Fig. 2A).

Peering into the future

Undoubtedly the tools showcased by the authors through the development of the ANISEED database, and the 3D Virtual Embryo in particular, are not just attractive for understanding ascidians. In fact, most investigators would welcome the availability of such a platform for navigating and interacting with multidimensional data in their system of interest. It is therefore only a matter of time until the application of this software, or variants of it, is expanded to encompass a larger repertoire of model organisms.

The use of vital fluorescent protein reporters, together with the development of new imaging protocols and databases that integrate data across platforms, provide the essential bridge between existing molecular data and emerging multidimensional anatomical data. It is likely that these developments will allow direct connections to be made between genetic perturbations and their cellular consequences, revealing
intricate details of the regulatory networks operating to generate different morphologies. This type of approach will inevitably call for high-resolution, high-throughput functional genomics methods. Furthermore, one might predict that live imaging, combined with the use of transgenesis for visualizing and/or perturbing gene function, will be performed on a large scale in the future.

Ultimately this type of problem enters the realm of systems biology, a goal of which is to understand the function of biological networks in generating 3D forms over time. An important aspect of systems biology is the integration of information obtained through reductionist approaches into a more global understanding at the organismal level. The development of interactive databases such as NISEED, that can take data from reductionist experiments and integrate them alongside large-scale genomic information, represents a necessary step towards achieving this goal.

Acknowledgments
We thank Yale Passamaneck and Jerry Rhee for images, valuable discussions and constructive comments.

References
What the papers say