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Generation of the germ layers along the animal-vegetal axis in *Xenopus laevis*

HITOYOSHI YASUO* and PATRICK LEMAIRE

Laboratoire de Génétique et Physiologie du Développement. Institut de Biologie du Développement de Marseille, CNRS-INSERM-Universite de la Méditerannée, Marseille, France

ABSTRACT After completion of gastrulation, typical vertebrate embryos consist of three cell sheets, called germ layers. The outer layer, the ectoderm, which produces the cells of the epidermis and the nervous system; the inner layer, the endoderm, producing the lining of the digestive tube and its associated organs (pancreas, liver, lungs etc.) and the middle layer, the mesoderm, which gives rise to several organs (heart, kidney, gonads), connective tissues (bone, muscles, tendons, blood vessels), and blood cells. The formation of the germ layers is one of the earliest embryonic events to subdivide multicellular embryos into a few compartments. In *Xenopus laevis*, the spatial domains of three germ layers are largely separated along the animal-vegetal axis even before gastrulation; ectoderm in the animal pole region; mesoderm in the equatorial region and endoderm in the vegetal pole region. In this review, we summarise the recent advances in our understanding of the formation of the germ layers in *Xenopus laevis*.

KEY WORDS: *Xenopus laevis, animal-vegetal axis, germ layers, cell and non-cell autonomy, VegT.*

Specification of the animal-vegetal axis during oogenesis

As discussed later, maternal information, which is distributed differentially along the animal-vegetal (A-V) axis during oogenesis, plays a critical role in formation of the embryonic germ layers. In this section, we summerise how this axis is established. In *Xenopus*, the animal-vegetal axis is established during oogenesis (reviewed in Gard, 1995). Several observations indicate that the A-V polarity is not dependent on external factors. First, the oocytes are oriented in the ovary at random with respect to gravity. In addition, the polarity of the A-V axis bears no relation to the ovarian walls or blood vessels. These observations suggest that the A-V axis is specified intrinsically. A primary oogonium goes through four incomplete mitotic divisions to form a nest of 16 oocytes, which remain connected at the centre of the nest by cytoplasmic bridges which result from incomplete cytokinesis. The postmitotic oocytes in the nest all exhibit a distinct polar arrangement of subcellular organelles, where Golgi structures locate closest to the centre of the nest and then mitochondria mass (which harbors the centriole) and nucleus are arranged progressively more distant from the centre. After completion of the pachytene stage of meiotic prophase, however, little evidence remains of this initial oocyte polarity. The mitochondria cloud, a mitochondria rich area also called the Balbiani body, is subsequently formed in mid-stage I oocytes. Some vegetally-localised maternal transcripts such as *Xcat2* localise to the mitochondria cloud in stage I oocytes and are later transported to the vegetal cortex (reviewed in King *et al.,* 1999). The position of the mitochondria cloud in the cytoplasm of stage I oocyte thus represents one of the earliest known markers of the definitive A-V axis. However, it remains to be addressed whether there is a relationship between the initial axis of younger oocytes and position of the mitochondria cloud of stage I oocytes.

As briefly described above, some maternal transcripts localise to the vegetal cortex of oocytes during *Xenopus* oogenesis. Two pathways have been described that target maternal RNAs to the vegetal pole; mitochondria cloud-dependent pathway and microtubule-dependent pathway (reviewed in King *et al.,* 1999). Most of the RNAs, which are targeted to the vegetal cortex through the mitochondria cloud-dependent pathway, are segregated with the germ plasm in primordial germ cells (PGCs) and likely encode proteins required for germ cell formation. In this pathway, RNAs localise to the mitochondria cloud in stage I oocytes. Then, the RNAs are segregated to a small domain at the vegetal cortex during stage II. In contrast, maternal transcripts for *Vg1* and *VegT*, which encode a

Abbreviations used in this paper: FGF, fibroblast growth factor; MBT, mid-blastula transition; TGFβ, transforming growth factor β.

***Address correspondence to:** Hitoyoshi Yasuo. Laboratoire de Biologie du Développement, Station Zoologique, Observatoire Océanologique, BP28, 06234 Villefranche-sur-mer, France. FAX: +33 (0)-493-763-792. e-mail: yasuo@obs-vlfr.fr

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TGFβ ligand and a T-box transcription factor, respectively, localise to the vegetal cortex through a microtubule-dependent pathway during stage III. From stage IV through VI (the terminal stage of oogenesis), these transcripts spread along the entire length of the vegetal cortex. Thus, these pathways allow some maternal transcripts encoding molecules critical for development to segregate to one pole of the oocyte. The discoveries of the two vegetally localised transcripts mentioned above, *Vg1* and *VegT*, have had significant impacts on our understanding of how the three germ layers are generated. In the next section, we provide an overview of mesoderm and endoderm formation in early embryos.

Overview of mesoderm and endoderm formation in *Xenopus laevis*

A series of initial experiments by Nieuwkoop suggested that a signal released by vegetal pole cells induces the overlying prospective ectoderm to form mesoderm in the marginal zone, thus generating the three germ layers of amphibian embryos (reviewed in Nieuwkoop, 1977) (Fig. 1A). He showed that isolated animal pole explants, which otherwise develop into epidermis, can be induced to form mesoderm when combined with vegetal pole explants. Following experiments indicated that cell-cell interactions are indeed required for endogenous mesoderm formation. Marginal zones dissected from mid or late blastula embryos express mesoderm markers (in this experiment, a possible contamination of presumptive endoderm cells was not examined). However, if marginal zones are dissociated, the expression of a pan-mesodermal gene, *Xbra*, is completely abolished by the early gastrula stage (Lemaire and Gurdon, 1994; Sokol, 1994). The TGFβ and FGF families of secreted growth factors mimic the mesoderm-inducing signal (Kimelman *et al.,* 1992). They are able to induce mesoderm when applied to animal pole explants. Conversely, several studies using dominant-negative forms of receptors for these secreted factors have shown that these signals are required for endogenous mesoderm formation.

Molecules of the TGFβ family also have an endoderm-inducing activity, and are required for normal endoderm formation (Gamer and Wright, 1995; Henry *et al.,* 1996). In *Xenopus*, the endoderm germ layer derives from a large area at the vegetal pole. Therefore, it is feasible to isolate prospective endoderm region without a contamination of the other regions, which lead to the following findings of the embryological basis of endoderm formation. The fate of vegetal pole blastomeres becomes restricted to endoderm around the mid-blastula stage (Heasman *et al.,* 1984). At this stage, vegetal pole blastomeres transplanted to an ectopic environment will adopt the fate of their new neighbours, indicating that they are not yet determined to an endodermal fate (Heasman *et al.,* 1984). During the late blastula period, however, an increasing number of vegetal pole blastomeres follow an endoderm differentiation pathway even when transplanted to an ectopic environment. In other words, the fate of vegetal pole blastomeres is progressively determined to endoderm (Wylie *et al.,* 1987). By the beginning of gastrulation, vegetal pole blastomeres all become determined to the endoderm fate. Determination implies that a certain embryonic blastomere has activated its genetic program and can develop autonomously into a determined fate. Vegetal pole blastomeres isolated and incubated *in vitro* continue the endodermal determination process only when an appropriate cell mass is present, suggesting that cell-cell communication is required for this process (Wylie *et al* 1987), which might be mediated by TGFβ signals.

The mesoderm-inducing signal has been reported to be present in vegetal pole cells as early as the 32-cell stage (Jones and Woodland, 1987), a few hours before the onset of zygotic transcription, which resulted in the widely accepted view that the endogenous mesoderm-inducing signal must be present as a maternal transcript or protein encoding a secreted factor. Vg1 has been considered to be a good candidate for the endogenous mesoderm- and endoderm-inducing factor, since it belongs to a TGFβ family, the processed form of Vg1 has mesoderm- and endoderm-inducing activity, and its maternal transcripts are localised to the vegetal cytoplasm during oogenesis (Weeks and Melton, 1987). However,

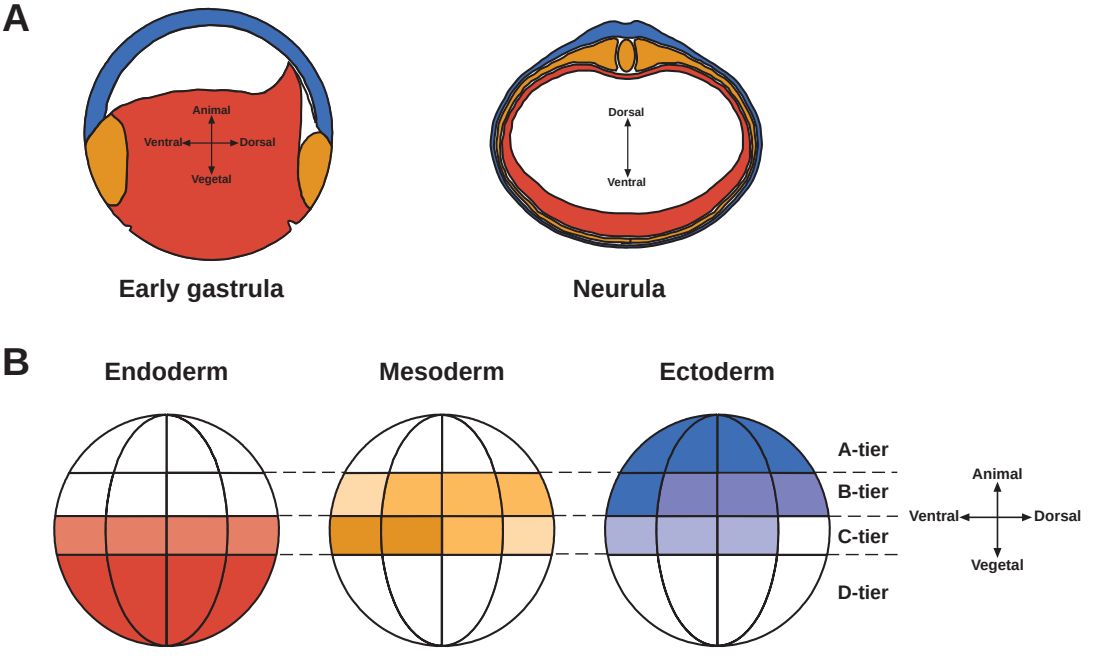

Fig. 1. Three germ layers and fate maps of the 32-cell embryo in *Xenopus laevis*. (A) *Spatial arrangement of the three germ layers in the early gastrula (left) and neurula (right) embryos. A midsagittal view of the early gastrula embryo is shown, together with a transverse view of the neurula embryo. Ectoderm is in blue, mesoderm in orange and endoderm in red.* **(B)** *Fate maps of the 32-cell Xenopus embryo. Each diagram illustrates a lateral view. The 32-cell embryos consist of four tiers of eight blastomeres, each of which is named the A-, B-, C-, and D-tier, respectively, along the animal-vegetal axis. Darker coloured blastomeres make a greater contribution to the germ layer than more lightly coloured blastomeres.*

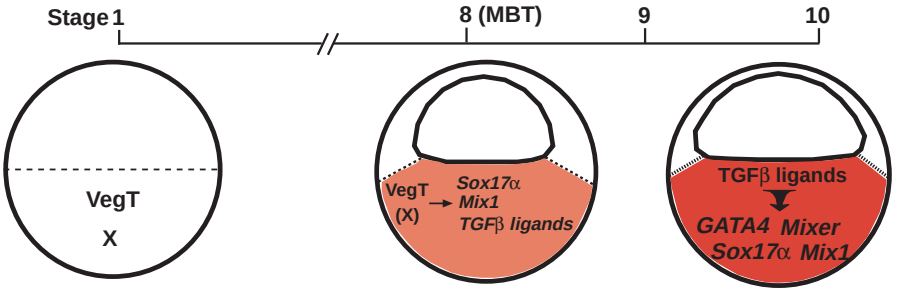

Fig. 2. Two step model for endoderm formation, *which consists of 1) cell autonomous gene activation at MBT and subsequently 2) non-cell autonomous gene activation and amplification during the late blastula period. MBT, mid-blastula transition. See text for detail.*

the mature form of Vg1 has never been detected *in vivo*, and wild type *Vg1* does not induce mesoderm or endoderm when ectopically expressed in the prospective ectoderm (Dale *et al.,* 1989; Tannahill and Melton, 1989). These results might indicate that the mature form of Vg1 is present only at undetectable levels *in vivo* and also that the processing of Vg1 precursor protein is tightly regulated. Experiments using a dominant-negative mutant of Vg1 have suggested that Vg1 is rather involved in formation of dorsal mesoderm and dorsal endoderm. Embryos expressing the mutant forms of Vg1 form lateral and ventral mesoderm (effects on the other parts of endoderm were not addressed) and also show normal expression pattern of a pan-mesoderm gene, *Xbra* (Joseph and Melton, 1998). These results suggest that other TGFβ family members must be involved in the formation of mesoderm and endoderm.

While the lack of a *bona fide* role for Vg1 in mesoderm and endoderm formation was somewhat disappointing, a newly discovered transcription factor bears many interesting properties. This factor is *VegT*, a T-box transcription factor, whose maternal transcripts are tethered to the vegetal cortex of *Xenopus* oocytes (Lustig *et al.,* 1996; Stennard, *et al.,* 1996; Zhang and King; 1996; Horb and Thomsen, 1997). Translated products are confined to the vegetal hemisphere of cleaving embryos (Stennard at al, 1999). Experiments involving depletion of maternal *VegT* transcripts have convincingly demonstrated the central role of VegT in establishing the mesoderm and endoderm germ layers (Zhang *et al.,* 1998; Kofron *et al.,* 1999). Embryos derived from *VegT*-depleted oocytes do not form any type of mesoderm and endoderm. Since zygotic transcription starts only at the mid-blastula stage (this embryonic event is called the mid-blastula transition; MBT), it is most likely that maternal VegT, a transcription factor, exerts its role through activating the expression of zygotic genes at MBT. Furthermore, it has been shown that vegetal pole explants derived from VegT-depleted embryos do not have a mesoderm-inducing capacity (Zhang *et al.,* 1998). These results challenged the widely accepted view of mesoderm induction by a maternal signalling molecule, and showed that zygotic products acting downstream of VegT are required for the endogenous mesoderm-and endoderm-inducing activities.

TGFβ (activin-like) signal and formation of mesoderm and endoderm: post-MBT events

Several data indicate that cell-cell interactions required for mesoderm and endoderm formation are mediated by activin-type and/or nodal-type TGFβ signals. Dominant-negative forms of activin type I or type II receptor block the expression of several endoderm and mesoderm markers (Hemmati-Brivanlou and Melton, 1992; Chang *et al.,* 1997; Gamer and Wright, 1995; Henry *et al.,* 1996; Yasuo and Lemaire, 1999; Chang and Hemmati-Brivanlou, 2000). Recently, the temporal profile as well as the spatial domain of endogenous activation of the activin-like signalling pathway have been revealed by taking advantage of an antibody specific for a phosphorylated (activated) form of Smad2, an intracellular mediator of activin-like signals (Faure *et al.,* 2000). The activin-like signalling pathway is activated after MBT in equatorial and vegetal regions in *Xenopus* embryos. Treatment of pre-MBT embryos with exogenous activin proteins resulted in activation of this pathway, indicating that downstream components are already present before MBT. Furthermore, inhibition of zygotic transcription by α-amanitin (inhibitor for RNA polymerase II) abolishes endogenous activation of this pathway, which can be rescued by addition of activin-like ligands. These results altogether strongly suggest that ligands activating the endogenous activin-like signalling pathway are of zygotic origin. Consistently, cell dissociation experiments have revealed that cell-cell interactions after MBT are required for the expression of the zygotic endoderm and mesoderm genes while pre-MBT cell contacts are dispensable (Yasuo and Lemaire, 1999; Chang and Hemmati-Brivanlou, 2000). Furthermore, Activin or Vg1, but not BMP2 or basic FGF rescue the expression of the zygotic endoderm genes in dissociated embryos, when provided after MBT (Chang and Hemmati-Brivanlou, 2000).

Several zygotic genes encoding activin-like ligands have been identified. Among them are *derrière*, *Xnr1*, *Xnr2* and *Xnr4*, which are transiently expressed in vegetal hemisphere of late blastula embryos, and *activinβb*, which is expressed ubiquitously (Sun *et al.,* 1999, Jones *et al.,* 1995; Joseph and Melton, 1997; Dohmann *et al.,* 1993; Clements *et al.,* 1999). In addition, ectopic expression experiments show that these TGFβ ligands are able to induce mesoderm and endoderm markers. Therefore, these zygotic TGFβ ligands are most likely responsible for the endogenous activation of the activin-like signalling pathway. A role of zygotic TGFβ signals has also been suggested by genetic studies in zebrafish and mouse. In zebrafish, double mutant embryos for the genes encoding nodal-related molecules, *cyclops* and *squint*, fail to develop both mesoderm and endoderm (Feldman *et al.,* 1998). Mouse mutant embryos for *nodal* display no morphological evidence for the formation of a primitive streak (Zhou *et al.,* 1993; Conlon *et al.,* 1994).

Endoderm specification: cell autonomous and non-cell autonomous aspects

The recent identification of zygotic endoderm genes has facilitated the drawing of a molecular pathway leading to endoderm formation. Zygotic endoderm genes include *Sox17α* and *β*, *Mix.1*, *Mixer*, *Milk* (also named *Bix2*), *Bix1/3/4*, *HNF1β* and *GATA4/5/6*. *Sox17s* encode HMG-domain-containing proteins (Hudson *et al.,* 1997), *Mix.1*, *Mixer*, *Milk* and *Bix1/3/4* are paired-like homeobox

proteins (Rosa, 1989; Henry and Melton, 1998; Ecochard *et al.,* 1998; Tada *et al.,* 1998), *HNF1β* is a divergent homeobox protein (Vignali *et al.,* 2000) and *GATAs* encode proteins with zinc-finger motifs (Jiang and Evans, 1996), therefore indicating that they are involved in transcriptional control. By the beginning of gastrulation, when vegetal pole blastomeres become determined to endodermal cell fate, the expression level of these genes reaches its peak. Ectopic expression experiments indicate that each of them has an endoderm-inducing activity (an endoderm-inducing activity of Bix3 and 4 have not yet been reported; Mix.1 is able to induce endodermal markers in the animal pole region only when co-injected with another homeobox gene, Siamois) and dominant-negative forms of Sox17s, Mix.1 and Mixer disrupt endoderm formation (Hudson *et al.,* 1997; Henry and Melton, 1998; Lemaire at al, 1998). Thus, in *Xenopus,* cell fate commitment of the vegetal pole blastomeres to endoderm coincides with the activation of the zygotic transcription factors which have an ability to promote the endoderm differentiation pathway. Requirement of these genes for endoderm formation has also been shown in genetic analyses of zebrafish mutants for *GATA5* (*faust*) and *Mix.1*-like gene (*bonnie and clyde*) (Kikuchi *et al.,* 2000; Reiter *et al.,* 1999).

When the post MBT cell-cell interactions are blocked by means of cell dissociation, expression of the zygotic endoderm genes as well as zygotic TGFβ genes is either completely abolished (*Mixer* and *GATA4*) or still activated but greatly reduced (*Sox17s*, *Mix.1*, *Xnr1*, *Xnr2*, *Xnr4* and *derrière*) (Yasuo and Lemaire, 1999; Clements *et al.,* 1999; Chang and Hemmati-Brivanlou, 2000). A similar effect is also observed when dominant-negative forms of activin type I or II receptor are overexpressed in vegetal pole region (Yasuo and Lemaire, 1999; Chang and Hemmati-Brivanlou, 2000). This suggests that transcription of *Mixer* and *GATA4* is regulated totally non-cell autonomously, while the latter genes are to some extent activated cell autonomously and their expression is maintained via cell-cell interactions. Furthermore, even in the absence of protein synthesis during post-MBT period, *Sox17α*, *Mix.1*, *Xnr1*, *Xnr2*, and *derriere* are still activated (expression of *Xnr4* has not yet been tested in this experimental condition) (Yasuo and Lemaire, 1999). This indicates that the molecules which activate these genes are maternally derived. Hence, the picture that emerges from the data so far described is that, at the onset of zygotic transcription, maternal cytoplasmic factors activate cell-autonomously the gene expression of some transcription factors (*Sox17s* and *Mix.1*) as well as TGFβ ligands (*derrière*, *Xnr1*, *Xnr2*, and *Xnr4*). Subsequently, during late blastula stage, TGFβ ligands activate *Mixer* and *GATA4* non-cell autonomously and also amplify the expression of all the genes (Fig. 2).

The best candidate for the maternal cytoplasmic factors is maternal VegT. As mentioned above, maternal VegT-depleted embryos form no endoderm and mesoderm (Zhang *et al.,* 1998; Kofron *et al.,* 1999). Since VegT seems to act as a transcriptional activator (Horb and Thomsen, 1997), the cell autonomous activation of zygotic endodermal genes could be mediated by maternal VegT. Accordingly, ectopic expression experiments have shown that VegT acts both cell autonomously for activation of zygotic endodermal genes such as *Sox17s*, *Mix.1*, *derrière*, *Xnr2* and *Xnr4* and non cell autonomously, through activin-like signals, for activation of *Mixer* and also for amplification of *Mix.1* and *Xnr2* (Yasuo and Lemaire, 1999; Clements *et al.,* 1999, Chang and Hemmati-Brivanlou, 2000). Upstream region analyses have shown that *Bix4* is a direct target of T-box transcription factors (Tada *et al.,* 1998; Casey *et al.,* 1999). Since expression of *Bix4* is first activated in the vegetal hemisphere, and furthermore the expression of *Bix4* is abolished in VegT-depleted embryos, it is most likely that *Bix4* is directly activated by maternal VegT in the presumptive endodermal region (Casey *et al.,* 1999). The upstream region of *Xnr1* also contains a VegT-binding site and a putative binding site for distinct T-box transcription factors, as well as two Wnt response elements (Hyde and Old, 2000; Kofron *et al.,* 1999). Experiments in which cell-cell interactions or protein synthesis during the post-MBT period is blocked, have shown that *Xnr1* is to some extent activated cell autonomously by maternal factors (Yasuo and Lemaire, 1999). These results suggest that *Xnr1* is a direct target of maternal VegT. Consistently, *Xnr1* is not activated in VegT-depleted embryos (Kofron *et al.,* 1999). However, the situation does not seem to be this simple. The upstream region of *Xnr1* which contains the two target sites for T-box transcription factors is not sufficient to drive expression of a reporter gene in the vegetal region, although VegT overexpressed in animal caps is able to promote expression of the reporter gene (Kofron *et al.,* 1999; Hyde and Old, 2000). This indicates that additional maternal factors could also be involved in the endogenous activation of *Xnr1*. Nonetheless, one of the most important aspects of endoderm formation is that the initial cell-autonomous activation of early endoderm genes by maternal determinants including, but not limited to VegT, is relayed by the action of zygotic TGFβ ligands such as Derriere or the Xnrs (Fig. 2).

Maternal VegT proteins disappear by the beginning of gastrulation, when vegetal pole blastomeres become committed to endoderm (Stennard *et al.,* 1999), and also endogenous activin-like signals start to decrease considerably after this time (Faure *et al* 2000). Consistently, expression of most of the early zygotic endodermal genes vanishes by the end of gastrulation, much before expression of region-specific endodermal genes, such as genes encoding the liver fatty acid binding protein (Henry and Melton, 1998), the intestinal fatty acid binding protein (Henry *et al.,* 1996; Shi and Hayes, 1994), and *Xlhbox-8* (also known as *Pdx1*), a pancreatic homeobox gene (Wright *et al.,* 1988). However, *Sox17α*, *HNF1β,* and *GATAs* continue to be expressed in the committed endoderm region even after gastrulation (Hudson *et al.,* 1997; Vignali *et al.,* 2000; Jiang and Evans, 1996), indicating establishment of a new transcriptional circuit for the expression of these genes. Therefore, these transcription factors could be involved both in initiation and maintenance of the endodermal lineage.

Mesoderm specification

After the seminal work by Nieuwkoop (Nieuwkoop, 1969), the widely accepted model for mesoderm formation has been that mesoderm is induced in the equatorial region by signals from vegetal pole cells. However, this model could be too simplistic. A fate map is available for the 32-cell stage of *Xenopus laevis* (Dale and Slack, 1987) (Fig. 1B). The 32-cell embryo consists of four tiers of eight blastomeres along the animal-vegetal axis. From the animal pole, they are named A, B, C and D, respectively. A large part of endoderm derives from the D-tier, while mesoderm originates mainly from the B- and C-tiers. The C-tier encompasses both mesoderm and endoderm fates. Since a fate map with a high

resolution does not exist for later stages, it is not known when these cell fates are segregated. Gurdon and colleagues showed that the subequatorial region, which should correspond to the C-tier, contains all components necessary for expression of a muscle gene (Gurdon *et al.,* 1985). Consistently, embryos lacking the D-tier form normal mesoderm (Kageura, 1995). However, the animal half of the 8-cell embryo, which subsequently divides to give rise to the A- and B-tiers, rarely adopts mesodermal fates (Kageura and Yamana, 1986). These results suggest that the vegetal pole cells (D-tier) are dispensable for mesoderm formation and also that mesoderm formation in B-tier descendants is dependent upon signals from C-tier descendants.

As mentioned earlier, post-MBT cell-cell interactions are required and sufficient for expression of pan-mesodermal gene, *Xbra*, (Yasuo and Lemaire 1999) and inhibition of activin-like as well as FGF signals results in defective mesoderm formation (Hemmati-Brivanlou and Melton, 1992; Chang *et al.,* 1997; Amaya *et al.,* 1991). Therefore, zygotic TGFβ ligands seem to be involved in mesoderm as well as endoderm formation. Some of the zygotic activin-like genes seem to be cell autonomously regulated by maternal VegT. However, VegT proteins are not detected in the animal half of embryos (Stennard *et al.,* 1999). Therefore, mesoderm formation in B-tier descendants would be mediated by a non-cell autonomous action of VegT, probably via the activity of zygotic TGFβ ligands and/or FGF (the developmental role of FGF is discussed later).

How is then mesoderm generated from C-tier, which normally gives rise to mesoderm as well as endoderm? In zebrafish, a cell fate mapping study has shown that marginal cells of zebrafish blastula embryos (40% epiboly) frequently gives rise to both mesodermal and endodermal derivatives (Warga and Nusslein-Volhard, 1999). Furthermore, marginal cells of the early blastula embryo (30% epiboly) express both endoderm (*gata5*) and mesoderm (*no tail*; *Brachyury* homologue) genes. By the beginning of gastrulation (50% epiboly), the expression domain of *no tail* extends more distant from the margin, while that of *gata5* is rather confined to the margin (Rodaway *et al.,* 1999). These results suggest that marginal cells are first specified as “mesendoderm” and the two cell fates are subsequently segregated during gastrulation. In *Xenopus* embryos, this may be also the case for formation of mesoderm and endoderm in C-tier descendants, where VegT may specify “mesendoderm” directly and/or indirectly through zygotic TGFβ ligands. Verification of this issue awaits detailed comparative analyses of gene expression patterns for early endoderm and mesoderm genes, as well as a fate map study with single cell resolution, in late blastula and early gastrula embryos.

The FGF signal: maintenance and/or amplification of mesoderm identity

Members of the FGF family of secreted growth factors were first identified as potential candidates for the mesoderm-inducing factor. bFGF is able to induce mesoderm in animal pole explants, although the induced mesoderm is primarily of ventral type (Kimelman and Kirschner, 1987; Slack *et al.,* 1987). A dominant-negative form of FGF receptor prevents formation of ventral as well as some dorsal mesoderm, leading to a lack of trunk and tail mesoderm in resultant embryos (Amaya *et al.,* 1991). Several observations have shown that FGF signalling is involved rather in maintenance and/or amplification of mesoderm identity but not in the first steps of mesoderm induction. Expression of *Xbra* in marginal zone tissues excised from early gastrula embryos is not maintained when the tissues are dissociated, but expression is restored by application of FGF protein (Isaacs *et al.,* 1994; Schulte-Merker and Smith, 1995). Furthermore, *Xbra* is transiently activated in activin-treated animal caps even when FGF signals are blocked (Schulte-Merker and Smith, 1995). Consistent with this view, *eFGF*, a member of the FGF family, is expressed in marginal zone of gastrulating embryos (Isaacs *et al.,* 1992; Casey *et al.,* 1998).

Several studies have suggested that maintenance and/or amplification of mesoderm identity by FGF signal are mediated by two T-box transcription factors, *Xbra* and *Antipodean* (*Apod*), and Derriere during gastrulation. *Apod* is a splicing variant of *VegT* and is zygotically expressed first in the entire marginal zone and then excluded from the involuting axial mesoderm (Stennard *et al.,* 1999). Expression domains of *derrière* include the vegetal hemisphere of late blastulae as well as marginal zone of gastrula embryos (Sun *et al.,* 1999). The later expression domain appears to be identical to that of *Apod*. Ectopic expression studies in animal pole explants showed that each of these molecules (eFGF, Xbra, Apod and Derrière) is able to activate expression of the other (Isaac *et al.,* 1994; Horb and Thomsen, 1997; Sun, *et al.,* 1999). Furthermore, a binding site for T-box transcription factors is present in the upstream region of *eFGF*(Casey *et al.,* 1998). Therefore, these factors are likely to establish an autoregulatory loop within the marginal zone of the gastrulating embryo, which might serve for maintenance and/or amplification of the initial mesoderm identity.

How to restrict mesoderm from endoderm

At the late blastula stage (stage 9-9.5), mesoderm genes such as *Eomesodermin* (*Eomes*) and *Xbra* are already expressed around the equatorial region, but not in the vegetal pole region (expression domain of *Eomes* expands more vegetally than that of *Xbra*) (Ryan *et al.,* 1996; Panitz *et al.,* 1998). Therefore, there must be a mechanism to restrict the initial activation of these genes to the equatorial region. Kimelman and colleagues challenged this issue by proposing that FGF signal might act as a competence factor which cooperates with activin-like signals to form mesoderm in the marginal zone (Cornell *et al.,* 1995). In their model, activin-like signals are restricted to the vegetal and equatorial regions, while FGF signals are restricted to the animal and equatorial regions. Thus an overlap of activin-like and FGF signals would specify mesoderm at the equatorial region. Consistently, FGF-treated vegetal pole explants express mesoderm genes and also repress expression of a late endoderm gene (Cornell *et al.,* 1995; Gamer and Wright, 1995). However, the issue of the presence of FGF signals acting in the blastula endoderm is still under dispute (LaBonne and Whitman, 1997; Christen and Slack, 1999) and loss of function studies will be essential to answer this question. Interestingly, promoter analyses of *Xbra* gene indicated that repressors in the vegetal and animal pole region prevent activation of *Xbra* in these region (Lerchner *et al.,* 2000). Further studies on transcriptional regulation of mesoderm genes will provide more details on the mechanisms underlying the spatial restriction of mesoderm gene expression in the equatorial region.

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