Anterior-posterior axis formation in Xenopus laevis

Jansen, H.J.

CHAPTER 6

Discussion

The research described in chapter 2 showed that in *Xenopus laevis* Hox genes are switched on in a collinear fashion in non-organiser mesoderm in the gastrula and that this coincides with the generation of anterior to posterior (A-P) identities in this tissue. The Spemann organiser was shown to have a role in A-P pattern formation by allowing ectodermal Hox gene expression, and by somehow stabilising the A-P identities formed in the mesoderm. Our findings following investigation of these phenomena led us to the formulation of a model, the time-space translator model, that describes how important A-P patterning events, like generation of A-P identities, cell movements, and neural induction, work together to pattern the A-P axis during gastrulation. Early in gastrulation a division of the mesoderm into a territory called the Spemann organiser, and the so-called non-organiser mesoderm becomes apparent (Fig 1).

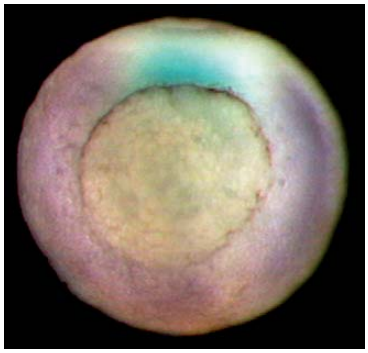


Fig. 1 *Spemann organiser and non-organiser mesoderm in the early gastrula*
In situ hybridisation showing expression of Chordin (turquoise) in the Spemann organiser and Hoxd-1 (magenta), indicating the non-organiser mesoderm.

The mesoderm is gradually internalised during gastrulation, and cell movements including involution and convergence and extension, cause it to become localised mainly under the future central nervous system. During gastrulation, new A-P identities are generated in the non-organiser mesoderm that is not yet involuted. Cells in this domain have a dynamic Hox-code, meaning that, during gastrulation, new, more posterior Hox genes are switched on in a temporally collinear sequence. When cells leave this domain, and become involuted, they lose the ability to express new Hox genes, and their A-P identity, and Hox-code become static. At the same time, the Hox code that is present in the involuted mesodermal cells, also appears in the overlying neurectoderm that is induced from embryonic ectoderm by signals from the Spemann organiser. This coexpression of the same Hox code in mesoderm and overlying ectoderm is associated with stabilising the Hox code, because without neurectodermal Hox expression, the mesodermal Hox expression loses its sharp anterior expression boundaries during later neurula stages (see also chapter 2 fig. 2 panel A, and unpublished observations).
Pattern formation in the A-P axis of the developing neurectoderm starts with neuralisation and generation of anterior identities (activation) and proceeds by subsequent generation of more posterior identities (transformation) (Nieuwkoop, 1952). It is clear that activation occurs via action on the embryonic ectoderm of signals from the organiser. And that transformation occurs via action on the activated neurectoderm of separate transformation signals. The region where the new A-P identities are generated (the source of the transformation signals) has been proposed to be in different parts of the embryo. Some have claimed that the Spemann organiser generates the A-P information (Eyal-Giladi, 1954; Mangold, 1933; Zoltewicz and Gerhart, 1997), while others pointed to the posterior mesoderm as a source for a transformation signal (Fainsod et al., 1994). In recent years a number of experiments have pointed to the non-organiser mesoderm as the tissue where A-P information is generated. In these experiments, embryos without a organiser but with

neural tissue were examined. These embryos all still show the emergence of an A-P pattern, fitting the idea that A-P information is generated in the non-organiser mesoderm. (Ang and Rossant, 1994; Ober and Schulte-Merker, 1999; Wacker et al., 2004a).

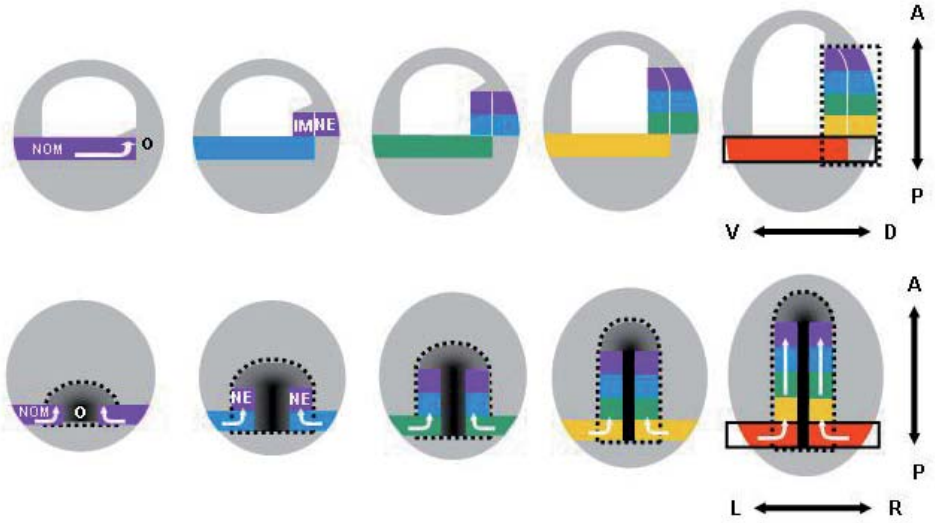
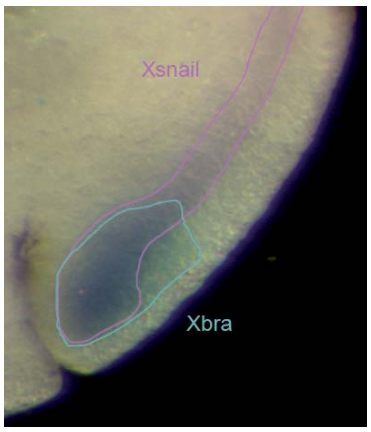


Fig. 2 The time-space translator model
The top row shows schematic embryos in sagittal sections. The bottom row shows a dorsal view of the same embryos. NOM: non-organiser mesoderm, NE: neurectoderm, O: Spemann organiser, A: anterior, P: posterior, D: dorsal, V: ventral, L: left, R: right. White arrows indicate cell movements.
The drawings in Fig. 2 illustrate this model. The differently coloured bars represent different A-P identities and the solid black bar, the Spemann organiser. The solid black line surrounds the (coloured) dynamic zone where new identities are being formed, and the stippled black line indicates the region within which A-P identities have become static. It possibly reflects the range of a stabilizing signal from the Spemann organiser



The expression of new Hox genes always starts in the same region which has been called the “Hox induction field” (Deschamps et al., 1999) or “opening zone” (Gaunt, 2000). Wacker *et al.* have shown that in Xenopus this region lies in the overlap between the gastrula stage domains of brachury expression and BMP4 signalling (Wacker et al., 2004b).

Fig. 3 Expression pattens of Xsnail and Xbra.
A dorsolateral view of mesoderm analysed for expression of Xbra (turquoise) and Xsnail (magenta). The domain of expression for each gene is indicated by a similarly colored line.

It is evident that Hox genes lie at the heart of axial patterning (McGinnis and Krumlauf, 1992). Their temporally colinear expression in the non-organiser mesoderm coincides with the generation of A-P identities in this tissue. How temporal colinearity in the Hox clusters is achieved is to not yet clear. Global control regions outside the Hox clusters might play a role in this (Kmita et al., 2002). Gradual opening of the chromatin in the Hox clusters has also been proposed as part of the mechanism to achieve temporal colineairity (Kmita and Duboule, 2003). At any rate, all who have speculated about the nature of Hox colinearity have assumed that this is regulated at the level of transcription. More recently, it became clear that the transcription of the Hox clusters is more complex than the simple expression of the individual Hox genes. Mainguy *et al.* have shown that the mouse and human Hox clusters generate many polycistronic transcripts and that large parts of them are transcribed both in the sense and the antisense directions (Mainguy et al., 2007). Differential splicing of large transcripts and sense-antisense pairing of mRNAs can also be ways by which the abundance of Hox gene transcripts are regulated, and temporal colinearity can be achieved. MicroRNAs are also known to regulate the expression other genes posttranscriptionally (see for review (Schier and Giraldez, 2006)). Genes encoding small non-coding RNA's of the Mir family have been found in the Hox clusters and have been shown to regulate the expression of Hox genes (Woltering and Durston, 2008; Yekta et al., 2004).

The research described in chapter 3 showed that neural induction is the only function of the Spemann organiser needed for A-P patterning, even though its other functions do greatly affect the shape and geometry of the embryo. In *Xenopus laevis,* the functions of the organiser have been divided into three categories (Harland and Gerhart, 1997). First, self-differentiation of the organiser generates a variety of mesodermal and endodermal tissues, including head mesoderm, notochord, and pharyngeal endoderm. Second, the organiser performs morphogenetic movements and, in addition, induces them in adjacent cells (e.g. convergence and extension in the presumptive notochord and in the somitic mesoderm). The organiser also has a influence on timing of mesodermal and endodermal internalization. Bottle cell formation, involution and vegetal rotation start up to two hours earlier on the organiser side than on the ventral side of the gastrula (Ibrahim and Winklbauer, 2001; Shih and Keller, 1994; Winklbauer and Schürfeld, 1999). Third, the organiser secretes signals which affect all three germ layers of the developing embryo. Most of these signals have been found to antagonize ventralizing signals like BMPs, Wnts, and Nodals (for review (De Robertis and Kuroda, 2004; Niehrs, 1999)). The inhibition of BMP signaling in the ectoderm in combination with FGF signaling leads to formation of a neural plate (Delaune et al., 2004; Linker and Stern, 2004).

The cell movements during gastrulation are an essential part of the time-space translation model. Due to these cell movements, cells are involuted at the blastopore lip. Convergence and extension movements direct cells from the ventral side to the dorsal side and elongate the embryo to make shape changes needed for it to become a tadpole.

With these movements, cells can leave the "Hox induction field" and thereby change their properties so that they do not switch on the expression of new Hox genes, and keep the Hox code they have at the moment that they left this field. The expression of this Hox code in involuted cells is not stable, because embryos with only mesodermal Hox expression show the loss of their anterior boundaries of Hox expression, and will express all Hox genes in

the involuted mesoderm (unpublished observations). This change in behaviour with respect to Hox expression occurs in the blastopore lip and is associated with an epithelial to mesenchymal transition (EMT). After their EMT, the involuted cells show separation behaviour that separates them from the blastocoel wall/roof, which allows the formation of Brachet's cleft and the migration of involuted cells across the blastocoel roof (Wacker et al., 2000). Involuted cells switch on the expression of new genes. One example is Snail, which is expressed in involuted cells but not in non-involuted cells. Its domain of expression overlaps partly with that of Xbra (Fig 3).

It has been shown in *Ciona intestinalis* and Xenopus that Snail is a inhibitor of brachyury expression (Fujiwara et al., 1998; Ibrahim, 2002). The repression of Xbra by Snail combined with the involution movements could be used by cells as a mechanism to fix their Hox code since expression of new Hox genes requires the presence of Xbra (Wacker et al., 2004b). Another possibility is that the involution movements carry cells away from the source of BMP signalling, which is located in the ventral and lateral blastopore lip (Wacker et al., 2004b). In both of these ways, cells are leaving the "Hox induction field".

Hox genes themselves also have influence on the time that the EMT takes place. Iimura and Pourquie showed that the time of ingression can be controled by overexpressing different Hox genes in the chick gastrula's paraxial mesoderm (Iimura and Pourquie, 2006). How all these mechanisms interact to make a spatial pattern out of a time sequence remains to be investigated.

When mesodermal cells are involuted and come to lie underneath the neural plate, expression of the same Hox genes as are expressed in this mesoderm starts in the neurectoderm. It has been debated whether planar signals within the neurectoderm or vertical signals between mesoderm and neurectoderm are responsible for this expression (Nieuwkoop, 1952; Ruiz I Altaba, 1992). Gradients of secreted molecules (FGFs, Wnts, retinoic acid) have been postulated to act in a planar way along the AP axis to define more posterior values (Cox and Hemmati-Brivanlou, 1995; Durston et al., 1989; Kiecker and Niehrs, 2001; Lamb and Harland, 1995).

There is clear evidence that mesoderm and neurectoderm signal to each other (Nieuwkoop and Weijer, 1978). In the experiments described in this paper the differentiation of transplanted gastrula mesoderm was affected by the overlying neurula ectoderm.

A candidate molecule for signalling from the mesoderm to the ectoderm is an active retinoid. Retinoic acid is synthesised in the mesoderm by RALDH2 (Niederreither et al., 1997). Its receptors are mainly found in the neurectoderm (Pfeffer and De Robertis, 1994). In chapter 4 we describe the loss of function of retinoid signalling by a receptor antagonist, and by targeted injection of Cyp26 and CRABP mRNA in the mesoderm. Both treatments fail to disrupt Hox expression in the mesoderm but these treatments dowregulate the early Hox expression in the neurectoderm. These experiments suggest that active retinoids can play a role as messengers that signal from mesoderm to ectoderm. Hox paralogue groups 1-5 respond differentially to retinoids (Dupe and Lumsden, 2001; Godsave et al., 1998). This differential response of pg 1-5 could be used to create different zones of Hox expression in the neurectoderm.

While Hox paralogue groups 1-5 can respond to active retinoids, Hox paralogue groups 6-13 are not retinoid sensitive. These genes could be regulated in the neurectoderm by Cdx genes, which are direct targets of FGF signalling (Isaacs et al., 1998). FGF4 is expressed during gastrulation in the mesoderm, and might play a role as a signalling molecule (Isaacs

et al., 1995). It remains to be investigated if the Cdx family of genes can differentially regulate Hox genes. In Xenopus these genes have largely overlapping expression domains and they do not show a graded expression along the A-P axis during gastrulation (Pillemer et al., 1998; Reece-Hoyes et al., 2002). FGF signalling itself also plays another role in signalling to the ectoderm. It is now clear that next to inhibition of BMP signalling by the Spemann organiser, a low dose of FGF signalling is also needed to induce a neural plate (Delaune et al., 2004; Linker and Stern, 2004).

Another class of candidates for the molecules mediating signalling between mesoderm and neurectoderm are the Hox proteins themselves. There is clear evidence that homeodomain proteins have the ability to travel from cell to cell and that these proteins are used as signalling molecules (Brunet et al., 2005; Derossi et al., 1994). Hooiveld *et al.* have shown that ectodermally overexpressed Hoxb-4 can induce Hoxb-4 and Hoxb-5 expression outside of the injected domain (Hooiveld et al., 1999). If Hox proteins could travel from the mesoderm to the ectoderm they could transfer the mesodermal Hox expression pattern to the neurectoderm by vertical signalling. This would be a simple, robust mechanism to pattern the neurectoderm, using the pattern already established in the mesoderm. With this type of signalling, coordinated expression of Hox genes between mesoderm and neurectoderm is guaranteed, and there is no need for a separate neurectodermal patterning mechanism. It is still to be determined whether Hox protein transfer is used to pattern the neurectoderm.

Apart from their possible role as signalling molecules, Hox genes evidently provide A-P identity to the tissues where they are expressed. Altered Hox expression by various methods in different animal model sytems clearly show a role in tissue specification (for review see (McGinnis and Krumlauf, 1992)). How tissue specification is achieved is only recently, with the advances in "genomics", becoming more clear. Recently microarray data has become available that sheds light on targets that are regulated by Hox genes (Hersh et al., 2007; Hueber et al., 2007; Rohrschneider et al., 2007; Schwab et al., 2006), Van den Akker in press, and this thesis, chapter 5).

In chapter 5 we compared the fly and the frog to identify conserved targets of Antp and/or Hoxc-6 at different developmental stages. The experimental approach chosen makes comparison of targets in very different model systems possible and should allow us to identify targets that are conserved in evolution (as is the A-P patterning role of Hox genes). Our results showed that there are indeed conserved targets of Hox genes. Some of the groups of targets identified can be linked to the visible phenotype of these embryos. The repression of transcription factors that are expressed in the head, and the genes that are involved with cell movements are examples of this. The influence of Antp/Hoxc-6 on cell cycle progression is also a conserved function of these genes. The validity of this appraoch is confirmed by the identification of Prickle as a Hox target in the zebrafish (Rohrschneider et al., 2007). Allthough the conserved targets are indicating important Hox functions, the not conserved Hox targets are also important to study since these point towards Hox functions that are more species specific. In Xenopus the notch signaling pathway is clearly affected by Hoxc-6 overexpression. We found several genes involved in this pathway up or downregulated. This finding is supported by recent findings where a connection was found between Hox genes, Delta-Notch signalling, and segmentation (Peres et al., 2006), Bardine in press).

When several stages are compared, more targets are found in the older stages (Hueber et al., 2007), chapter 5 this thesis). This might reflect the expression of secondary targets that are affected by the changed expression of primary targets. However, if the targets from different stages are compared, only a subset of targets is commonly regulated in different stages (Hueber et al., 2007), chapter 5 this thesis). This suggests that the transcriptional response to Hox genes is to some extent stage dependent.
The Hox response elements, through which Hox genes excert their function, do not show a high Hox specificity in vitro (Ekker et al., 1994). However, precise in vivo regulation of targets is observed, both in time and space (Hueber et al., 2007), chapter 5 this thesis). This suggests that the transcriptional response to Hox proteins depends on the presence of other factors. This is supprted by the expression data of Hoxc-6 targets after overexpression of Hoxc-6. The upregulation of several of these targets occurs within a limited domain even though Hoxc-6 was expressed ubiquitously (Fig. 1 chapter 5).
In this view Hox genes might not be seen as master genes that regulate identity along the A-P axis but as cofactors that act depending on other regulatory factors that are present in specific cells. This also allows for much more regulatory fine tuning, and adaptivity. Unraveling these highly complex regulatory networks of which Hox genes are only a part is the next challenge in understanding how specific structures in the embryo are made.

References

Ang, S. L. and Rossant, J. (1994). HNF-3 beta is essential for node and notochord formation in mouse development. Cell 78, 561-574.

Brunet, I., Weinl, C., Piper, M., Trembleau, A., Volovitch, M., Harris, W., Prochiantz, A. and Holt, C. (2005). The transcription factor Engrailed-2 guides retinal axons. Nature 438, 94-98.

Cox, W. G. and Hemmati-Brivanlou, A. (1995). Caudalization of neural fate by tissue recombination and bFGF. Development 121, 4349-5821.

De Robertis, E. M. and Kuroda, H. (2004). Dorsal-Ventral Patterning and Neural Induction in Xenopus Embryos. Annu.Rev.Cell Dev.Biol.

Delaune, E., Lemaire, P. and Kodjabachian, L. (2004). Neural induction in Xenopus requires early FGF signalling in addition to BMP inhibition. Development.

Derossi, D., Joliot, A. H., Chassaing, G. and Prochiantz, A. (1994). The third helix of the Antennapedia homeodomain translocates through biological membranes. J.Biol.Chem. 269, 10444-10450.

Deschamps, J., van den Akker, E., Forlani, S., De Graaff, W., Oosterveen, T., Roelen, B. and Roelfsema, J. (1999). Initiation, establishment and maintenance of *Hox* gene expression patterns in the mouse. Int.J.Dev.Biol. 43, 635-650.

Dupe, V. and Lumsden, A. (2001). Hindbrain patterning involves graded responses to retinoic acid signalling. Development 128, 2199-2208.

Durston, A. J., Timmermans, J. P., Hage, W. J., Hendriks, H. F., de Vries, N. J., Heideveld, M. and Nieuwkoop, P. D. (1989). Retinoic acid causes an anteroposterior transformation in the developing central nervous system. Nature 340, 140-146.

Ekker, S. C., Jackson, D. G., von Kessler, D. P., Sun, B. I., Young, K. E. and Beachy, P. A. (1994). The degree of variation in DNA sequence recognition among four Drosophila homeotic proteins. EMBO J. 13, 3551-3560.

Eyal-Giladi, H. (1954). Dynamic aspects of neural induction in amphibia. Arch.Biol.(Liege) 65, 179-259.

Fainsod, A., Steinbeisser, H. and De Robertis, E. M. (1994). On the function of BMP-4 in patterning the marginal zone of the Xenopus embryo. EMBO J 13, 5015-5025.

Fujiwara, S., Corbo, J. C. and Levine, M. (1998). The snail repressor establishes a muscle/notochord boundary in the *Ciona* embryo. Development 125, 2511-2520.
Gaunt, S. J. (2000). Evolutionary shifts of vertebrate structures and *Hox* expression up and down the axial series of segments: a consideration of possible mechanisms. Int.J.Dev.Biol. 44, 109-117.
Godsave, S. F., Koster, C. H., Getahun, A., Mathu, M., Hooiveld, M., van der Wees, J., Hendriks, J. and Durston, A. J. (1998). Graded retinoid responses in the developing hindbrain. Dev.Dyn. 213, 39-49.
Harland, R. M. and Gerhart, J. (1997). Formation and function of Spemann's organizer. Annu.Rev.Cell Dev.Biol. 13, 611-667.
Hersh, B. M., Nelson, C. E., Stoll, S. J., Norton, J. E., Albert, T. J. and Carroll, S. B. (2007). The UBX-regulated network in the haltere imaginal disc of D. melanogaster. Dev.Biol. 302, 717-727.
Hooiveld, M. H., Morgan, R., In der Rieden, P., Houtzager, E., Pannese, M., Damen, K., Boncinelli, E. and Durston, A. J. (1999). Novel interactions between vertebrate Hox genes. Int J Dev Biol 43, 665-674.
Hueber, S. D., Bezdan, D., Henz, S. R., Blank, M., Wu, H. and Lohmann, I. (2007). Comparative analysis of Hox downstream genes in Drosophila. Development 134, 381-392.
Ibrahim, H. Funktion und Regulation des Transkriptionsfaktors *Xsna* während der Embryonalentwicklung von *Xenopus laevis*. 2002. Köln, copy team Cologne.
Ref Type: Thesis/Dissertation
Ibrahim, H. and Winklbauer, R. (2001). Mechanisms of mesendoderm internalization in the *Xenopus* gastrula: lessons from the ventral side. Dev.Biol. 240, 108-122.
Iimura, T. and Pourquie, O. (2006). Collinear activation of Hoxb genes during gastrulation is linked to mesoderm cell ingression. Nature.
Isaacs, H. V., Pownall, M. E. and Slack, J. M. (1995). eFGF is expressed in the dorsal midline of Xenopus laevis. Int.J.Dev.Biol. 39, 575-579.
Isaacs, H. V., Pownall, M. E. and Slack, J. M. (1998). Regulation of Hox gene expression and posterior development by the Xenopus caudal homologue Xcad3. EMBO J 17, 3413-3427.
Kiecker, C. and Niehrs, C. (2001). A morphogen gradient of Wnt/beta-catenin signalling regulates anteroposterior neural patterning in *Xenopus*. Development 128, 4189-4201.
Kmita, M. and Duboule, D. (2003). Organizing axes in time and space; 25 years of colinear tinkering. Science 301, 331-333.
Kmita, M., Fraudeau, N., Herault, Y. and Duboule, D. (2002). Serial deletions and duplications suggest a mechanism for the collinearity of Hoxd genes in limbs. Nature 420, 145-150.
Lamb, T. M. and Harland, R. M. (1995). Fibroblast growth factor is a direct neural inducer, which combined with *noggin* generates anterior-posterior neural pattern. Development 121, 3627-3636.
Linker, C. and Stern, C. D. (2004). Neural induction requires BMP inhibition only as a late step, and involves signals other than FGF and Wnt antagonists. Development 131, 5671-5681.
Mainguy, G., Koster, J., Woltering, J., Jansen, H. and Durston, A. (2007). Extensive polycistronism and antisense transcription in the Mammalian hox clusters. PLoS.ONE. 2, e356.
Mangold, O. (1933). Über die Induktionsfähigkeit der verschiedenen Bezirke der Neurula von Urodelen. Naturwissenschaften 21, 761-766.
McGinnis, W. and Krumlauf, R. (1992). Homeobox genes and axial patterning. Cell 68, 283-302.
Niederreither, K., McCaffery, P., Drager, U. C., Chambon, P. and Dolle, P. (1997). Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene during mouse development. Mech.Dev. 62, 67-78.
Niehrs, C. (1999). Head in the WNT: the molecular nature of Spemann's head organizer. Trends.Genet. 15, 314-319.
Nieuwkoop, P. D. (1952). Activation and organisation of the central nervous system in amphibians. III. Synthesis of a new working hypothesis. J.Exp.Zool. 120, 83-108.
Nieuwkoop, P. D. and Weijer, C. J. (1978). Neural induction, a two-way process. Med.Biol. 56, 366-371.

Ober, E. A. and Schulte-Merker, S. (1999). Signals from the yolk cell induce mesoderm, neuroectoderm, the trunk organizer, and the notochord in zebrafish. Dev.Biol. 215, 167-181.
Peres, J. N., McNulty, C. L. and Durston, A. J. (2006). Interaction between X-Delta-2 and Hox genes regulates segmentation and patterning of the anteroposterior axis. Mech.Dev.
Pfeffer, P. L. and De Robertis, E. M. (1994). Regional specificity of RAR gamma isoforms in Xenopus development. Mech.Dev. 45, 147-153.
Pillemer, G., Epstein, M., Blumberg, B., Yisraeli, J. K., De Robertis, E. M., Steinbeisser, H. and Fainsod, A. (1998). Nested expression and sequential downregulation of the *Xenopus caudal* genes along the anterior-posterior axis. Mech.Dev. 71, 193-196.
Reece-Hoyes, J. S., Keenan, I. D. and Isaacs, H. V. (2002). Cloning and expression of the Cdx family from the frog Xenopus tropicalis. Dev.Dyn. 223, 134-140.
Rohrschneider, M. R., Elsen, G. E. and Prince, V. E. (2007). Zebrafish Hoxb1a regulates multiple downstream genes including prickle1b. Dev.Biol. 309, 358-372.
Ruiz I Altaba, A. (1992). Planar and vertical signals in the induction and patterning of the Xenopus nervous system. Development 116, 67-80.
Schier, A. F. and Giraldez, A. J. (2006). MicroRNA function and mechanism: insights from zebra fish. Cold Spring Harb.Symp.Quant.Biol. 71, 195-203.
Schwab, K., Hartman, H. A., Liang, H. C., Aronow, B. J., Patterson, L. T. and Potter, S. S. (2006). Comprehensive microarray analysis of Hoxa11/Hoxd11 mutant kidney development. Dev.Biol. 293, 540-554.
Shih, J. and Keller, R. (1994). Gastrulation in *Xenopus laevis*: involution - a current view. Semin.Dev.Biol. 5, 85-90.
Wacker, S., Grimm, K., Joos, T. and Winklbauer, R. (2000). Development and control of tissue separation at gastrulation in *Xenopus*. Dev.Biol. 224, 428-439.
Wacker, S. A., Jansen, H. J., McNulty, C., Houtzager, E. and Durston, A. J. (2004a). Timed interactions between the Hox expressing non-organiser mesoderm and the Spemann organiser generate positional information during vertebrate gastrulation. Dev.Biol. 268, 207-219.
Wacker, S. A., McNulty, C. L. and Durston, A. J. (2004b). The initiation of Hox gene expression in Xenopus is controlled by Brachyury and BMP-4. Dev Biol 266, 123-137.
Winklbauer, R. and Schürfeld, M. (1999). Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus*. Development 126, 3703-3713.
Woltering, J. M. and Durston, A. J. (2008). MiR-10 represses HoxB1a and HoxB3a in zebrafish. PLoS.ONE. 3, e1396.
Yekta, S., Shih, I. H. and Bartel, D. P. (2004). MicroRNA-directed cleavage of HOXB8 mRNA. Science 304, 594-596.
Zoltewicz, J. S. and Gerhart, J. C. (1997). The Spemann organizer of *Xenopus* is patterned along its anteroposterior axis at the earliest gastrula stage. Dev.Biol. 192, 482-491.