

Cdx2 is essential for axial elongation in mouse development

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Inactivation of *Cdx2* leads to preimplantation embryonic lethality. Rescue of the implantation defect by tetraploid fusion established that *Cdx2* is necessary for trophoblastic development, vasculogenesis in the yolk sac mesoderm, allantoic growth, and chorio-allantoic fusion. "Rescued" *Cdx2* mutants die at late gastrulation stages because of failure of placental development. *Cdx2* is also needed for the completion of the normal process of gastrulation and tail bud elongation. Presegmental paraxial mesoderm is severely restricted in amount and somites posterior to somite 5 are abnormal. The *Cdx2* mutation, like mutations impairing *Wnt* and *Fgf* signaling, causes posterior truncations and disturbs axial patterning of the embryonic structures, indicated by changes in the *Hox* expression domains. The gene appears to be important in the integration of the pathways controlling embryonic axial elongation, and anterior–posterior patterning.

The *Drosophila* homeotic gene *caudal* (*cad*) determines the fly's most posterior body segment (1). The three mouse *cad* homologues, *Cdx1*, *Cdx2* and *Cdx4*, (2–4) have developmental roles with overlap of expression patterns.

Cdx2 is expressed (5) at 3.5 days postcoitum (dpc) in the trophoctoderm but not in the inner cell mass. At 7.5 dpc (Theiler stage 11), it is present in the chorion, ectoplacental cone, mesoderm of the developing allantoic bud, and posterior primitive streak. At 8.5 dpc (Theiler stage 13), expression is seen in all three germ layers at the posterior end of the embryo extending into the mesodermal root of the allantois, in the endodermal epithelium of the hindgut rudiment, and in the neural tube; the presegmented paraxial mesoderm expresses the gene, but the somites, lateral plate, and intermediate mesoderm do not. By 9.5 dpc (Theiler stage 15), the caudal pole of the embryo remains positive, as does the posterior neural tube and posterior gut endoderm. At 12.5 dpc (Theiler stage 20), expression is confined exclusively to the gut endoderm posterior to the foregut/midgut junction (5). *Cdx1* (6) is not expressed in the trophoctoderm, but is expressed along the primitive streak at 7.5 dpc. It is demonstrable in the neural tube and somitic and lateral plate mesoderm, but not the early definitive endoderm. *Cdx4* (3) appears at 7.5 dpc in the posterior part of the primitive streak and allantois and persists until 10 dpc. It has been identified in posterior neurectoderm, presomite and lateral plate mesoderm, and hindgut endoderm. There is no information concerning expression at later stages.

In 8.5 dpc (Theiler stage 13) embryos the appearance is of a nested expression of the three *Cdx* genes in both the neural tube and mesoderm, suggesting a possible role in patterning the entire anterior–posterior axis. *Cdx1* expression extends most anteriorly, followed by *Cdx2* and *Cdx4*, respectively, with all three genes expressed posteriorly.

Cdx2-null mutant embryos die between 3.5 and 5.5 dpc, and heterozygotes have tail abnormalities and exhibit anterior homeotic shifts involving the cervical and upper thoracic vertebrae, ribs, and midgut endoderm (7). Subramanian *et al.* (8) reported similar homeosis in the anterior regions of the cervical spine in homozygous *Cdx1* mutants, but without an apparent gut phenotype.

We used *Cdx2*^{-/-} embryonic stem (ES) cells derived from heterozygote intercrossing to "rescue" the *Cdx2*^{-/-} phenotype by using tetraploid aggregation. *Cdx2*-null mutants developed to the point when a chorio-allantoic placenta normally begins to function, then died because of underdevelopment of the allantois, which failed to fuse with the chorion. The mutant embryos were grossly retarded posteriorly, showing a truncated body and a deficient yolk-sac circulation. Altered expression patterns of cognate genes involved in controlling developmental processes in these mutants showed that *Cdx2* is important in the constellation of genes essential for posterior tissue generation and axial patterning during gastrulation.

Materials and Methods

Tetraploid Aggregation. Morulae from *Cdx2* heterozygote intercrosses were aggregated with 160 tetraploid embryos generated by electrofusion at the two-cell stage. This technique has been used to rescue lethal mouse mutants caused by defective extraembryonic tissues (9). We show that it overcomes implantation block caused by defective trophoblast development. Tetraploid embryos were derived from two mouse strains (253 and B5/EGFP) expressing HMG-CoA-LacZ (10) and β -actin-CMV-EGFP (11) transgenes, respectively.

To increase numbers, tetraploid embryos were also aggregated with *Cdx2*-null ES cells to generate completely ES cell-derived embryos (12). Four *Cdx2*-null mutant ES cell lines were isolated from blastocysts of intercrossed *Cdx2*^{+/-} heterozygotes of mixed background (129SV \times C57BL/6J) using the protocol of Abbondanzo *et al.* (13). Homozygous mutant blastocysts did not form trophoblastic outgrowths, but development of the inner cell mass (ICM) made it possible to pick the colonies and culture ES cell lines. The tetraploid cells (marked by LacZ staining) did not contribute significantly to embryonic tissues, but they developed normally along the trophoblastic lineage.

Whole-Mount *in Situ* Hybridization. Whole-mount *in situ* hybridization was performed essentially as described (14) using probes for *Cdx1* (6), *Cdx4* (3), *Hoxb1* (15), *Hoxb8* (16), *Hoxd4* (17), *Wnt3a* (18), *Brachyury T* (19), *Mox1* (20), *Paraxis* (21), *Tbx6* (22), *mShh* (23), *Fgf8* (24), and *Hes5* (25).

Whole-Mount Immunohistochemistry. Material was fixed overnight in 4% paraformaldehyde and stained with rat anti-PECAM-1 antibody (clone MEC13.3, Pharmingen). Anti-rat IgG horseradish peroxidase (Biosource) was the secondary antibody.

Results

***Cdx2*^{-/-} Blastocysts Do Not Form Trophoblastic Outgrowths: Postimplantation Development Can Be Rescued by Aggregation with Wild-Type Tetraploid Embryos.** Homozygous null mutant embryos do not implant *in vivo* (7) and when explanted for the purpose of

Abbreviations: dpc, days postcoitum; ES, embryonic stem.

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isolating *Cdx2*^{-/-} ES cells, did not form trophoblastic outgrowths, indicating that the implantation defect is associated with defective trophoblastic development. We have taken advantage of the rescue of the trophoblastic defect to study the role of *Cdx2* in later embryonic development. Embryos produced by aggregation of diploid morulae from heterozygote intercrosses with wild-type tetraploid morulae were transferred to pseudo-pregnant females, which were killed at 7 days of nominal embryonic development. Forty-six resulting embryos were photographed and genotyped by using the PCR primers described in ref. 7. Of the 46 embryos, 19 were wild type, 20 were heterozygotes, and 7 were homozygous mutants (Fig. 4, which is published as supporting information on the PNAS web site). The null mutant/tetraploid embryo aggregates implanted in a similar manner to the wild-type and heterozygote aggregates. At 7 days, the embryos from null mutants were viable and appeared morphologically normal (Fig. 4), having reached Theiler stage 10 of development with clear evidence of mesoderm formation posteriorly. These results indicate that failure to implant in *Cdx2*^{-/-} embryos is not the result of failure of the inner cell mass, as suggested by Tamai *et al.* (26)

Because no gross defects were observed at a nominal age of 7 dpc, we next examined null mutants at later developmental stages.

Phenotype of *Cdx2*-Null Mutants. For these experiments ‘rescue’ was performed by tetraploid aggregation with null mutant ES cells. *Cdx2*^{-/-} embryos were generated by using two lines of mutant ES cells both of which yielded similar phenotypes. Tetraploid aggregation controls, generated by using wild-type ES cells did not exhibit any morphological abnormalities.

Mutant embryos only survived to a nominal age of 11.5 days, the time at which a functional chorio-allantoic placenta becomes the principal organ of maintenance. However, the developmental stage reached by the most advanced mutant embryos was 36–48 h behind their nominal age, whereas tetraploid aggregation controls were up to 24 h less developed than their nominal age.

Defects were first apparent at the late gastrulation/early somite stages at a nominal age of 8.5 dpc. At ≈7.5 dpc, the allantois of normal embryos forms as a bud of extraembryonic mesoderm continuous with the posterior end of the primitive streak. Allantoic growth up to the three-somite stage is effected by the addition of mesoderm from the posterior primitive streak. As the bud extends into the exocoelomic cavity, its outer cells differentiate into a mesothelium, making the tip of the allantois competent for fusion with the chorion. The allantois of rescued null mutants at 8.5 dpc was severely underdeveloped and never extended enough to fuse with the chorion, thus preventing the formation of a functional chorio-allantoic placenta (Fig. 1 *A* and *B* and Fig. 5 *A* and *B*, which is published as supporting information on the PNAS web site). Almost all mutants developed to between 15 and 17 somites (Theiler, stage 14), and in every case development of the allantois was rudimentary.

The yolk sac circulation was abnormal in null mutant embryos (Fig. 5 *C* and *D*). At a nominal age of 8.5 dpc, the fine honeycomb appearance of vessels seen in wild-type embryos (Fig. 2*C*) was replaced by a coarse plexus of enlarged channels, suggesting failure in vascular remodelling in the yolk sac (Fig. 2*D*). In the more mature embryos, the circulation was sluggish and the pericardial cavity was usually dilated.

In embryos that survived to day 10 or 11, the cranial region developed normally, but the posterior region was grossly curtailed. The hind limb bud was not developed and the maximum number of somites was 17 (Figs. 2*A* and *B* and 5*A* and *B*). From the fifth onwards, the somites became smaller and irregularly shaped compared with controls at the same stage of brain development. At this level, the neural tube often became irregular.

The oldest mutant embryos, which according to other devel-

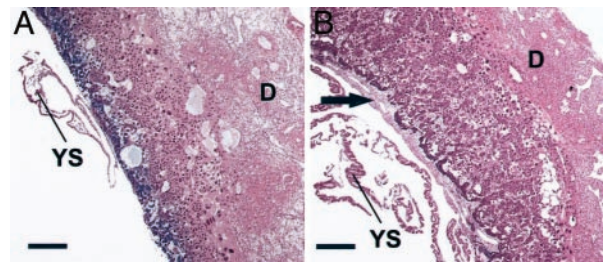


Fig. 1. Section through the placentae of mutant (*A*) and wild-type (*B*) mice at a nominal age of 11.5 dpc created by injection of ES cells into tetraploid wild-type blastocysts bearing a LacZ transgene. The allantoic component of the wild-type placenta is clearly seen (arrow) but is absent from the mutant specimen. YS, yolk sac; D, maternal decidua. The section was stained for β -galactosidase and with hematoxylin and eosin. (Bar = 100 μ m in *A* and 125 μ m in *B*.) The diffuse blue staining on the fetal side of the mutant placenta is an artefact due to leaching of β -galactosidase stain from the trophoblastic nuclei into the surrounding cytoplasm. The tetraploid nuclei are dark due to β -galactosidase staining, whereas those of the allantoic mesoderm and of the decidua (D) of maternal host origin do not have LacZ activity.

opmental landmarks should have 20+ (Theiler stage 14/15) but only had 17 somites, showed that many were beginning to exhibit widespread diffuse necrosis probably due to the disturbance of extraembryonic membrane function. Some embryos, however, were not yet moribund, and in these the phenotype was constant. They were truncated posteriorly beyond the forelimb bud, but the neural tube, notochord, and endodermal gut tube extended well toward the posterior tip. There was evidence of cell death in the posterior gut lining and in the surrounding splanchnopleuric mesoderm (Fig. 6, which is published as supporting information on the PNAS web site) and more general mesenchymal death toward the posterior tip of the specimen. The neural tube, notochord, and somites were reasonably well preserved. Somites extended to almost the posterior end of the embryo, and unsegmented paraxial mesoderm was hardly present.

Mesoderm and Neurectoderm Are Properly Specified in *Cdx2* Null Embryos but Are Grossly Deficient Posteriorly. Transcript distribution of various genes normally expressed in mesoderm and neurectoderm was investigated by whole mount *in situ* hybridization starting at stages preceding the inception of externally manifest abnormalities (Theiler stage 11, head fold stages).

Mox1 and *Paraxis* are markers of somitic mesoderm. Their expression levels at Theiler stages 13 and 14 (≈10 and 15 somites) was weaker in mutants and confirmed observations that somites caudal to somite 5 are smaller and irregular in mutants (*Mox1*, Fig. 3; *Paraxis*, not shown). Because these somite abnormalities and the arrest of axial development at ≈15 somites are rostral to tissues derived from the tail bud, it seems that somites anterior to and preceding in time of development those produced by axial extension of tail bud origin are also affected in mutant embryos. *Mox1*-labeled somites were apparent to the caudal extremity of the paraxial mesoderm in mutant embryos, indicating a reduction in amount of presomitic mesoderm.

Tbx6 is expressed in the primitive streak and nascent paraxial mesoderm, and is down-regulated at somite formation (22). Expression of *Tbx6* in mutants is reduced antero-posteriorly and is complementary to that of *Mox1*, in agreement with the reduction in the amount of unsegmented paraxial mesoderm (Fig. 3).

The early neural marker *Hes5* labeled the central nervous system to the caudal end of the spinal cord. No ectopic *Hes5* was detected in mutants (not shown), indicating that, in contrast to other caudally truncated phenotypes such as *T*, *Wnt3a*, *Fgfr1*, and

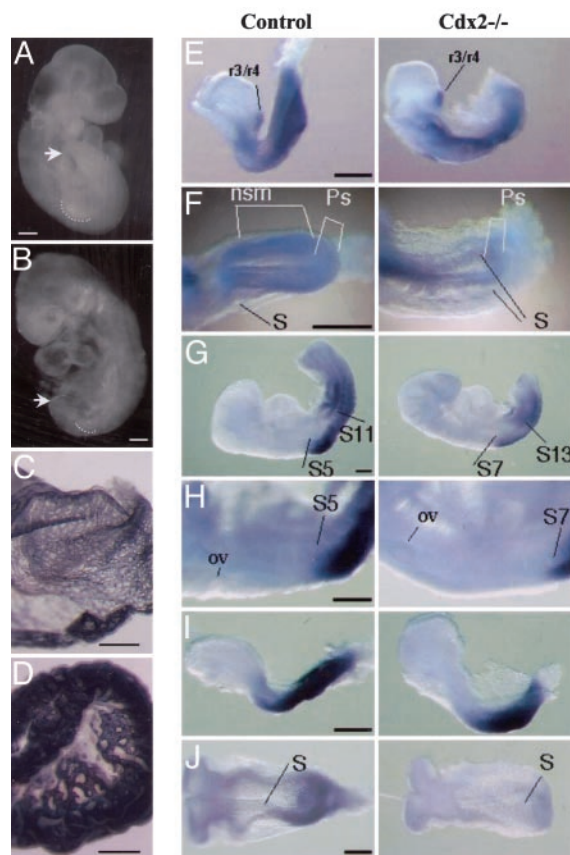


Fig. 2. Wild-type (A) and *Cdx2*^{-/-} (B) mouse embryos at a nominal age of 10.5 dpc. The rostral region of the mutant is normal, though somewhat retarded in development when compared with the wild-type embryo. Caudally there is gross truncation distal to the forelimb bud (marked) in the mutant embryo. Arrows mark the tail tip in both mutant and wild-type. (Bar = 250 μ m in A and 225 μ m in B.) (C and D) An 8.5-dpc yolk sac from wild-type (C) and mutant (D) embryos immunologically stained with a PECAM-1 antibody. A coarse plexus of enlarged vessels is visible in the mutant (D), compared with the fine honeycomb appearance in the control (C). (Bar = 500 μ m.) (E–J) Expression of *Hox* and *Cdx* genes in wild-type and mutant embryos. Whole-mount *in situ* hybridization of *Hoxb1* (E) and *Hoxd4* (F) transcripts at the five-somite stage in control and *Cdx2*^{-/-} embryos. Note, in the *Hoxd4* hybridized mutant embryo, the reduced amount of unsegmented mesoderm, the last somite being close to the anterior part of the streak. Expression of the more 5' *Hox* gene, *Hoxb8* (G and H) shows a posterior shift of the anterior boundary of the expression domain in the mesoderm (S13 instead of S11) and in the neural tube (level of S7 instead of S5) in the mutant. Expression levels of *Cdx1* (I) appear unchanged in *Cdx2* mutants at the early somite stage, whereas the expression level of *Cdx4* (J) is much reduced compared to wild-type embryos. (Bar = 200 μ m.) ov, optic vesicle; r, rhombomere; nsm, nonsegmented mesoderm; Ps, primitive streak; S, somite.

CYP26 mutants (27–30), *Cdx2* null mutants do not exhibit ectopic neural tube development.

Endodermal Development in Rescued *Cdx2*-Null Embryos. Posterior underdevelopment of mutant embryos is associated with delayed appearance of the hindgut diverticulum from four-somite (Theiler stage 12) to eight-somite (Theiler stage 13) stages. However, a distinct hindgut does eventually form (Figs. 6 and 7, which are published as supporting information on the PNAS web site). *Sonic hedgehog* (*Shh*) is endodermally expressed at the earliest stages of hindgut development (31), and mutants at seven somites (Theiler stage 12), which had not yet initiated hindgut invagination, did not exhibit *Shh* expressing endoderm in the caudal region, compared to control specimens (Fig. 3). By

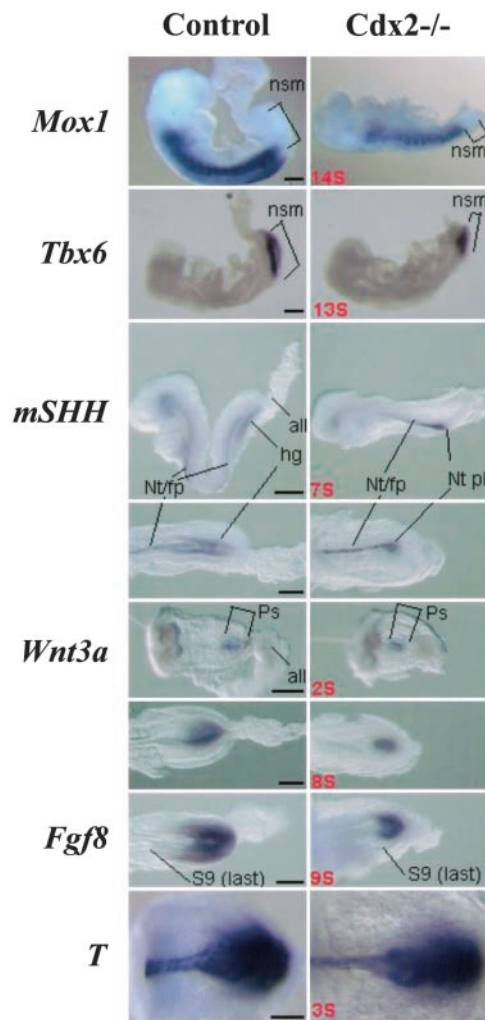


Fig. 3. Expression of mesoderm, neuroectoderm, and endoderm markers, and of *Wnt* and *Fgf* signals in *Cdx2* mutant embryos and controls. *Mox1* marks segmented paraxial mesoderm; note the important reduction in the amount of unsegmented mesoderm in the caudal aspect of the 14-somite *Cdx2*^{-/-} embryo. The expression domain of *Tbx6*, marking nonsegmented mesoderm, is reduced in *Cdx2*^{-/-} compared with controls. This pattern is complementary to that of *Mox1*. *Shh* is a marker of midline structures and of hindgut endoderm; a lateral view above, and a ventral view below both show delayed hindgut diverticulum formation in the mutant leading to an absence of *Shh* endodermal signal posteriorly in *Cdx2*^{-/-} embryos. *Wnt3a* was assayed at a stage earlier (two somites, above), or later (eight somite embryos, below) than the first manifestation of an altered phenotype in *Cdx2* mutant embryos. Expression of *Fgf8* is shown in a nine-somite mutant and a control. *Brachyury T* was expressed in the posterior mesoderm of both mutant and control at the three- and two-somite stage, respectively. all, allantois; nsm, nonsegmented mesoderm; ps, primitive streak; Nt/fp, Notochord, floorplate; Nt pl, notochordal plate; hg, hindgut; S9, somite 9, the last formed somite. (Bar = 200 μ m.)

contrast, *Shh* was expressed in the notochord and floor plate of both mutants and controls (Fig. 3 and data not shown). The territories of *Hoxd11/13* expression are not generated in *Cdx2* mutants, and we cannot therefore draw definitive conclusions concerning the effect of *Cdx2* on expression of these genes.

Disturbance of Posterior Elongation at Late Primitive Streak Stages. Signaling by *Wnt* and *Fgf* genes, and of the T box-containing transcription factors *Tbx6* and *Brachyury* (T) are essential for posterior axial elongation at late primitive streak and tail bud stages (27, 32–34)

Wnt3a-null mutants exhibit a posteriorly truncated phenotype similar to *Cdx2* null embryos (35). *Wnt3a* is similarly expressed in the primitive streak region of both controls and mutants at the beginning of somite formation (Fig. 3). Later, a decrease in the antero-posterior extent of the *Wnt3a* expression domain at Theiler stage 13 (seven to nine somites) (Fig. 3) reflects reduced posterior development of the mutants. We conclude that *Cdx2*-null mutation does not prevent early *Wnt3a* expression and that *Cdx2* is not required upstream of *Wnt3a*.

FgfR1 (the principal Fgf8 receptor) hypomorph mutations also lead to posteriorly truncated embryos (36). *Fgf8* is expressed in the unsegmented posterior region of the paraxial mesoderm of wild-type embryos, and Fgf signaling has an important role in convergence extension movements and exit of paraxial mesodermal cells from the primitive streak (28, 32). Expression of *Fgf8* in early somite *Cdx2*-null embryos is unaltered compared to controls, but at the seven- to nine-somite stage (Fig. 3) it is less extended antero-posteriorly in mutants, reflecting reduced posterior development but indicating that *Cdx2* is not required for *Fgf8* expression.

Brachyury (*T*) is down-regulated in the paraxial mesoderm precursor cells of *Wnt3a* mutants (27) and a feedback loop may exist between *Wnt* and *T* (reviewed in ref. 33). Although an apparently normal notochord develops in *Cdx2*-null embryos, the phenotype has much in common with that of *Brachyury* embryos. At early stages (between one and four somites, Theiler, early stage 12), *T* expression in the primitive streak and notochord of wild-type and mutant embryos was similar, although it seemed to be less widely extended laterally in the posterior part of its expression domain (Fig. 3). Importantly, specimens stained for *T* showed that anterior-posterior patterning at these early stages was similar in wild-type, *Cdx2*^{+/-}, and *Cdx2*^{-/-} embryos (Fig. 8, which is published as supporting information on the PNAS web site). On comparing *T* expression in 10 somite *Cdx2*-null mutants with 10- and 5-somite wild-type controls the posterior *T* expression domain in mutants was again restricted laterally (data not shown). In sections posterior to the node, neurectoderm, and mesoderm were considerably less abundant in the mutant (thus confirming the findings obtained by inspection of whole mounts) and hindgut invagination was rudimentary in 10 somite mutants, whereas hindgut was present in 5- and 10-somite controls.

Effect of *Cdx2* Mutation on *Hox* and Other *Cdx* Genes. Homeotic shifts in the skeletons of *Cdx2* heterozygotes (7), potentiation of this effect when in combination with the *Cdx1* mutation (37), and the reported regulatory activity of Cdx proteins on *Hox* gene expression (8, 38) suggested that *Cdx2*-null mutants undergo changes in the expression patterns of *Hox* genes.

Expression of *Hoxb1* at 11 somites (Theiler stage 13) showed normal anterior distribution in the neural tube, reaching the rhombomere 3/4 boundary in the neurectoderm (Fig. 2E) and a mesodermal level rostral to the first somite (Fig. 2E), though these rostral expression boundaries were somewhat fuzzy in the mesoderm in both mutants and controls. A reduced level of expression of *Hoxb1* and *Hoxd4* was apparent caudally in mutants (Fig. 2F; data not shown for *Hoxb1*). This contrasts with the strong notochordal expression of *T* seen at this level (see above), and therefore does not appear to be the result of a general decrease in caudal gene expression.

In the most advanced *Cdx2*^{-/-} mutant embryos (equivalent to ≥ 20 somites, Theiler stage 15) anterior *Hoxb8* expression boundaries were shifted posteriorly from somite 11 to 13 in the mesoderm and from the level of somite 5 to 7 in the neurectoderm (Fig. 2G and H).

Cdx genes are subject to feedback inhibition (39), and cross-inhibitory regulation has been shown to occur. Thus, overexpression of *Cdx2* down-regulates expression of *Cdx1* *in vitro* (40)

and *Cdx2* has been shown to inhibit β -catenin stimulated expression of *Cdx1* in human colon cancer cell lines (41). We therefore examined expression levels of the other two *Cdx* genes in *Cdx2*^{-/-} embryos. Expression was monitored at 7–10 somites (Theiler stages 12 and 13). *Cdx1* levels were not significantly altered in the trunk and along the primitive streak, although poor posterior development resulted in reduction posteriorly (Fig. 2I). The anterior extent of expression appeared unchanged, but the rostral boundaries were somewhat fuzzy (Fig. 2I), making the anterior limit of expression difficult to localize.

Unlike many of the cognate genes examined, near complete loss of *Cdx4* expression (Fig. 2J) is not caused by posterior truncation of the embryo and represented a significant finding. Taken together with the nested expression pattern of the *Cdx* genes described above at 8.5 dpc, there is a strong possibility that direct cross-regulatory activity exists between *Cdx2* and *Cdx4*.

Discussion

***Cdx2* Belongs to the Gene Network Playing a Pivotal Role in Embryonic Axial Elongation.** Early gastrulation in rescued homozygous *Cdx2* mutants takes place normally, but disturbances appear during the later development of the primitive streak. The first defects seen involve abnormalities of extraembryonic mesodermal development. Deficient allantoic outgrowth prevents fusion with the chorion so that a chorio-allantoic placenta fails to form. Furthermore, abnormal yolk sac vasculogenesis may be caused by defective extra-embryonic mesoderm produced by the posterior part of the streak. The longest surviving *Cdx2*^{-/-} embryos show failure in extension of the body axis beyond forelimb levels (about somite 17) and an alteration in somite morphology posterior to somite 5. The observation that the first five somites are unaffected accords with the fact that expression of *Cdx2*, spreading from posterior streak regions, only reaches the node region at late head fold stages (Fig. 9, which is published as supporting information on the PNAS web site), when cells making up the first five somites have left the node region (42). This is also consistent with the finding that *Wnt3a* and *Fgf8* expressions are only affected after the completion of the early, presomite stages of development in mutants and specifically in areas that normally express *Cdx2* in wild types.

It has been reported that *cad* homologues are downstream of *Fgf* in numerous vertebrate species (43–45), and our observations suggest that *Cdx2* operates as a transducer of *Fgf* and *Wnt* signals in all germ layers. However, the existing interaction between the *Wnt* and *Fgf* signaling cascades (32, 46), and the possible feedback loops between the genetic steps involved, complicate the elucidation of the hierarchical cascades impaired by the *Cdx2* mutation.

The *Cdx2*^{-/-} phenotype is somewhat similar to *Brachyury* (*T*), suggesting that both are involved (perhaps cooperatively) in controlling mesoderm formation at gastrulation, downstream of *Fgf* and *Wnt* signaling. However, the *Cdx2*-null mutant differs from *T*, *Tbx6*, *Wnt3a*, and *Fgfr1* mutants in several respects. First, in all of the mutants exhibiting caudal truncations so far described, the posterior paraxial mesoderm is partially transformed into ectopic neural tissue (27, 29, 30, 32, 47), and no such structures were detected in *Cdx2*-null embryos. Thus mesoderm specification is not disturbed in the *Cdx2*-null mutants. Second, the gene seems to be necessary for processes originating not only from the more anterior part of the streak (paraxial mesoderm, neurectoderm, endoderm), but also from derivatives of the posterior part of the streak (extraembryonic mesoderm), which is unaffected in *Wnt3a* and *Fgfr1* mutants but is compromised in *T* mutants. *Wnt3a*, *Fgfr1*, and *Cdx2* mutants are not impaired in axial mesoderm generation for, unlike *Brachyury T*, they generate a normal notochord. This indicates that there is some degree of specificity in the involvement of each of the participants in the constellation orchestrating posterior morphogenesis and patterning.

Cdx2 Function and Axial/Paraxial Progenitors in Caudalmost Regions of Mouse Embryos.

Posterior morphogenesis and balanced tissue generation making up the trunk and tail depend on the integrated activity of the *Fgf* and *Wnt* pathways and on the subsequent activity of T-box proteins. Our observations indicate that *Cdx2* is also centrally involved. Dubrulle *et al.* (48) and Vasiliauskas and Stern (49) suggest that high *Fgf* signaling around the anterior part of the primitive streak and anterior to the node maintains a population of self-renewing immature (nonsegmented) paraxial mesoderm cells. High *Fgf* signaling has also been shown to maintain an immature population of neural progenitors in a stem cell-like state in the growth region of the caudal neural plate (50). A feature accompanying posterior truncation in the *Cdx2*-null mutant phenotype is the reduced amount of unsegmented paraxial mesoderm present at late gastrulation. At Theiler stage 13, somites are present as far posteriorly as the node, whereas in wild-type controls a region of unsegmented mesoderm equivalent to seven somites is present rostral to the node (51).

The reduction in the amount of newly formed nonsegmented paraxial mesoderm, and the slowing down and arrest of mesoderm and neurectoderm formation in the posterior region of *Cdx2*-null mutants suggests a role for *Cdx2* in the mechanism of tissue generation. Because formation of endoderm also seems to be affected in posterior development of *Cdx2* mutant embryos, all three germ layers are posteriorly truncated "in register." *Cdx2* might thus be necessary for continued maintenance of prolifer-

ation at and around the anterior primitive streak, regulating tissue production as the axis elongates.

Cdx Genes and Integration of Antero-Posterior Patterning and Caudal Morphogenesis.

Cdx mutations produce homeotic-like transformation accompanied by changes in the distribution of *Hox* gene expression (8, 37, 44). In addition to causing axial truncation, loss of *Cdx2* also causes homeotic alterations along the body axis (7) and posterior shifts in *Hox* expression domains (this work). *Cdx* genes, as a class, may have a homeotic function of their own, being phylogenetically related to the *Hox* genes (52); for example, *Cdx2* deficiency produces an anterior homeotic alteration in the specification of midgut endoderm (53), a tissue in which *Hox* genes are not expressed (54).

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