A regulatory ‘landscape effect’ over the Hoxd cluster

Patrick Tschopp a, Denis Duboule a,b,*

a National Research Centre ‘Frontiers in Genetics’, Department of Zoology and Animal Biology, University of Geneva, Sciences III, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland
b National Research Centre ‘Frontiers in Genetics’, School of Life Sciences, Federal Institute of Technology (EPFL), Lausanne, Switzerland

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ABSTRACT

Faithful expression of Hox genes in both time and space is essential for proper patterning of the primary body axis. Transgenic approaches in vertebrates have suggested that this collinear activation process is regulated in a largely gene cluster-autonomous manner. In contrast, more recently co-opted expression specificities, required in other embryonic structures, depend upon long-range enhancer sequences acting from outside the gene clusters. This regulatory dichotomy was recently questioned, since gene activation along the trunk seems to be partially regulated by signals located outside of the cluster. We investigated these alternative regulatory strategies by engineering a large inversion that precisely separates the murine HoxD complex from its centromeric neighborhood. Mutant animals displayed posterior transformations along with subtle deregulations of Hox genes, indicating an impact of the centromeric landscape on the fine-tuning of Hox gene expression. Proximal limbs were also affected, suggesting that this ‘landscape effect’ is generic and impacts upon regulatory mechanisms of various qualities and evolutionary origins.

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Introduction

The patterning of animal body plans largely depends upon the HOX family of transcription factors. These gene products help to specify the various body segments, often through a combinatorial input of different HOX proteins (Lewis, 1978; Krumlauf, 1994). In many species, including all vertebrates, Hox genes are found clustered at distinct loci in the genome, an organization that bears important implications for their coordinated transcriptional regulation (Duboule, 2007). Both the transcription onset and the rostral to caudal extent of any genes’ expression domain are determined by the relative position of each Hox gene within its respective genomic cluster (‘temporal and spatial collinearities’, respectively; Kmíta and Duboule, 2003). Spontaneous and engineered regulatory mutations leading to the mis-expression of Hox genes can have spectacular effects upon morphological specification and hence their transcription during development needs to be tightly controlled.

In tetrapods, 39 Hox genes belonging to 13 groups of paralogy are distributed into four gene clusters (HoxA to HoxD), generated by two rounds of whole genome duplication (see Garcia-Fernandez, 2005). The presence of up to four paralogous genes has allowed for a substantial diversification in function, for example via the acquisition of novel expression domains in a variety of embryonic structures (Deschamps, 2007). However, the implementation of the collinear regulation during trunk development, which is considered as the most ancestral function for this gene family, is thought to be similar at all four genomic clusters. When located on a PAC clone, the human HoxD cluster rather faithfully reproduced the collinear distribution of its transcripts in the primary body axis of transgenic mouse embryos. In contrast, it failed to recapitulate expression domains, which were more recently co-opted during vertebrate evolution. Accordingly, it was proposed that the ancestral mechanism relies upon regulatory modalities intrinsic to the gene cluster, whereas more recently co-opted transcriptional controls are exerted from outside the locus itself (Spitz et al., 2001).

In the case of the HoxD cluster, structures involving vertebrate-specific regulatory modalities include the external genitalia (Dolle et al., 1991), the caecum (Zakany and Duboule, 1999), the metanephric kidneys (Di-Poi et al., 2007) and the proximal and the distal segments of paired appendages (Dolle et al., 1989; Nelson et al., 1996). Interestingly, global gene regulations required for the development of either proximal or distal limb structures are located on opposite sides of the gene cluster, suggesting their distinct evolutionary histories (Spitz et al., 2005). The former regulation (in both the arm and forearm, excluding digits) was assessed in some detail, using series of internal deletions and duplication at the locus in vivo. In this way, it was proposed that the nested expression patterns observed in the developing proximal limb (Dolle et al., 1989; Nelson et al., 1996), while initiated from the telomeric neighborhood of the gene cluster, were negatively modulated via a repressive effect elicited from the centromeric side (Tarchini and Duboule, 2006; Zakany et al., 2004). The deleterious effects of ectopic Hoxd13 expression on the developing forearm of Ulnaless mice illustrate this necessity to repress posterior Hoxd genes during early zeugopod development (Herault et al., 1997; Peichel et al., 1997).
By using a set of chromosome rearrangements at the HoxD locus, we recently assessed the early temporal activation of these genes during extension of the primary body axis and suggested that a similar negative regulatory influence, coming from flanking centromeric sequences, could be involved in fine-tuning the onset of the incipient expression domains (Tschopp et al., 2009). The hypothetical presence of a remote negative effect exerted over HoxD gene activation by the centromeric landscape echoed an earlier observation derived from a set of centromeric deletions extending into the HoxD cluster (Kondo and Duboule, 1999). However, all engineered alleles used so far to address this issue also compromised the integrity of the HoxD cluster itself, making a clear distinction between internal and external influences problematic.

Here, we clarify this issue by engineering a large inversion, which flips away the centromeric neighborhood of the gene complex, including an extended gene desert spanning approximately 500 Mb up to Atp5g3, while leaving the HoxD cluster intact. In this way, regulation[s] located centromeric to HoxD is expectedly abrogated. Animals carrying this inversion displayed skeletal phenotypes in both their trunk and proximal limbs, suggesting a gain of function for posterior Hox genes. Expression studies confirmed an up-regulation of these genes towards the end of their activation process. Altogether, our observations reveal the existence of a negative effect of the centromeric landscape on HoxD gene expression. We discuss both the potential impact of large genomic contexts on gene regulation, as well as the possibility that co-opted regulatory modalities may have been constrained by global mechanisms selected to fine-tune the ancestral function of these genes in the building of the main body axis.

Materials and methods

Mouse strains and crosses

The new inversion (Inv) allele was generated using the STRING approach (see Fig. S1 and Spitz et al., 2005). As parental alleles, we used a loxP site at the Iuga6 locus (Gimond et al., 1998), 3 Mb away from the HoxD cluster, and a HoxD11/lacZ-loxP transgene, targeted into the Evo2 to HoxD13 intergenic region (van der Hoeven et al., 1996).

After inversion, the HoxD11/lacZ-loxP transgene is removed from the cluster together with its immediate centromeric neighborhood. The allele was maintained on a B6/CBA F1 hybrid background. For embryo crosses, noon on the day of the vaginal plug was considered as E0.5.

Embryos were dissected in ice-cold PBS and fixed overnight in 4% PFA.

Genotyping

Genotyping was performed on isolated ear punch or yolk sac DNA using a duplex PCR protocol (see Fig. S1B). Oligo sequences were as follows:

Oligo 1: 5′-CGCTCAATGCCTGTGTTACCC-3′

Oligo 2: 5′-GGACACCAATGAAATCCTTAATGG-3′

Oligo 3: 5′-GCTGCTTCCATGTAAGAAGACTG-3′

Southern blot analysis was done following standard protocols. The centromeric probe was PCR-subcloned into a pGEM-T easy vector (Promega), using oligos 5′-CTCGGTGCTCCCTCTGTTAAGG-3′ and 5′-AAGGAAAAACCGCCACATTGGACCCG-3′. The telomeric probe was an 800 bp XbaI-BglII fragment, telomeric to the Nsi site used to target the HoxD11/lacZ transgene. Both fragments were released by restriction digest, gel-purified and labeled using DIG-High prime (Roche).

In situ hybridization, X-Gal staining and skeletal preparation

Whole-mount in situ hybridization (WISH) was performed according to standard protocols, with both mutant and control embryos processed in the same well to maintain identical conditions throughout the procedure. Probes were as described elsewhere.

Results

Separation of the HoxD cluster from its centromeric neighborhood

To evaluate a potential influence of the genomic context on the transcriptional regulation of HoxD genes, we engineered a novel allele where the adjacent centromeric neighborhood was inverted, without disturbing the integrity of the gene cluster. This inversion disconnected HoxD genes from a large gene desert, which contains a range of highly conserved non-coding DNA sequences (Lee et al., 2006). The inversion was generated in vivo, using the STRING approach (Fig. S1A; Spitz et al., 2005). As parental alleles, we used a loxP-containing modification of the Iuga6 locus (Gimond et al., 1998), located 3 Mb centromeric to the HoxD cluster, and a HoxD11/lacZ-loxP transgene, introduced into the Evo2 to HoxD13 intergenic region, i.e. right next to the gene cluster (van der Hoeven et al., 1996). After breeding, recombinant F2 offspring containing both loxP sites in cis were further crossed into HprtCre mice (Tang et al., 2002). Once the inversion had occurred, the HprtCre allele was segregated out (Fig. S1B) and the integrity of both centromeric and telomeric breakpoints was verified by Southern blot analysis (Fig. S1C and D).

Expression of the translocated HoxD11/lacZ transgene

As a result of the inversion, the HoxD11/lacZ transgene present upstream HoxD13 in the parental allele, was translocated 3 Mb far from the HoxD cluster, to the Iuga6 locus, along with the gene desert. We looked at the expression of this transgene before and after inversion, to evaluate potential differences due to the two different genomic contexts (Fig. 1A). In 12.5 days old embryos (E12.5) carrying the non-inverted configuration, i.e. where the HoxD11/lacZ transgene is near the HoxD cluster, β-gal activity was detected in a pattern resembling the endogenous HoxD11 gene, with rather faithful anterior limits of expression in both the axial mesoderm and the spinal cord. In E12.5 embryos carrying the inversion, however, this Hox-like lacZ expression was lost in both mesoderm and neural tube, while still observed in the most caudal aspect of the embryo, the tail bud.

In addition, the inversion induced the ectopic transcription of the transgene in the central nervous system (CNS), as anterior as into the midbrain (Fig. 1D), reminiscent of the expression of the neighboring Evc2 gene in V0 interneurons (Fig. 1H; asterisk; Dolle et al., 1994; Moran-Rivard et al., 2001). We concluded that in the non-inverted configuration, the HoxD cluster prevents the HoxD11/lacZ transgene from responding to this Evc2-associated regulation, likely as a side-effect of a general strategy to avoid the deleterious transcription of Hox genes into this particular type of neurons (see Kmita et al., 2002). After inversion, this negative effect was alleviated, due to the absence of the HoxD cluster, and the V0 regulation readily co-opted by the transgene.

Expression in developing appendages was generally as expected (Fig. 1C). Developing forelimbs of Inv embryos completely lacked transgene expression in the most proximal domain (Fig. 1E and F).
Fig. 1. A centromeric inversion, which separates a Hoxd11/lacZ transgene targeted right upstream of the HoxD cluster. (A) Floxed allele, with the Hoxd11/lacZ-loxP transgene targeted into the Evx2 to Hoxd13 intergenic region and a second loxP site, in a reverse orientation, replacing exon 25 of the Itga6 gene (see Fig. S1 for details). (B) Exposure to the Cre recombinase induces inversion of the floxed interval, thereby moving both Evx2 and the Hoxd11/lacZ transgene 3 Mb away from the HoxD complex. (C and D) E12.5 embryos stained with X-Gal to assess the activity of the Hoxd11/lacZ transgene either before (C) or after (D) inversion. Transgene expression in the primary body axis changes from a Hox-like pattern in the floxed allele (C) to an Evx2-like pattern in the Inv (D). (E and F) Forelimbs of the embryos depicted under (C) and (D). While the proximal domain is lost in Inv limbs (E and F, arrowhead), the distal domain extends into presumptive digit 1 (E and F, arrow). (G and H) Dorsal view of embryos in (C) and (D). Expression in the mesoderm up to somite level 27 (G, arrowhead) and in the spinal cord up to somite level 25 are lost in Inv embryos (H). In contrast, Hoxd11/lacZ is transcribed in the spinal cord (H, asterisk), likely in V0 interneurons, up into the midbrain (D and H). Scale bar is 1 mm in C and D, and 500 µm in E–H.

arrowhead). In contrast, expression in the distal part not only persisted, but was even slightly expanded into presumptive digit 1 (Fig. 1E and F, arrow). This expansion of Hoxd11/lacZ expression was likely due to a decrease in promoter competition for the centromeric-located global digit enhancers, all activity now being re-routed exclusively towards both the transgene and Evx2. Similar effects have been reported for various alleles wherein the HoxD cluster was modified (Montavon et al., 2008). A complete absence of transgene expression was also scored in proximal hindlimbs (Fig. 1G and H, arrowheads) along with the loss in somitic mesoderm (Fig. 1G and H, arrows), supporting the proposal that activation of HoxD genes in both the primary body axis and the proximal limb domain depends in part on regulatory modalities located at (or influenced by) more telomeric positions (Tarchini and Duboule, 2006; Tschopp et al., 2009).

Phenotypes of Inv mutant animals

Homozygous Inv animals were born at the expected Mendelian ratio. Their skeletons were prepared at P0 and analyzed in details. We first compared the axial skeletons of both heterozygous and homozygous Inv mutant versus wild-type control litters. We observed no difference between control and mutant skeletons, when 223 the most anterior body levels were considered, i.e. at the cervical level and in the beginning of the thoracic region (data not shown). However, a significant reduction in the average number of lumbar vertebrae was scored for both heterozygous and homozygous mutant animals, with some homozygous mutant animals displaying only four lumbar vertebrae (Fig. 2A–C).

In addition, several homozygous mutant skeletons showed a distinct reduction of the last pair of ribs (on the 13th thoracic vertebra; Fig. 2C; T13, arrows), up to a unilateral agenesis, in the most severe cases. Mutant animals also showed a slight, yet significant, reduction in the number of caudal vertebrae (Fig. 2D). Altogether, Inv mutant animals suffered from several partial and/or complete posterior transformations, at different levels along the primary body axis, thereby causing an overall reduction in the number of skeletal elements.

Up-regulation of posterior Hoxd genes

We looked for changes in endogenous Hoxd gene expression, as induced by the inversion, which could provide an explanation for the observed phenotypic effects. We first analyzed those genes located next to the inversion breakpoint, i.e. belonging to the posterior groups of paralogy. Whole-mount in situ hybridization for both late (E12.5) and early (E8.5) stages did not reveal any drastic change in gene expression (data not shown). However, careful investigations of intermediate stages of axial elongation revealed either premature activation, or up-regulation for several posterior Hoxd genes. In E10 control embryos, Hoxd13 was already expressed around the proctodeum region, whereas transcripts were not yet detected in the presomatic mesoderm. Inv heterozygous embryos, in contrast, showed a clear up-regulation of Hoxd13 transcripts at their caudal ends (Fig. 3A). The ectopic activation was observed in presomatic mesoderm while the expression around the proctodeum remained largely unchanged (Fig. 3B and C).
weak mesomelia, i.e. a shortening in the length of zeugopodial elements (forearm and foreleg; Fig. 4A and B). Morphometric analyses were carried out on both Inv and wild-type animals, using for normalization the scapula and pelvic girdle, as these two elements were not affected in the double-inactivation of the HoxA and HoxD clusters (Kmita et al., 2005).

While the humerus did not show any significant variation in length, both the radius and ulna of Inv mutant animals were clearly mesomelic, with a decrease in length of about 15% with respect to wild-type. A similar, although slightly weaker, reduction was scored for the tibia in the hindlimb (Fig. 4C). Expectedly, the inversion of the centromeric landscape also separated Hoxd genes from the global digit enhancers (Gonzalez et al., 2007) necessary for their transcription in the developing autopods (hands and feet). This led to a reduction of autopodal skeletal elements (Fig. 4A and B and Fig. S2A and B), resembling the phenotypes observed in the combined deletions in cis of the three posterior-most Hoxd genes (Zakany and Duboule, 1996).

Regulatory re-allocations in developing limbs

Two fundamentally different regulatory processes organize the expression of Hox genes in developing limbs. In early budding appendages, a balance between a centromeric-located repression and a telomeric-located activation governs the nested patterns observed in the proximal part of both the developing fore- and hindlimbs (Fig. 5B; Tarchini and Duboule, 2006). Subsequently, a group of centromeric enhancers activate the most posterior genes in the presumptive autopods (Fig. 5A; Gonzalez et al., 2007). At E10, Inv heterozygous embryos showed a severe reduction in the autopodial expression for both Hoxd13 and Hoxd12 transcripts, as one dose of these enhancers was relocated to remote centromeric positions (Fig. 5C and E). Homozygous embryos displayed an almost-complete absence of Hoxd transcripts from the autopodal domain in mid-gestation embryos, as shown in both fore- and hindlimbs (Fig. 5C–H). The inversion-induced relocation of these digit enhancers thereby led to a de facto loss of function of Hoxd genes in the autopodal domain (Fig. 5G).

In contrast, more proximal regions seemed unaffected at early stages. However distinct changes in gene expression profiles in the proximal domain became apparent in slightly older embryos. In particular, ectopic Hoxd13 transcripts were scored in the posterior part of the putative forearm domain (Fig. 5D, arrowhead), whereas the proximal domain of Hoxd12 transcription was expanded towards the anterior margin in Inv mutant embryos (Fig. 5F). Hoxd11 and Hoxd10 profiles remained spatially unchanged, yet steady-state transcript levels for both genes appeared clearly elevated in the proximal domain (data not shown). Therefore, the inversion of the centromeric landscape led to the alleviation of a repressive influence, which in turn allowed for an anterior expansion of the expression domains in the presumptive forearm (Fig. 5H).

Discussion

A landscape effect modulates Hox gene expression

Previous work had suggested that several transgenes containing single Hox transcription units could be transcribed rather faithfully when introduced randomly in the genome. This led to a view whereby the regulation(s) necessary for the correct expression of a given individual Hox transcription unit along the developing AP axis would lie in close genomic proximity (e.g. Sharpe et al., 1998). Using a PAC containing the human Hoxd gene complex, this observation was further extended to the level of the gene cluster itself, as mouse embryos harboring this large transgenic DNA showed spatial collinear expression of the human genes (Spitz et al., 2001). However, expression of these genes in structures that are more recent,
evolutionary speaking (for example the limbs), were not scored in this context, suggesting that such co-opted modes of regulation are implemented from outside the gene cluster, rather than being interspersed between Hox genes.

Our centromeric inversion at the HoxD locus shows that this regulatory dichotomy should be considered with more caution, as gene expression in the developing major body axis is also fine-tuned by sequences located outside of the gene complex. It is not surprising that transgenic experiments overlooked the importance of the cluster neighborhoods for proper regulation, since their readout was mostly at the transcriptional, rather than functional level. Our inversion allele, however, clearly shows that the centromeric vicinity of the gene cluster exerts a negative effect upon the expression of several posterior Hox genes. While this inversion-induced de-repression had only a subtle impact upon the expression levels, the effect was strong enough to lead to phenotypic consequences reflecting ectopic actions of several posterior Hox genes (Carapuco et al., 2005; Wellik and Capecchi, 2003; Young et al., 2009).

Fig. 3. Up-regulation of posterior Hoxd gene expression in the presomitic mesoderm of Inv mutant embryos. Expression of Hoxd13 (A–C), Hoxd12 (D–F) Hoxd11 (G) and Hoxd10 (H) in E9 to E10 control and Inv heterozygous embryos. Mutant embryos are on the right, next to a representative wild-type control. (A–F) Hoxd13 and Hoxd12 are activated prematurely in Inv presomitic mesoderm, whereas transcription around the proctodeal region remains unchanged. (B, C, E, and F) Caudal ends of embryos shown in A and D. (G and H) Transcript levels of Hoxd11 (G) and Hoxd10 (H) are elevated in the caudal end of Inv embryos. (I and J) Perturbation of the regulatory balance in Inv embryos. (I) In wild-type, the sequential activation of Hox genes in the primary body axis depends on a balance between a repression (red) established from the centromeric side, and an activation (green) from the opposite side. (J) After inversion, posterior Hox genes escape the repressive influence to become up-regulated in the presomitic mesoderm. Scale bar is 500 μm in A, D, G, and H, and 250 μm in B, C, E, and F.
This negative effect could be caused either by a single, sequence-specific element mediating the activity of repressor molecules or, instead, by a global repressive influence elicited by the entire centromeric DNA interval containing multiple entities to modulate transcriptional efficiency in the cluster. The centromeric neighborhood of HoxD, a region of extended synteny amongst vertebrates (Lee et al., 2006), contains range of conserved, non-coding sequences. A scanning deletion approach had previously suggested a candidate region wherefrom such a negative effect could originate (between the Rel3 and Rel2 breakpoints in Kondo and Duboule, 1999). However, since a set of nested deletions extending into the HoxD complex were used, we could not ascertain whether the proposed negative effect was implemented by a single sequence located between these two breakpoints or, alternatively, whether the largest deletion removed a combination of sequences capable to negatively influence transcription over the HoxD cluster in a synergistic manner. While these results confirm the presence of a negative influence outside the HoxD cluster, our strategy does not allow us to map it precisely within this large DNA interval.

A similar situation was reported to prevent the same posterior Hoxd genes from being mis-expressed in CNS derivatives, by blocking the action of enhancers controlling the transcription of Evx2 in V0 interneurons throughout the AP axis. In this context, a combination of DNA segments was found necessary to implement this insulation, as shown by progressively larger deletions into the gene complex (Kmita et al., 2002). A large part of this insulating activity was subsequently associated to a small DNA fragment located between Evx2 and the breakpoint we used in the present study to introduce our Hoxd11/lacZ reporter transgene (Yamagishi et al., 2007). Here, we show that after inversion, this insulation is lost and the transgene becomes expressed ectopically in the CNS, even though it is inverted along with the proposed enhancer-blocking sequence. A position effect of the ‘landing site’ in this de-repression is unlikely, as insulation in V0 interneurons is maintained when a larger piece of DNA is inverted, while using the same centromeric breakpoint (Tschopp et al., 2009).

We conclude that the short ‘insulator’ sequence (Yamagishi et al., 2007) may not be sufficient and likely works in combination with several other DNA fragments to act either as an insulator, or as a repressor (Kmita et al., 2002).

Repressive mechanisms at work in CNS cells may not be comparable to those implemented during trunk extension. Nevertheless, in the latter case too, some global properties of the HoxD centromeric neighborhood, rather than a specific DNA sequence, may elicit the observed negative influence. This could be, for instance, to the synergistic effect of several DNA fragments and/or to a global 3D configuration of this extended genomic landscape, imposing some constraints over a fully efficient transcriptional activity of the gene cluster itself. Upon inversion of the centromeric fragment, this regulatory balance may tip and thus release some of these negative effects. The impact of DNA flanking sequences over the behavior of transgenes randomly inserted into various genomic sites is usually qualified as a ‘position effect’. We propose to use the

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term ‘landscape effect’ whenever a large DNA segment likely impacts over the general transcriptional status of several genes via its mere intrinsic organization.

The evolution of landscape effects

It is questionable as to whether or not such a landscape effect may have represented an adaptive value in all the different tissues or structures where it is observable. This question also applies to those contexts where it induces phenotypic consequences when inverted. Indeed, it is possible that this particular negative effect was critical in one particular cell type and was subsequently implemented, via a bystander effect, in other contexts. For instance, the apparent necessity not to have posterior Hox genes expressed in anterior V0 interneurons may have consolidated this repressive modality, thereby enabling it to impact upon other domains (e.g. in paraxial mesoderm), without any major evolutionary constraint attached to these latter contexts.

The functional diversification of the murine Hox clusters, which accompanied the two-genome duplications at the basis of the vertebrate radiation, is illustrated by the many cluster-specific functions scored during development. In several instances, the evolution of the required regulatory modules (sequences, enhancers) occurred outside the gene clusters themselves, presumably to prevent interferences with the ancestral, cluster-internal collinear mechanisms at work during trunk extension. This phenomenon can likely be associated with the presence of gene deserts, generally present on either sides of Hox clusters and containing series of non-coding conserved DNA elements with potential regulatory capacities (Lee et al., 2006). The evolution of new regulations in this set-up may not have happened completely de novo, but may rather have built upon generic elements and locus conformations already at work during primary axis elongation (‘regulatory priming’ in Gonzalez et al., 2007).

While this could have facilitated the emergence of novel regulatory specificities, it may also have imposed important constraints regarding

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their modes of operation. In the case described here, the negative influence of the centromeric landscape may have contributed to the delay of activation of the most posterior genes Hoxd13 and Hoxd12 during the extension of the trunk, thus allowing the caudal region of the embryonic axis to grow further (Young et al., 2009). As a consequence of this regulatory strategy, the activation of the same genes is delayed during proximal limb development, which contributes to the elongation of zegopod elements.

Because such landscape effects may inherently rely on extended genomic neighborhoods, preferably in gene-poor regions, the possibility exists for a considerable evolutionary flexibility in the modulation of any such regulation. In the case of posterior Hox genes, such a repressive mechanism could be of different magnitude in various species and hence may contribute to the increased diversity that is found in terminal body structures when compared to more anterior regions (e.g. Goodrich, 1913). From a phylogenetic viewpoint, it could thus be of interest to investigate whether other evolutionary innovations patterned by secondary (co-opted) sites of Hox expression are also modulated in concert with more ancestral morphological features dependent upon Hox gene activity. For example, in structures like limbs, changes in patterning across different vertebrate taxa may be associated with distinct modifications in axial skeletons.

Human ‘landscape syndromes’

The shortening of forelimbs we describe upon inversion of the centromeric landscape is reminiscent of human mesomelia, a variety of genetic syndromes that negatively impacts upon the length of proximal limb elements. Interestingly, several such conditions were associated with genomic rearrangements at or around the human HoxD cluster, including deletions, inversions and duplications (Dlugoszewska et al., 2006; Kantaputra et al., 2010; Mitter et al., 2010). Accordingly, the molecular aetiology of these syndromes was tentatively explained by the impact of these large rearrangements upon previously described elements controlling Hox genes during limb development (e.g. Kantaputra et al., 2010). In support of this view, copy number variations (CNVs) are arguably the cause of several human diseases, potentially through their interference with regulatory mechanisms (Henrichsen et al., 2009). In addition, ectopic expression of Hoxd13 in the developing proximal limb induces mesomelic dysplasia in Ulnaless mice carrying a large inversion of the HoxD cluster (Spitz et al., 2003).

Here, we demonstrate that a destabilization of a regulatory landscape can lead to imbalances in gene regulation, even if the rearrangement neither deletes any target genes, nor the major regulatory sequences responsible for the expression of these genes in the developing forelimbs. In this context, rearrangements of all kinds can slightly modify the global outcome of such long-range regulations, leading to transcriptional variations, even over large distances. The search for such ‘landscape effects’ as causes of particular syndromes or pathologies will call for careful consideration of the nuclear organization of large DNA intervals, for example by using technologies to visualize spatial chromosome conformations (van Steensel and Dekker, 2010). In addition, this may not be readily reproducible using model systems, as such chromosomal architectures may rely upon intrinsic, species-specific features that may vary even in regions of high synteny, as well as displaying cell type specific behaviors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ydbio.2010.12.034.

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Kantaputra et al., 2010.


