Hox gene function in vertebrate gut morphogenesis: the case of the caecum

Giovanna Zacchetti1, Denis Duboule1,2,* and Jozsef Zakany1

The digestive tract is made of different subdivisions with various functions. During embryonic development, the developing intestine expresses combinations of Hox genes along its anterior to posterior axis, suggesting a role for these genes in this regionalization process. In particular, the transition from small to large intestine is labelled by the transcription of all Hoxd genes except Hoxd12 and Hoxd13, the latter two genes being transcribed only near the anus. Here, we describe two lines of mice that express Hoxd12 ectopically within this morphological transition. As a consequence, budding of the caecum is impeded, leading to complete agenesis in homozygous individuals. This effect is concurrent with a dramatic reduction of both Fgf10 and Pitx1 expression. Furthermore, the interactions between ‘anterior’ Hox genes and ectopic Hoxd12 suggest a model whereby anterior and posterior Hox products compete in controlling Fgf10 signalling, which is required for the growth of this organ in mice. These results illuminate components of the genetic cascade necessary for the emergence of this gut segment, crucial for many vertebrates.

KEY WORDS: Hox target genes, Budding morphogenesis, Genetic analysis, Gut regionalization, Mouse organogenesis

INTRODUCTION

The caecum is a pouch of the digestive tube, located at the junction between the small and the large intestine, which is essential for many vertebrate species to digest dietary cellulose. In herbivorous species, where it represents a crucial gastrointestinal (GI) organ, the relative size of the adult caecum is much larger than that of carnivores. In addition, there is variation in the presence or absence of the caecum even among mammals (Langer, 2001), hence discovering genetic determinants of caecum growth may contribute to diverse types of investigations into both ontogenesis and phylogensis of the gastrointestinal system.

The role of Hox genes in patterning the mammalian GI tract in addition to the skeleton, the nervous system and the genitals has been documented for some time, in particular with respect to the differentiation of both the muscular layer and the epithelium. While systematic analyses of expression patterns in mice have revealed a coordinated expression strategy (Sekimoto et al., 1998; Pitera et al., 1999; Kawazoe et al., 2002), more recent studies employing various methodologies of gene expression profiling have also supported the involvement of many Hox genes, including those in the HoxD cluster, in regionalization (Bates et al., 2002; Choi et al., 2006).

Furthermore, examination of mice with modified Hox gene expression levels has provided decisive evidence for their function during development, as ranges of anatomical defects were discovered along the anteroposterior axis of the GI tract. Hox deficiencies due to the inactivation of single genes such as Hoxc4 and Hoxa5, as well as overexpression of either Hoxc8 or Hoxa4, were shown to affect the oesophagus, stomach or intestine, respectively (Boulet and Capecchi, 1996; Aubin et al., 2002; Pollock et al., 1992; Wollgemuth et al., 1989). We had previously shown that, in the absence either of all Hoxd genes, or of the Hoxd4 to Hoxd13 genomic interval, the genesis of both the ileo-caecal and anal sphincters was severely impaired, even though the gross anatomy was normal (Zakany and Duboule, 1999; Zakany et al., 2001). Furthermore, targeted inactivation of Hoxd12 or Hoxd13 affected the proper morphology of the anal sphincter selectively (Kondo et al., 1996).

The caecum forms at the limit between the ileum and the colon; in mice, it begins to grow at day 10 of embryonic development, and one day later it protrudes out of the abdominal cavity and is included in the intestinal hernia. A large number of Hox genes are co-expressed in posterior midgut, in a region that coincides with the future budding of the caecum (Dolle et al., 1991; Kawazoe et al., 2002; Levin et al., 1997; Pitera et al., 1999; Roberts et al., 1995; Sekimoto et al., 1998). By contrast, the expression of the most ‘posterior’ Hox genes, such as Hoxd12 and Hoxd13 is excluded from this precise region (Dolle et al., 1991; Kmita et al., 2000). Interestingly, the expression of the HoxD cluster genes in this particular region, the transition from the ileum to the colon, did not appear to follow the rule of collinearity, unlike that seen for the expression of these genes in other axial structures. Indeed, several genes belonging to the HoxD cluster were reported to be co-expressed at around the position of the future caecum, probably in response to a global regulatory mechanism located in 3’ of (telomeric to-) the cluster (Kmita et al., 2000; Spitz et al., 2005), suggesting that these transcription factors may be instrumental in the development of this organ.

In this report, we further investigate the importance of the embryonic Hox expression domains for the proper formation of the ileo-caecal transition. First, we confirm that HoxD cluster genes are excluded from the anterior small bowel and we show that all Hoxd genes, with the exception of Hoxd12 and Hoxd13, are heavily co-expressed in a limited segment of the posterior midgut. Next, by investigating novel mutant lines involving partial deficiencies of the HoxD cluster, we show that a robust gain of expression of Hoxd12 in the posterior midgut correlates, in time and place, with the absence of caecum budding originating from this region. In these foetuses, however, specific expression of Hoxa genes was maintained. We also show that Hoxd12 gain of function inhibits the outgrowth of the caecum, probably by interfering with fibroblast

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growth factor signalling, in particular Fgf10, which normally depends upon the activity of anterior Hox gene products. These results strongly suggest that several Hox gene(s) are required for the proper formation of the ileum-to-colon transition and concurrence budding of the caecum.

MATERIALS AND METHODS

Mouse stocks, TAMERE, crosses and genotyping

In order to obtain the various genotypes shown in Table 1, mice heterozygous for the HoxD<sup>Del(1-10)</sup> allele (Zakany et al., 2004) [referred to as ‘Del(1-10)’] were crossed with either del(1-13) (Zakany et al., 2001), del(4-13) (Zakany and Duboule, 1999), del(8i-13) (Tarchiani et al., 2005) or del(11-13) (Zakany and Duboule, 1996). To produce the novel Del(4-11) allele, we used targeted meiotic recombination (TAMERE) (Hérault et al., 1998) after a cross between the del(4-13) allele and the md11f allele (Becker and Duboule, 1998). Heterozygous Hoxd1lac mice (Zakany et al., 2001) were crossed together in order to monitor the expression of Hoxd1lac reporter gene by X-Gal assay or to detect lacZ transcript accumulation. For the production of the novel Del(4-11) allele, ‘transloker’ males were produced containing the two HoxD alleles del(4-13) and md11f, along with the SceYCRE transgene. Three recombinant pups were obtained after genotyping 171 progeny (1.7%), one of which carried the intended allele, and the two others the predicted reciprocal allele (see Fig. 3).

PCR primers used for genotyping were as follows: Inv1 (5’-CCAC-CCTGCTAAATACCGCT-3’) and 5’d1b (5’-GGTTGCCCTTTTCTCTTCTC-3’) to detect the wild-type HoxD allele; Inv1 and 3’d1b (5’-CTATTCAAGGTGGGGGAGCAGCTC-3’) to detect the Del(1-10)

Table 1. Range of caecum defects scored in newborn mice of HoxD mutant stocks

<table>
<thead>
<tr>
<th>HoxD genotype</th>
<th>Phenotypic classes</th>
<th>A</th>
<th>H</th>
<th>D</th>
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<tr>
<td>Del(1-10)/del(1-13)</td>
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<td>10</td>
<td>2</td>
<td>15</td>
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<tr>
<td>Del(1-10)/del(8i-13)</td>
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<td>11</td>
<td>3</td>
<td>21</td>
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<tr>
<td>Del(1-10)/del(11-13)</td>
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<td>4</td>
<td>4</td>
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<td>8</td>
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<tr>
<td>del(1-13)/del(1-13)</td>
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<td>16</td>
<td>18</td>
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<tr>
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<td>6</td>
<td>8</td>
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<td>del(8i-13)+</td>
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<tr>
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Genotypes are listed on the left and graphically represented in the middle; the incidence of the respective phenotypic classes is indicated on the right. Different colors signal the differential influence of ‘posterior’ and ‘anterior’ Hox genes on caecum morphogenesis. Del(1-10)-associated Hoxd12 and Hoxd11 gain-of-functions are in red, referring to their ectopic expression in caecum bud. In the other alleles, Hoxd1, 3, 4, 8, 9, and 10 genes are in green, referring to their buffering activity against the activity of more-posterior genes. The Hoxd1/lac reporter transgene (whose expression in caecum is documented in Fig. 4A,A’,D) is in blue. The four phenotypic classes were defined as follows. Class A: absent (agenesis; see Fig. 4A for illustration) or very short caecum bud without epithelial invasion (atresia; see Fig. 4A’,B for illustration). Class H: short caecum, of less than half the normal length (hypoplasia). Class D: thin caecum, not overly shorter than normal (dysplasia; see Fig. 4D,E for illustration of this mildly abnormal morphology). Class N: caecum having normal proportions and substantial lumen (normal; see Fig. 4F,H for illustration). The vast majority of wild-type (wt) specimens belonged to class N or occasionally to class D. For this reason, data from classes A and H were combined as ‘abnormal’ and those from classes N and D were combined as ‘normal’ for the purposes of statistical hypothesis testing (see below). Del(1-10)+ heterozygous mice fitted mostly into class D, rarely into the more severe class H. In a few cases, specimens were normal and thus assigned to class N. Del(1-10)/Del(1-10) homozygous mice represented the most abnormal group. The caecum was absent with 100% penetrance, assigning these mutants to class A. Although not included in this table, Del(4-11)/Del(4-11) homozygotes were also completely caecum-less and would thus qualify as class A. Del(1-10)/Del(1-13) compound mutants never showed normal caecum. Instead, caecum agenesis, atresia and severe hypoplasia were most often represented. Del(1-10)/del(4-13) and Del(1-10)/del(8i-13) showed the same abnormal distribution, with minor repartition between the phenotypic classes. Del(1-10)/del(8i-13) compound mutants were able to survive to adulthood, allowing recording of the postnatal caecum morphology (see Fig. 4A, A’, D) versus del(1-13) allele (Beckers and md11f) and the two others the predicted reciprocal allele (see Fig. 3).
or del(1-13) alleles; Inv1 and 5′d3b (5′-GGGATGTCAATCTTCTTGGAGTG-3′) to detect the del(1-13) allele; Inv1 and 5′d4b (5′-TGGCAACCAACGGTTCCTTC-3′) to detect the del(8i-13) allele; Xfw (5′-TACCCCTGCTTACCTCCGGTGTG-3′) and Xlew (5′-TGTGTCCCTGTCCCTGTTATCCG-3′) to detect the mid11A allele and Xfw (5′-GACGCTCTCAGAGATCTGT-3′) to detect the Del(4-11) allele.

Gastrointestinal tract dissection and whole-mount in situ hybridization

The morning of the recovery of vaginal plug was counted as day 0 of embryonic development. Foetuses were collected at gestational days 11, 12 or 13 and full-length gastrointestinal tracts were dissected in PBS then fixed and processed according to standard procedures. The RNA probes used to detect HoxD expression were the following: Hoxd1 (Zakany et al., 2001), Hoxd3 (Condie and Capel, 1993), Hoxd4 (Featherstone et al., 1988), Hoxd8 (Izpisua-Belmonte et al., 1990), Hoxd9 (Zappavigna et al., 1991), Hoxd10 and Hoxd11 (Gerard et al., 1996), Hoxd12 (Izpisua-Belmonte et al., 1991), Hoxd13 (Dolle et al., 1991). The remaining RNA probes were Hoxa6 (Sekimoto et al., 1998), Hoxa10 (Favier et al., 1996), Fgfl0 (Belluscio et al., 1997) and Pitx1 (Logan et al., 1998). For the complementation assay, newborns were recovered, their full GI was dissected, documented and genotyped.

RESULTS AND DISCUSSION

Posterior specificity of the HoxD cluster

We first established the expression pattern of all nine gene members of the HoxD cluster in wild-type embryos at mid-gestation (E12), at a time when the caecum is located in the intestinal hernia (Fig. 1A,B). Consistent with earlier observations, we found that Hoxd4, Hoxd8 and Hoxd9 are co-expressed in the developing caecum, in addition to Hoxd1, Hoxd3, Hoxd10 and Hoxd11. From Hoxd1 to Hoxd10, expression was detected up to the ileo-caecal transition. By contrast, Hoxd11 transcripts were restricted to the posterior half of the caecum bud (Fig. 1E), whereas the more ‘posterior’ genes Hoxd12 and Hoxd13 (Fig. 1C,D) were transcribed only in the most caudal part of the GI tract (Dolle et al., 1991; Kondo et al., 1996).

Despite the expression of most Hoxd and other Hox genes in the developing caecum, foregut derivatives appeared to be devoid of Hoxd transcripts. For instance, while expression of all three Hox4 paralogous genes was scored in E12 stomach mesenchyme (Kawazoe et al., 2002; Pitera et al., 1999), we were unable to detect either Hoxd1, or Hoxd4, transcripts in embryonic stomach. Because of the rapid degradation of Hoxd1 mRNA (Zakany et al., 2001), we performed in situ hybridization with a Hoxd1 probe on wild-type foetuses, and explored lacZ-specific transcript accumulation in embryos carrying the Hoxd1/lacZ knock-in allele. In contrast to the robust lacZ expression in the caecum (Fig. 1C), staining was not seen in stomach. Similarly, Hoxd3 was weakly expressed in stomach, compared with midgut, indicating a relative restriction of Hoxd gene expression to the posterior gut.

Ectopic expression of Hoxd genes

Over recent years, a collection of mouse lines carrying rearrangements at the HoxD locus were produced by targeted meiotic recombination (TAMERE) (Hérault et al., 1998), in order to study gene regulation at this locus. In several lines harbouring deletions of one or multiple Hoxd genes, the remaining genes usually changed their expression patterns, in agreement with their new respective position within the Hox cluster. Accordingly, mice carrying such deletions usually showed both loss-of-function and gain-of-function phenotypes. In particular, severe alterations were obtained when ‘posterior’ Hoxd genes such as Hoxd12 or Hoxd13 were expressed in more anterior territories, either in the trunk (Kimita et al., 2000), or in the limbs (Zakany et al., 2004), due to the antagonizing effect of the most posterior HOX products over anterior ones, a property referred to as ‘posterior prevalence’ (Duboule, 1991; Duboule and Morata, 1994).

Mice homozygous for a deletion of the anterior part of the cluster, from Hoxd1 to Hoxd10 including [the Del(1-10) allele], were born in mendelian proportions and newborns appeared overtly normal,
yet none of them survived due to acute respiratory failure. Interestingly, all homozygous animals showed a severe agenesis of the caecum. We investigated whether this defect was due to the combined loss of function of several Hox genes in cis by analysing mice homozygous for a complete deficiency of the HoxD cluster [the del(1-13) allele]. In del(1-13) homozygous mice, however, the caecum was never absent. This observation indicated that the absence of caecum in Del(1-10) homozygous individuals was caused by a gain-of-function mechanism involving either Hoxd11, Hoxd12 or Hoxd13, rather than by a combined loss of function.

To determine which one(s) of these three genes could be causative of this phenotype, we performed RNA whole-mount in situ hybridization on dissected GI tracts of E12 embryos, from the lower oesophagus to the rectum (Fig. 1B). Strikingly, the expression patterns of both Hoxd11 (Fig. 2B) and Hoxd12 (Fig. 2E) changed, in heterozygous mutants, to become similar to that of Hoxd10 (Fig. 1F). Both Hoxd11 and Hoxd12 transcripts were readily detected in the most posterior part of the ileum as well as in the caecum. In such heterozygous animals, a marked delay in the progression of caecum budding was clearly scored (Fig. 2B,E,H), whereas no budding at all was visible in homozygous littermates (Fig. 2C,F,I). Therefore, both Hoxd11 and Hoxd12 became ectopically expressed in the mesenchyme of the whole caecum up to the ileo-caecal transition, and this gain-of-function condition correlated with the suppression of caecum budding in homozygous Del(1-10) embryos. By contrast, no ectopic Hoxd13 expression could be seen in the digestive tract (Fig. 2H,I).

We then investigated whether the absence of caecum was due to a deficit in budding or to a more global problem of gut (mis-)specification, due to aberrant regulation of those Hox genes labelling the ileum-to-colon transition. To this aim, we used the Hoxa10 and Hoxa6 probes. In wild-type animals, Hoxa10 is expressed in the anterior colon up to the ileo-caecal valve, including the budding caecum. In Del(1-10) heterozygous animals, Hoxa10 expression was not importantly modified and still labelled the ileum-to-colon transition, reminiscent of the ectopic patterns of both Hoxd11 and Hoxd12 (Fig. 2I-L), suggesting that caecum agenesis was not due to a transcriptional effect of the gained genes over other Hox genes transcription. In contrast to Hoxa10, Hoxa6 signal is normally restricted to the budding caecum. Whereas in heterozygous Del(1-10) embryos, the signal was expectedly reduced, homozygous mutant GI tracts still showed a Hoxa6 signal, but only in a small group of cells located at the expected position for the caecum bud (Fig. 2M-O). From these observations we conclude that the overall molecular GI tract specification, as indicated by the HoxA-cluster-specific probes, was maintained even in homozygous mutants that did not develop a caecum. Consequently we searched for other genetic constitutions that result in caecum agenesis or hypoplasia, but without known involvement of general regionalization.

The development of the caecum is strongly impaired in mice, where either fibroblast growth factor genes (Fairbanks et al., 2004; Zhang et al., 2006) or receptors (Burns et al., 2004) are inactivated, in particular Fgf10, which is selectively expressed in the mesenchyme of the wild-type budding caecum (Fairbanks et al., 2004) (Fig. 2P). In the Del(1-10) mutant embryos, we found that Fgf10 transcript accumulation was reduced in heterozygotes and almost completely absent in homozygotes, leaving a small cluster of Fgf10-expressing cells in the ileo-colonic loop (Fig. 2Q-R). These observations suggest that caecum outgrowth is under the control of Fgf10, the expression of which may require the activity of several Hox genes in a defined region of the developing intestinal tract. In the absence of all Hox genes [del(1-13)], Hox genes from other clusters can still instruct presumptive cells to activate Fgf10 signalling, thus leading to the budding of a caecum. By contrast, the presence of ectopic Hoxd12 in this precise intestinal segment will abrogate the functions of more ‘anterior’ gene products from all clusters, via posterior prevalence. Accordingly, Fgf10 will fail to be produced and caecum budding will be suppressed. Interestingly, this situation is analogous to that recently reported to happen during...
hybridization signals for correlated with robust (H) are always detectable. Caecum bud growth is correlated with extremely reduced of bud mesenchyme. Absence of bud growth in homozygous specimens is heterozygous (C,F,I) and homozygous (D,G,J) posterior midguts at (Del wild-type control (B,E,H), hybridization of Del (could abrogate the Hoxd13 early limb budding, where ectopic expression of both Hoxd12 and Hoxd13 could abrogate the Fgf10-dependent growth of forelimb buds (Zakany et al., 2007).

In this scenario, the ectopic expression of Hoxd12 plays the key role via its concurrent deleterious effect upon the functions of other Hox genes. We challenged this hypothesis by producing and analysing yet another HoxD cluster deletion allele; Del(4-11) (Fig. 3A). In Del(4-11) F2 newborn progeny, all three genotypes were present in mendelian proportions. Strikingly, all homozygous embryos completely lacked the caecum. In situ hybridization analysis revealed a massive ectopic expression of Hoxd12 in posterior midgut mesenchyme (Fig. 3B-D), mimicking the normal expression pattern of Hoxd10 (Fig. 1F). The presumptive caecum of homozygous mice was consistently reduced to a small deformation of the gut, right inside the ectopic Hoxd12 expression domain. As for the case of Del(1-10), Hoxd13 was not gained in this presumptive caecum area in Del(4-10) mutant intestines at E12 (not shown), but the level of Fgf10 transcript was reduced (Fig. 3E-G). We also looked at the expression of the Pitx1 gene, whose transcripts are found both in the epithelium and mesenchyme of the developing gut (Lancot et al., 1997) and, as shown here, accumulate selectively in the mesenchyme of the growing caecum (Fig. 3H). Here again, this specific expression of Pitx1 was severely reduced in caecum mesenchyme of heterozygotes, whereas it was absent from homozygous specimens (Fig. 3I-J). Posterior midgut development was thus similarly compromised in both Del(1-10) and Del(4-11) homozygous animals. There was a robust correlation between all aspects of the caecum defect, on the one hand, and the ectopic expression of Hoxd12 and concurrent dose-dependent suppression of Fgf10 and Pitx1 transcripts in prospective caecum bud mesenchyme, on the other hand. We thus concluded that the induction and/or growth of the caecum are affected by ectopic expression of Hoxd12. Whether the loss of Fgf10, Hoxa6 and Pitx1 expression reflects the loss of the corresponding ‘presumptive caecal cells’ or, alternatively, the downregulation of these genes in these cells remains to be addressed. We did not fully assess the genetic cascade underlying the suppressive effect of HOX proteins on Pitx1 and Fgf10 transcription in developing caecum mesenchyme. However, Pitx1 expression was gained in the second branchial arch of mice lacking Hoxa2 following Hox interference with Fgf signalling (Bobola et al., 2003). Also, the data from the genetic and molecular embryological analysis presented here, together with those concerning early limb budding (Zakany et al., 2007), suggest that the Hox genes Fgf10 and possibly Pitx1 are components of a mesenchyme-specific genetic hierarchy that controls caecum budding.

These observations support an instructive role for ‘anterior’ Hox genes in the definition of a restricted territory from where the caecum will emerge. This precise area corresponds to an important morphological transition in the intestine, the position of which is probably also dependent upon the coherent expression of these same Hox genes. Induction of caecum budding and its elongation require a localized source of growth factors, as provided by Fgf10 signalling, downstream of Hox gene expression. We interpret our results in the context of posterior prevalence, according to which the function of a given Hox gene may be impeded by the presence of more posterior Hox product in the same cells (Duboule and Morata, 1994), in particular from the most posterior Hoxd12 and Hoxd13 groups. Because the absence of the whole HoxD cluster induced only a relatively mild posterior midgut malformation, we think that the expression of Hox genes left in the other clusters is equally capable of promoting posterior midgut development. However, in the case of internal HoxD cluster deletions, the ectopic expression of Hoxd12 abrogates the functions of several co-expressed ‘anterior’ Hox genes, leading to the inability to transcribe Fgf10 and consequent absence of budding.

Hoxd genes and the posterior midgut

In order to further document this conclusion, we produced a set of genetic configurations to fine-tune the doses of various Hox gene products. Because the Del(1-10) allele arguably delivers less ectopic activity of Hoxd12 than the Del(4-11) allele, we used the former together with selected HoxD cluster deficiencies, which by themselves do not induce ectopic gene expression. The rationale of these crosses was to manipulate doses of ‘anterior’ genes on the top of a fixed, standard level of ectopic Hoxd12 in the presumptive region for caecum budding (Table 1). First, we produced compound
mutants with the del(1-13) allele, i.e. a full deletion of the HoxD cluster. Interestingly, a proportion of Del(1-10)/del(1-13) trans-heterozygous individuals showed a phenocopy of the Del(1-10) homozygous phenotype, pointing to a strong influence of gene dose balance: in the absence of one haplotype of the HoxD cluster, half the dose of ectopic Hoxd12 gene product was sufficient to induce caecum agenesis. The occurrence of caecum agenesis in Del(1-10)/del(1-13) mice, compared with Del(1-10)/+ heterozygous mice, demonstrated that caecum development depends on the presence of ‘anterior’ Hox genes, capable of counterbalancing the deleterious effect of ectopic Hoxd12. In other words, higher doses of anterior HOXD gene products make a full posterior prevalence by HOXD12 deletions as the other allele; Del(1-10)/del(8i-13) and Del(1-10)/del(11-13) (Fig. 4A-I). Out of these combinations, Del(1-10)/del(11-13) embryos were the only ones to develop a normal caecum (Fig. 4D-F), similarly to Del(1-10)/+ heterozygous individuals. This genetic analysis, through a quantitative measurement, revealed the equivalence of one dose of Del(1-10)-associated ectopic Hoxd12, with one haplotype of Hoxd1, Hoxd3, Hoxd4, Hoxd8, Hoxd9 and Hoxd10.

Two doses of ectopic Hoxd12, in Del(1-10) homozygous, were capable of inactivating all the non-HoxD-derived caecum-promoting Hox activity, in all homozygous animals tested. Furthermore, even a single dose of gained Hoxd12 was capable of abrogating the HoxA, HoxB and HoxC gene function in a number of Del(1-10)/del(8i-13) individuals, in addition to an activity possibly provided by Hoxd1, Hoxd3 and Hoxd4 (Fig. 4A-C). From this, we conclude that in posterior midguft, the HoxA, HoxB and HoxC clusters together provide no more function than a single haplotype of the HoxD cluster does.

Hox function and postnatal growth

Quantitative modulation in the balance between ‘anterior’ Hox genes and ectopic Hoxd12 led to a phenotypic series involving more or less affected individuals, some of which survived for several weeks. In particular, most Del(4-11) heterozygous and some Del(1-10)/del(8i-13) compound mutants survived postnatally having either no, or reduced, caeca (Fig. 4B). In the Del(4-11) pedigree, we noticed a marked variation, and heterozygous mice proved lighter than their wild-type littermates. We took individual body mass readings of four litters sired by the same third generation backcross male with wild-type C57Bl6 females, and two litters of heterozygous parents. Of a total of 39 typed progeny, 17 were wild type and 22 were of Del(4-11) heterozygous genotype. At 4 weeks of age, the average body mass was 10.9 and 9.2 g, respectively, indicating approximately 20% deficit in Del(4-11) heterozygotes. This statistically significant body mass deficit persisted into adulthood.

Similar observations carried out on mice heterozygous for a full HoxD deficiency showed less than 10% body mass reduction, a figure statistically non-significant. In conclusion, Del(4-11) heterozygous mice do not thrive as well as wild-type littermates, which may indicate reduced digestion efficacy due to a shorter gut. We believe that this effect would be even more substantial on a less complete and mostly vegetal chow, as caecum and upper colon are sites of bacterial cellulose decomposition of nutritional importance. Therefore, these HoxD cluster mutants represent a valuable genetic resource to investigate gut patterning in general, and postnatal adaptive responses to environmental factors in particular (Wostmann and Bruckner-Kardoss, 1959), as well as the concurrent effects on body mass control (Backhed et al., 2004; Samuel and Gordon, 2006).

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