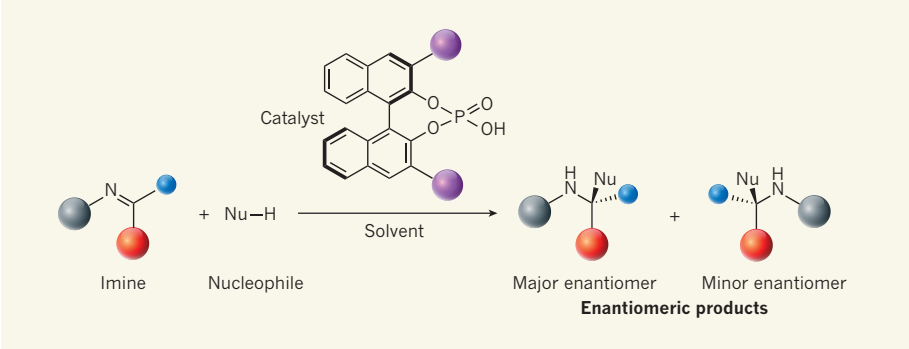


**Figure 1 | Model reactions.** Reid and Sigman[1] report a computational model that predicts the outcome of reactions when a wide range of nucleophilic molecules react with imines in the presence of a catalyst, accounting for factors such as molecular structure and solvent. More specifically, the model reports the magnitude of the enantioselectivity of the reactions — a measure of the ratio of the two mirror-image isomers (enantiomers) of the product formed in the reaction. Spheres represent a variety of chemical groups; bonds shown in bold or as solid wedges project above the plane of the page; broken wedges project below the plane of the page. Nu represents a range of groups or molecular structures.

one side by the chiral catalyst[4], so that any reaction has to occur on the other side. The third reaction component (a nucleophile), can therefore be varied substantially in the model. But the main reason is that the authors made a huge effort to produce a comprehensive training set of 367 individual reactions, each of which required multiple calculations to describe all the components, including the variability in shape (the conformations) of each component. It is highly encouraging to see that holistic reaction models can be produced by using such a wide training set.

Where next? A dream for reactivity modellers is to build an ultimate tool that accurately predicts the products of any reaction from the reaction components, thereby allowing computational screening of new reactions. Modellers have a long way to go to achieve this, but Reid and Sigman have shown that they can accurately predict outcomes for groups of related reactions, rather than having to model one type of reaction at a time. Other machine-learning methods are being tested on even bigger data sets[5].

The broadening of reaction scope demonstrated in the current work will encourage the search for more-general models, and might eventually enable models that predict the outcomes of reactions very different from those used for training. For now, making such predictions is still the domain of humans, but synthetic chemists will increasingly rely on theoretical tools to guide their work. I, for one, look forward to a future in which the tedious trial and error of synthetic chemistry is removed, and in which chemists can cut to the chase by carrying out only successful reactions. ■

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1. Reid, J. P. & Sigman, M. S. *Nature* **571**, 343–348 (2019).
2. Brown, J. M. & Deeth, R. J. *Angew. Chem. Int. Ed.* **48**, 4476–4479 (2009).
3. Reid, J. P. & Sigman, M. S. *Nature Rev. Chem.* **2**, 290–305 (2018).
4. Reid, J. P., Simón, L. & Goodman, J. M. *Acc. Chem. Res.* **49**, 1029–1041 (2016).
5. Segler, M. H. S., Preuss, M. & Waller, M. P. *Nature* **555**, 604–610 (2018).

EVOLUTION

# A deep dive into sea-squirt development

**An analysis of gene expression in sea-squirt embryos at different stages of development deepens our understanding of how the body plans of vertebrates might have evolved from those of less complex animals.** SEE ARTICLE P.349

NORIYUKI SATOH

Sea squirts such as *Ciona intestinalis* are the closest living invertebrate relatives of vertebrates. Their tadpole-like larvae feature some of the same organs and tissues as those found in developing vertebrates. On page 349, Cao *et al.*[1] use gene-expression data to examine the embryonic development of *C. intestinalis* larvae and to compare its development with that of other chordate animals, including vertebrates and cephalochordates, to reveal fresh insights into the evolution of vertebrates.

Single-cell analyses of gene expression have revolutionized various biological subdisciplines[2]. Such analyses at different stages of embryonic development have revealed how cells give rise to the various cell types that perform distinct functions and make up specific parts of the embryo[3,4]. As examples, studies of frog and zebrafish embryos have demonstrated that the three layers of cells that form these embryos — the ectoderm, endoderm and mesoderm — contain at least 50 cell types that have similar gene-expression profiles[3,4]. Studies into how different species develop often unveil clues to their evolutionary origins.

There are several advantages to studying embryonic development in sea squirts — which are also known as ascidians. As the closest relatives of vertebrates, they provide a reference for understanding the evolution of vertebrate body plans (Fig. 1). In *C. intestinalis*, embryogenesis — that is, the period of development that begins when cells are initially reorganized into a multilayered body of cells called a gastrula, and ends with larval hatching — takes just a day to complete. A *Ciona* larva comprises only about 2,500 cells, which make up distinctly differentiated organs and systems, including bilateral muscle, the central nervous system (CNS) and the notochord — a rod-like structure that gives rise to the backbone in vertebrates, and which is a defining characteristic of all chordate animals.

The cell lineages that comprise ascidian embryos have long been described[5]; the developmental fate of cells is restricted early in embryogenesis, at around the 110-cell stage. The *C. intestinalis* genome has been sequenced[6], and a network of genes and regulatory molecules that provides the blueprint for the body plan of all chordate animals has been characterized in *C. intestinalis*[7].

Cao *et al.* profiled the gene expression of more than 90,000 single cells from *C. intestinalis* at 10 developmental stages, from gastrulae to swimming larvae. The authors used these gene-expression data — carefully considering the expression of molecular markers of different cell types and lineages — to construct developmental trajectories of individual cell types. Whereas the larvae of *C. intestinalis* were previously thought to have approximately 20 cell types[8], Cao and colleagues' analysis identified 60 distinct cell types. A similarly comprehensive profiling of larval and embryonic cell types in vertebrates

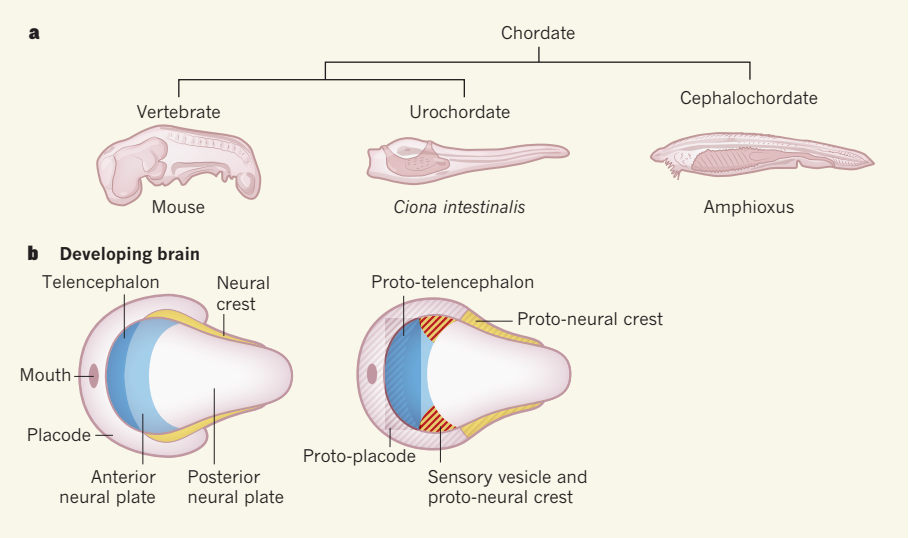


**Figure 1 | Evolution of the chordate body plan.** **a**, Vertebrates evolved from a common ancestor shared with urochordates, such as ascidians (including the sea squirt *Ciona intestinalis*), and the cephalochordate amphioxus. **b**, Whereas the amphioxus has no structures comparable to the vertebrate brain, cells in the sensory vesicle of the developing *C. intestinalis* nervous system express sets of regulatory genes that are also expressed in the neural crest and placode (structures of the developing vertebrate nervous system), suggesting that the simple brain of ascidian larvae contains prototypes of these regions. Cao *et al.*[1] measured gene expression in single cells of developing *C. intestinalis* embryos, and describe a cell-lineage map that reveals a prototype of the telencephalon (a part of the vertebrate brain that, in more complex vertebrates, is required for cognition) at the front of the larval ascidian brain. The neural plate gives rise to the brain and the spinal cord (in vertebrates) or the nerve cord (in urochordates). The position of the eventual mouth is also shown. In both panels, anterior is left, and posterior is right. (Embryo images adapted from ref. 15; CC BY 4.0.)

and cephalochordates would not currently be feasible.

Vertebrates and their sister group, the urochordates — which include the ascidians — are thought to share a common ancestor with cephalochordate animals, such as amphioxus (Fig. 1a). Cao and colleagues' study provides at least two insights into the evolution of vertebrates from this common ancestor: one concerning the notochord, and the other concerning the CNS, which becomes especially complex in vertebrates.

Amphioxus larvae are fish-like, and their notochord consists of stiff, coin-shaped muscle cells. By contrast, the notochords of ascidian larvae and vertebrates lack muscle-like properties, and instead consist of cells containing fluid-filled vacuoles that provide stiffness for muscle-driven movements of the tail. How these distinct notochord types evolved has been unclear. Cao *et al.* provide gene-expression evidence that the *C. intestinalis* notochord exhibits properties of both types. Specifically, the anterior part of the notochord is typical of that of ascidians and vertebrates, whereas the posterior part consists of cells that have muscular properties, as in amphioxus larvae. However, how *C. intestinalis* produces the anterior and posterior parts independently and combines them into a single organ is not clear[9].

According to the 'new head' hypothesis[10], the evolution of vertebrates can be largely ascribed to the emergence of placodes and the neural crest, which are developmental populations of cells that give rise to most of the tissues in the head and jaw. Previous evolutionary–developmental studies have shown that *C. intestinalis* possesses rudimentary versions of these two key vertebrate innovations[11,12]. In contrast to the CNS of amphioxus larvae, which is not very well organized, the CNS of ascidian larvae resembles a prototype of the vertebrate brain (Fig. 1b). Cao and colleagues identified 41 neural cell types in *C. intestinalis* larvae, including peripheral sensory cells and interneuronal cells, and showed that each type mapped to a specific region of the CNS, including the sensory vesicle (the anterior part of the CNS in urochordates), the motor ganglion (a cluster of neurons that control movement) and the nerve cord (the bundle of neuronal fibres that runs along the length of the body of chordate animals).

Cao and co-workers' data also help to elucidate the evolutionary origins of the telencephalon of the vertebrate brain; in many higher vertebrates, the telencephalon is enlarged and is crucial for perception and cognition. The gene-expression profiles and developmental trajectories of cells in the anterior-most regions of the neural plate (a developmental structure that gives rise to the CNS) revealed that these regions, particularly the sensory cells of the palps (protrusions of ectoderm tissue at the front of the larva) and the pro-anterior sensory vesicle (located at the anterior of the developing nervous system) are the invertebrate counterparts of the vertebrate telencephalon. The vertebrate telencephalon is thus likely to have arisen through the incorporation of non-neural ectoderm into anterior regions of the developing nervous system.

The finding of a prototype of the vertebrate telencephalon in the ascidian larva raises the question of how the complicated structure of the vertebrate brain evolved. Similar genetic and developmental trajectories are probably shared by ascidians and vertebrates, but the much more complex architecture of vertebrate brains means that they can perform more-sophisticated functions. Further experiments using single-cell analysis on amphioxus larvae will be needed to help determine how the complex architecture of the vertebrate brain arose.

Over the past two decades, there have been great advances in our understanding of the molecular, cellular and developmental mechanisms involved in the origins of chordates[13] and the evolution of vertebrates[14]. As shown by Cao *et al.*[1], single-cell analyses of gene expression deepen our understanding of the evolutionary emergence of cell types that confer vertebrate-specific properties. This line of research also highlights the importance of genomic information and the wide-ranging scope of analyses of gene regulatory networks. Mechanisms of vertebrate evolution revealed by evolutionary–developmental studies will increasingly be based on detailed and precise data from gene regulatory networks in individual cells, tissues and organs, and data acquired using other new techniques, such as those that probe the architecture of DNA complexes in the nuclei of individual cells, and sophisticated computational tools. ■

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1. Cao, C. *et al. Nature* **571**, 349–354 (2019).
2. Stuart, T. & Satija, R. *Nature Rev. Genet.* **20**, 257–272 (2019).
3. Farrell, J. A. *et al. Science* **360**, eaar3131 (2018).
4. Briggs, J. A. *et al. Science* **360**, eaar5780 (2018).
5. Conklin, E. G. *J. Acad. Nat. Sci. Philadelphia* **13**, 1–119 (1905).
6. Dehal, P. *et al. Science* **298**, 2157–2167 (2002).
7. Imai, K. S., Levine, M., Satoh, N. & Satou, Y. *Science* **312**, 1183–1187 (2006).
8. Satoh, N. *Developmental Genomics of Ascidians* (Wiley Blackwell, 2014).
9. Harder, M., Reeves, W., Byers, C., Santiago, M. & Veeman, M. *Dev. Biol.* **448**, 136–146 (2019).
10. Gans, C. & Northcutt, R. G. *Science* **220**, 268–273 (1983).
11. Stolfi, A., Ryan, K., Meinertzhagen, I. A. & Christiaen, L. *Nature* **527**, 371–374 (2015).
12. Horie, R. *et al. Nature* **560**, 228–232 (2018).
13. Satoh, N. *Chordate Origins and Evolution* (Academic, 2016).
14. Gee, H. *Across the Bridge: Understanding the Origin of the Vertebrates* (Univ. Chicago Press, 2018).
15. Inoue, J. & Satoh, N. *Mol. Biol. Evol.* **35**, 914–924 (2018).

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