Development 136, 2133 (2009) doi:10.1242/dev.039172

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There was an error published in *Development* **136**, 1591-1604.

Table 1 should also have cited Dunty et al., 2008 in the Wnt signalling section describing *β-catenin$^{f/f}$; T-Cre* mice.

Dunty, W. C., Jr, Biris, K. K., Chalamalasetty, R. B., Taketo, M. M., Lewandoski, M. and Yamaguchi, T. P. (2008). Wnt3a/beta-catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation. *Development* **135**, 85-94.

The authors apologise to readers for this mistake.

Development 136, 1591-1604 (2009) doi:10.1242/dev.021246

Stem cells, signals and vertebrate body axis extension

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The progressive generation of chick and mouse axial tissues – the spinal cord, skeleton and musculature of the body – has long been proposed to depend on the activity of multipotent stem cells. Here, we evaluate evidence for the existence and multipotency of axial stem cells. We show that although the data strongly support their existence, there is little definitive information about their multipotency or extent of contribution to the axis. We also review the location and molecular characteristics of these putative stem cells, along with their evolutionary conservation in vertebrates and the signalling mechanisms that regulate and arrest axis extension.

Introduction

Vertebrate embryos display a highly characteristic spatial patterning of tissues, including the arrangement of the neural tube, the somitic mesoderm and the notochord along the rostrocaudal (head-tail) length of the body axis (Fig. 1). Not only is this overall arrangement conserved, but the manner in which these axial tissues are produced is similar across vertebrate species. Much of the early patterning of the embryo is orchestrated during gastrulation by signals from a midline structure, known as the primitive streak in chick and mouse embryos. The postcranial axis (i.e. tissue caudal to the head) is then generated over an extended period in a rostral-to-caudal sequence by cells that are derived from the primitive streak and the adjacent epiblast cells, which together eventually form the tail bud. The area encompassing the primitive streak and the adjacent epiblast, and the later-forming tail bud, are the source of the neural tube and mesoderm over the entire period of body axis elongation (Fig. 2).

Detailed lineage analysis and fate-mapping studies have revealed that subdomains exist within these primordia. The primitive streak in chick and mouse embryos is organised such that the notochord emerges from its rostral tip, known as the node, and more caudal portions of the streak generate successively more lateral mesodermal tissues (Cambray and Wilson, 2002; Psychoyos and Stern, 1996; Selleck and Stern, 1991; Wilson and Beddington, 1996). A region that comprises the caudal end of the node and the rostral 5-10% of the primitive streak has been termed the 'axial-paraxial hinge', or 'region C' in chick (Charrier et al., 1999), and the 'node-streak border' in mouse (Cambray and Wilson, 2002; Cambray and Wilson, 2007). Cells from this region contribute to the neural tube and the somites, as well as to the notochord. Here, we will use the term 'node-streak border' (NSB) to refer to this cell population (Fig. 2A,B). The ventral midline of the neural tube is produced exclusively by the dorsal part of the node (Charrier et al., 1999; Selleck and Stern, 1991). The progenitors of the lateral and dorsal neural tube, and of some somitic tissue, are found in an arc of epiblast tissue on either side of the primitive streak. These progenitors have a rostral limit at the NSB and extend caudally for about 50% of the length of the streak in chick at the 1- to 2-somite stage (Brown and Storey, 2000; Catala et al., 1996; Schoenwolf, 1992; Spratt, 1952), and for about 80% of streak length in mouse at the 2- to 6-somite stage (Cambray and Wilson, 2007) (Fig. 2A,B). This region has been termed the caudal neural plate (Brown and Storey, 2000), the stem zone (Mathis et al., 2001) and the lateral epiblast (Cambray and Wilson, 2007; Iimura and Pourquie, 2006). Here, we will refer to this region as the 'caudal lateral epiblast' (CLE), as it does not give rise to exclusively neural tissue and is a caudally located epiblast cell population (Fig. 2A,B). The spatial map of prospective tissues in the tail bud is very similar to that in and adjacent to the primitive streak in early (2-6 somite) embryos (Fig. 2C-D′). In mouse and chick, the derivative of the NSB (with a minor contribution from the CLE), the 'chordo-neural-hinge' (CNH) (Cambray and Wilson, 2007; Catala et al., 1995; Charrier et al., 1999), contains progenitors for the ventral neural tube, somites and notochord (Cambray and Wilson, 2002; McGrew et al., 2008). The CNH is continuous with the most recently formed neural tube and notochord (Fig. 2C-D′). By contrast, the tissue immediately caudal to the CNH exclusively produces somites in mouse and chick (McGrew et al., 2008).

As the body axis elongates, a transition occurs from primary neurulation, during which the neural plate rolls up to form the neural tube, to secondary neurulation, which occurs following the formation of the tail bud and which involves the cavitation of a rod of tail bud mesenchyme. This switch indicates a significant change in the cellular and molecular mechanisms that operate in trunk and tail regions, and occurs at different times during axis extension in chick and mouse embryos: the tail bud arises at the 22-somite stage [Hamburger and Hamilton stage 14 (HH14)] in the chick (Criley, 1969; Hamburger and Hamilton, 1951) and at the 30-somite stage [embryonic day 9.5-10 (E9.5-E10)] in the mouse (Schoenwolf, 1984). However, many reports also suggest that a set of stem cells generates axial tissues (neural tube, somites, notochord) in these organisms. These cells are proposed to reside in the primitive streak region, and to be incorporated into the tail bud at a later stage of development. The self-renewing nature of stem cells implies that the axial tissues produced by such cells would be generated in a single continuous process rather than by separate cell populations (see Fig. 3). Here, we consider the accumulating evidence for the presence, persistence and multipotency of self-renewing stem cells during the elongation of the mouse and chick body axis and, crucially, identify equivalent cell populations in these two animals. We further review the molecular characteristics of cells in regions likely to contain such axial stem cells and the signalling pathways that regulate their behaviour, including signalling changes that lead to the arrest of body axis extension and to the determination of body length. Finally, we discuss the evidence for the existence of axial stem cells in fish and frog, and for the conservation of signalling mechanisms across species.

Evidence for the stem cell origin of axial tissues

Stem cells are classically defined by two characteristics: the ability to self-renew, giving rise to exact copies of themselves, and the capacity to give rise to one or more differentiated cell types. For a

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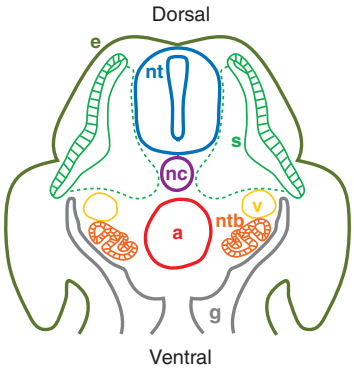

Fig. 1. Transverse section through a typical vertebrate embryo. Schematic showing the stereotyped positions of key tissues in a vertebrate embryo. The central neural tube (nt, blue) is flanked by somitic mesoderm (s, green), and below the embryo's ventral midline is the notochord (nc, purple), an axial mesodermal tissue that lies above the aorta (a, red), gut (g, grey), veins (v, yellow), and nephric tubules (ntb, orange). A covering of surface ectoderm/epidermis (e, dark green) encases all of these tissues.

putative axial stem cell, self-renewal implies retention in the progenitor region throughout axial elongation. An axial stem cell must meet two additional criteria: (1) individual cells should normally contribute descendants to both rostral and caudal regions of the axis in vivo; and (2) the cells at late developmental stages should retain the capacity to generate rostral tissues as well as the caudal ones that they are normally fated to produce. Importantly, neither of these tests requires that the putative stem cells be multipotent. In principle, non-stem cell progenitors could be multipotent, and stem cells could contribute to single or multiple lineages.

In both mouse and chick, experimental evidence supports the notion that stem cells contribute to the generation of the notochord, neural tube and somites. The first support for this idea came from single-cell labelling experiments, which used a fluorescent dye, lysine-rhodamine dextran, in the chick node and primitive streak (Selleck and Stern, 1991). These studies revealed that single cells in the node contribute in a periodic fashion to the notochord with an interval of two somite lengths (Selleck and Stern, 1992; Selleck and Stern, 1991). This periodicity might be accounted for by a stem cell that divides in the node at intervals to generate daughter cells that then contribute to the notochord (Selleck and Stern, 1992; Selleck and Stern, 1991). Indeed, a rostrally located cell cluster in the notochord has more cells and is less heavily labelled than the next (more caudally located) cluster. This is consistent with each cluster being derived from a single stem cell daughter, where rostral clusters have had more time to divide than their later-born, more caudal, siblings. However, it should be noted that although regular stem cell divisions could produce such a pattern, this is not the only possible means by which a reiterated pattern of cell contribution could be generated. For example, a population of labelled cells might undergo a stereotyped pattern of intercalation with unlabelled cells. Furthermore, such a pattern is not a prerequisite for these clusters having a stem cell origin: variations in stem cell cycle time, periods where a given stem cell does not contribute cells to the axis, and irregular patterns of cell division in transient amplifying populations could all produce distortions in a regular contribution pattern from stem cells.

The presence of resident notochord progenitors in the node has also yet to be demonstrated (Selleck and Stern, 1991). Live imaging of single cells in the chick CLE at stages later than 10 somites (Mathis et al., 2001) did reveal that, over a short culture period, some daughter cells remained resident in this region, whereas others contributed to the elongating neural axis (Mathis et al., 2001). In the early mouse embryo, studies have also identified cells in the rostral tip of the primitive streak that remain in the node after a period of embryo culture (Forlani et al., 2003; Lawson et al., 1991). However, owing to the short culture periods employed in these experiments, they do not address the question of whether cells reside in the node for the whole period of axial elongation.

The first indication that some axial progenitors must persist over long periods of time was inferred from retrospective clonal analyses of the mouse myotome (the muscle progenitor compartment of the

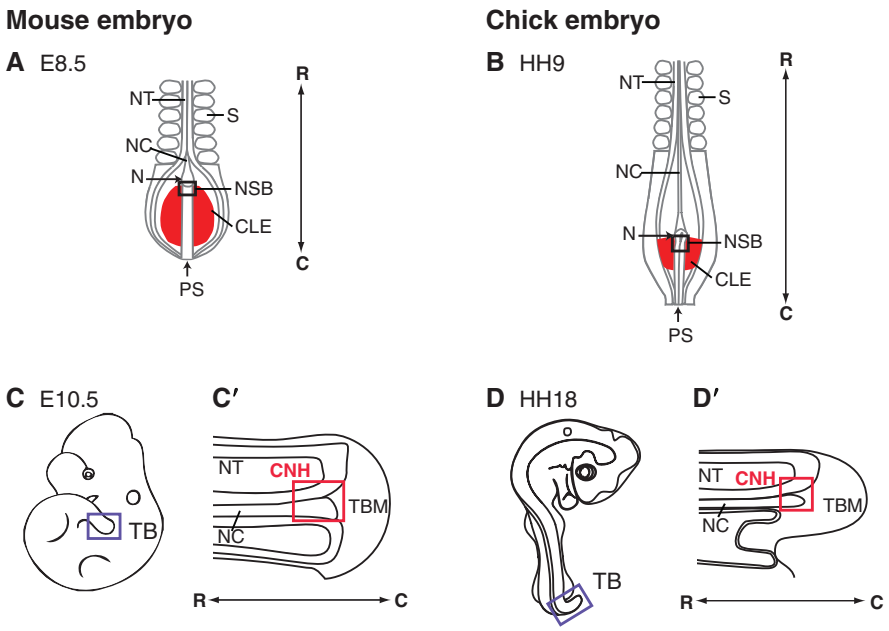

Fig. 2. Location of key tissues and caudal progenitor cell populations in mouse and chick embryos. (**A-D′**) Schematics showing the location of key tissues in (A,B) early (six somites; mouse E8.5, chick HH8) and (C-D′) late (35 somites; mouse E10.5, chick HH18) embryos. At the six-somite stage, the primitive streak (PS), node (N), notochord (NC), neural tube (NT) and somites (S) are visible in both (A) mouse and (B) chick embryos. Regions that contribute to the extending body axis, namely the node-streak border (NSB) and caudal lateral epiblast (CLE), are also indicated. (C-D′) Both of these cell populations also contribute to the later-forming chordo-neural hinge (CNH; red box) located in the tail bud (TB; blue box) at the junction of the notochord and neural tube, and rostral to the tail bud mesoderm (TBM). C′ and D′ represent longitudinal sections through the tail bud. C, caudal; E, embryonic day; R, rostral.

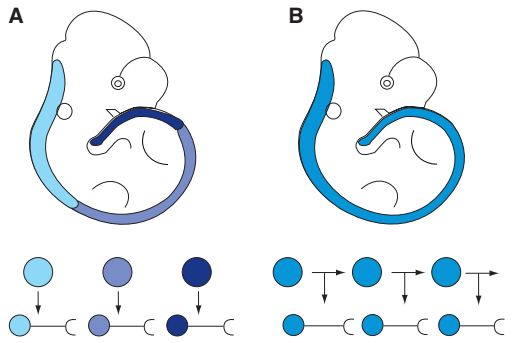

Fig. 3. Possible mechanisms for generating the body axis. Putative stem cell and non-stem cell mechanisms to generate axial tissues. (**A**) Separate populations of cells that generate discrete portions of the axis, and whose births predate axial extension, could generate the spinal cord. (**B**) Alternatively, stem cells present throughout axial elongation could continue to generate axial tissue. Different colours represent different clonally-derived populations. The embryo diagrams show the eventual position of the cells that are depicted schematically below the embryo. Upper row of cells, progenitors (stem cell or non-stem cell); lower row of cells, differentiated derivatives at their final axial destination.

somite) (Nicolas et al., 1996) and spinal cord (Mathis and Nicolas, 2000). These studies exploited a modified form of the *lacZ* reporter gene, termed *laacZ*, which contains a duplication in its coding sequence, resulting in a truncated, non-functional β-galactosidase protein. Reversion to functional *lacZ* occurs at random, thus labelling individual revertant cells and their descendants and allowing the visualisation of clones generated in vivo over the whole period of axial elongation. By using *laacZ* under the control of a myotome-specific or a neuronal-specific promoter, the patterns of cell division that generate these tissues have been deduced. In the myotome, clones that contribute to an axial length greater than seven somite segments ('long' clones) have a rostral limit anywhere along the axis, but tend to continue as far as the most caudal limit of promoter activity, indicating that they were generated by cells that contributed to myotomes in a stem cell-like manner (Nicolas et al., 1996) (see Fig. 4A,B). This class of clone has also been visualised in the neural tube, but fewer of these clones were obtained and therefore this stem cell contribution pattern, in which large clones extend to the caudal region, was less obvious (Mathis and Nicolas, 2000). It is, however, important to note that the promoters in these studies drive expression in specific lineages (myotomal or neuronal); hence, these data provide no information about the putative multipotency of such axial stem cells.

Analysing the contribution of the myotome to axial extension (Nicolas et al., 1996) reveals that, although long clones display a strong tendency to contribute from a variable rostral limit up to as far as the caudal end of the embryo, several long clones do not fit this pattern, but stop well short of the caudal end (Fig. 4C,D). Such clones might arise in several ways. As the authors suggest, developmental variability between litters leads to a different total number of somites in which the promoter is active. However, several other possibilities could also explain this result: (1) because the myotome progenitors in the tail bud were not labelled in this study, the lack of caudal labelling might in reality represent a very large unlabelled gap that indicates erratic stem cell contribution; (2) not all long-clone progenitors are stem cells; or (3) individual stem cells may exit the stem cell compartment. The limitations of the study, namely the use of a tissue-specific promoter (which precludes visualisation of the progenitors) and the relatively small number of long clones, do not allow for the exclusion of any of these possibilities. Therefore, even though some long clones support the existence of persisting stem cells that contribute along the entire rostrocaudal length of the axis, these studies leave open the possibility that not all axial cells derive from stem cells.

A more recent study of *laacZ*-revertant clones in the spinal cord further indicates that different contribution patterns characterise the rostral and the caudal regions of this tissue (Roszko et al., 2007). In this study, the pattern of clones observed in the spinal cord caudal to somite 20 fitted well with a constant probability of labelling a given rostrocaudal domain, which was interpreted as an indication of stem cell activity. By contrast, the greater and less regular intervals between labelled cells that were evident within clones in rostral regions suggested that other mechanisms might operate here. These might include a non-stem cell mechanism, such as the intercalation of existing short-term progenitors, but this pattern does not necessarily exclude that a stem cell mechanism generates the rostral spinal cord, which then undergoes differential growth after cells have exited the stem cell compartment. Conversely, as noted above, a reiterative pattern of contribution is not necessarily diagnostic of a stem cell contribution. This study does, however, highlight that different mechanisms operate in the rostral and caudal spinal cord, and raises the possibility that stem cells might make different contributions to axial extension in distinct rostrocaudal regions.

Although these retrospective lineage studies indicate that the progenitors of the myotome and possibly also of the spinal cord are generated by stem cell divisions, they do not provide information on where such cells are located or on whether cells in the tail bud retain the capacity to contribute to rostral axial levels. Tam and Tan (Tam and Tan, 1992) showed that cells in the mouse tail bud, when transplanted to earlier primitive streaks, could contribute to axial tissues. In addition, Cambray and Wilson (Cambray and Wilson, 2002) showed that the only progenitors that could contribute over long axial distances in this assay were those in the CNH. Groups of CNH-derived cells could be passaged through multiple primitive streaks and still retain the capacity to contribute to both mesodermal and neural tissue over long distances, with some cells remaining in the CNH. More recently, another study (McGrew et al., 2008) has demonstrated the same property for chick CNH cells. Furthermore, this study has shown that cells caudal to the CNH in the tail bud in both mouse and chick could contribute to somites over long axial distances, but, unlike cells from the CNH, these could not be serially passaged. This demonstrates that CNH cells are unique in their ability to contribute to axial tissues over an extended period of time. It is, however, crucial to bear in mind that all of these studies were performed using groups of cells, and currently no study directly links the cells that can be experimentally manipulated to behave like stem cells to the stem cell-like progenitors identified by the retrospective lineage analyses.

Evidence for multipotency in axial stem cells

The clonal studies described above that indicate the existence of axial stem cells do not provide information about the potency of these progenitors – such cells might be separate neural and mesodermal progenitors or multipotent cells. Selleck and Stern showed in the chick that single node cells could contribute to more than one tissue (somite and notochord, or notochord and ventral neural tube/floor plate) (Selleck and Stern, 1991), demonstrating that at least some cells in the region where stem cells are predicted to reside are multipotent. Single-cell labelling of the mouse node

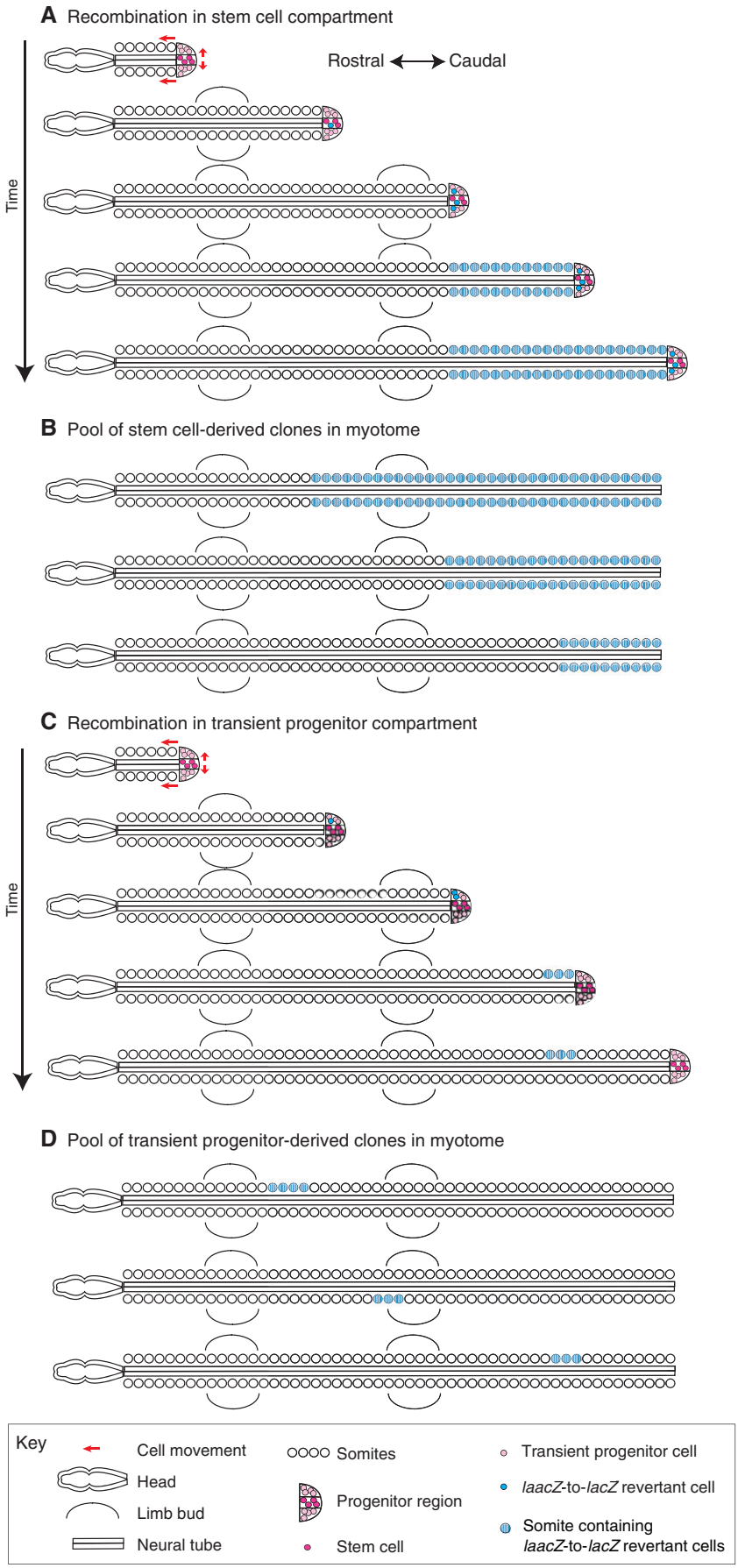

Fig. 4. Predicted patterns of axial tissue contribution from candidate parent populations by retrospective clonal analysis. Schematic of the predicted patterns of contribution to the axis arising in clones originating from either axial stem cells or transient progenitors, as seen by retrospective clonal analysis in developing mouse embryos. Adapted, with permission, from Nicolas et al. (Nicolas et al., 1996). (**A**) Pattern expected from a single recombination event in a stem cell that contributes to somites. The progenitor region is shown as consisting of three hypothetical compartments, one containing stem cells (dark pink circles), and two, on either side of the midline, containing transient progenitors (light pink circles). The latter might in reality include, but not be limited to, the presomitic mesoderm. Arrows represent cell movements and concurrent differentiation events from stem cell to transient progenitor to differentiated somites. If a *laacZ*-to-*lacZ* reversion event occurs in a stem cell during axis elongation (blue circle), its descendants will exit first to the transient progenitor compartment, and from there to the somites. Stripes represent a situation where a stem cell and its descendants are members of a population and therefore contribute only a fraction of the total cell number of a somite. During development, if a stem cell remains in the progenitor region for long periods, it can in principle contribute descendants to all axial levels formed by the population after the reversion event. (**B**) When a large group of clones label the myotome (represented without the progenitor region to show that only the differentiated somite is labelled) the diagnostic test for whether a stem cell population has contributed to the somite is that large clones will have a variable rostral limit, corresponding to the time of initiation of the clone, and a caudal limit at the caudal-most end. (**C**) By contrast, transient progenitor-derived clones will label smaller stretches of somites. (**D**) Transient progenitor-derived clones, which would initiate at random and are by definition not retained in the progenitor region, are not expected to show the polarised pattern seen in B.

region between late-streak and head-fold stages also indicates that some cells can contribute to the neural tube and somites, or to the notochord and somites (Forlani et al., 2003). Similar neural and somitic contributions were also observed by focally labelling up to three epiblast cells close to the chick node (Brown and Storey, 2000). It is important to note that in all prospective clonal labelling studies, only a small proportion of the total cells in the region of interest is labelled, and the accuracy of the single-cell labelling technique used prior to culture is often not reported. Reports of multipotency in progenitors must therefore be interpreted and generalised with caution, and ideally should be confirmed by more than one independent labelling method. However, the independent observations of the presence of apparently multipotent cells in the node and caudal lateral epiblast by several investigators (Brown and Storey, 2000; Forlani et al., 2003; Lawson et al., 1991; Selleck and Stern, 1991) indicate that at least some progenitor cells are multipotent.

As noted above for tissue-specific lineages, there is little information about the persistence of multipotent cells at later stages. Focal labelling in the chick CLE at head-fold stages (HH5-HH7) did not result in any remaining labelled cells in the tail bud after culture (Brown and Storey, 2000). This, however, might be because too few cells were labelled, because a dilution of dye occurred following multiple cell divisions, or because axial stem cells arise later in this cell population. Evidence that supports this latter possibility comes from the change in the contribution pattern of labelled cells in different rostrocaudal regions of the spinal cord, as revealed by the previously mentioned retrospective labelling study (Roszko et al., 2007).

A mixed origin for axial tissues

Whereas the data discussed above indicate where axial stem cells might reside, fate-mapping studies during early gastrulation stages in both mouse and chick show that there is a net movement of epiblast cells towards the primitive streak, with most cells passing through this region and with few epiblast cells apparently residing there (Joubin and Stern, 1999; Lawson et al., 1991; Lawson and Pedersen, 1992; Psychoyos and Stern, 1996; Quinlan et al., 1995; Tam, 1989). At least some of these lateral epiblast cells appear to have an origin that is distinct from the putative stem cells in the early gastrulation epiblast (Hatada and Stern, 1994; Lawson et al., 1991; Tam, 1989). This is difficult to reconcile with a model in which stem cells are the sole contributors to axis elongation. Furthermore, it is noteworthy that cells transit through the chick organiser region before the head process forms (Joubin and Stern, 1999), but that this net flow then ceases, just as the CLE becomes molecularly distinct (see below), which raises the possibility that a suitable niche for resident axial stem cells arises at this point. When traced from about this time, there is also evidence that medial somite progenitors, which are initially located in the NSB region, are retained in the later tail bud, whereas lateral somite progenitors are cleared from this region after two days (Iimura et al., 2007). This latter work corroborates previous studies which also suggested that medial, but not lateral, somite components have a stem cell origin (Selleck and Stern, 1991; Freitas et al., 2001) (reviewed by Tam and Trainor, 1994). Hence, it is possible that the medial, but not the lateral, somite component originates from stem cells.

In conclusion, even though there is compelling evidence that axial stem cells exist in both the mesodermal and neural lineages, the definitive identification of such cells requires further single-cell analysis to demonstrate both the ongoing contribution of individual cells to axial tissues and their long-term residence in the CLE/NSB and tail bud/CNH. These experiments should establish whether all, or merely some, axial tissue is derived from a stem cell population, and whether this corresponds to the multipotent cells observed at earlier stages. In particular, the use of a ubiquitously expressed promoter that drives *laacZ* would provide robust evidence for the existence of long-lived multipotent axial stem cells that can contribute to both mesodermal and neural lineages.

Axial stem cells and extension in lower organisms

The strong evidence that stem cells contribute to axial elongation in mouse and chick, and the conservation of the vertebrate body plan suggest that this mechanism should be conserved between vertebrates. However, reports in fish and amphibians do not usually invoke stem cell participation in axis development. Iimura and Pourquié have proposed that the separate origin of medial and lateral somites is, in fact, conserved amongst all vertebrate model organisms (Iimura and Pourquié, 2007). Clonal lineage analyses in zebrafish and *Xenopus* consistently show that cells close to or overlapping with the organiser (the equivalent of the node) contribute to mostly medial regions of the somites all along the axis, whereas cells progressively more distant from the organiser contribute to successively more caudal somites and to more lateral regions of the somite (Dale and Slack, 1987; Hirsinger et al., 2004; Keller, 1976; Kimmel et al., 1990; Lane and Sheets, 2000). Therefore, it is plausible that in all vertebrates, both organiser cells (i.e. putative stem cells) and separate populations of cells distant from the organiser contribute to somitic tissue. Organiser-derived somite cells constitute a minority population and thus may have been largely overlooked in lower vertebrates.

A discrepancy between higher and lower vertebrates exists, however, in that in *Xenopus* and zebrafish, the rostrocaudal differences in somite contribution from cells originating near to and far from the organiser are very large and are initiated very early: in these organisms, it is possible to label cells at pre-gastrulation stages that will only contribute to the caudal trunk and tail (Dale and Slack, 1987; Kimmel et al., 1990). In mouse and chick, by contrast, the rostrocaudal offset between labelled cell contribution to medial and lateral somite regions that results from labelling cells close to and distant from the organiser represents only a few somite lengths and can be detected only when cells are labelled after the end of gastrulation (Cambray and Wilson, 2007; Iimura and Pourquie, 2006). Furthermore, in chick and mouse, cells in the pre-gastrulation embryo do not contribute exclusively to caudal, rather than rostral, somites. The exclusive contribution of ventral cells to the caudal somites in fish and frog might therefore reflect a much earlier specification of these cells in lower than in higher organisms.

Because, as discussed above, there is evidence for multipotent cells in the region of the organiser, it is of interest to establish whether fate maps of the organiser in lower vertebrates also indicate the presence of multipotent somite, notochord or neural cells. In zebrafish, the fate mapping of single-cell progenitors of the caudal body shows that both the dorsal and ventral components of the shield (the zebrafish equivalent of the node) contribute to the tail somites, but that only the dorsal component produces descendants in the spinal cord and notochord (Kanki and Ho, 1997). Interestingly, no single cell contributes descendants to more than one tissue type. It is possible, however, that multipotent progenitors were missed in this study owing to the small sample of cells labelled (n=105), or that the location of the labelled cells did not coincide with mixed-fate progenitors. Clonal labelling of cells at earlier gastrulation stages in the organiser produced a small minority of multipotent cells (Kimmel et al., 1990; Melby et al., 1996). A fate-mapping study

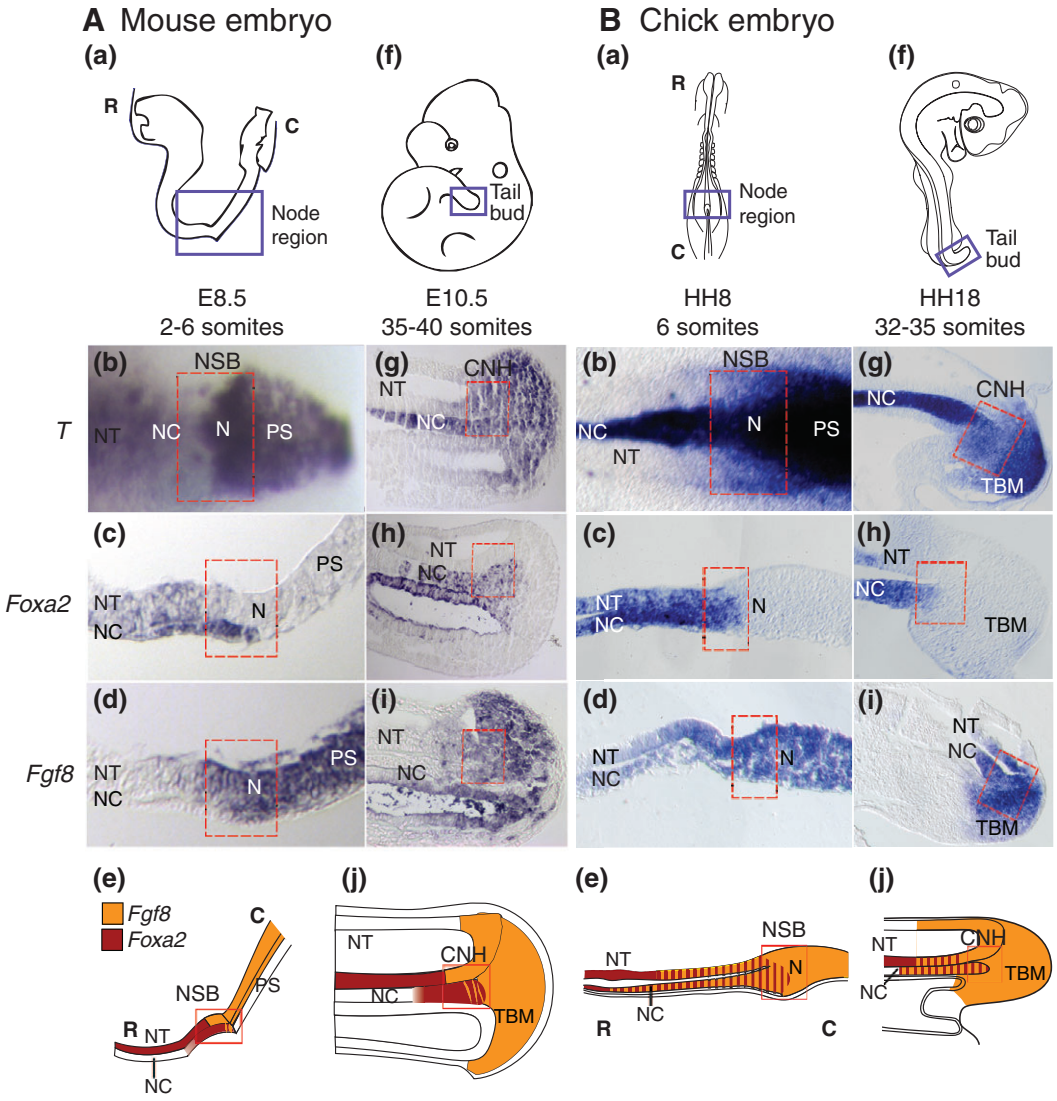

Fig. 5. Defining the node-streak border, caudal lateral epiblast and chordo-neural hinge. (**A**,**B**) Schematics of (A) mouse and (B) chick 2- to 6-somite (Aa,Ba) and 35- to 40-somite (Af,Bf) embryos, depicting the node and the tail bud regions (blue boxes) shown below. (Ab-Ad,Ag-Ai,Bb-Bd,Bg-Bi) Whole-mount in situ hybridisation of selected genes expressed in the node-streak border (NSB, red boxes in early embryos) and chordo-neural hinge (CNH, red boxes in late embryos): *Bra* (Ab,Ag,Bb,Bg); *Foxa2* (Ac,Ah,Bc,Bh) and *Fgf8* (Ad,Ai,Bd,Bi). Ab and Bb, ventral view; Ac,Ad,Ag-Ai,Bc,Bd,Bg-Bi, sagittal sections. Both *Bra* and *Fgf8* are expressed in the NSB and the CNH in both species, but their expression extends further rostrally in the chick than in the mouse. Thus, in the chick, they cannot be considered markers of the NSB or CNH. *Foxa2* labels the end of the neural tube and notochord in both species and can be used to localise the NSB and the CNH. (Ae,Aj,Be,Bj) Summary diagrams showing the similar topography of *Foxa2* (dark red) and *Fgf8* (orange) gene expression in the mouse and chick NSB and CNH. C, caudal; N, node; NC, notochord; NT, neural tube; PS, primitive streak; TBM, tail bud mesoderm; R, Rostral.

using focal labelling (Davis and Kirschner, 2000) in *Xenopus* also suggests that some cells in the later CNH may be multipotent. However, this result must be interpreted with caution, as up to three cells were labelled per embryo, and therefore apparent multipotency may result from the labelling of individual progenitors of restricted potency. Hence, in contrast to mouse and chick, there is little definitive evidence to support the presence of multipotent progenitors in *Xenopus* and zebrafish any later than early gastrulation.

Molecular characterisation of axial progenitors

The analysis of gene expression patterns in the primitive streak, the caudal lateral epiblast and in the tail bud has identified many genes that mark distinct subpopulations of cells from late gastrulation, as the head process emerges and somitogenesis commences, until tail bud stages in both mouse and chick (see Fig. 5). A group of primitive streak marker genes, including fibroblast growth factor 8 (*Fgf8*) and *Wnt3a*, are expressed in the primitive streak, in the upper (epiblast) layer of the NSB and in the CLE from late gastrulation stages in mouse and chick embryos (Fig. 5A,B); these genes are also expressed in the tail bud (Fig. 5A,B) (Cambray and Wilson, 2007; Chapman et al., 2002; Gofflot et al., 1997) (K.G.S. and I.O.-M., unpublished). The boundary between the rostral part of the node and the NSB is marked in mouse by a transition in the epiblast domain between a region of *Fgf8* expression and an epiblast-specific expression domain of the forkhead transcription factor *Foxa2* (Fig. 5A), which might mark the beginning of floor plate formation. Interestingly, in chick, the *Fgf8* expression domain appears to extend

further rostrally into the node itself and overlaps with the epiblast-specific *Foxa2* domain (Fig. 5B). Hence, although the expression of key marker genes in early caudal progenitor and tail bud cell populations is conserved, there are some species-specific differences in the precise spatial relationship of these genes.

The CLE becomes molecularly distinct from the rest of the neural plate as the head process and the notochord emerge from the node at the 1- to 2-somite stage. In the chick, this is indicated by the expression of the basic helix-loop-helix (bHLH) proneural gene homologue *Cash4* and an Nkx transcription factor, *Sax1* (Henrique et al., 1997; Spann et al., 1994). *Sax1* expression is conserved in the mouse (*Nkx1-2* – Mouse Genome Informatics) and similarly distinguishes the CLE from rostral neural plate regions (Schubert et al., 1995). Unlike the rest of the neural plate, the CLE cell population expresses both early pan-neural genes, such as *Sox2*, and brachyury (*T*), which marks prospective as well as nascent paraxial mesoderm (Cambray and Wilson, 2007; Delfino-Machin et al., 2005; Kispert and Herrmann, 1994; Kispert et al., 1995). In both chick and mouse embryos, this overlap between early neural and mesodermal genes persists into tail bud stages and includes cells in the CNH (Cambray and Wilson, 2007; Kispert and Herrmann, 1994; Kispert et al., 1995; Knezevic et al., 1998; Schubert et al., 1995; Spann et al., 1994). These expression patterns are therefore consistent with the cell labelling studies described above, which indicate that the CLE contributes to neural and mesodermal lineages, and with the possible existence of multipotent neural and/or mesodermal axial stem cells in the caudal lateral epiblast/upper layer of the NSB and the CNH.

Importantly, although there are many similarities between gene expression patterns in the caudal lateral epiblast/NSB and the tail bud, they should not be viewed as being exactly equivalent. The primitive streak does not express caudal Hox genes, whereas the tail bud does. McGrew and colleagues showed that *Hoxa10*, expressed in the tail bud, is dramatically downregulated a few hours after tail bud cells are transplanted to the primitive streak of earlier stage embryos, which are *Hoxa10* negative (McGrew et al., 2008). *Hoxa10* and *Hoxc10* then become appropriately expressed as the cells contribute to the axis. Thus, not only do CLE/NSB and tail bud cells change their Hox gene expression profile over time, but these axial progenitors retain the capacity to adapt this profile to more rostral and developmentally younger environments, indicating that, at least for these caudal-most Hox genes, the change in expression is a reversible process. These findings underscore the general observation that the signalling environment that progenitor cells experience influences their pattern of gene expression.

Signals promoting body axis extension

Despite these differences in signalling environment across developmental stages, the activity of a number of signalling pathways, including that of the FGF, Wnt and Notch pathways, is known to maintain the undifferentiated cell state of progenitors in the caudal region. Principal among these is FGF signalling, and numerous FGFs are expressed in chick and mouse embryos in the rostral primitive streak, in the CLE and in the presomitic mesoderm (Boettger et al., 1999; Crossley and Martin, 1995; Karabagli et al., 2002; Mahmood et al., 1995; Ohuchi et al., 2000; Riese et al., 1995; Shamim and Mason, 1999). The emergence of the presomitic mesoderm from the primitive streak is important for the maintenance of *Cash4* and *Sax1*, as the expression of these genes is lost on the removal of this mesoderm in the chick (Diez del Corral et al., 2002), and mice that lack paraxial mesoderm owing to a mutation of *T* also lose *Sax1* expression (Schubert et al., 1995). FGF signalling maintains the expression of these genes in the CLE (Diez del Corral et al., 2002; Henrique et al., 1997), and a direct requirement for FGFR signalling for *Sax1* expression in these epiblast cells has been demonstrated (Delfino-Machin et al., 2005). FGF signalling is also required for the movement of epiblast cells through the primitive streak to form paraxial mesoderm (Ciruna and Rossant, 2001; Partanen et al., 1998; Yamaguchi et al., 1994; Yang et al., 2002) (see Table 1). *Fgf8* expression is downstream of *Wnt3a* (Aulehla et al., 2003) in the mouse, and *Fgfr1* and *Wnt3a* mutant mice both form ectopic neural tissue at the expense of paraxial mesoderm (Takada et al., 1994; Yamaguchi et al., 1994; Yamaguchi et al., 1999b; Yoshikawa et al., 1997) (see Table 1), which is consistent with the possibility that these tissues arise from a common progenitor. These observations, together with the finding that low-level FGFR signalling promotes neural cell fate in the *Xenopus* embryo (Delaune et al., 2005; Launay et al., 1996; Linker and Stern, 2004; Sasai et al., 1996), support the idea that high levels of or prolonged exposure to FGF signalling promotes mesoderm formation, whereas low levels elicit a neural fate (reviewed by Stern, 2005). Interestingly, it is the epiblast cells closest to the primitive streak that actively transcribe *Fgf8*, whereas only mature *Fgf8* mRNAs are detected in the caudal paraxial mesoderm (Dubrulle and Pourquié, 2004). The CLE might therefore be a region of heightened FGF signalling – as further supported by high levels of Erk1/2 mitogen-activated protein kinase activity (Corson et al., 2003; Lunn et al., 2007) – that can provide a suitable niche for multipotent stem cells, which might contribute to neural or mesodermal lineages. Neural progenitors might be exposed to lower levels of FGF signalling for shorter time periods, exiting the NSB/CLE for the neuroepithelium and downregulating *Fgf8* (the loss of which is promoted by retinoid signalling, see below), whereas cells ingressing through the primitive streak continue to be exposed to high levels of Fgf8 for longer, and hence give rise to mesoderm (Fig. 6).

FGF signalling is also required for the expression of the Notch ligand *Delta1* in epiblast cells closest to the primitive streak in the chick (Akai et al., 2005) (Fig. 6), although this regulatory relationship does not appear to exist in the mouse caudal paraxial mesoderm (Wahl et al., 2007). In chick, Notch signalling in this medial part of the CLE is required to maintain cell proliferation, and this role is also consistent with the axial truncation that is seen in many mouse mutants that lack Notch signalling [such as in Delta-like 3, Notch 1, Rbpj and lunatic fringe (Lfng) mutants] (de la Pompa et al., 1997; Donoviel et al., 1999; Evrard et al., 1998; Shen et al., 1997; Wong et al., 1997) (see Table 1). Other signals have also been shown to maintain cell proliferation in caudal regions. Wnt5a acts in the paraxial mesoderm and the primitive streak, but through a distinct mechanism that does not involve conventional or canonical Wnt signalling (Yamaguchi et al., 1999a). Interestingly, Wnt5a mutants, like those of Wnt3a, fail to extend the tail after E10.5 (Table 1), but segmentation continues into their tail tip, as if the embryo simply failed to form enough mesoderm rather than switching to a neural fate, as occurs in Wnt3a mutant mice (see Table 1).

The findings discussed above suggest that a combination of FGF, Wnt and Notch signalling acts to promote proliferation and to support the less-differentiated cell state that is characteristic of tail end tissues. Furthermore, differential exposure to Wnt/FGF signalling at the tail end might additionally help to resolve neural and mesodermal cell fates in the extending axis. Retinoid signalling is also implicated in this process, as excess retinoic acid (RA) at the tail end not only causes axis truncation (see below), but also generates a phenotype similar to that of Wnt3a mutant mice, with

Table 1. Examples of mutations in signalling pathway components that cause axis truncations in the mouse

Mutant gene	Phenotype	References
Fgf signalling		
Fgfr1$^{-/-}$	Die at gastrulation, accumulation of cells at the caudal streak, severe reduction in paraxial mesoderm formation.	Deng et al., 1994; Yamaguchi et al., 1994
Fgfr1$^{-/-}$ and wild-type chimaeric embryos	Caudal truncations; tail distortion; spina bifida; ectopic neural tubes.	Ciruna et al., 1997; Deng et al., 1997; Ciruna and Rossant, 2001
Fgfr1$^{f/f}$*;T-Cre**	Axial truncations in the sacral and tail regions; cervical vertebrae normal, thoracic and lumbar progressively fused.	Wahl et al., 2007
Fgf8$^{-/-}$	Cells move into the streak but do not come out, no mesoderm forms.	Sun et al., 1999
Wnt signalling		
Vestigial tail (*Wnt3a* hypomorph) mutant	Axis truncation, distal to first caudal vertebrae; caudal NT closure defects; crossed to *Wnt3a* null, less Wnt3a, more rostral vertebral defects.	Greco et al., 1996; Gruneberg and Wickramaratne, 1974; Takada et al., 1994; Yoshikawa et al., 1997
Wnt3a$^{-/-}$	Axis truncation, distal to the forelimb, no tailbud forms; one ectopic ventral NT.	Takada et al., 1994; Yamaguchi et al., 1999b
Wnt5a$^{-/-}$	Axis truncation from sacral vertebrae 0-4, no caudal vertebrae; vertebral fusions, and vertebrae are also shorter than in wild type.	Yamaguchi et al., 1999a
Lrp6$^{-/-}$	Axis truncated distal to lumbar vertebrae; caudal neuropore closure defects; die at birth; Wnt3a down, mesoderm missing, excess neural tissue.	Pinson et al., 2000
Dkk1$^{d/d-}$ (doubleridge mutant)	Lower Dkk1 expression, hypomorphic allele; kinked tails (vertebral fusions).	MacDonald et al., 2004
Axin (fused mutant)	Kinking and shortening of the tail.	Reed, 1937; Zeng et al., 1997
β-catenin$^{f/f}$*;T-Cre*†	Severe axis truncation after a few somites.	Aulehla et al., 2008
Notch signalling		
Notch1$^{-/-}$	Developmental arrest around E9.	Conlon et al., 1995; de la Pompa et al., 1997
Rbpj$^{-/-}$	Developmental arrest around E9.	de la Pompa et al., 1997
Lfng$^{-/-}$	Axis truncation, sacral and caudal vertebrae missing; vertebrae fused and malformed in cervical, thoracic and lumbar.	Evrard et al., 1998; Shifley et al., 2008; Zhang and Gridley, 1998
Dll3$^{-/-}$ (pudgy mutant)	Tail truncation, vertebrae missing from caudal 5-10; skeletal disorganisation from vertebra cervical 1.	Dunwoodie et al., 2002
Ps1$^{-/-}$	Some caudal vertebrae missing; vertebrae fused.	Wong et al., 1997; Donoviel et al., 1999
Retinoid signalling		
Cyp26a1$^{-/-}$	Axis truncation around hindlimb level; cervical and thoracic vertebrae caudal transformations, vertebrae behind L1 missing or so malformed that they are impossible to identify; caudal NT open.	Abu-Abed et al., 2001
Por$^{-/-}$	Die at E9.5; caudal truncation from around the first caudal vertebrae.	Otto et al., 2003; Ribes et al., 2007
Gcnf$^{-/-}$	Die at 10.5; halt in somitogenesis after 13 somites; open NT; tail bud develops ectopically outside the yolk sac.	Chung et al., 2001
BMP signalling		
Nog$^{-/-}$	Axis truncation, loss of caudal vertebrae; open NT; shortened axis.	Goldman et al., 2000; McMahon et al., 1998
FGF and Wnt targets		
Brachyury (*T* mutant)	Tail truncated in heterozygous mice, after a few caudal vertebrae.	Dobrovolskaia-Zavadskaia, 1927; Herrmann et al., 1990; Yamaguchi et al., 1999b
Tc (*T* antimorph)	Post-anal tail truncated.	MacMurray and Shin, 1988
Cdx2$^{+/-}$	Mild axis truncation, vertebrae missing from C26-28; caudal transformations.	Chawengsaksophak et al., 1997
Cdx1$^{+/-}$*;Cdx2*$^{+/-}$	Axis truncation, vertebrae missing from C15-20; caudal transformations.	van den Akker et al., 2002
Cdx1$^{-/-}$*;Cdx2*$^{+/-}$	Axis truncation, vertebrae missing from C6-11; caudal transformations of vertebrae along the length of the spinal cord.	van den Akker et al., 2002

*Conditional null homozygous for Fgfr1 floxed in a T-Cre background.
†Conditional null homozygous for β-catenin floxed in a T-Cre background.
NT, neural tube.

DEVELOPMENT

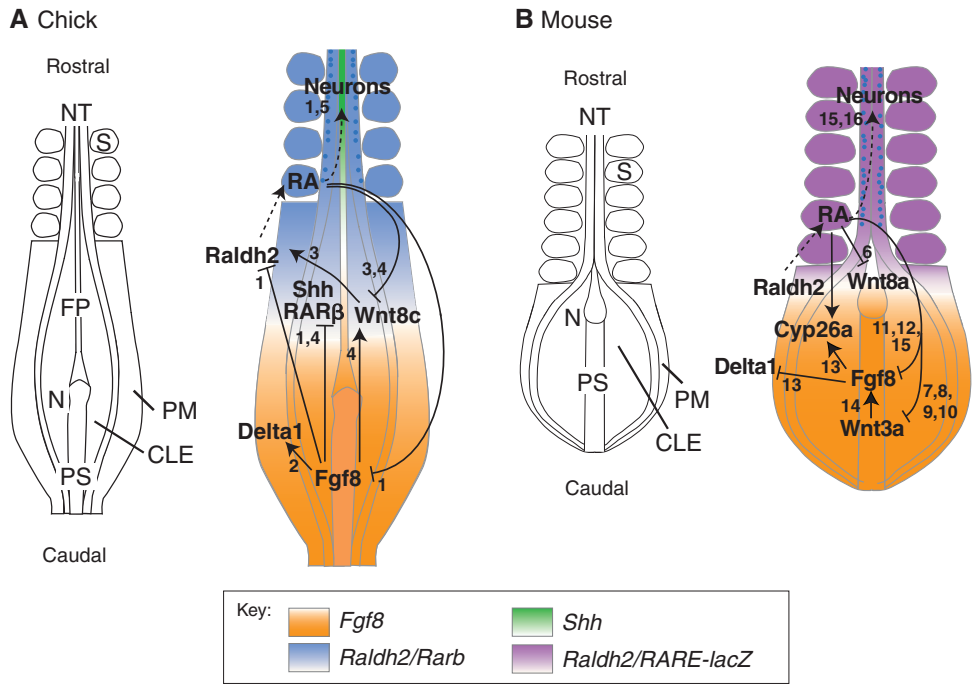

Fig. 6. Signals that regulate axis extension in chick and mouse embryos. (A,B) Schematics of the molecular interactions that regulate differentiation in the extending body axis in chick and mouse, with reference to the supporting published data (indicated by the numbers on the schematics and below). (**A**) In the HH10 chick, Fgf8 inhibits the onset of expression of the retinoic acid (RA)-synthesizing enzyme Raldh2 in the presomitic mesoderm (1) and the expression of *Rarb* in the neuroepithelium (4), thus preventing RA from triggering differentiation in the CLE and the caudal-most paraxial mesoderm (1,5). In addition, Fgf8 inhibits sonic hedgehog (*Shh*) expression in the floorplate, controlling the onset of ventral patterning genes (1). FGF signalling is also required for expression of *Delta1* in the medial CLE (2) and promotes expression of *Wnt8c* (4). As *Fgf8* decays in the caudal paraxial mesoderm (Dubrulle and Pourquié, 2004), Wnt signalling, most likely provided by Wnt8c, now acts to promote *Raldh2* in the adjacent presomitic mesoderm (4). RA produced by Raldh2 activity represses *Fgf8* (1) and *Wnt8c* (3,4), and the expression of both these genes is increased in vitamin A-deficient quails (1,4). (**B**) In the E8.5-E9.5 mouse, *Fgf8* is maintained by Wnt3a, as indicated by *Fgf8* loss in the Wnt3a hypomorph vestigial tail (14). Loss of signalling through Fgfr1 specifically in the *T*-expressing domain leads to loss of *Cyp26a* (13). Loss of such signalling also leads to increased *Delta1* expression in the emerging paraxial mesoderm (13). Excess RA signalling by RA treatment (7,8) or loss of *Cyp26a* (9,10) leads to a reduction of caudal Wnt3a expression. *Raldh2* mutant mice exhibit an expanded domain of caudal *Fgf8* (11,12,15) and *Wnt8a* (6). RA is also required for the onset of neuronal differentiation and for expression of patterning genes in the neural axis (15,16). CLE, caudal lateral epiblast; FP, floorplate; N, node; NT, neural tube; PM, paraxial mesoderm; PS, primitive streak; S, somite. References that support the interactions shown: 1 (Diez del Corral et al., 2003), 2 (Akai et al., 2005), 3 (Dupe and Lumsden, 2001), 4 (Olivera-Martinez and Storey, 2007), 5 (Novitch et al., 2003), 6 (Niederreither et al., 2000), 7 (Iulianella et al., 1999), 8 (Shum et al., 1999), 9 (Sakai et al., 2001), 10 (Abu-Abed et al., 2001), 11 (Vermot et al., 2005), 12 (Sirbu and Duester, 2006), 13 (Wahl et al., 2007), 14 (Aulehla et al., 2003), 15 (Molotkova et al., 2005), 16 (Ribes et al., 2008).

neural tissue forming at the expense of paraxial mesoderm (Abu-Abed et al., 2001; Sakai et al., 2001). As cells leave the tail end of the embryo, an attenuation of FGF signalling is required for the onset of expression of differentiation genes in both neural and paraxial mesodermal tissue (Diez del Corral et al., 2003; Dubrulle et al., 2001). The progressive loss of FGF signalling as cells leave the caudal paraxial mesoderm is thought to constitute a wave-front, which, in combination with the Notch-mediated periodic expression of so-called ‘clock genes’, determines the position of somite boundaries in this tissue (for a review, see Dequeant and Pourquié, 2008).

Cessation of axis elongation

The arrest of body axis elongation seems intimately associated with the differentiation process, as both involve the downregulation of FGFs and Wnts. A key signalling pathway that regulates both processes is that mediated by RA. During somitogenesis stages, cells are exposed to endogenous RA as they leave the CLE and the NSB or later tail bud. This is provided by the activity of the RA synthesising enzyme *Raldh2*, which is expressed in the newly segmenting mesoderm. RA signalling drives the expression of neural and mesodermal differentiation genes in axial tissues (Diez del Corral et al., 2003; Molotkova et al., 2005; Moreno and Kintner, 2004; Ribes et al., 2008). This includes neuronal differentiation genes, which promote neuron production, the floor-plate expression of sonic hedgehog (*Shh*), the key orchestrator of ventral patterning and hence of neuronal cell-type specification (Diez del Corral et al., 2003), and mesodermal differentiation genes such as *Mesp2*, a key segmentation gene that helps to define new somite borders (Morimoto et al., 2005).

RA promotes differentiation in part by inhibiting *Fgf8* expression as cells move out of the CLE and the primitive streak in chick and mouse embryos (Diez del Corral et al., 2003; Sirbu and Duester, 2006; Vermot et al., 2005). It can also accelerate *Fgf8* loss in the chick caudal presomitic mesoderm (Diez del Corral et al., 2003), where *Fgf8* mRNA is not actively transcribed (Dubrulle and Pourquie, 2004). This action thus also implicates RA signalling in the paraxial mesoderm in the positioning of the somite boundary and hence in

determining somite size, given that the distance travelled by the falling level of Fgf signalling in the presomitic mesoderm during one oscillation of the segmentation clock defines where each somite boundary will form (Dubrulle et al., 2001) (reviewed by Dequeant and Pourquié, 2008). Consistent with this role, smaller (recently formed) somites are found in retinoid-deficient animals (Diez del Corral et al., 2003). However, in later-stage mouse embryos, retinoid signalling is not detected in the presomitic mesoderm and might only be required for early segment formation in this context (Sirbu and Duester, 2006). In mice, *Fgf8* is maintained by Wnt3a (Aulehla et al., 2003), and in chick Fgf8 in turn promotes the expression of *Wnt8c* (the orthologue of mouse *Wnt8a*) in the forming neural axis (Olivera-Martinez and Storey, 2007). The expression of all three genes is lost upon RA exposure in both chick (Diez del Corral et al., 2003; Dupe and Lumsden, 2001; Olivera-Martinez and Storey, 2007) and mouse (Iulianella et al., 1999; Niederreither et al., 2000; Shum et al., 1999) (see below and Fig. 6).

Normally, the embryo deploys a number of mechanisms to protect the tail end from retinoid signalling. The ones uncovered so far are all downstream effects of FGF signalling (see Fig. 6). FGF signals inhibit the onset of *Raldh2* expression in the paraxial mesoderm (Diez del Corral et al., 2003) and also repress the expression of retinoic acid receptor β (*Rarb*) in the neuroepithelium (Olivera-Martinez and Storey, 2007). In addition, conditional *Fgfr1* loss in the *T*-expressing domain results in the loss of a major RA-metabolising enzyme called Cyp26a, which is expressed at the mouse tail end (Abu-Abed et al., 2001; Sakai et al., 2001; Wahl et al., 2007). This regulatory relationship is conserved in *Xenopus* (Moreno and Kintner, 2004). Furthermore, in zebrafish, Notch signalling is upstream of *Cyp26a* expression in the tail bud (Echeverri and Oates, 2007). Crucially, the loss of Cyp26a (a cytochrome P450 oxidoreductase), of Por (a cytochrome P450 reductase, which donates electrons to P450 enzymes during the breakdown of RA) or of germ cell nuclear factor (Gcnf), also known as retinoid receptor-related testis-associated receptor (RTR or Nr6a1; a mediator of retinoid signalling in ES cells), all lead to axial truncations in the mouse (Abu-Abed et al., 2001; Chung et al., 2001; Gu et al., 2005; Otto et al., 2003) (see retinoid signalling mutants in Table 1).

Significantly, and consistent with the above phenotypes, exposure to exogenous RA causes axial truncations in many vertebrate embryos, including the mouse (Griffith and Wiley, 1991; Kessel, 1992). This involves the rapid inhibition of *Wnt3a* (Shum et al., 1999). Indeed, the mouse mutant vestigial tail, which is a *Wnt3a* hypomorph, displays caudal agenesis (Greco et al., 1996; Gruneberg and Wickramaratne, 1974; Takada et al., 1994; Yoshikawa et al., 1997), and both RA-treated embryos and vestigial tail mutants exhibit extensive cell death in the tail bud (Shum et al., 1999) (see Table 1). *Wnt3a* and *Fgf8* expression can both promote the expression of the transcription factor T (Galceran et al., 2001; Yamaguchi et al., 1999b). *T* mutant embryos also exhibit dramatic axial truncation (Chesley, 1935) (see Table 1). This can be rescued by the *T* gene in a dose-dependent manner (Stott et al., 1993) and its loss is also characterised by precocious cell death in the mouse primitive streak (Chesley, 1935). Taken together, these findings suggest that Wnt3a and Fgf8 signalling upstream of *T* and *Cyp26a* expression promote cell survival in the tail bud.

As the presomitic mesoderm shortens during axial elongation (Gomez et al., 2008; Sanders et al., 1986), RA from the most recently formed somites might now be able to reach tail bud cells, thereby ending this process. This possibility is supported by the downregulation of *Fgf8* in the mouse tail by E12.5 (Cambray and Wilson, 2007) and in the chick at HH26/HH27 (I.O.-M. and K.G.S., unpublished) just prior to the end of axis elongation. This might elicit a slowing down of the cell cycle and the eventual cell cycle exit of progenitor cell populations. Conversely, the loss of *Fgf8* also coincides with high levels of cell death in the late-stage chick tail bud (Hirata and Hall, 2000; Mills and Bellairs, 1989; Sanders et al., 1986; Yang et al., 2006), raising the possibility that a local increase in endogenous RA triggers apoptosis to terminate axis extension.

Although premature cell death can produce axial truncations, it is unlikely to be the sole cause of this phenotype. Mice that lack the expression of the transcription factors caudal type homeobox 1 (*Cdx1*) and *Cdx2* have axial truncations similar to those seen in *Wnt3a*, *Lef/Tcf1* double mutants (Galceran et al., 1999), *Fgfr1* hypomorphs (Partanen et al., 1998) and heretozygous *T* mutants (Herrmann et al., 1990; van den Akker et al., 2002) (Table 1). However, cell death does not appear to increase in the tail bud of these embryos, although complete Cdx null mice have yet to be examined (J. Deschamps, personal communication). Cdx genes are regulated by FGF and Wnt in the chick caudal lateral epiblast (Bel-Vialar et al., 2002; Nordstrom et al., 2006; Wang and Shashikant, 2007) and have been proposed to regulate proliferation in this tail bud context (van den Akker et al., 2002). Indeed, an increased dose of Cdx2 protein also leads to axis truncation, but in this case neurulation is defective and excessive mesodermal tissue forms in a bulbous mass at the tail end, as if cells over-proliferate and fail to exit this region to commence differentiation (Gaunt et al., 2008). This suggests that an imbalance between the maintenance of progenitors and their differentiation might be an alternative cause of axial truncation.

Signals from the ventral ectodermal ridge (VER), an ectodermal thickening that runs along the underside of the tail bud (Gruneberg, 1956), are also required for the normal elongation and segmentation of the tail (Goldman et al., 2000; Liu et al., 2004; Ohta et al., 2007). However, VER removal does not appear to induce cell death (Goldman et al., 2000). The VER maintains expression of the bone morphogenetic protein (BMP) antagonist noggin (*Nog*) in the underlying tail bud mesenchyme (Goldman et al., 2000), and *Nog* mutant mice display axial truncations from E10.5 (McMahon et al., 1998) (Table 1). These mutants lack the VER and display neither cell death nor proliferation defects in the tail bud (Ohta et al., 2007). However, they do exhibit ectopic cell ingression from the outer ectoderm mediated by BMP signalling (Ohta et al., 2007). The VER therefore exerts its influence, at least in part, by maintaining the correct level of BMP signalling. This seems to be crucial for normal cell movements in the tail bud, which might be a further requirement for normal axial elongation.

It will be interesting to discover how these different signalling pathways act on distinct tail bud progenitor populations, and how they interact to arrest axis extension and to define body length. The impact of these signals on the expression of cell identity genes, including Cdx and Hox genes, such as Hoxb13, the loss of which promotes excessive axis extension (Economides et al., 2003), will also further elucidate how positional identity is linked to cell behaviour. Some of these signals that regulate body axis extension in chick and mouse embryos are also implicated in the related process of tail induction in lower vertebrates.

Conservation of signalling mechanisms regulating axis extension

The comparison of the regulation of axis extension in higher vertebrates with tail induction in lower vertebrates might reveal evolutionarily conserved mechanisms. Tucker and Slack proposed a model for tail development in *Xenopus* in which the cells that

remain in the blastopore (equivalent to the primitive streak remnant) at the end of gastrulation are composed of three separate populations that interact to initiate tail elongation: the neural precursors in the neural plate (the N region); the muscle progenitors immediately behind them in the ectoderm (the M region); and the underlying notochord (the C region) (Tucker and Slack, 1995). These three regions are then incorporated into the CNH of the tail bud. The topological equivalent of the N-M-C junction would therefore be the NSB in mouse and chick.

In zebrafish, blastopore closure has been proposed to bring the dorsal cells (the organiser, expressing nodal-related genes and Bmp2/Bmp4 antagonists) close to the ventral involuting cells that express Bmp2/Bmp4 (Agathon et al., 2003). This ventral tissue has a 'tail organiser' activity, i.e. it promotes tail outgrowth without contributing to all tail tissues through the combinatorial activity of the BMP pathway with Wnt and Nodal signalling. This agrees with data in *Xenopus* that show that BMP signalling is essential for tail somitogenesis (Beck et al., 2001), and that the induction of tail outgrowth requires Xwnt3A (Beck and Slack, 2002). In the zebrafish tail induction studies, FGF signalling was not specifically investigated. In *Xenopus*, however, the species in which the mesoderm-inducing properties of FGF signalling were first shown (Slack et al., 1988), blocking FGF signalling leads to axial truncations, showing that FGF is involved in axial elongation (Amaya et al., 1991). Furthermore, retinoid signalling also attenuates FGF signalling in the frog body axis, supporting the idea of conservation of the signalling mechanism that regulates differentiation onset during axis elongation (Moreno and Kintner, 2004). Notch signalling is also important for *Xenopus* tail bud outgrowth (Beck and Slack, 1999; Beck and Slack, 2002). Therefore, in all vertebrates studied, axial elongation seems to involve similar signalling pathways. However, the N-M-C model for tail bud induction in *Xenopus* is proposed to act at stage 13 (early neurula stage), well before the beginning of somite segmentation at stage 17 (Hausen, 1991; Nieuwkoop and Faber, 1967). Similarly, in zebrafish, tail bud induction is proposed to take place at, or just before, the onset of somitogenesis (Kimmel et al., 1995), which is essentially the equivalent of mouse late head-fold/early somite stage or chick HH5-HH7. Therefore, the mechanisms that are proposed to induce the tail in *Xenopus* and zebrafish might act at mouse and chick late primitive streak stages, and it is possible that, in these organisms, they ensure continued axis elongation during early somitogenesis stages rather than producing the tail bud per se.

Interestingly, during node regression in the mouse, the node approaches the caudal end, such that once the tail fold is formed the ventral BMP-expressing tissue is closely apposed to the organiser. Therefore, the phase of somitogenesis that occurs after node regression in the mouse, i.e. the formation of the axis caudal to the forelimb bud, might depend on the close apposition of BMP, Wnt and Nodal signalling. Nodal expression completely disappears from the mouse axis by E9.5 (i.e. between the 8- and 22-somite stages) and is therefore unlikely to play a part in the formation of the post-anal tail. This lends further support to the idea that, if signalling mechanisms similar to those described above for lower vertebrates operated in chick and mouse, they would act not in the formation of the tail, but more rostrally in the axis.

Conclusions

The evidence discussed above suggests that vertebrate axis elongation is likely to depend on contributions from a mixture of the output of a retained stem cell population and of transient progenitors. This stem cell contribution has only been studied in mesodermal and neural tissues, and the mechanism of extension of the third germ layer, the endoderm, has received little attention. Because all three layers extend along the whole axis, it is likely that mechanisms exist to ensure their coordinated extension. This is supported by the observation that in mouse mutants that display axial truncations (such as the *Cdx2* mutant) (Chawengsaksophak et al., 2004) gut endoderm fails to form from the same level as neural and mesodermal tissues. However, evidence for a stem cell population in the tail bud region effecting gut elongation is currently lacking. This is in part because long-term clonal analysis has yet to be carried out in this tissue. The extent to which axial stem cells contribute to body extension might vary during the course of this process, and between higher and lower vertebrate model embryos. It is now important to identify the precise location(s) and molecular characteristics of the axial stem cell sub-population, as well as the signalling niche that specifies and maintains this distinct cell type. Current data suggest that these cells reside in the NSB, but possibly also at the edge of the primitive streak in the CLE and in the later-forming CNH. These cells might be maintained by a combination of high FGF, Wnt and Notch signalling, and perhaps by the expression of P450 enzymes, such as Cyp26a, which together promote proliferation, maintain an uncommitted progenitor cell state and provide protection from retinoid-mediated differentiation. Axial stem cells might share some properties with recently identified epiblast stem cells derived from the pre-gastrula epiblast, which self-renew, in mouse, when exposed to a combination of FGF and activin and, in human, in response to FGF alone (Brons et al., 2007; Rossant, 2008; Tesar et al., 2007). A key challenge for the future is to understand the essential changes that take place as cells progress along the apparent continuum from an embryonic stem cell to an epiblast stem cell-like state and, later, to a potential axial stem cell state.

We are grateful to Pamela Halley for cryostat sections of chick embryos and for the photographs shown in Fig. 5Bb-d,g-i, and to anonymous reviewers for helpful comments. V.W., K.G.S. and I.O.-M. are supported by the MRC. V.W. is also supported by the Association for International Cancer Research.

References

Abu-Abed, S., Dolle, P., Metzger, D., Beckett, B., Chambon, P. and Petkovich, M. (2001). The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures. *Genes Dev.* **15**, 226-240.

Agathon, A., Thisse, C. and Thisse, B. (2003). The molecular nature of the zebrafish tail organizer. *Nature* **424**, 448-452.

Akai, J., Halley, P. A. and Storey, K. G. (2005). FGF-dependent Notch signaling maintains the spinal cord stem zone. *Genes Dev.* **19**, 2877-2887.

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant-negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66**, 257-270.

Aulehla, A., Wehrle, C., Brand-Saberi, B., Kemler, R., Gossler, A., Kanzler, B. and Herrmann, B. G. (2003). Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev. Cell* **4**, 395-406.

Aulehla, A., Wiegraebe, W., Baubet, V., Wahl, M. B., Deng, C., Taketo, M., Lewandoski, M. and Pourquie, O. (2008). A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nat. Cell Biol.* **10**, 186-193.

Beck, C. W. and Slack, J. M. (1999). A developmental pathway controlling outgrowth of the Xenopus tail bud. *Development* **126**, 1611-1620.

Beck, C. W. and Slack, J. M. (2002). Notch is required for outgrowth of the Xenopus tail bud. *Int. J. Dev. Biol.* **46**, 255-258.

Beck, C. W., Whitman, M. and Slack, J. M. (2001). The role of BMP signaling in outgrowth and patterning of the Xenopus tail bud. *Dev. Biol.* **238**, 303-314.

Bel-Vialar, S., Itasaki, N. and Krumlauf, R. (2002). Initiating Hox gene expression: in the early chick neural tube differential sensitivity to FGF and RA signaling subdivides the HoxB genes in two distinct groups. *Development* **129**, 5103-5115.

Boettger, T., Wittler, L. and Kessel, M. (1999). FGF8 functions in the specification of the right body side of the chick. *Curr. Biol.* **9**, 277-280.

Brons, I. G., Smithers, L. E., Trotter, M. W., Rugg-Gunn, P., Sun, B., Chuva de

Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A. et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448**, 191-195.
Brown, J. M. and Storey, K. G. (2000). A region of the vertebrate neural plate in which neighbouring cells can adopt neural or epidermal cell fates. *Curr. Biol.* **10**, 869-872.
Cambray, N. and Wilson, V. (2002). Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. *Development* **129**, 4855-4866.
Cambray, N. and Wilson, V. (2007). Two distinct sources for a population of maturing axial progenitors. *Development* **134**, 2829-2840.
Catala, M., Teillet, M. A. and Le-Douarin, N. M. (1995). Organization and development of the tail bud analyzed with the quail-chick chimaera system. *Mech. Dev.* **51**, 51-65.
Catala, M., Teillet, M. A., De Robertis, E. M. and Le Douarin, M. L. (1996). A spinal cord fate map in the avian embryo: while regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. *Development* **122**, 2599-2610.
Chapman, S. C., Schubert, F. R., Schoenwolf, G. C. and Lumsden, A. (2002). Analysis of spatial and temporal gene expression patterns in blastula and gastrula stage chick embryos. *Dev. Biol.* **245**, 187-199.
Charrier, J. B., Teillet, M. A., Lapointe, F. and Le Douarin, N. M. (1999). Defining subregions of Hensen's node essential for caudalward movement, midline development and cell survival. *Development* **126**, 4771-4783.
Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F. and Beck, F. (1997). Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* **386**, 84-87.
Chawengsaksophak, K., de Graaff, W., Rossant, J., Deschamps, J. and Beck, F. (2004). Cdx2 is essential for axial elongation in mouse development. *Proc. Natl. Acad. Sci. USA* **101**, 7641-7645.
Chesley, P. J. (1935). Development of the short-tailed mutant in the house mouse. *Exp. Zool.* **70**, 429-459.
Chung, A. C., Katz, D., Pereira, F. A., Jackson, K. J., DeMayo, F. J., Cooney, A. J. and O'Malley, B. W. (2001). Loss of orphan receptor germ cell nuclear factor function results in ectopic development of the tail bud and a novel posterior truncation. *Mol. Cell. Biol.* **21**, 663-677.
Ciruna, B. and Rossant, J. (2001). FGF signalling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. *Dev. Cell* **1**, 37-49.
Conlon, R. A., Reaume, A. G. and Rossant, J. (1995). Notch1 is required for the coordinate segmentation of somites. *Development* **121**, 1533-1545.
Corson, L. B., Yamanaka, Y., Lai, K. M. and Rossant, J. (2003). Spatial and temporal patterns of ERK signaling during mouse embryogenesis. *Development* **130**, 4527-4537.
Criley, B. B. (1969). Analysis of the embryonic sources and mechanisms of development of the posterior levels of chick neural tubes. *J. Morphol.* **128**, 465-502.
Crossley, P. H. and Martin, G. R. (1995). The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development.* **121**, 439-451.
Dale, L. and Slack, J. M. (1987). Fate map for the 32-cell stage of Xenopus laevis. *Development* **99**, 527-551.
Davis, R. L. and Kirschner, M. W. (2000). The fate of cells in the tailbud of Xenopus laevis. *Development* **127**, 255-267.
de la Pompa, J. L., Wakeham, A., Correia, K. M., Samper, E., Brown, S., Aguilera, R. J., Nakano, T., Honjo, T., Mak, T. W., Rossant, J. et al. (1997). Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* **124**, 1139-1148.
Delaune, E., Lemaire, P. and Kodjabachian, L. (2005). Neural induction in Xenopus requires early FGF signalling in addition to BMP inhibition. *Development* **132**, 299-310.
Delfino-Machin, M., Lunn, J. S., Breitkreuz, D. N., Akai, J. and Storey, K. G. (2005). Specification and maintenance of the spinal cord stem zone. *Development* **132**, 4273-4283.
Deng, C. X., Wynshaw-Boris, A., Shen, M. M., Daugherty, C., Ornitz, D. M. and Leder, P. (1994). Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev.* **8**, 3045-3057.
Deng, C., Bedford, M., Li, C., Xu, X., Yang, X., Dunmore, J. and Leder, P. (1997). Fibroblast growth factor receptor-1 (FGFR-1) is essential for normal neural tube and limb development. *Dev. Biol.* **185**, 42-54.
Dequeant, M. L. and Pourquie, O. (2008). Segmental patterning of the vertebrate embryonic axis. *Nat. Rev. Genet.* **9**, 370-382.
Diez del Corral, R., Breitkreuz, D. N. and Storey, K. G. (2002). Onset of neuronal differentiation is regulated by paraxial mesoderm and requires attenuation of FGF signalling. *Development* **129**, 1681-1691.
Diez del Corral, R., Olivera-Martinez, I., Goriely, A., Gale, E., Maden, M. and Storey, K. (2003). Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. *Neuron* **40**, 65-79.
Dobrovolskaia-Zavadskaia, N. (1927). Sur la mortification spontanee de la queue chez la souris nouveau-nee et sur l'existence d'un caractere hereditaire "non viable". *C. R. Hebd. Soc. Biol.* **97**, 114-116.
Donoviel, D. B., Hadjantonakis, A. K., Ikeda, M., Zheng, H., Hyslop, P. S. and Bernstein, A. (1999). Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Dev.* **13**, 2801-2810.
Dubrulle, J. and Pourquie, O. (2004). fgf8 mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. *Nature* **427**, 419-422.
Dubrulle, J., McGrew, M. J. and Pourquie, O. (2001). FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. *Cell* **106**, 219-232.
Dunwoodie, S. L., Clements, M., Sparrow, D. B., Sa, X., Conlon, R. A. and Beddington, R. S. (2002). Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene Dll3 are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development* **129**, 1795-1806.
Dupe, V. and Lumsden, A. (2001). Hindbrain patterning involves graded responses to retinoic acid signalling. *Development* **128**, 2199-2208.
Echeverri, K. and Oates, A. C. (2007). Coordination of symmetric cyclic gene expression during somitogenesis by Suppressor of Hairless involves regulation of retinoic acid catabolism. *Dev. Biol.* **301**, 388-403.
Economides, K. D., Zeltser, L. and Capecchi, M. R. (2003). Hoxb13 mutations cause overgrowth of caudal spinal cord and tail vertebrae. *Dev. Biol.* **256**, 317-330.
Evrard, Y. A., Lun, Y., Aulehla, A., Gan, L. and Johnson, R. L. (1998). lunatic fringe is an essential mediator of somite segmentation and patterning. *Nature* **394**, 377-381.
Forlani, S., Lawson, K. A. and Deschamps, J. (2003). Acquisition of Hox codes during gastrulation and axial elongation in the mouse embryo. *Development* **130**, 3807-3819.
Freitas, C., Rodrigues, S., Charrier, J. B., Teillet, M. A. and Palmeirim, I. (2001). Evidence for medial/lateral specification and positional information within the presomitic mesoderm. *Development* **128**, 5139-5147.
Galceran, J., Farinas, I., Depew, M. J., Clevers, H. and Grosschedl, R. (1999). Wnt3a$^{-/-}$-like phenotype and limb deficiency in Lef1$^{-/-}$Tcf1$^{-/-}$ mice. *Genes Dev.* **13**, 709-717.
Galceran, J., Hsu, S. C. and Grosschedl, R. (2001). Rescue of a Wnt mutation by an activated form of LEF-1: regulation of maintenance but not initiation of Brachyury expression. *Proc. Natl. Acad. Sci. USA* **98**, 8668-8673.
Gaunt, S. J., Drage, D. and Trubshaw, R. C. (2008). Increased Cdx protein dose effects upon axial patterning in transgenic lines of mice. *Development* **135**, 2511-2520.
Gofflot, F., Hall, M. and Morriss-Kay, G. M. (1997). Genetic patterning of the developing mouse tail at the time of posterior neuropore closure. *Dev. Dyn.* **210**, 431-445.
Goldman, D. C., Martin, G. R. and Tam, P. P. (2000). Fate and function of the ventral ectodermal ridge during mouse tail development. *Development* **127**, 2113-2123.
Gomez, C., Ozbudak, E. M., Wunderlich, J., Baumann, D., Lewis, J. and Pourquie, O. (2008). Control of segment number in vertebrate embryos. *Nature* **454**, 335-339.
Greco, T. L., Takada, S., Newhouse, M. M., McMahon, J. A., McMahon, A. P. and Camper, S. A. (1996). Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev.* **10**, 313-324.
Griffith, C. M. and Wiley, M. J. (1991). Effects of retinoic acid on chick tail bud development. *Teratology* **43**, 217-224.
Gruneberg, H. (1956). A ventral ectodermal ridge of the tail in mouse embryos. *Nature* **177**, 787-788.
Gruneberg, H. and Wickramaratne, G. A. (1974). A re-examination of two skeletal mutants of the mouse, vestigial-tail (vt) and congenital hydrocephalus (ch). *J. Embryol. Exp. Morphol.* **31**, 207-222.
Gu, P., LeMenuet, D., Chung, A. C., Mancini, M., Wheeler, D. A. and Cooney, A. J. (2005). Orphan nuclear receptor GCNF is required for the repression of pluripotency genes during retinoic acid-induced embryonic stem cell differentiation. *Mol. Cell. Biol.* **25**, 8507-8519.
Guo, Q. and Li, J. Y. (2007). Distinct functions of the major Fgf8 spliceform, Fgf8b, before and during mouse gastrulation. *Development* **134**, 2251-2260.
Hamburger, H. and Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Exp. Morphol.* **88**, 49-92.
Hatada, Y. and Stern, C. D. (1994). A fate map of the epiblast of the early chick embryo. *Development* **120**, 2879-2889.
Hausen, P. R. (1991). *The Early Development of Xenopus laevis: An Atlas of the Histology*. New York: Springer-Verlag.
Henrique, D., Tyler, D., Kintner, C., Heath, J. K., Lewis, J. H., Ish Horowicz, D. and Storey, K. G. (1997). Cash4, a novel achaete-scute homologue induced by Hensen's node during generation of the posterior nervous system. *Genes Dev.* **11**, 603-615.
Herrmann, B. G., Labeit, S., Poustka, A., King, T. R. and Lehrach, H. (1990).

Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343**, 617-622.
Hirata, M. and Hall, B. K. (2000). Temporospatial patterns of apoptosis in chick embryos during the morphogenetic period of development. *Int. J. Dev. Biol.* **44**, 757-768.
Hirsinger, E., Stellabotte, F., Devoto, S. H. and Westerfield, M. (2004). Hedgehog signaling is required for commitment but not initial induction of slow muscle precursors. *Dev. Biol.* **275**, 143-157.
Iimura, T. and Pourquie, O. (2006). Collinear activation of Hoxb genes during gastrulation is linked to mesoderm cell ingression. *Nature* **442**, 568-571.
Iimura, T. and Pourquie, O. (2007). Hox genes in time and space during vertebrate body formation. *Dev. Growth Differ.* **49**, 265-275.
Iimura, T., Yang, X., Weijer, C. J. and Pourquie, O. (2007). Dual mode of paraxial mesoderm formation during chick gastrulation. *Proc. Natl. Acad. Sci. USA* **104**, 2744-2749.
Iulianella, A., Beckett, B., Petkovich, M. and Lohnes, D. (1999). A molecular basis for retinoic acid-induced axial truncation. *Dev. Biol.* **205**, 33-48.
Joubin, K. and Stern, C. D. (1999). Molecular interactions continuously define the organizer during the cell movements of gastrulation. *Cell* **98**, 559-571.
Kanki, J. P. and Ho, R. K. (1997). The development of the posterior body in zebrafish. *Development* **124**, 881-893.
Karabagli, H., Karabagli, P., Ladher, R. K. and Schoenwolf, G. C. (2002). Comparison of the expression patterns of several fibroblast growth factors during chick gastrulation and neurulation. *Anat. Embryol. (Berl.)* **205**, 365-370.
Keller, R. E. (1976). Vital dye mapping of the gastrula and neurula of Xenopus laevis. II. Prospective areas and morphogenetic movements of the deep layer. *Dev. Biol.* **51**, 118-137.
Kessel, M. (1992). Respecification of vertebral identities by retinoic acid. *Development* **115**, 487-501.
Kimmel, C. B., Warga, R. M. and Schilling, T. F. (1990). Origin and organization of the zebrafish fate map. *Development* **108**, 581-594.
Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* **203**, 253-310.
Kispert, A. and Herrmann, B. G. (1994). Immunohistochemical analysis of the Brachyury protein in wild-type and mutant mouse embryos. *Dev. Biol.* **161**, 179-193.
Kispert, A., Ortner, H., Cooke, J. and Herrmann, B. G. (1995). The chick Brachyury gene: developmental expression pattern and response to axial induction by localized activin. *Dev. Biol.* **168**, 406-415.
Knezevic, V., De Santo, R. and Mackem, S. (1998). Continuing organizer function during chick tail development. *Development* **125**, 1791-1801.
Lane, M. C. and Sheets, M. D. (2000). Designation of the anterior/posterior axis in pregastrula Xenopus laevis. *Dev. Biol.* **225**, 37-58.
Launay, C., Fromentoux, V., Shi, D. L. and Boucaut, J. C. (1996). A truncated FGF receptor blocks neural induction by endogenous Xenopus inducers. *Development* **122**, 869-880.
Lawson, K. A. and Pedersen, R. A. (1992). Clonal analysis of cell fate during gastrulation and early neurulation in the mouse. *Ciba Found. Symp.* **165**, 3-21; discussion 21-26.
Lawson, K. A., Meneses, J. J. and Pedersen, R. A. (1991). Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Development* **113**, 891-911.
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.
Liu, C., Knezevic, V. and Mackem, S. (2004). Ventral tail bud mesenchyme is a signaling center for tail paraxial mesoderm induction. *Dev. Dyn.* **229**, 600-606.
Lunn, J. S., Fishwick, K. J., Halley, P. A. and Storey, K. G. (2007). A spatial and temporal map of FGF/Erk1/2 activity and response repertoires in the early chick embryo. *Dev. Biol.* **302**, 536-552.
MacDonald, B. T., Adamska, M. and Meisler, M. H. (2004). Hypomorphic expression of Dkk1 in the doubleridge mouse: dose dependence and compensatory interactions with Lrp6. *Development* **131**, 2543-2552.
MacMurray, A. and Shin, H. S. (1988). The antimorphic nature of the Tc allele at the mouse T locus. *Genetics* **120**, 545-550.
Mahmood, R., Kiefer, P., Guthrie, S., Dickson, C. and Mason, I. (1995). Multiple roles for FGF-3 during cranial neural development in the chicken. *Development* **121**, 1399-1410.
Mathis, L. and Nicolas, J. F. (2000). Different clonal dispersion in the rostral and caudal mouse central nervous system. *Development* **127**, 1277-1290.
Mathis, L., Kulesa, P. M. and Fraser, S. E. (2001). FGF receptor signalling is required to maintain neural progenitors during Hensen's node progression. *Nat. Cell Biol.* **3**, 559-566.
McGrew, M. J., Sherman, A., Lillico, S. G., Ellard, F. M., Radcliffe, P. A., Gilhooley, H. J., Mitrophanous, K. A., Cambray, N., Wilson, V. and Sang, H. (2008). Localised axial progenitor cell populations in the avian tail bud are not committed to a posterior Hox identity. *Development* **135**, 2289-2299.
McMahon, J. A., Takada, S., Zimmerman, L. B., Fan, C. M., Harland, R. M. and McMahon, A. P. (1998). Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes Dev.* **12**, 1438-1452.
Melby, A. E., Warga, R. M. and Kimmel, C. (1996). Specification of cell fates at the dorsal margin of the zebrafish gastrula. *Development* **122**, 2225-2237.
Mills, C. L. and Bellairs, R. (1989). Mitosis and cell death in the tail of the chick embryo. *Anat. Embryol.* **180**, 301-308.
Molotkova, N., Molotkov, A., Sirbu, I. O. and Duester, G. (2005). Requirement of mesodermal retinoic acid generated by Raldh2 for posterior neural transformation. *Mech. Dev.* **122**, 145-155.
Moreno, T. A. and Kintner, C. (2004). Regulation of segmental patterning by retinoic acid signaling during Xenopus somitogenesis. *Dev. Cell* **6**, 205-218.
Morimoto, M., Takahashi, Y., Endo, M. and Saga, Y. (2005). The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. *Nature* **435**, 354-359.
Nicolas, J. F., Mathis, L., Bonnerot, C. and Saurin, W. (1996). Evidence in the mouse for self-renewing stem cells in the formation of a segmented longitudinal structure, the myotome. *Development* **122**, 2933-2946.
Niederreither, K., Vermot, J., Schuhbaur, B., Chambon, P. and Dolle, P. (2000). Retinoic acid synthesis and hindbrain patterning in the mouse embryo. *Development* **127**, 75-85.
Nieuwkoop, P. D. and Faber, J. (1967). *Normal table of* Xenopus laevis *(Daudin)*. Amsterdam: North-Holland Publishing Company.
Nordstrom, U., Maier, E., Jessell, T. M. and Edlund, T. (2006). An early role for wnt signaling in specifying neural patterns of Cdx and Hox gene expression and motor neuron subtype identity. *PLoS Biol.* **4**, e252.
Novitch, B. G., Wichterle, H., Jessell, T. M. and Sockanathan, S. (2003). A requirement for retinoic acid-mediated transcriptional activation in ventral neural patterning and motor neuron specification. *Neuron* **40**, 81-95.
Ohta, S., Suzuki, K., Tachibana, K., Tanaka, H. and Yamada, G. (2007). Cessation of gastrulation is mediated by suppression of epithelial-mesenchymal transition at the ventral ectodermal ridge. *Development* **134**, 4315-4324.
Ohuchi, H., Kimura, S., Watamoto, M. and Itoh, N. (2000). Involvement of fibroblast growth factor (FGF)18-FGF8 signaling in specification of left-right asymmetry and brain and limb development of the chick embryo. *Mech. Dev.* **95**, 55-66.
Olivera-Martinez, I. and Storey, K. G. (2007). Wnt signals provide a timing mechanism for the FGF-retinoid differentiation switch during vertebrate body axis extension. *Development* **134**, 2125-2135.
Otto, D. M., Henderson, C. J., Carrie, D., Davey, M., Gundersen, T. E., Blomhoff, R., Adams, R. H., Tickle, C. and Wolf, C. R. (2003). Identification of novel roles of the cytochrome p450 system in early embryogenesis: effects on vasculogenesis and retinoic Acid homeostasis. *Mol. Cell. Biol.* **23**, 6103-6116.
Partanen, J., Schwartz, L. and Rossant, J. (1998). Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. *Genes Dev.* **12**, 2332-2344.
Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J. and Skarnes, W. C. (2000). An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* **407**, 535-538.
Psychoyos, D. and Stern, C. D. (1996). Fates and migratory routes of primitive streak cells in the chick embryo. *Development* **122**, 1523-1534.
Quinlan, G. A., Williams, E. A., Tan, S. S. and Tam, P. P. (1995). Neuroectodermal fate of epiblast cells in the distal region of the mouse egg cylinder: implication for body plan organization during early embryogenesis. *Development* **121**, 87-98.
Reed, S. C. (1937). The inheritance and expression of fused, a new mutation in the house mouse. *Genetics* **22**, 1-13.
Ribes, V., Otto, D. M., Dickmann, L., Schmidt, K., Schuhbaur, B., Henderson, C., Blomhoff, R., Wolf, C. R., Tickle, C. and Dolle, P. (2007). Rescue of cytochrome P450 oxidoreductase (Por) mouse mutants reveals functions in vasculogenesis, brain and limb patterning linked to retinoic acid homeostasis. *Dev. Biol.* **303**, 66-81.
Ribes, V., Stutzmann, F., Bianchetti, L., Guillemot, F., Dolle, P. and Le Roux, I. (2008). Combinatorial signalling controls Neurogenin2 expression at the onset of spinal neurogenesis. *Dev. Biol.* **321**, 470-481.
Riese, J., Zeller, R. and Dono, R. (1995). Nucleo-cytoplasmic translocation and secretion of fibroblast growth factor-2 during avian gastrulation. *Mech. Dev.* **49**, 13-22.
Rossant, J. (2008). Stem cells and early lineage development. *Cell* **132**, 527-531.
Roszko, I., Faure, P. and Mathis, L. (2007). Stem cell growth becomes predominant while neural plate progenitor pool decreases during spinal cord elongation. *Dev. Biol.* **304**, 232-245.
Sakai, Y., Meno, C., Fujii, H., Nishino, J., Shiratori, H., Saijoh, Y., Rossant, J. and Hamada, H. (2001). The retinoic acid-inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterio-posterior axis within the mouse embryo. *Genes Dev.* **15**, 213-225.
Sanders, E. J., Khare, M. K., Ooi, V. C. and Bellairs, R. (1986). An experimental and morphological analysis of the tail bud mesenchyme of the chick embryo. *Anat. Embryol.* **174**, 179-185.
Sasai, Y., Lu, B., Picolo, S. and De Robertis, E. M. (1996). Endoderm induction

by the organizer-secreted factors chordin and noggin in Xenopus animal caps. *EMBO J.* **15**, 4547-4555.
Schoenwolf, G. C. (1984). Histological and ultrastructural studies of secondary neurulation in mouse embryos. *Am. J. Anat.* **169**, 361-376.
Schoenwolf, G. C. (1992). Morphological and mapping studies of the paranodal and postnodal levels of the neural plate during chick neurulation. *Anat. Rec.* **233**, 281-290.
Schubert, F. R., Fainsod, A., Gruenbaum, Y. and Gruss, P. (1995). Expression of a novel murine homeobox gene Sax-1 in the developing nervous system. *Mech. Dev.* **51**, 99-114.
Selleck, M. A. and Stern, C. D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* **112**, 615-626.
Selleck, M. and Stern, C. D. (1992). Evidence for stem cells in the mesoderm of Hensen's node and their role in embryonic pattern formation. In *Formation and Differentiation of Early Embryonic Mesoderm* (ed. J. W. Lash, R. Bellairs and E. J. Sanders), pp. 23-31. New York: Springer.
Shamim, H. and Mason, I. (1999). Expression of Fgf4 during early development of the chick embryo. *Mech. Dev.* **85**, 189-192.
Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J. and Tonegawa, S. (1997). Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* **89**, 629-639.
Shifley, E. T., Vanhorn, K. M., Perez-Balaguer, A., Franklin, J. D., Weinstein, M. and Cole, S. E. (2008). Oscillatory lunatic fringe activity is crucial for segmentation of the anterior but not posterior skeleton. *Development* **135**, 899-908.
Shum, A. S., Poon, L. L., Tang, W. W., Koide, T., Chan, B. W., Leung, Y. C., Shiroishi, T. and Copp, A. J. (1999). Retinoic acid induces down-regulation of Wnt-3a, apoptosis and diversion of tail bud cells to a neural fate in the mouse embryo. *Mech. Dev.* **84**, 17-30.
Sirbu, I. O. and Duester, G. (2006). Retinoic-acid signalling in node ectoderm and posterior neural plate directs left-right patterning of somitic mesoderm. *Nat. Cell Biol.* **8**, 271-277.
Slack, J. M., Isaacs, H. V. and Darlington, B. G. (1988). Inductive effects of fibroblast growth factor and lithium ion on Xenopus blastula ectoderm. *Development* **103**, 581-590.
Spann, P., Ginsburg, M., Rangini, Z., Fainsod, A., Eyal Giladi, H. and Gruenbaum, Y. (1994). The spatial and temporal dynamics of Sax1 (CHox3) homeobox gene expression in the chick's spinal cord. *Development* **120**, 1817-1828.
Spratt, N. T. (1952). Localisation of the prospective neural plate in early chick blastoderm. *J. Exp. Zool.* **120**, 109-130.
Stern, C. (2005). Neural Induction: old problem, new findings, yet more questions. *Development* **132**, 2007-2021.
Stott, D., Kispert, A. and Herrmann, B. G. (1993). Rescue of the tail defect of Brachyury mice. *Genes Dev.* **7**, 197-203.
Sun, X., Meyers, E. N., Lewandoski, M. and Martin, G. R. (1999). Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* **13**, 1834-1846.
Takada, S., Stark, K., Shea, M., Vassileva, G., McMahon, J. and McMahon, A. (1994). Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8**, 174-189.
Tam, P. P. (1989). Regionalisation of the mouse embryonic ectoderm: allocation of prospective ectodermal tissues during gastrulation. *Development* **107**, 55-67.
Tam, P. P. and Tan, S. S. (1992). The somitogenetic potential of cells in the primitive streak and the tail bud of the organogenesis-stage mouse embryo. *Development* **115**, 703-715.
Tam, P. P. and Trainor, P. A. (1994). Specification and segmentation of the paraxial mesoderm. *Anat. Embryol. (Berl.)* **189**, 275-305.
Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L. and McKay, R. D. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196-199.
Tucker, A. S. and Slack, J. M. (1995). Tail bud determination in the vertebrate embryo. *Curr. Biol.* **5**, 807-813.
van den Akker, E., Forlani, S., Chawengsaksophak, K., de Graaff, W., Beck, F., Meyer, B. I. and Deschamps, J. (2002). Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation. *Development* **129**, 2181-2193.
Vermot, J., Gallego Llamas, J., Fraulob, V., Niederreither, K., Chambon, P. and Dolle, P. (2005). Retinoic acid controls the bilateral symmetry of somite formation in the mouse embryo. *Science* **308**, 563-566.
Wahl, M. B., Deng, C., Lewandoski, M. and Pourquie, O. (2007). FGF signaling acts upstream of the NOTCH and WNT signaling pathways to control segmentation clock oscillations in mouse somitogenesis. *Development* **134**, 4033-4041.
Wang, W. C. and Shashikant, C. S. (2007). Evidence for positive and negative regulation of the mouse Cdx2 gene. *J. Exp. Zoolog. B Mol. Dev. Evol.* **308**, 308-321.
Wilson, V. and Beddington, R. S. (1996). Cell fate and morphogenetic movement in the late mouse primitive streak. *Mech. Dev.* **55**, 79-89.
Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H. and Sisodia, S. S. (1997). Presenilin 1 is required for Notch1 and Dll1 expression in the paraxial mesoderm. *Nature* **387**, 288-292.
Yamaguchi, T., Harpal, K., Henkemeyer, M. and Rossant, J. (1994). Fgfr-1 is required for embryonic growth and mesoderm patterning during mouse gastrulation. *Genes Dev.* **8**, 3032-3040.
Yamaguchi, T. P., Bradley, A., McMahon, A. P. and Jones, S. (1999a). A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **126**, 1211-1223.
Yamaguchi, T. P., Takada, S., Yoshikawa, Y., Wu, N. and McMahon, A. P. (1999b). T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* **13**, 3185-3190.
Yang, H. J., Wang, K. C., Chi, J. G., Lee, M. S., Lee, Y. J., Kim, S. K., Lee, C. S. and Cho, B. K. (2006). Cytokinetics of secondary neurulation in chick embryos: Hamburger and Hamilton stages 16-45. *Childs Nerv. Syst.* **22**, 567-571.
Yang, X., Dormann, D., Munsterberg, A. E. and Weijer, C. J. (2002). Cell movement patterns during gastrulation in the chick are controlled by positive and negative chemotaxis mediated by FGF4 and FGF8. *Dev. Cell* **3**, 425-437.
Yoshikawa, Y., Fujimori, T., McMahon, A. P. and Takada, S. (1997). Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev. Biol.* **183**, 234-242.
Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vasicek, T. J., Perry, W. L., 3rd, Lee, J. J., Tilghman, S. M., Gumbiner, B. M. and Costantini, F. (1997). The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* **90**, 181-192.
Zhang, N. and Gridley, T. (1998). Defects in somite formation in lunatic fringe-deficient mice. *Nature* **394**, 374-377.

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