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Review

Heading in a new direction: Implications of the revised fate map for understanding *Xenopus laevis* development

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Abstract

Amphibian embryos have served as a model system for vertebrate axial patterning for more than a century. Recent changes to the *Xenopus laevis* fate map revised the assignment of the embryonic dorsal/ventral (back-to-belly) axis in pre-gastrula embryos and allowed the assignment of the rostral/caudal (head-to-tail) axis for the first time. Revising the embryonic axes after many years of experimentation changes our view of axial patterning in amphibians. In this review, we discuss the revised maps and axes, and show by example how the new map alters the interpretation of three experiments that form the foundations of amphibian embryology. We compare the revised amphibian fate map to the general maps of the protochordates, and discuss which features of the maps and early development are shared by chordates and which distinguish vertebrates. Finally, we offer an explanation for the formation of both complete and incomplete axes in the rescue assays routinely used to study axial patterning in *Xenopus*, and a model of amphibian axial patterning.

Keywords: Amphibian; Axial patterning; Chordates; Fate map; Organizer; Vertebrate development; *Xenopus laevis*

Introduction

Fate maps are the projection of advanced developmental stages of an organism back to an earlier stage, and are a primary tool of embryologists. A fate map reveals what cells residing in a particular region of an embryo become in the course of normal development, and they are used routinely in both classical and molecular embryology. It is crucial for the design and interpretation of experiments that fate maps be accurate.

Vogt published the first comprehensive amphibian fate maps in 1929, and many additional maps have since been constructed (including Keller, 1975, 1976; Dale and Slack, 1987a,b; Moody, 1987 and numerous others). Amphibian maps are unique among metazoan maps in that investigators historically assigned only two embryonic axes—the dorsal/ventral (D/V, e.g., back-to-belly) and left/right (L/R) axes, but not the rostral/caudal (R/C, e.g., head-to-tail) axis. This is especially problematic for researchers for two reasons. First, the most rostral structure in an amphibian is its complex head, which is a novel, vertebrate-specific structure (Gans and Northcutt, 1983; but also see Northcutt, 2005). Although amphibian embryologists routinely refer to the head as dorsal or dorsoanterior, it is not an extension of the dorsal tissues found in all chordates. It is rostral by anatomical definition, and the source of the tadpole head should be assigned as rostral in a fate map. Second, the body plans of all vertebrates form in a pronounced rostral-to-caudal sequence. This means rostral regions of the embryo develop before caudal, so rostral regions must be distinct from non-rostral regions beginning very early in development. If vertebrate researchers seek to describe development accurately, they need to define rostral, and they can discern this information by constructing fate maps that reveal the origins of the head.

Recently a new *Xenopus* fate map determined the origin of the head and designated the "missing" R/C axis (Lane and Sheets, 2000; Lane et al., 2004). This map raised questions about the interpretation of previous amphibian maps. Controversy arose because the R/C axis was assigned to what was called previously "D/V", and the D/V axis was reassigned to the animal/vegetal axis. Re-orienting the axes after many years of experimentation is controversial because changing the axes changes our view of an embryo that has served as a model for

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vertebrate embryonic development for over one hundred years. In 1924, Spemann and Mangold defined "Spemann's organizer", a group of cells near the upper blastoporal lip of a gastrula stage embryo, which induces an ectopic embryonic axis when grafted to the opposite side of a host embryo. All vertebrate embryos have an organizer equivalent (e.g., the node in the mouse and chick and the shield in fish) that influences embryonic patterning. Many modern molecular and cell biological studies still focus on the organizer phenomenon, with the primary goal of defining the chemical nature of Spemann's organizer. As a result, a list of molecules that evoke secondary axes when expressed ectopically includes wild type and/or dominant-negative versions of many growth factors, transcription factors, and signaling molecules. In recent years, reviews of amphibian development have grown so complex that understanding how an egg becomes a tadpole is becoming an increasingly difficult problem. However, the revised fate and axis orientation maps may offer alternative and much simpler explanations for amphibian developmental patterning, and make frog development a more tractable problem.

Now is an appropriate time to step back from the established models and the molecular details and reconsider the early events that establish the vertebrate body plan. The time is ripe for two reasons. First, revisions of the *Xenopus* fate map alter the interpretation of many classical experiments, including the seminal experiment of Spemann and Mangold (1924, discussed below; see Lane et al., 2004). Second, our knowledge of the morphogenetic movements that construct the vertebrate body plan has grown extensively, but few developmental biologists understand the movements that shape the amphibian embryo. In the quest to understand axial patterning, nothing compensates for a thorough understanding of where cells move during construction of the body plan. Acceptance of the new fate and axis orientation maps will help investigators and students understand morphogenesis better. In turn, this will give them a deeper understanding of the molecular expression patterns observed in both normal and manipulated embryos, and the altered body plans frequently observed after experimental manipulations.

In this review, we will (1) review the old and new views of D/V and R/C axial patterning in *Xenopus*; (2) show with three examples how the new map alters the interpretation of experiments; (3) consider a generalized chordate fate map and the vertebrate elaborations that complicate the map; (4) describe a model to explain complete vs. incomplete axis formation in patterning assays; and (5) propose a revised model to describe axis determination in amphibians.

Two conflicting views of axial patterning in amphibians: the historical view and a revised, modern view

The tasks of fate mapping are to find the origins of various tissues and abstract the orientations of the embryonic axes from the observed tissue distributions. The tissue distributions in the revised *Xenopus* fate map differ very little from previous maps—the principal change is in the distributions of dorsal and ventral mesoderms. But this difference is significant because it alters the assignment of the D/V axis, and allows for the first assignment of the R/C axis in pre-gastrula embryos. The new axis orientations have major repercussions for how we think about axial patterning. To help readers understand how the new map changes our view of the embryo, we will first summarize the common features of the old and new views, and then highlight the differences.

Both views agree that an unfertilized amphibian egg is radially symmetric about its animal/vegetal axis. The germinal vesicle occupies the heavily pigmented animal hemisphere, while yolk platelets are concentrated in the lightly pigmented vegetal hemisphere (Fig. 1A). Fertilization initiates reorganization of the egg cytoplasm, by triggering massive cytoskeletal and cytoplasmic rearrangements during the first cell cycle. This process is called cortical rotation, and it involves directional transport of maternal determinants and an *en masse* shift of the cytoplasm relative to the cortex. Shearing between the cortex and cytoplasm lightens the pigmentation on one side of the animal hemisphere (Fig. 1B). Cortical rotation breaks radial symmetry, establishing two axes. At this point the two views diverge as to which two axes are determined (discussed below). However, both views agree that by gastrulation, the three germ layers are set up with ectoderm in the animal region, mesoderm in the marginal zone and endoderm in the vegetal region (Fig.

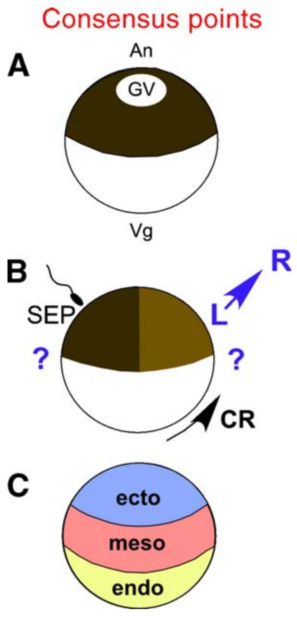

Fig. 1. (A–C) Consensus points between the traditional and revised views of amphibian axial patterning. Both views concur that the egg (A) is radially symmetric about its animal/vegetal (*An/Vg*) axis, and that (B) fertilization establishes two axes, by initiating cortical rotation (*CR*). The sperm entry point (*SEP*) organizes the cytoskeleton for the rotation, which results in the animal hemisphere being lightly colored on the side opposite the SEP after CR. The SEP serves as one pole of an axis and on the opposite side of the embryo, organizers and signaling centers will form later in development. This axis is disputed by the two views. In the traditional view, it is the ventral-to-dorsal axis but in the revised view, it is the caudal-to-rostral axis. The second axis established by CR is the left/right (*L/R*) axis. The left side of the embryo is shown in all panels. (C) Both views agree on the arrangement of the germ layers. Ectoderm (blue) maps to the animal region, mesoderm (red) maps to the equatorial region and endoderm (yellow) maps to the vegetal region.