Anterior-posterior axis formation in Xenopus laevis

Jansen, H.J.

CHAPTER 1

Introduction

Introduction

Our understanding of the developmental processes that lead from a zygote to a multicellular embryo with patterned axes has increased enormously over the past hundred years. Researchers started to use the accessable embryos of sea urchins, frogs and salamanders to investigate this problem during the early part of the last century. Their findings revealed that cell movements massively reorganize the embryo during gastrulation, at the time when the embryonic axis is being formed (Vogt, 1925; Vogt, 1929). More exciting yet was that their grafting experiments also revealed that certain embryonic tissues influence other parts of the embryo (Mangold, 1933; Spemann and Mangold, 1924). These grafting experiments led to the concept of the axis-inducing Spemann organiser. Many details are now known about the behaviour of cells during gastrulation (Keller, 2005). At the molecular level, our understanding of patterning processes in the embryo has increased exponentially. Several of the different signaling pathways that were discovered have been shown to be involved in specifying the embryo's body plan, for example, its anterior-posterior axis. Ideas about how embryos pattern their anterior-posterior (A-P) axis have been put forward (Doniach et al., 1992; Nieuwkoop, 1952; Ruiz I Altaba, 1992). However, a model that explains the emergence of an A-P pattern and incorporates all the processes, like cell movements and active signalling pathways, that are active in the embryo during gastrulation is still lacking. This thesis is an attempt to formulate such a model.

The Spemann organiser

The dorsal blastopore lip of the gastrula stage in amphibian embryos is called the Spemann organiser. It is capable of recruiting cells that do not normally participate in making a dorsal anterior-posterior (A-P) axis, into such an axis (Spemann and Mangold, 1924). Similar structures with similar properties were later found in other model species (shield in fish, and (Hensen's) node in chicken and mouse). The organiser has several functions. It is involved in changing the fate of part of the embryo's ectoderm from epidermal to neural. It is also involved in regulating cell movements, causing cells to start involute earlier on the dorsal side then on the ventral side of the gastrula. In addition to that, it also induces convergence and extension movements that elongate the anterior to posterior body axis. Later self differentation of organiser cells will give rise to head mesoderm, notochord, and pharyngeal endoderm (for a review see (Harland and Gerhart, 1997). In an early model for A-P patterning, the organiser was thought to contain, and induce in other tissues, different anterior to posterior identities (Eyal-Giladi, 1954; Mangold, 1933). Gradients of secreted molecules, like FGF's Wnt's and retinoic aid, that act in a planar way along the AP axis have also been postulated to have a role in AP patterning (Cox and Hemmati-Brivanlou, 1995; Durston et al., 1989; Kiecker and Niehrs, 2001; Lamb and Harland, 1995). The prevalent assumption was that these molecules are secreted by the organizer and diffuse in a planar fashion through the embryo's neurectoderm. See below.

Activation transformation

A refinement of the model above is the activation transformation model formulated by Pieter Nieuwkoop and collegues (Nieuwkoop, 1952; Nieuwkoop and van Nigtevecht, 1954). This model proposed that neuralising signals from gastrula mesoderm induce

embryonic ectoderm to form neural tissue, which initially has, by default, an anterior identity (activation). In a subsequent step, this anterior neural tissue is posteriorised (transformation). Anterior to posterior morphogen gradients are a possible candidate responsible for the transformation step (Cox and Hemmati-Brivanlou, 1995; Durston et al., 1989; Kiecker and Niehrs, 2001; Lamb and Harland, 1995). Cells sense their position in these gradients and respond by taking on a more posterior identity according to the concentration of the posteriorising morphogen that they experience..

An alternative way to posteriorise the neural tissue is by position specific vertical signalling from the underlying mesoderm. Mangold showed that dorsal mesodermal grafts from different positions (and with different A-P identities) along the A-P axis of a newt embryo induced corresponding identities in neural tissue induced from ventral (non neural) ectoderm derived from the host. (Mangold, 1933). Xenopus embryos in which the contact between mesoderm and ectoderm is inhibited by exogastrulation show only very posterior AP identities in their neurectoderm (Ruiz I Altaba, 1992) suggesting strongly that vertical signalling plays a role in AP patterning of the neural plate.

Hox genes

In 1978 Lewis showed that several mutations that caused homeotic transformations in *Drosopila melanogaster* lay close together in a gene complex (Lewis, 1978). This marked the starting point for the discovery of the Hox genes and their role in pattern formation (for review see (McGinnis and Krumlauf, 1992)). The Hox genes are transcription factors that determine the A-P positional fates of the cells in which they are expressed. More anteriorly expressed Hox genes give rise to more anterior structures. The cascades by which Hox genes exert their functions are largely unknown. Only recently, with the aid of transcriptome analysis, are more Hox target genes becoming known (Hueber et al., 2007; Rohrschneider et al., 2007); Van den Akker, in press; chapter 5 of this thesis).

In vertebrate species, Hox genes lie in an ordered fashion on chromosomes, in chromosomal clusters. The number of Hox clusters has, due to genome dublications during evolution, increased from one in invertebrates to four in vertebrates, and due to a extra duplication, to eight in teleost fish (Hoegg and Meyer, 2005). The position of each gene in a hox cluster determines the timing of its start of expression and its anterior border of expression along the a-p axis (temporal and spatial colineairity). Hox genes that lie 3' in a cluster start their expression early and end up by being expressed in an anterior position in the embryo while 5' genes start their expression later and have a posterior expression domain. Temporal and spatial colineairity are tightly linked and a mechanism by which the temporally colinear Hox sequence is translated into a spatial anterior-posterior sequence is described in this thesis. The temporal Hox sequence is the force generating new and more posterior identities in the part of the mesoderm that still has to be patterned, and it is therefore an important force that drives A-P patterning. How temporal colinearity is achieved is unclear, but it seems to involve global control regions (enhancers) outside of the Hox clusters (Kmita et al., 2002). Sequential opening of the chromatin of the clusters is also suspected to be a part of the mechanism of temporal colineairity (Kmita and Duboule, 2003).

A model for A-P patterning

In chapter 2, our description of the earliest expression of Hox genes in *Xenopus laevis* embryos emphasized the importance of a subdivision of the gastrula mesoderm into (dorsal) organiser and (ventral) non-organiser mesoderm. Hox genes start their expression in the gastrula's non-organiser mesoderm. Organiser mesoderm is void of Hox gene expression. We analyzed hox gene expression in embryos without organisers or without non organizer mesoderm and in heterochronic organiser transplant experiments into ventralised embryos, containing only non organizer mesoderm, These experiments showed that the A-P pattern is generated in the non-organiser mesoderm, and by interaction between the non-organiser mesoderm, and the Spemann organizer, becomes stabilised in the neurectoderm. These findings led to a model: the time-space translation model.

In this model A-P identities are generated in the non-organiser mesoderm. When mesodermal cells involute, and by convergence extention movements are dorsalised, this identity appears also in the neurectodermal cells directly above these mesodermal cells. For this step the Spemann organiser is an indispensible component.

Manipulated embryos are also capable of forming an A-P axis, when no Spemann organiser is present (Ang and Rossant, 1994; Ober and Schulte-Merker, 1999). In all of these experiments, the embryos still had neural tissue, implying that neural induction is a important function of the Spemann organiser. See below

The role of the Spemann organiser

In chapter 3, we further studied the facilitating role of the Spemann organiser by using embryos without an organiser and bringing back a single organiser function. We showed that the only requirement from the organiser for it to be able to play its part in generating an A-P pattern is that it mediates neural induction. Embryos that contain only non-organiser mesoderm could pattern their ectoderm if this was given a neural fate by injection of a BMP inhibitor combined with FGF, resulting in a radially symmetric A-P patterned embryo. This clarifies one aspect of the time-space translator model. The facilitating role of the Spemann organiser is to induce neurectoderrm that can subsequently be patterned by signals from the non-organiser mesoderm.

The role of active retinoids

The time-space translator model depends on vertical signalling to transfer positional information from the non organizer mesoderm to the neurectoderm. This mesoderm is known to secrete several molecules that might act as signalling molecules. Several Wnt ligands, including Wnt-8, and Wnt 3a ,are expressed in the non-organiser mesoderm. BMP is also secreted by this tissue. Another molecule secreted by the non-organiser mesoderm is an active form of vitamin A (active retinoid).

The teratogenic effects of active retinoids have been known for a long time. Gain of function experiments showed severe phenotypes (Durston et al., 1989). However the precise role of retinoids in A-P pattern formation became clearer only recently. Loss of function mutants in mouse and zebrafish showed a small defect in the hindbrain. In chapter 4 we describe loss of retinoid function in the frog, obtained using a synthetic high affinity antagonist hat blocks all RAR retinoid receptors without activating them. We show that this

reagent blocks expression of certain (the more anterior) hox genes in neurectoderm from the end of gastrulation. It does not block early mesodermal hox gene expression in the gastrula. We also showed that specifically blocking retinoid function in the mesoderm blocks neurectodermal hox gene expression. These findings indicate that retinoids are involved in the signalling from non-organiser mesoderm to neurectoderm, and can thus play a role in the time-space tranlator model by signalling, or facilitating signalling of A-P identities from mesoderm to neurectoderm.

How do the hox genes function?

Patterning of the A-P axis consists initially of the establishment of A-P identities which can be defined by the expression of Hox genes. The regulation of genes by Hox proteins will in the end lead to development of different body parts. The question of how Hox genes exert their functions is thus of extreme interest. High throughput analysis has recently enabled progress in investigating this question. Hox targets were identified in zebrafish after Hoxb1 expression (Rohrschneider et al., 2007); Van den Akker, in press) and Drosophila after overexpression of six Hox genes (Hueber 2007). In chapter 5, we investigated how a Hox gene exerts its function by using microarrays to identfy transcriptional targets after its overexpression. To have an indication of which targets are evolutionarily conserved we performed Antp/Hoxc-6 gain of function experiments in *Drosophila melanogaster* and *Xenopus laevis*. We identified a limited number of early conserved upregulated functions, amongst which are cell cyle control, cell movement control, ubiquitin pathway, and GTPase mediated signalling, and in general a downregulation of genes expressed in the head territory.

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