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Region-Specific Regulation of Posterior Axial Elongation During Vertebrate Embryogenesis

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Background: The vertebrate body axis extends sequentially from the posterior tip of the embryo, fueled by the gastrulation process at the primitive streak and its continuation within the tailbud. Anterior structures are generated early, and subsequent nascent tissues emerge from the posterior growth zone and continue to elongate the axis until its completion. The underlying processes have been shown to be disrupted in mouse mutants, some of which were described more than half a century ago. Results: Important progress in elucidating the cellular and genetic events involved in body axis elongation has recently been made on several fronts. Evidence for the residence of self-renewing progenitors, some of which are bipotential for neurectoderm and mesoderm, has been obtained by embryo-grafting techniques and by clonal analyses in the mouse embryo. Transcription factors of several families including homeodomain proteins have proven instrumental for regulating the axial progenitor niche in the growth zone. A complex genetic network linking these transcription factors and signaling molecules is being unraveled that underlies the phenomenon of tissue lengthening from the axial stem cells. The concomitant events of cell fate decision among descendants of these progenitors begin to be better understood at the levels of molecular genetics and cell behavior. Conclusions: The emerging picture indicates that the ontogenesis of the successive body regions is regulated according to different rules. In addition, parameters controlling vertebrate axial length during evolution have emerged from comparative experimental studies. It is on these issues that this review will focus, mainly addressing the study of axial extension in the mouse embryo with some comparison with studies in chick and zebrafish, aiming at unveiling the recent progress, and pointing at still unanswered questions for a thorough understanding of the process of embryonic axis elongation. *Developmental Dynamics 243:88–98, 2014.* © 2013 Wiley Periodicals, Inc.

Key words: vertebrate axial growth; posterior body elongation; axial progenitors for trunk tissues; transcription factors and signaling pathways in axial growth

Key findings:

- Morphogenesis of anterior to posterior body regions depends on different rules
- Bipotent self-renewing axial progenitors ensure the growth of trunk tissues
- These progenitors cannot be visualized by unique markers
- The niche of these progenitors is key to their properties
- The genetic network underlying axial growth comprises transcription factors such as T Brachyury, Sox2 and Hox-like proteins, and signaling pathways by Wnt, Fgf and RA.

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PROGRESSIVE ANTERIOR TO POSTERIOR BODY DEVELOPMENT IN VERTEBRATES

Vertebrates as most bilaterians develop progressively from anterior to posterior. The cephalic structures and the rostral trunk develop first from the anterior epiblast and the mesoderm and endoderm that have arisen early from the primitive streak. At the late primitive streak stage (embryonic day 7.2, E7.2) in the mouse and at definitive streak stage in the chicken (stage 4 according to Hamburger and Hamilton, HH), the streak region will continue contributing descendants for the extending trunk and tail (Schoenwolf, 1977; Kinder et al., 1999). This process corresponds to what has been called the primary body formation. Around closure of the posterior neuropore in both mouse (around E10.0, after some 30 somite pairs have been generated) and chick embryos (at about 14 HH, and 22 somite pairs), tissue emergence will take place from the tailbud rather than from the primitive streak that becomes internalized. The anterior streak/node region subsists as chordo-neural hinge (CNH), and the rest of the streak as ventral ectodermal ridge (VER). Tissue generation for the posteriorly extending lumbosacral region and tail then occurs from the CNH. This phase has been called secondary body formation. Some of the morphogenetic movements during these processes, and gene expression associated with this phase of body elongation, suggest that axial extension from the tailbud is the continuation of the earlier process of trunk elongation (Gont et al., 1993; Benazeraf and Pourquie, 2013). In agreement with this idea, mutations affecting the process of axial elongation were shown to affect both the primary and secondary body formation (see below). However, secondary body elongation differs from the primary phase by the fact that the underlying morphological mechanisms rely less on convergence extension and ingression than on the expansion of the three embryological derivatives from progenitors residing in the CNH niche. For instance, the extending neural tube arises by cavitation rather than by lateral elevation and closure of a neural plate. In the following sections, we review extensive evidence that the modalities of vertebrate development differ during the laying down of anterior and more posterior tissues, and that there are different rules behind tissue morphogenesis in the successive subregions of the axis.

AXIAL PROGENITORS IN THE EMBRYONIC GROWTH ZONE

Cell labeling in the node and primitive streak of the chick embryo have long suggested the existence of stem cells at these locations (Selleck and Stern, 1991, 1992). Subsequent experiments following the contribution of single cells in the early somite chick embryo by time-lapse imaging confirmed that stem cell–like progenitors remained in the posterior epiblast whereas their descendants found themselves more anteriorly in the extending neural axis (Mathis et al., 2001). In the mouse, clonal analysis of single epiblast cells in the gastrulating embryo revealed cases of epiblast cells in the anterior primitive streak that contributed descendants along the axis, including some that remained in the node region after a one-day culture period (Lawson et al., 1991; Forlani et al., 2003). Some of these progenitors just posterior to the node were found to give descendants in both neurectoderm and mesoderm (Forlani et al., 2003). Longer-term studies by retrospective lineage analysis indicated that some axial progenitors give rise to descendants in differentiated tissues spanning a large rostro-caudal distance and extending back to the node region (Nicolas et al., 1996; Mathis and Nicolas, 2000). The last 10 years have seen major progress in the characterization of axial tissue generation from the primitive streak and tailbud. Serial grafting experiments in early somite mouse and chick embryos demonstrated that stem cell–like progenitors reside in a stem zone located in a small region between the node and the anterior primitive streak (node-streak border, NSB; Fig. 1) and subsequently in the tailbud CNH (Cambray and Wilson, 2002, 2007; McGrew et al., 2008). These cells contribute descendants for long periods of embryogenesis, to all levels of the elongating trunk in the neural tube and mesoderm. Heterotopic grafts suggested that the properties of these stem cell–like axial progenitors were conferred by the embryonic position rather than being inherent to the cells (Cambray and Wilson, 2007; McGrew et al., 2008). Some cells of the caudolateral epiblast (CLE, Fig. 1) indeed will contribute to the stem cell–like axial progenitor population after they have been moved to the NSB by the morphogenetic movement of gastrulation (Cambray and Wilson, 2007). A recent retrospective clonal analysis in the mouse revealed that the only progenitors generating clonal cell populations colonizing most levels of the axis including the stem zone/CNH are bipotent progenitors for both mesoderm and neurectoderm, which reside in the posterior part of the embryo (Tzouanacou et al., 2009). In addition to questioning the dogma concerning the order of derivation of the three germ layers during gastrulation, this study demonstrated that the mode of generation of mesoderm and neural descendants from bipotent progenitors remains the same all along the trunk. These data constitute a solid argument in favor of tailbud development being a continuation of the gastrulation process. Nevertheless, several subclasses of clones descending from single neuro-mesodermal progenitors could be distinguished, revealing a change in composition of the axial progenitor pool active during trunk to tail development (Tzouanacou et al., 2009). Most of the clones contribute descendants to the CNH region where the progenitors reside, but the anterior border of the contribution is variable. The number of clones with an anterior limit of the position of their descendants in the trunk region was higher than that at head and tail levels. This points to an increase in the number of neuromesodermal progenitors between

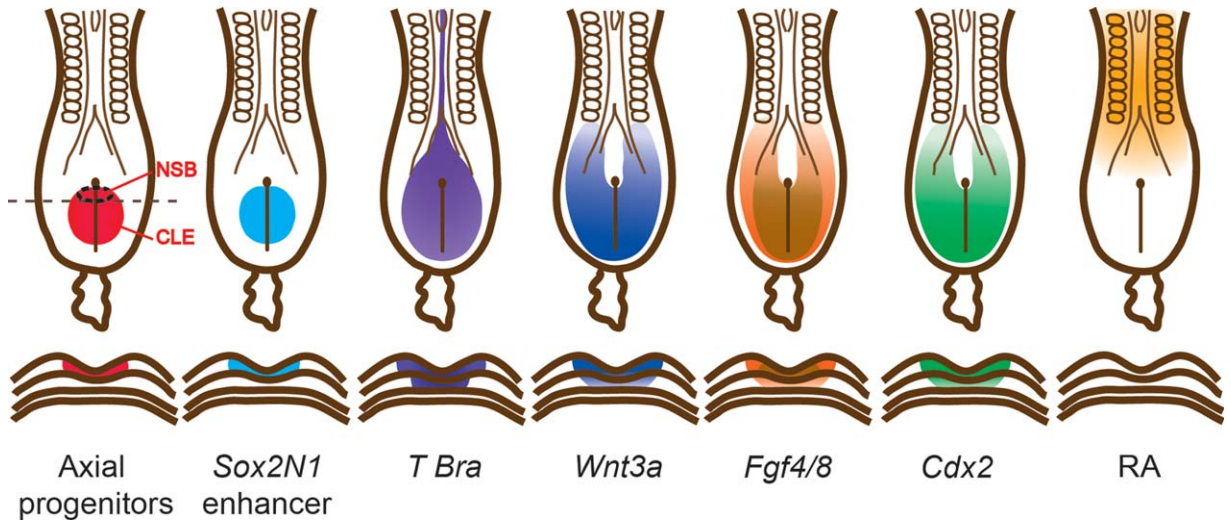

Fig. 1. Localization of axial progenitors and expression of molecular players in posterior growth during elongation of the embryonic trunk. Top: Schematic renderings of an E8.5 (10 somite pairs) mouse embryo (dorsal view, anterior is up). The primitive streak is represented by a vertical black line, with a black dot at its anterior end, representing the node. From left to right: the red-colored area specifies the epiblast cells contributing to the stem cell–like population eventually releasing descendants in mesoderm and neurectoderm of the growing axis; the anterior-most part of the domain (circled) corresponds to the "node-streak border" (NSB), and the rest to the caudo-lateral epiblast (CLE) (Cambray and Wilson, 2007; Wilson et al., 2009); the turquoise blue area corresponds to the activity domain of the *Sox2 N1* enhancer (Takemoto et al., 2011; Kondoh and Takemoto, 2012); purple corresponds to transcription of *T Bra* (Kispert and Herrmann, 1994) and dark blue to the expression of *Wnt3a* (Yoshikawa et al., 1997; Nowotschin et al., 2012); orange with brown sub-domain stands for expression of *Fgf8* (orange) and *Fgf4* (brown), based on Molotkova et al. (2005), Naiche et al. (2011), and L.A. Naiche and coworkers, personal communication; *Cdx2* expression is shown in green (Young et al., 2009), and the domain of retinoic acid (RA) signaling is shown in ochre, based on RARβ-driven reporter gene expression and Raldh2 activity (Rossant et al., 1991; Niederreither et al., 1997). Bottom schemes: The localization of progenitors, gene expression, and signals corresponding to the cross-section of the embryos (top) at the level of the anterior primitive streak (dashed line). The three germ layers are shown, of which the upper layer is epiblast, the middle layer mesoderm, and the bottom the primitive endoderm.

gastrulation (when head and neck are laid down) and later stages (early somite stages, when the trunk is generated) (Tzouanacou et al., 2009). It also indicates a decrease in the progenitor pool at tailbud stages when the tail will be formed. The subclasses of clones descending from single neuro-mesodermal progenitors, therefore, seem to correspond well with the different anatomical regions of the mouse embryo. These data raise the possibility that the composition of the neuro-mesodermal progenitor population changes with time, and thus with the axial level of tissue extension. The rearrangement of the pool of axial stem cells at certain axial levels might correspond to changes in activity of genes essential for modulating these populations. Candidate genes for this modulation are genes the mutation of which impairs axial elongation in a dosage-dependent way, as will be discussed in the following sections. The progenitor pool appears to be depleted in the tailbud at the end of axial extension (Tzouanacou et al., 2009).

Several aspects of axial tissue elongation from the progenitor zone have yet to be elucidated. One of the remaining questions results from the indication that not all tissues at a given axial level derive from the stem cell reservoir localized in the anterior streak/CLE, later the CNH in the tailbud. Tzouanacou and colleagues (Tzouanacou et al., 2009) have demonstrated that this mode of derivation is used by part of the paraxial mesoderm and the neural tube. Regarding the paraxial mesoderm, previous experiments suggested that only the medial parts of the somites arise by a stem cell mode (Selleck and Stern, 1991; Eloy-Trinquet and Nicolas, 2002; Iimura et al., 2007). Cells that will contribute to the lateral part of the somites emerge from a more caudal part of the primitive streak and its flanking epiblast, and are thought to get "organized" subsequently by their medial somitic counterparts when somites are formed (Freitas et al., 2001; Wilson et al., 2009). No evidence for a stem cell–like mode of generation of the lateral plate mesoderm has been found. Homotopic grafting of labeled subregions in and along the primitive streak of early somite embryos subsequently cultured for two days (Cambray and Wilson, 2007) confirmed the origin of lateral plate mesoderm at the posterior streak levels established in previous studies (Tam and Beddington, 1987; Kinder et al., 1999). In embryos grafted with cells of the posterior streak regions, no resident progenitor was left in the primitive streak. This lateral plate mesoderm must, therefore, emerge from the posterior primitive streak from fully ingressing proximal epiblast. The endoderm also follows its own mode of elongation. Only the early and anterior-most definitive endoderm emerges from the node region (Lawson et al., 1991), whereas the trunk endoderm expands from this early endoderm without stemming from progenitors in the posterior growth zone. Moreover, an intercalating contribution of the primitive endoderm to the gut epithelial endoderm has been demonstrated (Kwon et al., 2008). The expansion process of lateral plate mesoderm and endoderm thus differs from that of the stem cell–derived medial mesoderm and neurectoderm. The

emergence of all these tissues nevertheless obeys at least some common rules since they are all affected by mutations arresting posterior axial elongation (to be discussed in the next section).

In summary, at least part of the paraxial mesoderm and neurectoderm of the trunk and tail is generated from a population of bipotent progenitors residing in the NSB within the posterior growth zone, with some contribution of the CLE (Fig. 1). The precise population and physical extent of this progenitor zone remains to be defined, and the production mode of trunk lateral mesoderm and definitive endoderm remains to be better understood.

TRANSCRIPTION FACTORS AND SIGNALS MODULATING THE AXIAL STEM CELL NICHE

Spontaneous and induced mutations have long been described (*T Brachyury, T Bra*, Dobrovolskaïa-Zavadskaïa, 1927; *Danforth Short Tail, Sd*, Danforth, 1930; Dunn et al., 1940) that cause posterior truncation in the mouse embryo. The gene encoding the T-box transcription factor T Bra was identified a long time ago, and it was cloned in the late 1980s (Herrmann et al., 1990). The genetic event causing dysgenesis of posterior embryonic tissues in the *Sd* mutant was characterized only very recently, and shown to be a retrotransposon insertion event leading to down regulation of *T Bra* (Lugani et al., 2013; Vlangos et al., 2013). An extensive amount of information has been gathered on the cellular processes and gene expression affected by the *T* gene in the mouse embryo (Beddington et al., 1992; Wilson and Beddington, 1997). *T Bra* is expressed in and along the entire primitive streak and in the notochord (Fig. 1). *T Bra* null epiblast cells are impaired in migrating out of the primitive streak during gastrulation, and their accumulation compromises the process of axial elongation. The defect is dependent on the *T* gene dosage (Wilson and Beddington, 1997). Although *T Bra* is the earliest sign of mesoderm formation in the embryo, *T Bra* null mutants generate anterior mesoderm and form the first 6 somites. This points to a dichotomy between tissue generation at anterior and posterior levels that we will discuss later on. Other experimental investigations made it clear that the Wnt pathway is involved upstream of *T Bra*, in controlling both transcriptional initiation of the gene in the streak epiblast (Rivera-Perez and Magnuson, 2005; Tortelote et al., 2013) and maintenance of its expression (Yamaguchi et al., 1999; Galceran et al., 2001). A break-through discovery was made in studies on the function of *T Bra* (together with a second *T Bra* gene) in zebrafish. The action of T Bra in embryonic axial elongation was demonstrated to be mediated by Wnt signaling, meaning that the canonical Wnt pathway acts downstream of T Bra upon a process of positive feedback regulation (Martin and Kimelman, 2008). T Bra thus acts by maintaining Wnt signaling in the posterior part of the growing embryo. Accordingly, exogenous Wnt signals rescued the posterior truncation of zebrafish *T Bra* mutants (Martin and Kimelman, 2008). These findings are in line with the fact that mouse *Wnt3a* null mutants are as severely posteriorly truncated as *T Bra* null embryos (although some phenotypical features differ between these mutants). A spontaneous hypomorphic mutation of *Wnt3a* (*Vestigial tail, Vt)*, believed to disrupt a regulatory element of the *Wnt3a* gene (Greco et al., 1996), also causes posterior axial truncation, albeit in a much lesser extent than the full gene inactivation. Posterior axial growth is, therefore, dependent, in a dosage-dependent way, on the Wnt signaling strength in the embryonic growth zone.

The Wnt pathway is not the only stimulatory effector controlling axial elongation. Mutations inactivating Fgf receptor 1 (FgfR1) cause early lethality and truncation of the posterior embryonic regions (Deng et al., 1994; Yamaguchi et al., 1994) among other defects. Hypomorphic mutations in *FgfR1* also lead to posterior truncation of the embryos (Partanen et al., 1998). Active transcription of *Fgf8* in the posterior embryonic tissues takes place exclusively in the primitive streak region. Work in the chicken has shown that transcripts remain present more anteriorly due to their stability (Dubrulle and Pourquie, 2004). During the production of nascent axial tissues extending the axis, a decreasing gradient of *Fgf8* mRNA and protein is thereby generated with a minimum playing the role of wave front specifying a new somite boundary in the presomitic mesoderm (PSM) (Dubrulle and Pourquie, 2004). *Fgf8* transcripts and proteins in chicken and mouse embryos are thus present more widely than where the gene is transcribed (Fig. 1). Conditional inactivation of the *FgfR1* receptor or depletion of both *Fgf4* and *Fgf8* using a Cre recombinase driven by promoters active in the primitive streak also precludes complete axial extension and leads to the downregulation of *T Bra* (Wahl et al., 2007; Naiche et al., 2011; Boulet and Capecchi, 2012). Experiments using chimeric embryos documented that epiblast cells fully deprived of FgfR1 fail to ingress and instead accumulate in the primitive streak (Ciruna et al., 1997). Later inactivation of Fgfs seems to impair the maintenance of progenitor populations for axial elongation from the tailbud (Boulet and Capecchi, 2012). Wnt and Fgf signaling have both been proposed to act upstream of one another (Aulehla et al., 2003; Wahl et al., 2007), making it difficult to unambiguously build a network involving them together with T Bra in axial elongation.

The discovery of the importance of the niche for progenitor maintenance has been a crucial advance in understanding axial elongation. In the mouse, this importance was highlighted by the experiments of Wilson and colleagues. They showed that the CLE, a region normally generating mesoderm for a limited extent of the axis, acquires the properties of contributing to both mesoderm and neurectoderm for long periods of time if grafted to the residence of axial stem cells in the anterior-most part of the streak (Cambray and Wilson, 2007). The lack of molecular definition of the niche of axial stem cells, the very specific area at the anterior extremity of the primitive streak supporting the maintenance of the long-term axial progenitors (NSB and CNH), has puzzled the field for years. In the

absence of unique markers, attempts to identify genetic features for this zone have kept the attention on important players in axial extension such as *Wnt3a* and *Fgf8*, and on the co-expression of genes associated with early pluripotency and with early mesoderm differentiation such as *Sox2* and *T Bra*, respectively, in the mouse (Cambray and Wilson, 2007; Wilson et al., 2009) and in the chicken embryo (Delfino-Machin et al., 2005; Olivera-Martinez et al., 2012). However, all these players are each expressed more broadly than the territory defined for the axial stem cell niche (Fig. 1). The transcriptional activity of an early enhancer (*N1*) of the *Sox2* gene seems to overlap with the anterior streak and CLE, and is therefore also not restricted to the long-term axial progenitor zone (Takemoto et al., 2011) (Fig. 1). The identification of long-term axial progenitors has remained elusive, as it has not been possible so far to isolate self-renewing axial stem cells and define their properties and transcriptome. Co-expression studies with Sox2 and T Bra by immuno-histochemistry are so far the most likely to define a subpopulation of cells that can be considered as multipotent axial stem cells, as has been recently shown in the chick tailbud by Olivera-Martinez et al. (2012).

CELL FATE DECISION AND GERM LAYER EXPANSION DURING AXIAL EXTENSION

Cell fate choice and appropriate differentiation are as crucial for axial growth as progenitor self-renewal. The evidence for long-term neuromesodermal progenitors residing in the anterior streak/CLE (later the CNH) (Cambray and Wilson, 2002, 2007; McGrew et al., 2008), and the recent discovery of the close kinship between neurectoderm and mesoderm among the derivatives of these axial progenitors (Tzouanacou et al., 2009; Kondoh and Takemoto, 2012; Martin and Kimelman, 2012) were key to understanding the control of cell fate choice during axial extension. All mutants mentioned in the previous sections, which compromise posterior axial elongation, exhibit ectopic neurectoderm tissues at locations where mesoderm normally ends up. *T Bra* mutant embryos (Yamaguchi et al., 1999), *Wnt3a* mutants (Yoshikawa et al., 1997), and chimeric embryos containing *FgfR1* mutant epiblast cells (Ciruna et al., 1997) all formed ectopic neural tubes at their posterior-most axial levels. The molecular genetic mechanism underlying this deviation from the normal behavior, and the interdependence between mesoderm and neurectoderm differentiation has been recently elucidated (Takemoto et al., 2011; Kondoh and Takemoto, 2012). The early *N1* enhancer of the *Sox2* gene, active in the axial stem cells of the CLE, is normally turned off as soon as cells ingress through the primitive streak (Takemoto et al., 2011) under the repressive influence of Tbx6 in the mesoderm. If Tbx6 is absent, *N1* remains active upon cell ingression through the streak, and neural structures develop instead of PSM and somites. In that case, axial extension fails because of an arrest of paraxial mesoderm production. Work in zebrafish showed that canonical Wnt signaling is required for mesodermal differentiation of the bipotent neuromesoderm axial progenitors (Martin and Kimelman, 2012). Inactivating Wnt signaling in these progenitors causes the descendants to form neural tissue exclusively and to remain in the epiblast instead of undergoing epithelium-mesenchymal transition (EMT). Recent work using genetic analysis of single and double mouse mutants proposed ordering in at least some of these events involved in axial elongation. Wnt3a was suggested to regulate the process of ingression by EMT, and Tbx6 was proposed to act at a hierarchically lower level in post-ingression mesoderm (Nowotschin et al., 2012). Maintenance of the axial stem cell population by the Wnt pathway is also a functional component of axial growth, which would not distinguish itself phenotypically very much from a function in EMT. Arrest of epiblast ingression through the primitive streak and exhaustion of the axial stem progenitor pool at the streak level are, therefore, expected to be difficult to tell apart.

In summary, progress has been made in understanding cell fate decision during derivation of posterior neural and mesodermal tissues from the axial stem cells. It has become clear that the Wnt and Fgf pathways modulate the mesoderm/neurectoderm differentiation potential of the long-term progenitors. This modulation affects the balance between tissue production and differentiation regulating the extension of the body axis.

CDX AND HOX GENES IN AXIAL ELONGATION

The involvement of additional transcription factors in steering axial extension via stimulation of Wnt and Fgf signaling recently added a level of complexity in the underlying genetic network. Cdx genes (orthologs of *Drosophila caudal*) are expressed in the posterior growth zone similarly to *Wnt3a* and *Fgf8* (Young et al., 2009) (Fig. 1). Mutations inactivating the three Cdx genes impair axial extension with a severity that depends on the combination of alleles inactivated (Savory et al., 2009, 2011; Young et al., 2009; van de Ven et al., 2011; van Rooijen et al., 2012). Failure to complete the elongation of posterior tissues in Cdx mutants is at least partially rescued by a genetic gain of function of the Wnt pathway, or by exogenous Fgf added to the mutant embryos in culture (Young et al., 2009; van Rooijen et al., 2012). The realm of action of the Cdx genes encompasses the mouse embryonic trunk and tail extent of the axis. Again, this points to the difference, mentioned in an earlier section, in the genetic mechanism underlying the generation of the posterior axial tissues relatively to the more anterior cephalic and occipital structures. The loss of all three Cdx genes is accompanied by extinction of *T Bra* expression after 5 somites have been generated (van Rooijen et al., 2012). Although Cdx-binding sequences were identified in the proximal upstream region of the *T Bra* gene (Savory et al., 2009), *T Bra* is normally initiated, and remains expressed until early somite stages in Cdx null mutants (van Rooijen et al., 2012). These data might indicate that Cdx proteins work on maintaining *T Bra* transcription in the posterior growth zone

during trunk development, but are dispensable for the initial expression of the T gene in the primitive streak at the beginning of gastrulation. Alternatively, the loss of Cdx might cause the exhaustion of cells with progenitor properties in the streak region, causing the disappearance of the tissue that expresses *T Bra* in the primitive streak. The expression of *T Bra* in the notochord is not affected in Cdx null embryos (van Rooijen et al., 2012). Like mutations in *T Bra*, and impairment of the Wnt and Fgf pathways, Cdx deficiencies affect the neural/mesoderm differentiation choice, since partial Cdx mutants exhibit ectopic neural tissues (van de Ven et al., 2011). The data collected on the Cdx mutants, therefore, ascertain that Cdx proteins affect the activity of the self-renewing bipotent axial progenitors, acting on their maintenance and on their differentiation choice during axial growth. T Bra, Cdx, Wnt, and Fgf are all needed in a dosage-dependent way to promote posterior axial elongation. These gene products thus possess the properties expected from regulators fine tuning the pool of neuro-mesodermal axial progenitors and their differentiation, as proposed by Tzouanacou et al. (2009).

Essential questions remain to be answered in order to understand the precise molecular interactions between the Cdx transcription factors and the key effector Wnt and Fgf pathways. Reporter assays with *Wnt3a* promoter fragments in transfected cells in culture suggested a direct role of Cdx in stimulating *Wnt3a* transcription (Savory et al., 2009). However, no specific impact of Cdx mutations on components of the Wnt and Fgf pathways has been demonstrated so far, in spite of several transcriptome analyses in posterior tissues of Cdx mutant embryos (van de Ven et al., 2011).

Another complication concerns the functional relationship between Cdx and their relatives, the Hox genes (Young et al., 2009). Hox genes are also expressed in the posterior growth zone during the laying down of the trunk and tail (Young et al., 2009). It was shown that Hox genes of the middle of the clusters (*Hoxb8* for instance) can rescue the posterior truncation of Cdx mutants. It is not known so far whether these Hox genes and Cdx activate the same or overlapping series of downstream targets genes. Striking in any case is the fact that the last genes of the Hox clusters exert an opposite influence on axial growth. A precocious activation of these Hox13 genes is not tolerated in the posterior growth zone (Young et al., 2009). Hox13 genes exert a dominant-negative effect on the activity of Cdx and on earlier Hox cluster members, antagonizing their function. This situation fits the principle of temporal collinearity of action of the Hox genes (Duboule, 1994, 2007; Iimura and Pourquie, 2006; Tschopp et al., 2009). Hox13 genes are candidates to normally initiate the slowing down of axial extension at the trunk/tail transition, foreshadowing the arrest of posterior growth. In the genetic network underlying the control of axial elongation, the Hox/Cdx genes would bring in the notion of timing and positional identity of the axial tissues that are being generated. Cdx and Hox genes would integrate the spatial and temporal components that control axial growth.

The elucidation of the role of Cdx and Hox genes in the interacting network of players at work during axial growth and tissue differentiation will have to await a more complete molecular genetic analysis of the available single and multiple mouse mutants. It will also need a more exhaustive mapping of the molecular interactions between the different transcription factors and their genomic targets.

CONTROL OF BODY LENGTH DURING EVOLUTION

The experiments of Cambray and Wilson (2002) have shown that the long-term neuro-mesodermal progenitors start losing their activity at the trunk/tail transition, upon a genetically programmed decrease of Wnt and Fgf signaling in the "aging" progenitor niche in growing mouse embryos. This transition corresponds to the shrinking of the PSM, which was shown in the chick to anticipate the termination of somitogenesis and posterior tissue addition (Gomez et al., 2008). This transition also correlates with a milestone in the activation of clustered Hox genes: the activation of Hox13 genes. These genes are believed to promote the termination of axial growth (Young et al., 2009). Comparative analysis of Hox gene expression during embryogenesis of the corn snake and shorter body animals has revealed that two of the four Hox13 paralogs are not expressed in the growth zone in snake embryos, whereas all four are expressed in the related shorter body squamate, the whiptail lizard (Di-Poi et al., 2010). These findings suggest that loss of expression of two of the Hox13 genes might correlate with a delay in unleashing the slowing down of axial extension, which would result in an extended trunk elongation. The presence of a high number of DNA repeats within the snake Hox clusters, which are not found in the mammalian clusters (Di-Poi et al., 2010), suggest that relaxing the tightly controlled temporal progression of Hox gene expression has played a role in the emerging of long body vertebrates during evolution.

The role of the Hox-related Cdx genes in body axis growth seems to have been evolutionarily conserved. The requirement of *caudal*/Cdx for generating axial tissues posterior to the head was not only demonstrated in mice, but also in short (the flour beetle) and intermediate (the cricket) germ band insects, and in the crustacean *Artemia* (Copf et al., 2004; Shinmyo et al., 2005). The ancestors of these phylogenetically distant species thus already involved Cdx transcription factors to construct their trunk and posterior structures. This demonstrates that axial extension from a posterior growth zone is the basal mode of body axis elongation in bilateria (Akam, 1989; Jacobs et al., 2005) and that Cdx/Wnt/Fgf constitutes an evolutionarily conserved genetic toolkit for trunk and tail generation (Copf et al., 2004; Shinmyo et al., 2005; Beermann and Schroder, 2008; Beermann et al., 2011).

ROLE OF RETINOIDS IN THE WNT/FGF CONTROL OF AXIAL LENGTHENING

A signaling route not involved so far in this overview is that of retinoic

acid (RA) and its pathway components. Tight regulation of this pathway is known to be essential for well-controlled axial growth, and to allow interaction of the pathway with every single effector mentioned above. The transcription factor–encoding genes *T Bra* and Cdx, and Wnt and Fgf signaling all were shown to be affected by loss or gain of RA biosynthesis (Abu-Abed et al., 2001; Sakai et al., 2001; Diez del Corral and Storey, 2004; Vermot et al., 2005; Ribes et al., 2009; Zhao and Duester, 2009). RA signaling in the early embryo is spatially restricted by RA synthesizing and metabolizing enzymes, Raldh2 and Cyp26a1, respectively. RA-responsive domains are highly dynamic during the time course of posterior development from primitive streak stages and somitogenesis to axis termination.

At early somite stages, complementary domains of RA biosynthesis and degradation are generated in the posterior part of the embryo. Somites and anterior PSM express Raldh2, which generates RA that then diffuses in the overlying neural plate (Sirbu and Duester, 2006; Ribes et al., 2009). These activities of source and sink create a gradient of RA concentrations with a maximum in the somites, fading out posteriorly to a minimum in the primitive streak and stem zone region, where *Cyp26a1* is expressed. The RA distribution is thus opposite to the *Fgf8* gradient mentioned in an earlier section of this review. During trunk formation, the dichotomy between a posterior stem cell zone expressing Fgf, Wnt, *T Bra*, Cdx, and *Cyp26a1*, and a differentiation zone with high RA bioactivity, is therefore very clear. Cells leaving the posterior progenitor zone become exposed to higher RA and lower Fgf8 levels. They then undergo differentiation into the caudal neural plate, or segment into somites. RA is cleared in the stem cell niche by Cyp26a1, the expression of which depends on Fgf signaling (Wahl et al., 2007; Boulet and Capecchi, 2012) and on Cdx (Savory et al., 2009; Young et al., 2009; van Rooijen et al., 2012) and *T Bra* (Martin and Kimelman, 2010; Vidigal et al., 2010). Exposure of the embryos to supra-physiological levels of RA by maternal administration, or following a defective RA degradation, causes severe axial truncations (Kessel and Gruss, 1991; Shum et al., 1999; Abu-Abed et al., 2001; Sakai et al., 2001). Transient RA treatment during somitogenesis restricts Fgf and Wnt signaling and expression of *T Bra* in the progenitor zone (Iulianella et al., 1999; Shum et al., 1999; Diez del Corral et al., 2003; Wilson et al., 2009). Reciprocally, Fgf antagonizes RA signaling, at least in part by repressing *Raldh2* expression in the region where these players overlap (Diez del Corral et al., 2003; Wahl et al., 2007; Olivera-Martinez et al., 2012). Thus, a large amount of experimental evidence shows that RA is necessary to induce neural differentiation from the axial progenitor descendants, and that RA clearance in the growth zone is required to maintain the genetic program of progenitors during trunk formation.

At the trunk/tail transition (E10.0), Fgf and Wnt signals decline, the PSM shrinks (Gomez et al., 2008), and the RA-producing domain then abuts the progenitor zone in the tailbud. *Cyp26a1* levels decrease posteriorly at this stage, in both chicken and mouse embryos. Surprisingly, *Raldh2* was found to become transiently induced in a localized area of the tailbud (Tenin et al., 2010; Olivera-Martinez et al., 2012). RA at this location was reported to be biologically active in the chick, as it leads to a decline in the expression of Fgf, Wnt, and *T Bra*, apoptosis and axis termination (Olivera-Martinez et al., 2012). In co-culture experiments, chick tailbuds have been shown to activate *RARE-lacZ* in a reporter cell line, whereas mouse tailbuds did not appear to be an endogenous source of RA in the same assay (Tenin et al., 2010). It might be that RA does not play a role in the termination of the mouse body axis. In *Raldh2* mutant embryos transiently treated with RA to overcome early developmental lethality, posterior *Fgf8* is downregulated and axial elongation is arrested normally (Cunningham et al., 2011). The mechanism of axial termination is thus possibly different in these two organisms. It is not known how the differences in the mechanism of axial termination have arisen during evolution. Altogether, the data again emphasize the differential regulation of the distinct anatomical regions of the body axis.

CONCLUSIONS AND ADDITIONAL FUTURE PROSPECTS

Trunk and tail tissues in vertebrates stem from the embryonic posterior growth zone that harbors a niche for the maintenance of axial progenitors, some of which are self-renewing and bipotent for mesoderm and neurectoderm. The progenitor niche ages at mid-gestation, upon genetically programmed decrease of Wnt and Fgf signaling. These processes foreshadow the termination of axial growth. The aging process can be reversed, as the niche and its progenitors can be rejuvenated upon transplantation into the corresponding region of a younger embryo (Cambray and Wilson, 2002). The molecular genetic characterization of the long-term progenitor niche, within the region of overlap between the activity domains of the key effectors of axial growth, is an important remaining goal in future research. Given the fact that the effectors in the progenitor niche, Wnt and Fgf, are regulated by the transcription factors *T Bra* and Cdx/Hox in feed-back loop relationships (Martin and Kimelman, 2008; Young et al., 2009; van Rooijen et al., 2012), the transcriptional control of these transcription factors in the growth zone is a major steering component in axial growth. Understanding the regulation of these regulators might shed light on the upstream command of key events of posterior morphogenesis such as the emergence of the Fgf8 gradient that is crucial for posterior axial growth (Diez del Corral and Storey, 2004; Dubrulle and Pourquie, 2004; Naiche et al., 2011; Boulet and Capecchi, 2012). A pioneer study regarding the transcriptional regulation of the *Fgf8* locus has just been published (Marinic et al., 2013) and promises to lead to unveiling regulatory events driving posterior embryonic growth. The establishment of the epistatic relationship between the players in the network driving axial extension, and the precise mapping of the productive binding events

of the key transcription regulators at their genomic targets, will be essential to fully understand the genetic program underlying axial tissue growth and patterning.

A conclusion of the data reviewed above is that the rules governing axial elongation anterior to the trunk, within the trunk and at the termination level of the tail, follow different principles. The phenotype of *T Bra* and Cdx null mutants indicates that the corresponding gene products are needed for axial elongation posterior to the 5/6 most anterior somites. This suggests a scenario whereby early Wnt signaling (Wnt3) is enough to ensure extension of the anterior axial structures, whereas trunk and posterior elongation requires prolonged Wnt signaling (Wnt3a) maintained by *T Bra* and Cdx. Early lethality of the *Wnt3* mutants that fail to undergo gastrulation (Liu et al., 1999) precludes assessing the validity of this hypothesis. The same may hold true for Fgf signaling. Early *Fgf4* and *Fgf8* may suffice for occipital tissue elongation but fall short of supporting more posterior trunk and tail growth when they are inactivated shortly thereafter by *T Bra*-driven Cre recombination (Naiche et al., 2011). Fgf and Wnt may, therefore, play a continued role in maintaining the progenitors for axial extension in the different windows of developmental time. Boosting of both pathways by Cdx and T Bra might mark the occipital/post-occipital transition. An unknown in this scenario is whether anterior tissues are laid down at all from a population of long-term neuro-mesodermal precursors.

Tissue morphogenesis in body regions at different axial levels has been shown earlier to obey distinct rules and to differentially depend on specific unravelled molecular genetic circuits. The oscillatory activity of genes inherent to the segmentation clock such as Lunatic fringe is differentially required for thoraco-lumbar and sacro-caudal body patterning of the presomitic mesoderm (Shifley et al., 2008; Stauber et al., 2009). Shifley and colleagues suggest that the process of primary versus secondary body formation may impact on the regulation of rostro-caudal patterning by the segmentation clock during the various stages of anteroposterior axis development. Not only the sclerotome, but also the myotome, seems to be differentially controlled during primary and secondary body formation: PDGFα receptor mutants were found to exhibit myotome defects in the 20 most rostral somites and to appear normal in the caudal somites (Soriano, 1997). Myogenesis has indeed been shown to be controlled by different strategies during ontogenesis of the different regions of body development. Trunk and head muscles are generated by paraxial mesoderm that is, respectively, segmented and unsegmented (at least not segmented in the same way as the trunk) into somites (reviewed by Sambasivan et al., 2011). Cranial and somitic myogenesis clearly depends on distinct genetic networks (Sambasivan et al., 2011). Comparison of these processes in different chordates suggests that the neck muscles appeared at a transition zone between the cranial and trunk mesoderm and ancestral vertebrates do not seem to have possessed neck muscles (Sambasivan et al., 2011). Strikingly the endoderm of the developing embryonic body has been recently shown also to differentially depend on well-defined signaling pathways. Wnt/β-catenin signaling is crucially important for the formation of definitive endoderm of the mid- and hindgut, whereas it is dispensable for foregut formation (Engert et al., 2013).

Another difference between the progenitors of the anterior-most, and trunk part of the axis concerns their sensitivity to RA. The early axial progenitor niche at primitive streak stages expresses *Raldh2* (Vermot et al., 2005; Ribes et al., 2009) whereas the presence of RA in the growth zone during trunk elongation is detrimental to the progenitors (see above). The biphasic response of embryonic axial progenitors exhibiting initial compatibility of RA exposure with self-renewal, and later drifting towards differentiation in the presence of RA was reported to be observed as well in murine embryonic stem cells in culture (Stavridis et al., 2010). These dynamic changes in niche composition with developmental time make it even more difficult than appreciated so far to understand the essential properties of the axial progenitors and their niche.

Many aspects of tissue generation from the progenitor zone remain to be elucidated, as set out in the different sections above. In addition, the recent arousal of interest for additional parameters to be considered in the control of morphogenesis is expected to bring new light to the phenomenon of posterior embryonic growth. Besides the involvement of cell adhesion, known to be controlled during gastrulation, cell flow and fluidity change in posterior tissues during axial elongation have recently been evoked as potential regulators of axial growth in zebrafish embryos (Lawton et al., 2013). The authors found that the movement of cells from the dorsomedial zone, the remnant site of gastrulation, to the PSM depends on decreasing Fgf and Wnt signal concentrations. This migration within the tailbud is accompanied by a change in cell flow and tissue fluidity. Previous work in chicken embryos showed that high Fgf signaling promotes cell motility in the posterior PSM, that this motility decreases as cells approach the segmentation boundary in the low values of the Fgf gradient, and that disruption of the motility gradient results in slowing down of axial elongation (Benazeraf et al., 2010). The distribution of non-canonical Wnt and Fgf signaling, known to affect cell migration directly or indirectly, would generate a balanced equilibrium between the cell flow rate, the emergence of differentiated derivatives, and the coherence of collective cell migration within the tailbud. Disrupting the Wnt and Fgf gradients would introduce chaos in cell flux, aberrant elongation of the trunk, and in some cases inappropriate localization of tissue derivatives such as neurectoderm. It is evident that we are far from completely apprehending the complexity of signaling regulating the morphogenetic event of posterior axial growth. Future progress in these respects is anticipated. The design of non-intrusive methods to genetically label selected groups of cells in developing embryos in culture, and the development of live imaging technology during progressing embryogenesis in vivo should allow for precise clarification

of the role of each effector in the process of axial elongation.

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