

Pulling forces acting on *Hox* gene clusters cause expression collinearity

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ABSTRACT The development of normal patterns along the primary and secondary vertebrate axes depends on the regularity of early *Hox* gene expression. During initial stages, these expression events form a sequential pattern of partially overlapping domains along the anteroposterior axis in coincidence with the 3' to 5' order of the genes in the *Hox* cluster (spatial collinearity). In addition, the genes are activated one after the other in the 3' to 5' order (temporal collinearity). These features are poorly understood within the framework of Molecular Genetics. A model was proposed according to which physical forces act on *Hox* clusters as a result of signaling from morphogen gradients. The model can explain the collinearity of *Hox* gene expression along the primary and secondary body axes. The increase in the concentration of morphogen is accordingly followed by an increase of the force acting on the cluster. The genes are sequentially translocated, in the 3' to 5' order, toward the interchromosome domain where they are exposed to transcription factors for activation. The above geometrodynamics approach reproduces most collinearity data. Recent experiments verify the above prediction of sequential 3' to 5' *Hox* gene translocations in the interchromosome domain. Furthermore, it seems that these translocations, combined with cluster decondensations, are caused by attractive forces acting on the 3' end of the cluster and pulling the genes out of the chromosome territory. Additional experiments are proposed in order to specify the origin of the forces.

KEY WORDS: *collinearity, Hox gene expression*

Introduction

In 1978, after a long series of classical genetic studies in *Drosophila*, E. B. Lewis established a correlation between the activation pattern of the genes of the bithorax complex (*BX-C*) along the antero-posterior axis of the embryo and the proximo-distal location of these genes along the chromosome (Lewis, 1978). This astonishing correlation (coined collinearity) proved to be a property extending to orthologous genes of all metamerical animals, humans included (McGinnis and Krumlauf, 1992). It turns out that these genes contain a conserved sequence of 180 bp, the so-called homeobox, which was first discovered in the *Antennapedia* and the other homeotic genes of *Drosophila* (McGinnis and Krumlauf, 1992; Krumlauf, 1994). Compared to *Antennapedia*, numerous homologue genes were found in many other genomes. These were named *Hox* genes and it turns out that they are grouped in complexes called *HOX* clusters. As in the *Drosophila*, *Hox* gene mutations cause severe homeotic transformations and malformations of the embryonic body plan. Because of their importance in axial patterning, several homologue clus-

ters have been formed by duplication in the course of evolution and their genes play significant and complementary roles in development. While *Drosophila* has only one homeotic complex (*HOM-C*), vertebrates have four such paralogous clusters *Hoxa*, *Hoxb*, *Hoxc* and *Hoxd* each one located in a different chromosome (Krumlauf, 1994). Every paralogous cluster has a variable number of genes (9 to 11) numbered from 1 to 13 in their physical order along the 3' to 5' direction on the chromosome. (In every cluster some genes of the above numbering are missing).

It was soon realized that the anteroposterior boundaries of *Hox* gene expression along the axis of the mouse embryo followed a collinear relationship similar to the *BX-C* correlation established by Lewis in *Drosophila* (Duboule and Dollé, 1989; Graham *et al.*, 1989). Namely, the anterior boundary of every expression was shifted along the posterior direction following the order 1, 2, 3, ... of the genes in the cluster (Krumlauf, 1994). This was characterized

Abbreviations used in this paper: BX-C, bithorax complex; CT, chromosome territory; ICD, interchromosome domain; np, nuclear periphery; PM, polar molecule; PPM, positive polar molecule; TF, transcription factor.

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as *spatial collinearity* for the primary axis whereas a modified spatial collinearity was also observed for the mouse and chick limb buds (Dollé *et al.*, 1989; Yokouchi *et al.*, 1991) (Fig. 1). In this case, spatial collinearity takes the following form at the initial stages of activation: for the *Hoxa* genes at the 5' end of the cluster, the expression domains create a nested pattern of partially overlapping regions along the proximo-distal axis of the bud. The expression domain of the last gene, *Hoxa13*, is limited at the distal tip of the bud. A similar pattern of nested expression domains is also observed for the 5' genes of *Hoxd* cluster with the last gene, *Hoxd13*, being expressed at the posterior-distal boundary of the bud (Nelson *et al.*, 1996).

Another interesting observation in vertebrate development concerns the time dependence of *Hox* gene expression: the initiation of expression follows the physical order of the genes in the cluster. Gene1 is expressed first followed by gene2 etc. This sequence in time of gene activation is termed *temporal collinearity* (Izpisua-Belmonte *et al.*, 1991). A third kind of *Hox* collinearity results from the fact that the intensity at the anterior part of an

expression domain is strong compared to the posterior expression region (McGinnis and Krumlauf, 1992). In particular and in contrast to the anterior boundary, the posterior expression boundary is faint and unclear. Therefore, at every location the expression of a posterior gene is stronger and dominates over any other overlapping anterior gene (Fig. 1). This property can be related to the *posterior prevalence* if we assume that the protein dominance reflects the dominance in strength of its gene expression (McGinnis and Krumlauf, 1992). One can think of an equivalence between *posterior prevalence* and *quantitative collinearity* observed for the 5' gene expressions of the *Hoxd* cluster in the limb bud (Dollé *et al.*, 1991; Kmita *et al.*, 2002): it turns out that distally in the bud the expression intensity systematically increases following the order *Hoxd10*, ..., *Hoxd13*.

The above features of *Hox* gene collinearity have been meticulously analyzed for more than 25 years in many organisms ranging from *Drosophila* to humans. Many attempts have been made to justify these surprising regularities but, up to now, no convincing solution has been possible based on molecular genetics and biochemical processes. Furthermore, the changes of a gene location in the cluster are associated with systematic alterations of its expression mode (Kmita *et al.*, 2002). This leads to the hypothesis that the chromatin deforming forces in the cluster may be responsible for the expression pattern of *Hox* genes. I think therefore that the mechanistic nature of collinearity phenomena requires a geometrodynamical approach. I have put forward a broad framework of physical principles combined with well founded biological facts which can reasonably reproduce the observed collinearity data (Papageorgiou, 2001; Papageorgiou, 2004). The guiding dogma in this pursuit is that *what happens is what can happen*. Phenomena that have been observed in other occasions are adopted and applied in the present formulation as long as they support the central hypothesis. This is legitimate since, in this way, the constructed model is compatible with well established facts and principles. The details of the mechanisms involved can only be determined and confirmed by experiment. It is instructive to give an example of how this has to be done. The model, in its broad formulation, proposes that collinearity is the result of physical forces translocating the *Hox* cluster toward a specific region where gene activation can occur. The nature, direction and application point of the forces cannot be known *a priori* since several possibilities can produce the expected result. After the formulation of the model an experiment has confirmed these translocations during gene activation of the *Hoxb* cluster (Chambeyron and Bickmore, 2004). Furthermore, this experiment has indicated where and how the force should apply on the cluster. This is helpful since it allows to write down, in the following, a detailed and more concrete version of the model. A short description is given below.

A physical model for *Hox* gene collinearity

The model functions at two levels in space: 1) At a multicellular ('macroscopic') level, a morphogen gradient is established over several cell diameters in morphogenetic fields of linear size approaching 1 mm (Wolpert, 1996; Summerbell *et al.*, 1973) as shown in Fig. 1 for the developing chick limb bud. For many years, persistent efforts aimed to explain how these gradients are created (Green, 2002). Some recent findings corroborate a mecha-

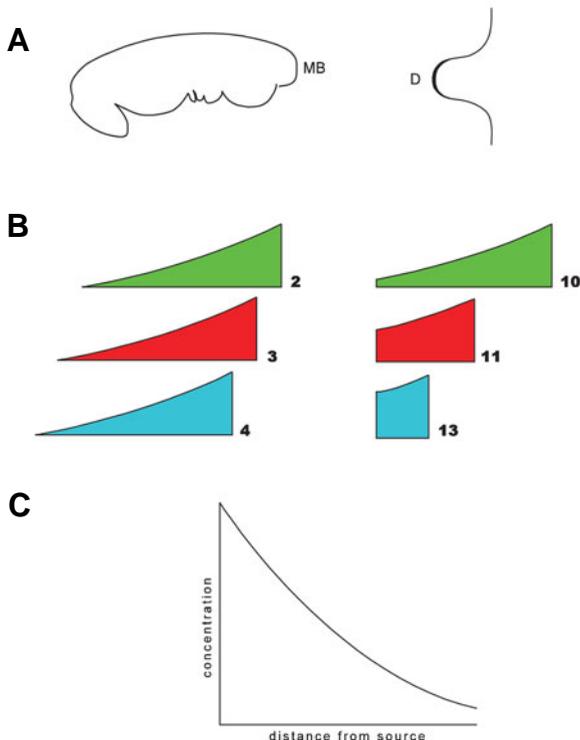


Fig. 1. Diagrams of embryos and morphogen gradients. (A) On the left, a 12 day mouse embryo is represented with the midbrain (MB) to the right boundary of the embryo axis and the posterior end to the left. On the right, the limb bud is represented with the distal tip (D) to the left. (B) On the left, the schematic expression domains of *Hoxb2*, *Hoxb3* and *Hoxb4* are shown along the primary axis of the embryo. Expression is stronger anteriorly (right side) and the expression domains are shifted along the anterior-posterior axis following the order 2, 3, 4 (posterior prevalence). On the right, the expression domains of *Hoxa10*, *Hoxa11* and *Hoxa13* in the limb bud at the initial stages (around 24) are depicted. The domains form a nested pattern while distally the intensity of expression increases in the order *Hoxa10*, *Hoxa11*, *Hoxa13* (quantitative collinearity). (C) A morphogen gradient with a peak at the posterior end of the vertebrate embryo or the distal tip of the limb bud.

nism of local morphogen production and subsequent extracellular spreading and degradation of the morphogen. The spreading is diffusion-based associated with or without endocytosis depending on the specific developing system (Scholpp and Brand, 2004; Belenkaya *et al.*, 2004). Passive diffusion may be combined with secondary procedures like morphogen transport through membranes (Kerszberg and Wolpert, 1998) or it may operate in parallel with processes like growth (Smith and Gurdon, 2004). Such secondary effects might hide the basic features of diffusion and, in order to determine the actual signal propagating mechanism, it is useful to compare the observed data with the features of signal traveling and their dependence on time and source intensity as expected in the case of pure diffusion (Papageorgiou, 1998; Vargesson *et al.*, 2001). For simplicity here it is assumed that at the posterior end of the vertebrate embryo axis (or the distal end of the limb bud) a source starts producing a morphogen which is spread in the morphogenetic field through a mechanism based on diffusion, as described above. After a transient time interval of morphogen net production and degradation in every cell, a decreasing steady state distribution is reached of exponential form with its peak at the source area (Papageorgiou, 1998; Papageorgiou and Almirantis, 1996) (Fig. 1).

2) At the nuclear ('microscopic') level, it is assumed that the tightly packaged *Hox* clusters take the shape of an elongated rigid body whose length is of the order of 500 nm (Papageorgiou, 2001). This is consistent with the findings that at interphase chromatin fibers are supercoiled into larger chromonema fibers whose diameters are about 80 nm (Belmont and Bruce, 1994; Tumber *et al.*, 1999) as shown in Fig. 2. These tightly clustered *Hox* complexes, when inactive, they are embedded inside the chromosome territory (CT) with their regulatory regions inacces-

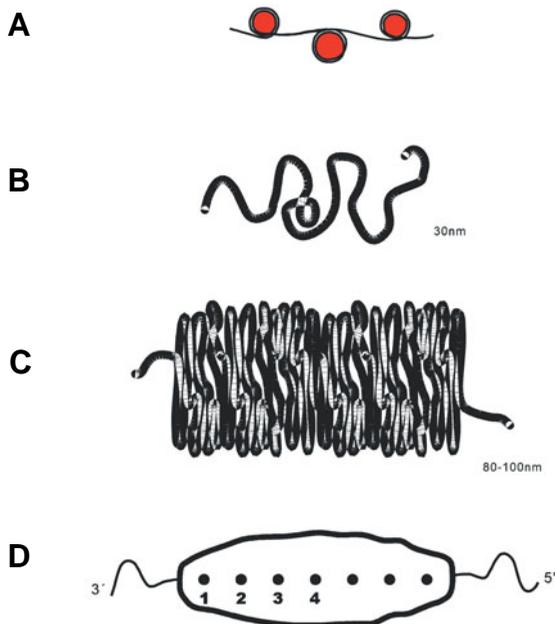


Fig. 2. Hierarchical levels of DNA packaging. (A) Nucleosomes (11 nm) arranged as beads-on-a-string. **(B)** Folded chromatin fiber of 30 nm diameter. **(C)** Condensed chromatin fiber (chromonema) of 100 nm diameter. **(D)** A *Hox* cluster schematically shown as a rod whose length is about 500 nm.

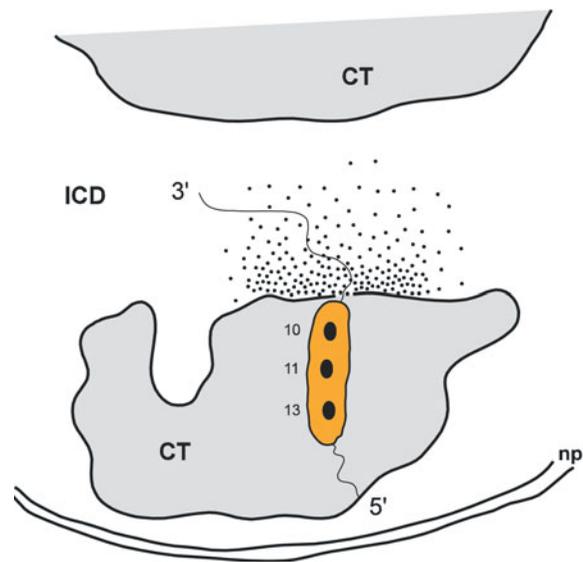


Fig. 3. Schematic drawing of a nuclear section. The single Chromosome Territory (CT) is traversed by the Interchromosome Domain (ICD) which forms a set of channels around and through the chromosome (Visser *et al.*, 2000). Transcription factors (black spots) and other regulating molecules circulate in the ICD. Before activation, the *Hox* cluster is hidden inside the Chromosome Territory. At the bottom is the envelop of the nuclear periphery (np).

sible for transcription (Cremer and Cremer, 2001; Schloscherr *et al.*, 1994). There is strong evidence that, with chromatin restructuring, gene activation occurs at the surface of the chromosome territories when genes enter in the interchromosome domain (ICD) where they can be reached by the transcription factors (Volpi *et al.*, 2000; Cheutin *et al.*, 2003; van Driel *et al.*, 2003) (Figs. 3,4). The CT forms a meandering dense structure from which decondensing chromatin fibers extend (Fig. 4) (Volpi *et al.*, 2000; Visser *et al.*, 2000). The ICD consists of a network of channels around and through CTs. These channels are connected with nuclear pores at the nuclear surface (Visser *et al.*, 2000). Transcription factors (TF) activating *Hox* genes are confined in ICD together with other mobile regulatory molecules (Cremer and Cremer, 2001). It is furthermore assumed that the density of TFs decreases away from the chromosome surface. This agrees with the observation of a concentration gradient of transcription factor Stat1 imported in the nucleus via a carrier-free (diffusion) process (Marg *et al.*, 2004).

The morphogen-signal of the macroscopic level is transduced and uniformly amplified inside the cell so that the product molecules [PM] are produced and they act on the microscopic nuclear level. The transportation of SMAD2 inside the nucleus is an example of such a transduction and amplification (Bourillot *et al.*, 2002; Shimizu and Gurdon, 1999). Furthermore, it was recently observed that Smad regulation in the nucleus is achieved through a physical interaction between the inner nuclear membrane MAN1 and R-Smads (Pan *et al.*, 2005). The case of SMAD2 is not unique. Many other molecules are imported and deposited in specific locations inside the nucleus as for instance the DSH protein which is the product of Wnt signal transduction (Itoh *et al.*, 2005). Thus, the morphogen concentration acting macroscopically is associated to a corresponding concentration of [PM]

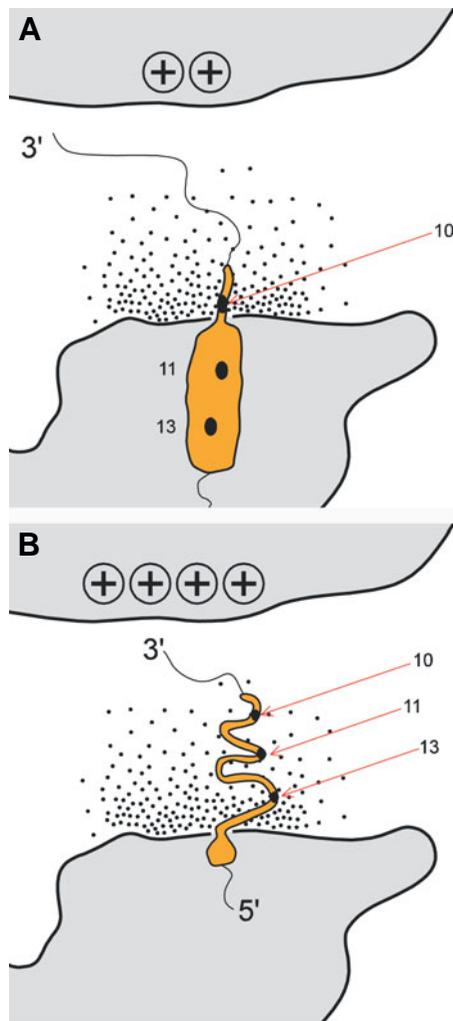


Fig. 4. Detail of Fig. 3 around the exit pore of *Hoxa* cluster. **(A)** A small electrophoretic force from the opposite Chromosome Territory surface pulls *Hoxa10* out of the Chromosome Territory. **(B)** A stronger force stretches the fiber and pulls all three *Hoxa* genes inside the Interchromosomal Domain.

endowed with suitable physicochemical properties. For example, [PM] can be polar molecules that bind on the chromosome surface and collectively create an electric field (Papageorgiou, 2001; Papageorgiou, 2004). The resulting Coulomb force (attractive or repulsive) acts then on the (negatively charged) *Hox* cluster (Fig. 4).

The case of electric repulsion pushing the cluster out of CT has been worked out and the existing data of spatial, temporal and quantitative collinearity have been well reproduced (Papageorgiou, 2001; Papageorgiou, 2004). Furthermore, the results of genetic deletions and duplications and the associated modifications of *Hoxd* expressions in the mouse limb bud (Kmita *et al.*, 2002) were also well described (Papageorgiou, 2004).

Attractive electric forces

Chambeyron and Bickmore have induced gene transcription of the *Hoxb* cluster during the differentiation of murine ES cells

(Chambeyron and Bickmore, 2004). When *Hoxb1* and *Hoxb9* are inactive they are located inside the CT. After induction with retinoic acid, the chromatin is decondensed and an extrusion of *Hoxb1* from the CT follows in association with *Hoxb1* expression while *Hoxb9* remains inside CT. Later *Hoxb9* is also shifted from inside the CT and it stays in the ICD but close to the CT surface. At the same time *Hoxb1* and the decondensed chromatin fiber performs a 'choreographed looping' in ICD with an overall movement toward the centre of the nucleus (Chambeyron and Bickmore, 2004). This behaviour of *Hoxb1* and *Hoxb9* is in agreement with the fiber translocation according to the model.

Instead of a repulsive force applied on the 5' end of the cluster as described above, the observation of Chambeyron and Bickmore could be understood as the result of an attractive force that acts on the 3' end of *Hoxb* cluster and pulls the fiber toward ICD. This situation, in principle, is similar to the experiment of Cui and Bustamante who measured chromatin extensions (decondensations) as the result of pulling forces for a wide range of variation of the stretch modulus up to 25 pN (Cui and Bustamante, 2000). It is assumed therefore that, in response to the extracellular morphogen signals, positive polar molecules [PPM] are deposited opposite the 3' end of the cluster as shown in Fig. 4. An appropriate electric field is then acting on the negatively charged *Hox* cluster. The resulting electric force pulls the chromatin fiber toward the ICD and the packaged cluster is decondensed with the chromatin tethered and stretched out of the CT. Note that the same physical principles govern electrophoresis in experiments of stretching or active transport of DNA molecules and other biopolymers (Stigter and Bustamante, 1998). Electrophoretic forces are widely used to measure the elastic properties and large-scale conformational changes of chromatin fibers (Ferree and Blanch, 2003). The deformations of tethered DNA in an electric field are similar to the stretching deformations due to hydrodynamic flow (Long *et al.*, 1996; Ferree and Blanch, 2004) and it turns out that this chromatin remodeling is necessary for DNA transcription (Katritch *et al.*, 2000).

In cells exposed to low morphogen concentrations the resulting electrophoretic forces are weak and the *Hox* cluster translocations are accordingly small (Fig. 4A). A sequential increase of the morphogen concentration will respectively produce an increasing dragging force and the *Hox* genes will be translocated into ICD following their physical order: 1, 2, 3... (Fig. 4B). It is assumed that the density of transcription factors decreases away from the CT surface and this generates quantitative collinearity (Fig. 4). According to Fig. 4A proximally only *Hoxa 10* is expressed and the intensity of expression is strong since the gene is close to the CT surface. Distally, all genes are expressed with increasing intensity (*Hoxa 10*, *Hoxa 11*, *Hoxa 13*) because of their relative position in ICD (Fig. 4B) in agreement with quantitative collinearity of Fig. 1B.

Correlation of morphogen thresholds and gene translocations

The present model can correlate morphogen thresholds with gene translocations and transcription. As an example, in Fig. 5 is shown the normal morphogen gradient M which controls the activation of the 5' genes of *Hoxa* cluster in the limb bud. The horizontal lines L and H represent the lower and higher morpho-

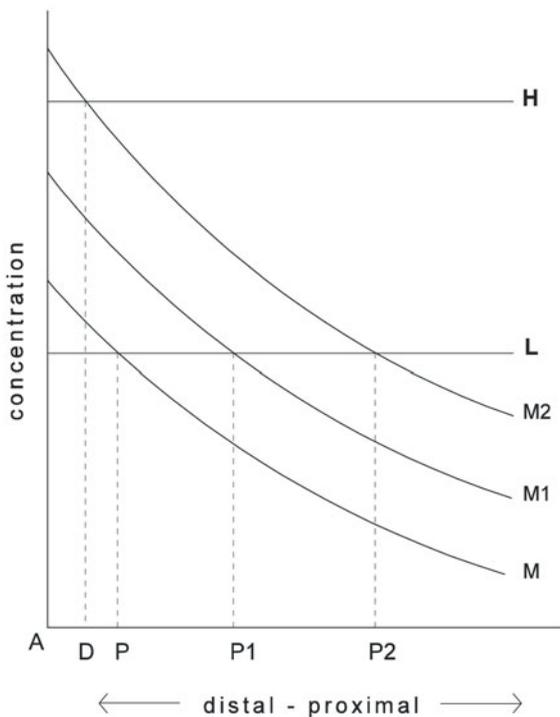


Fig. 5. Morphogens and thresholds. Normal morphogen gradient *M* with *L* (lower) and *H* (higher) thresholds for *Hoxa13* activation. Additional morphogen sources at the tip increase *M* to *M1* or *M2*.

gen thresholds respectively for *Hoxa13* activation in the distal region of the bud. For *M* the expression domain of *Hoxa13* is (AP). When an additional morphogen source is inserted at the distal tip, the gradient increases to *M1* and the *Hoxa13* activation domain expands to (AP1). The region (PP1) represents the domain of ectopic gene expression. If an even stronger morphogen source is inserted, the gradient increases further to *M2* exceeding eventually the higher threshold *H* at the tip. In this case the ectopic expression reaches the proximal boundary *P2* but in the region (AD), where the morphogen concentration is higher than *H*, the gene expression disappears.

It is instructive to interpret the above gene response to morphogen thresholds in terms of the geometry and dynamics of cluster translocations. For simplicity *Hoxa13* is isolated from the cluster and it is depicted in Fig. 6 for the different stages of gene activation. When *Hoxa13* is hidden in the interior of CT (Fig. 6A) the gene is inactive and this case corresponds to proximal cells where the morphogen level is below *L* in Fig. 5. When the morphogen concentration increases, the associated electrophoretic force pulls the gene just above the chromosome surface (Fig. 6B). For the normal gradient *M* this occurs in proximal cells of the domain (AP) in Fig. 5. When the morphogen concentration increases further, the gene is accordingly pulled stronger and it is shifted toward the border of the transcription factor region (Fig. 6C). This corresponds to the distal boundary *D* which is reached for the gradient *M2* of Fig. 5. The morphogen level there is just below *H*. Finally, for *M2* the domain (AD) has morphogen concentrations higher than *H*, therefore the associated gene translocations are beyond the border of the transcription factor region and gene expression disappears (Fig. 6D). Such an expression

attenuation has been observed for *Hoxa13* in the chick limb when a FGF4 bead was inserted at the distal tip of the bud (Vargesson *et al.*, 2001). However, in this case no proximal ectopic expression was observed (PP2 in Fig. 5), as would be expected according to the model. A possible explanation of this failure is that, at st21, proximal cells in the bud are not yet competent to respond to signals for *Hoxa13* expression. An experiment in progress, when completed, will test the validity of this explanation (Papageorgiou, 2004).

Conclusions and predictions

The present model does not merely describe the collinearity data but its aim is to explain the origin of collinearity in terms of fundamental and well documented processes. Specific experiments have to determine the particular features of the mechanisms involved. In this spirit, different mechanisms cooperate at several levels: a) At the multicellular level and depending on the case, the study of modified diffusion with or without endocytosis has clarified how morphogen gradients can be established (Green, 2002; Scholpp and Brand, 2004; Belenkaya *et al.*, 2004; Smith and Gurdon, 2004). b) Signal transduction has been determined with accuracy leading to the production and transportation of particular molecules in specific locations inside the nucleus (Shimizu and Gurdon, 1999; Bourillot *et al.*, 2002; Pan *et al.*, 2005; Itoh *et al.*, 2005). c) At the subcellular level, physical forces are produced causing translocations of the *Hox* clusters in the nucleus and as a plausible candidate an electric force (attractive or repulsive) was analyzed in more detail (Papageorgiou, 2001; Papageorgiou, 2004). The subsequent results of gene transcription in murine ES cells not only are compatible with this hypothesis but, at the same time, they specify some features of the attractive force involved (Chambeyron and Bickmore, 2004). Furthermore, some recent experiments *in vivo* at different stages of embryonic mouse development (Chambeyron *et al.*, 2005) confirm the above findings and provide further support to the present model. It is still possible that, in addition to the attraction, a repulsive force may also act on the posterior end of the cluster (Papageorgiou, 2001; Papageorgiou, 2004). However, the origin of the force remains still undetermined and a direct experiment is crucial for its specification.

To the above end, a change of the electric charge of the cluster and the consequent modification of the expression pattern would be an undisputable test of the electric hypothesis. The combination of genomic rearrangement techniques (Herault *et al.*, 1998) with the insertion methods of charged oligonucleotides in the genome (Pitard *et al.*, 2004) could eventually serve this purpose. Accordingly, if negative charges are inserted at the 3' end of *Hoxb* cluster (increasing the total negative charge of the cluster) the pulling electrophoretic force will increase and the expressions of *Hoxb* genes will be shifted anteriorly (to the right in Fig. 1B). On the contrary, if positive charges are inserted the expressions will be shifted to the left toward the posterior end of the embryo axis.

In the present framework collinearity is the outcome of cooperation of physical principles and biochemical mechanisms. This formulation allows a unification of *posterior prevalence* of expressions along the embryo axis and *quantitative collinearity* of 5' gene expressions in the limb since, in both cases, *Hox* gene transcription obeys the same rules. In Fig. 5 the distance between

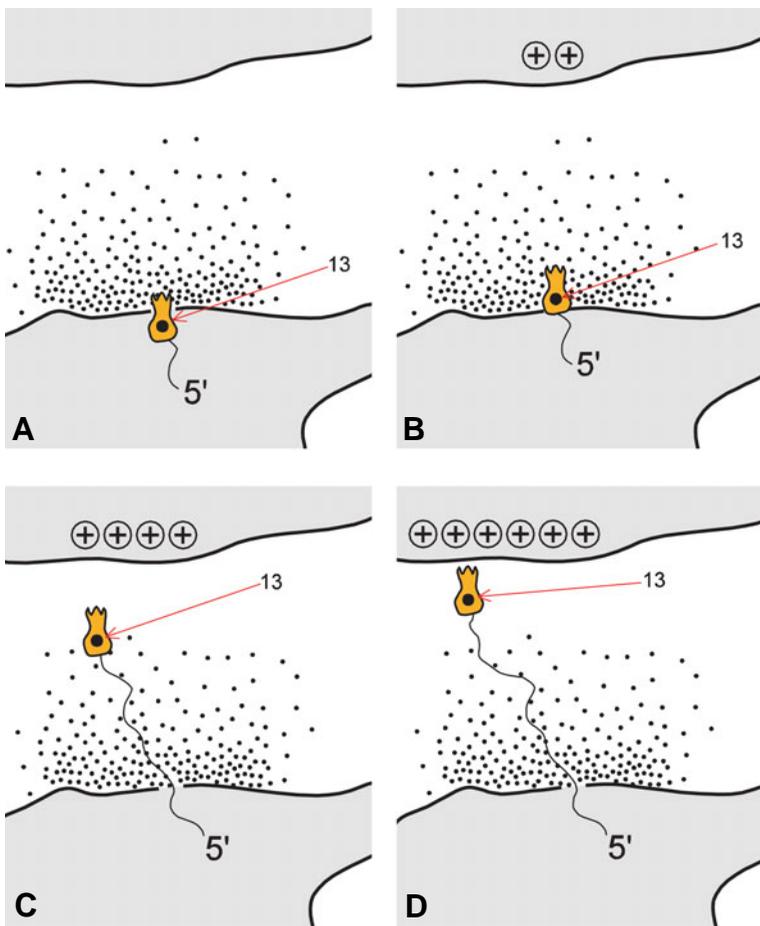


Fig. 6. Locations of *Hoxa13*. (A) Inside the chromosome territory; (B) just over the surface of the chromosome territory; (C) at the outer border of a Transcription Factor area and (D) beyond the Transcription Factor area.

L and H determines a 'window between thresholds' for *Hoxa13* gene expression. This is associated to the fiber stretching inside the TF area (Fig. 4) and corresponds to a 'window of gene expression' contained between the spatial boundaries (proximal and distal). In this respect, the difference between partially overlapping and nested expression domains is only accidental: the 3' genes in the primary embryo axis cannot be accommodated in the TF area and for every cell only a subset of these gene expressions fits in. In the limb bud, when extruded in the ICD, the 5' genes can be contained in the TF area and they form a nested pattern. In both cases the posterior gene expression dominates compared to the effect of the overlapping more anterior gene expressions. Furthermore, an FGF4 bead inserted distally in the bud will increase the morphogen level above H at the tip area (Fig. 5) and accordingly *Hoxa13* gene will be shifted beyond the window of gene expressions (Fig. 6). The verification of the gradual *Hoxa13* attenuation distally (Vargesson, 2001) indicates that, as in the primary axis, the nested expression pattern can become partially overlapping as a result of appropriate gene translocations.

If the electric hypothesis proves correct, many specific questions could be asked: how reversible is the decondensed *Hox* fiber toward the initial condensed state inside the CT when the gene activation is turned off? In this spirit, a regression of *Hoxb1* was

noticed from the ICD toward the CT at later stages of activation (Chambeyron and Bickmore, 2004).

There are some experiments where the collinearity picture is violated. For instance when the anterior *Hoxb1* is ectopically relocated at the posterior end of the *HoxD* cluster near *Hoxd13* the following expressions were observed (Kmita *et al.*, 2000): normally *Hoxb1* is expressed in the fourth rhombomere of the developing nervous system. The expression of the relocated *Hoxb1* transgene is totally abolished in agreement with the present model since the posterior *Hoxd* genes are not expressed in this rhombomere. However the transgene expression in the mesoderm was unexpectedly not suppressed. This probably indicates that the normal collinearity behaviour depends on the developing stage, the tissue and the transient structure of the chromatin architecture. Thus, it seems that in the early mesoderm the chromatin is not dense enough and the factors of *Hoxb1* activation can recognize the ectopic transgene inducing a local opening in the neighborhood of *Hoxd13* (Kmita *et al.*, 2000). In the same article the authors describe some specific mechanisms to this end (Kmita *et al.*, 2000).

In *Drosophila* the developmental fate of the domains along the anterior-posterior axis is specified by the repression of the homeotic gene expressions (Busturia and Bienz, 1993). Apart from the obvious differences from the present formulation, the ABD-B protein spreads across several parasegments in the posterior- anterior direction in a manner 'vaguely reminiscent' of the spreading of the posterior *Hox* genes (Abd-B-like genes) in the developing vertebrate limb (Dollé *et al.*, 1989; Busturia and Bienz, 1993).

Physical forces are involved in many developmental processes (Gordon, 1999). In the case of *Hox* gene collinearity I think it is impossible to explain the observed regularities without using a physical principle, for the following reason: as exposed above, the chemical (molecular) cue of positional information is transformed into a chemical signal at the chromatin level. One biochemical response to this signaling is the high increase in histone H3 acetylation and methylation (Chambeyron and Bickmore, 2004). However, these histone modifications are not sufficient to produce the *HoxB* cluster decondensation which is the necessary first step for gene activation (Chambeyron and Bickmore, 2004). Only physical forces can cause such mechanical modifications of large molecular complexes (e.g. decondensation, stretching, translocation, shearing etc) and the observed collinearity reflects the correlation between the measure of the applied force and the degree of chromatin (cluster) deformation. The explanation of collinearity therefore should be based on appropriate physical mechanisms generating suitable forces acting on the nuclear macromolecules. Gene enhancers, inhibitors, *cis*-regulators and the other tools of the biochemical machinery, although crucial, cannot explain only by themselves all aspects of collinearity (Papageorgiou, 2004). This inability and the accumulation of new complicated data led to the assumption that no single and universal mechanistic explanation may exist for these correlated phenomena (Kmita and Duboule, 2003). Instead, tinkering without underlying logic other than the attainment of the final goal might be responsible for the observed

characteristic gene expressions. In contrast, according to the present formulation, collinearity is unique and universal since it is the result of physical laws. However, in every patterning pathway along axes, the backbone of collinearity is covered by the features of the particular geometry (macroscopic and microscopic) combined with the molecular specificity of the by-side genetic activation. For example, collinearity in the primary vertebrate axis generates partially overlapping expressions while in the limb bud the expressions form a nested pattern (Fig. 1B). In other cases, the chromosome environment of the cluster does not fulfill the prerequisites for the proper appearance of *Hox* gene collinearity (Kmita *et al.*, 2000). Whenever the universal phenomenon of collinearity is at work, the phenotypes in each case are modulated by geometry and the concurrent molecular and genetic processes. Future experiments will clarify whether tinkering or the 'dressed' core of collinearity is responsible for the observed variety of axial developmental events.

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