A biophysical model can explain the multiscale phenomenon of collinearity of clustered HOX genes

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By

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Lectures on ‘Embryogenesis explained’

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History

Bateson (1894). Systematically studied homeotic mutations (***homeosis***).

E.B. Lewis discovered **collinearity** in *Drosophila* bithorax complex (1978)
(Nobel prize 1995).

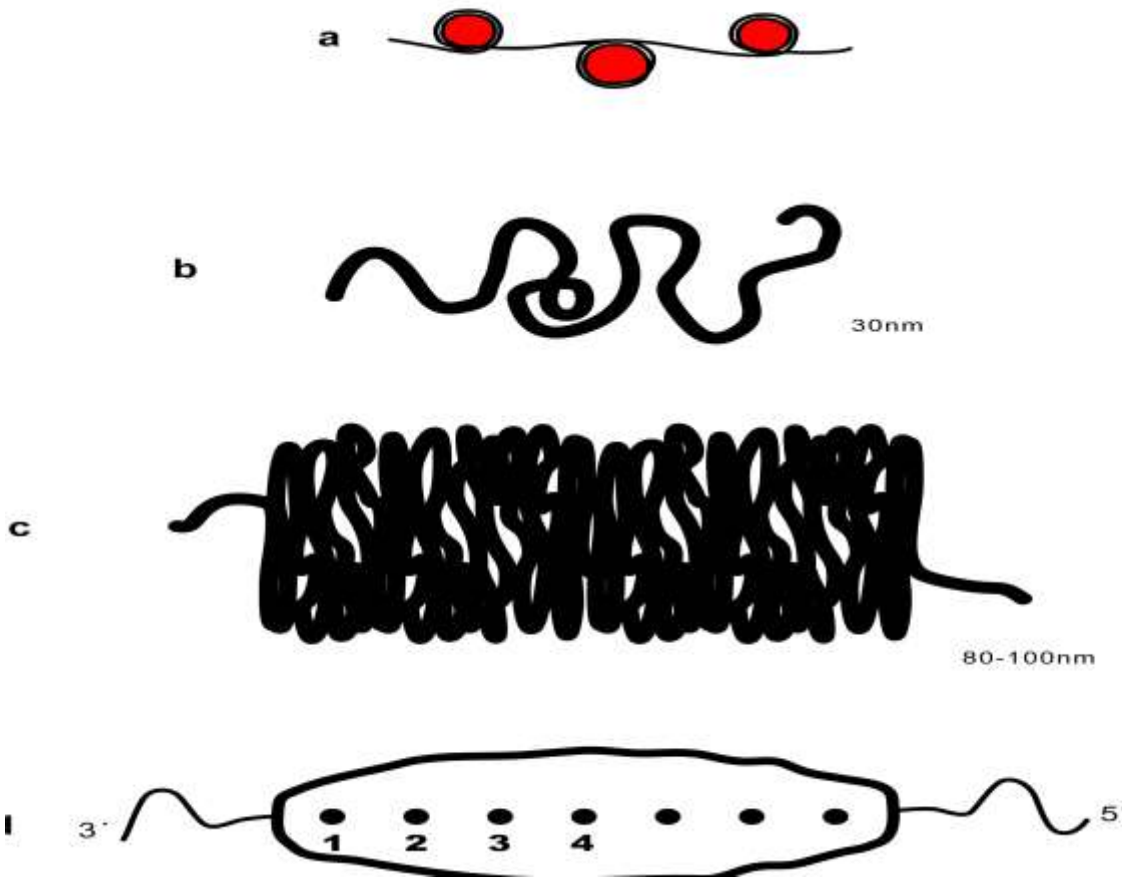
Universality of HOX genes in animal and plants.

Homeiosis: Development of an organ in the place of some other organ (*Antennapedia*)

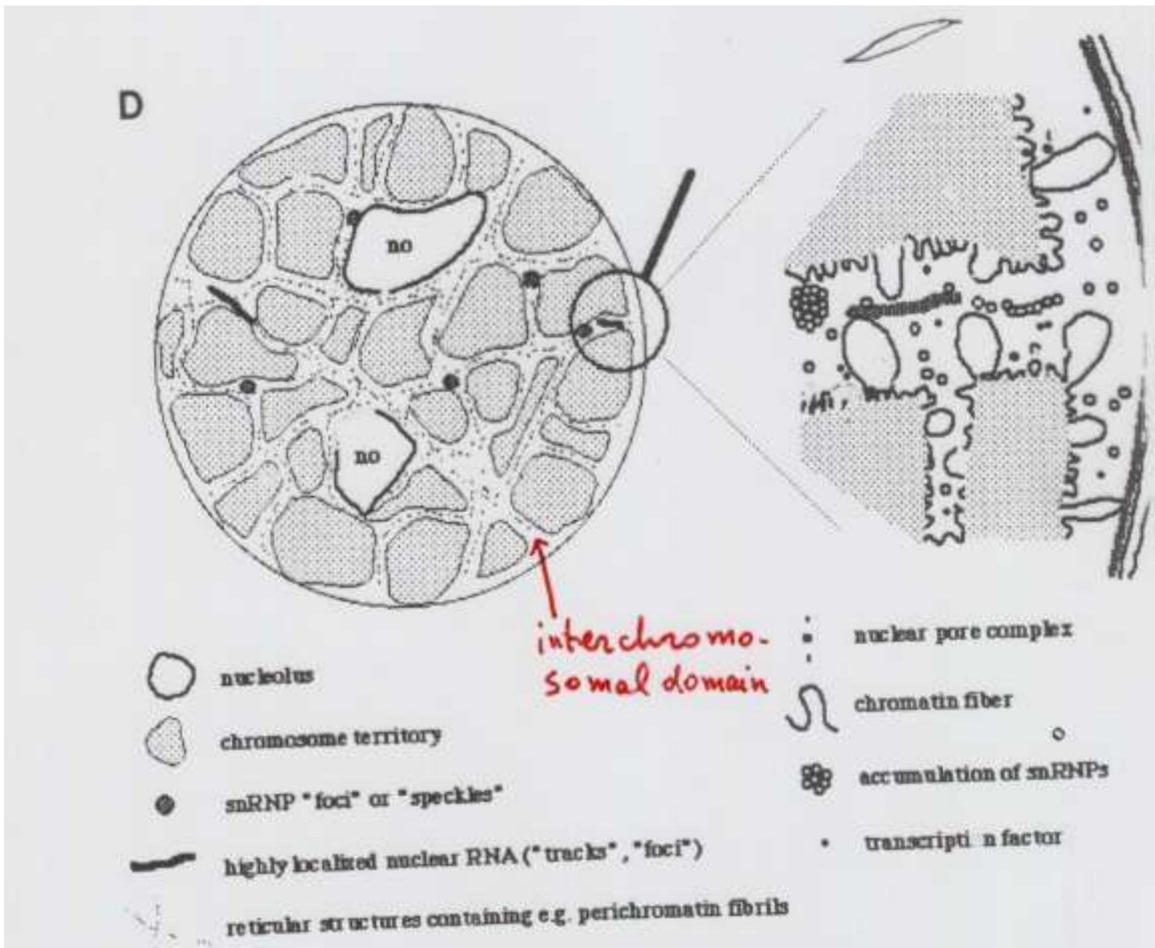
Homeotic box- 180bp

- After the molecular revolution (cloning, sequencing etc) the homeotic genes were completely determined.
- Some homeotic genes are clustered- HOX genes
- 1 cluster in *Drosophila*
- 4 homologue clusters in vertebrates.

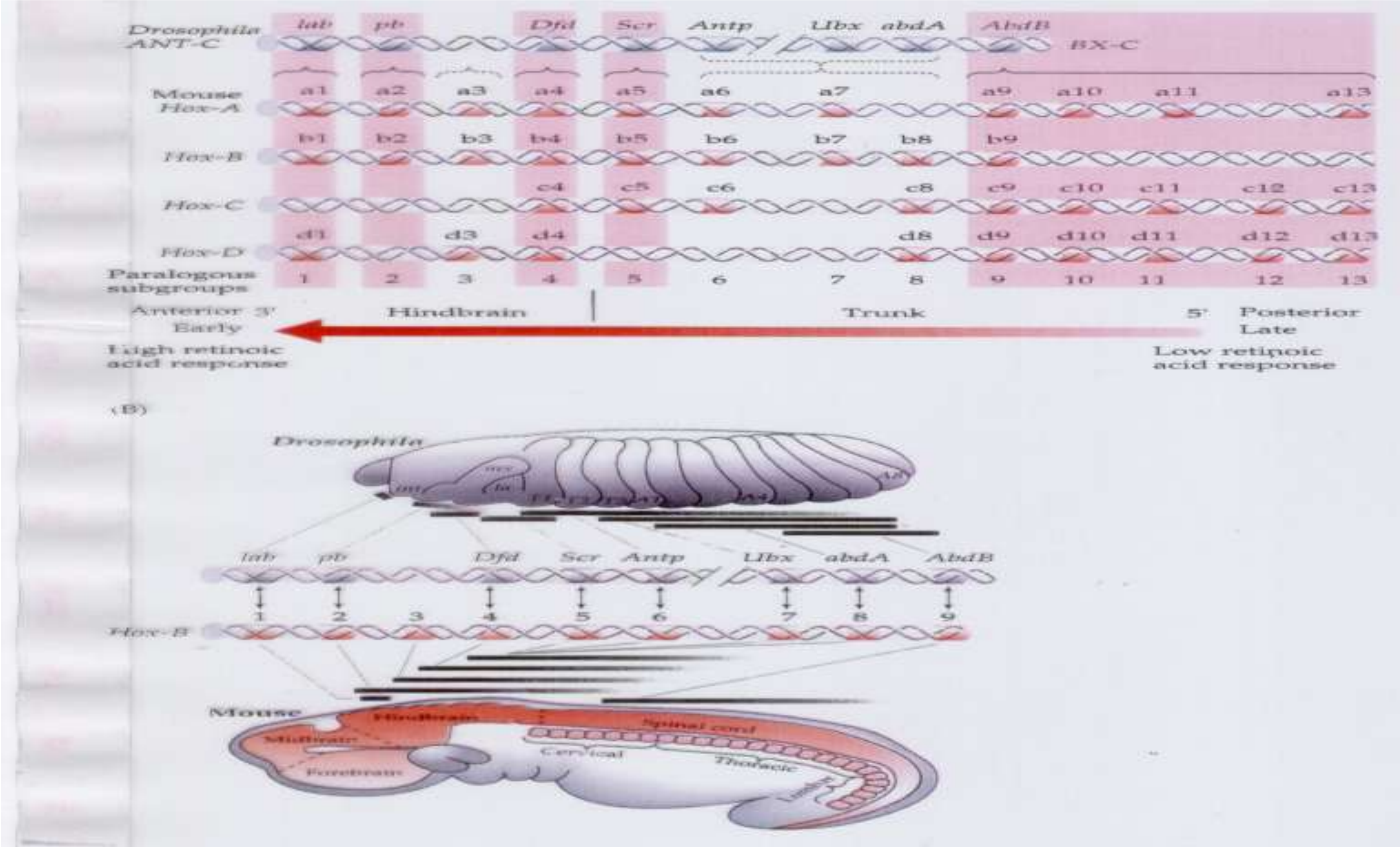
HOX gene clustering



Chromatin territory (CT)
Interchromosome domain (ICD)



1 Hox cluster in Drosophila
4 Hox clusters in Vertebrates



Collinearity features

- Spatial collinearity
- Temporal collinearity
- Quantitative collinearity

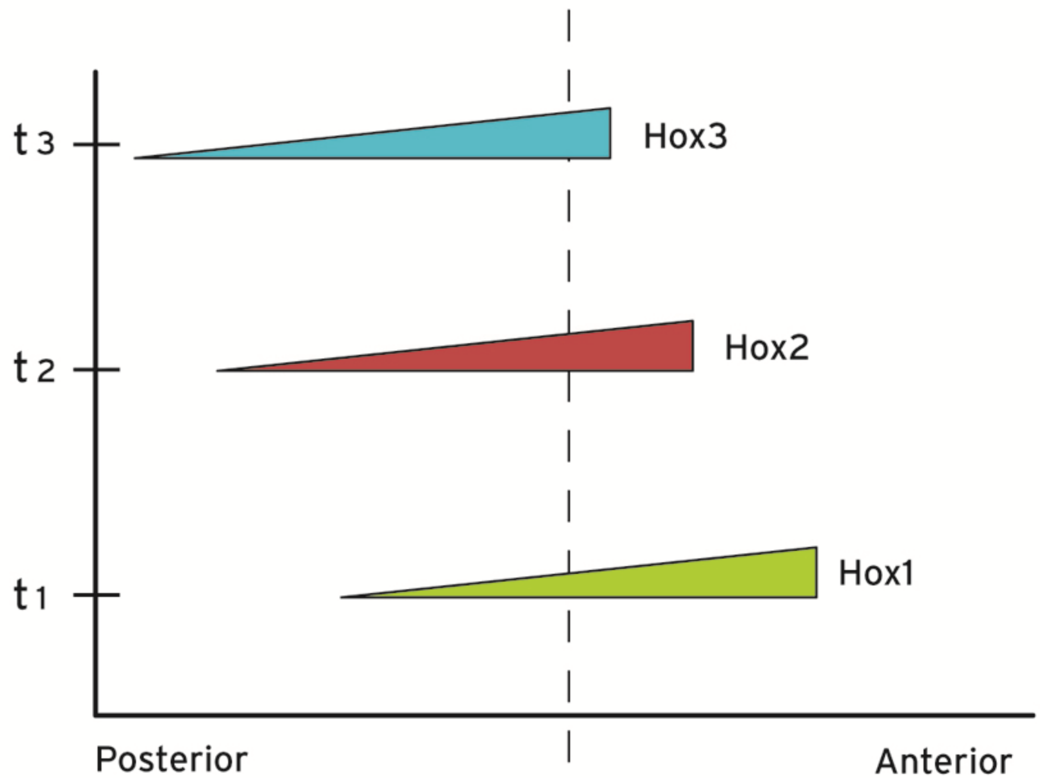


Figure 1

Schematic representation of HOX collinearity

Two-wave model (Duboule *et al*.)

First wave for the early bud

Second wave for the late bud

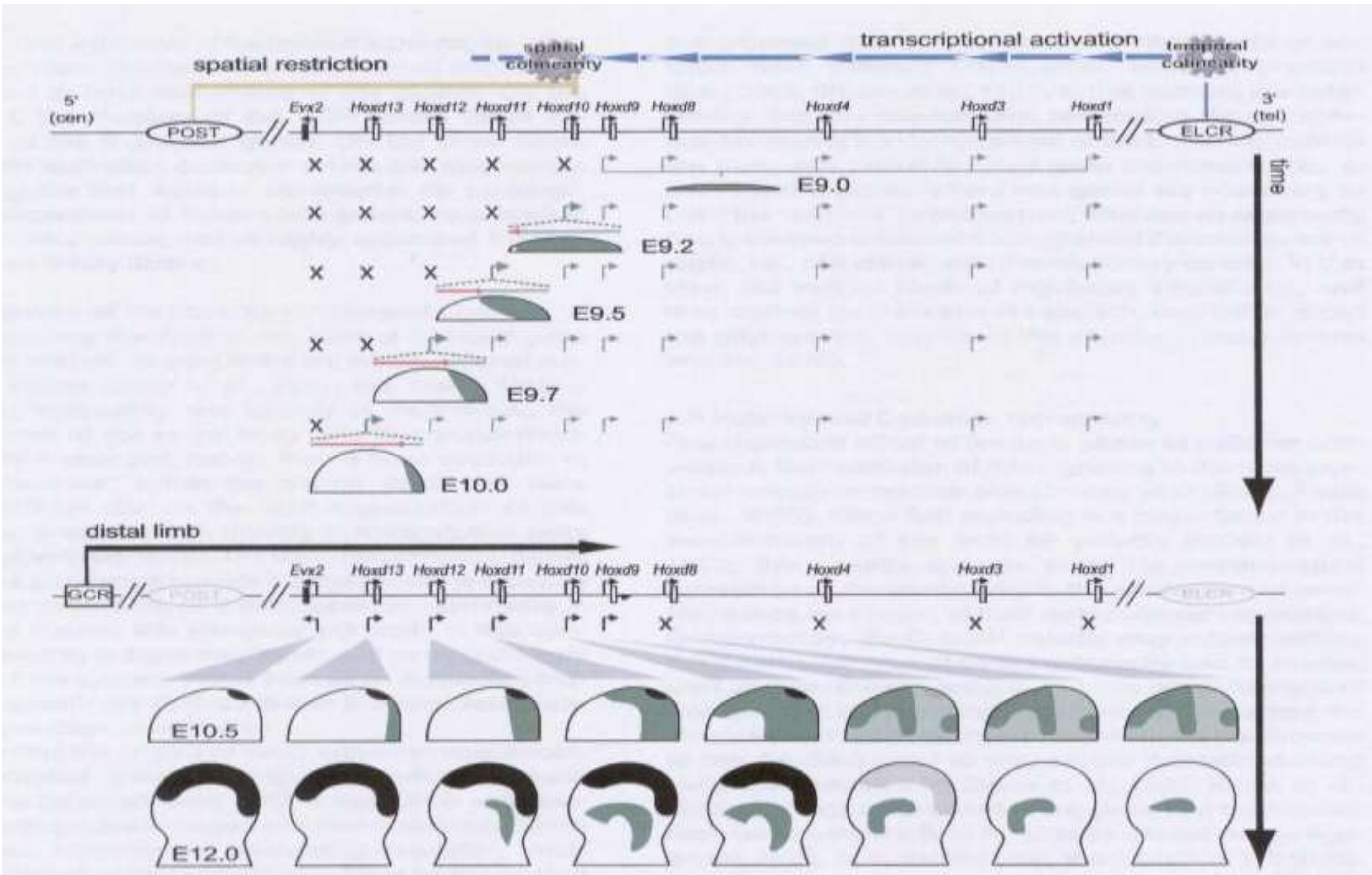
Early bud (Two regulatory mechanisms)

1.One telomeric (3’) activating mechanism (ELCR) Early Limb Control Regulation responsible for temporal collinearity.

2. An inhibitory regulation located posteriorily (5’) (POST) responsible for spatial collinearity.

Two-wave Model (TW M)

P Tschopp *et al. PLoS Genet. 5* (3), (2009)



<u>Biophysical Model (B M)</u>

S. Papageorgiou

Dev. Growth & Different. **53,** 1 (2011)

- **<u>Physical forces pull sequentially the HOX cluster from inside the chromatin territory (CT) toward the interchromosome domain (ICD)</u>**
- <u>A possible realization</u>: Coulomb forces.
- The cluster is negatively charged (N).
- Positive polar molecules (P) are deposited opposite the cluster. Force **<u>F=P*N</u>**

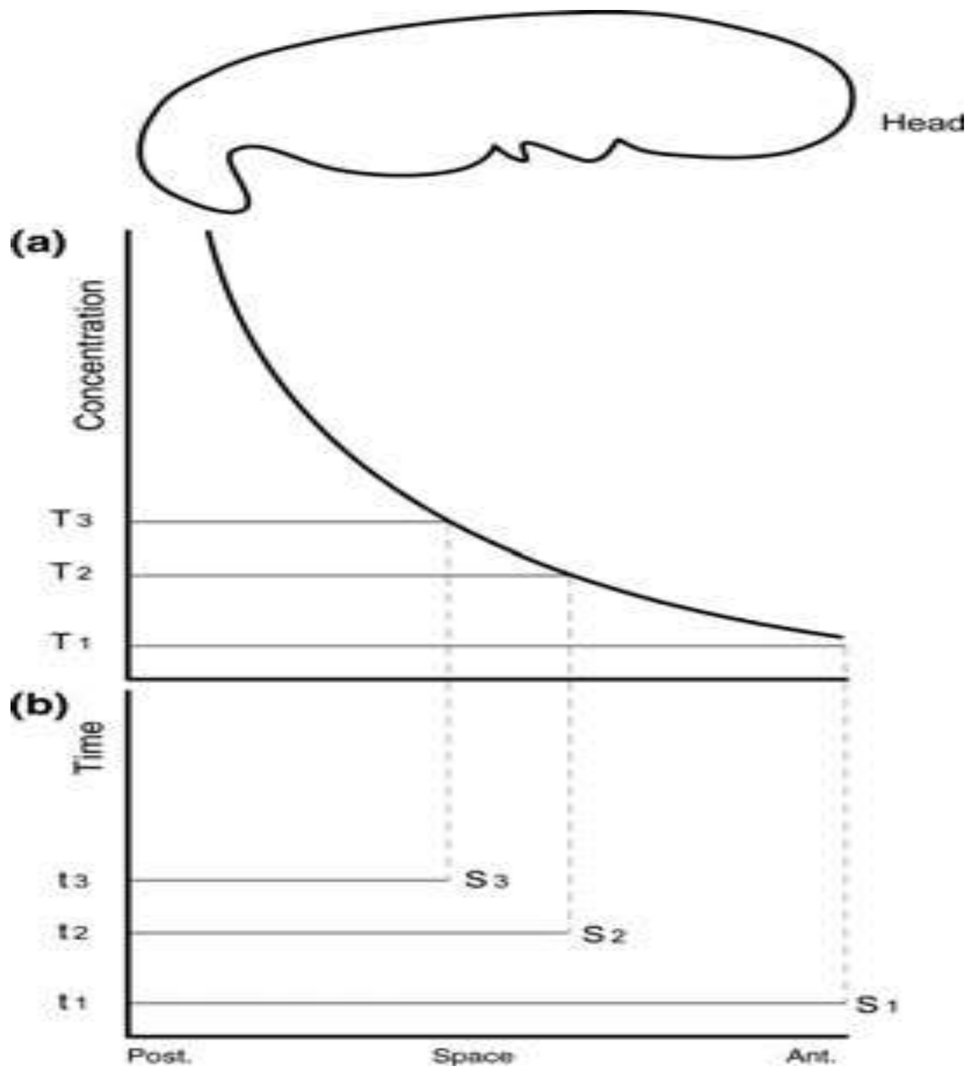
Evidence for apposition of P molecules

- Signals from a morphogen gradient are transduced and amplified inside the cell

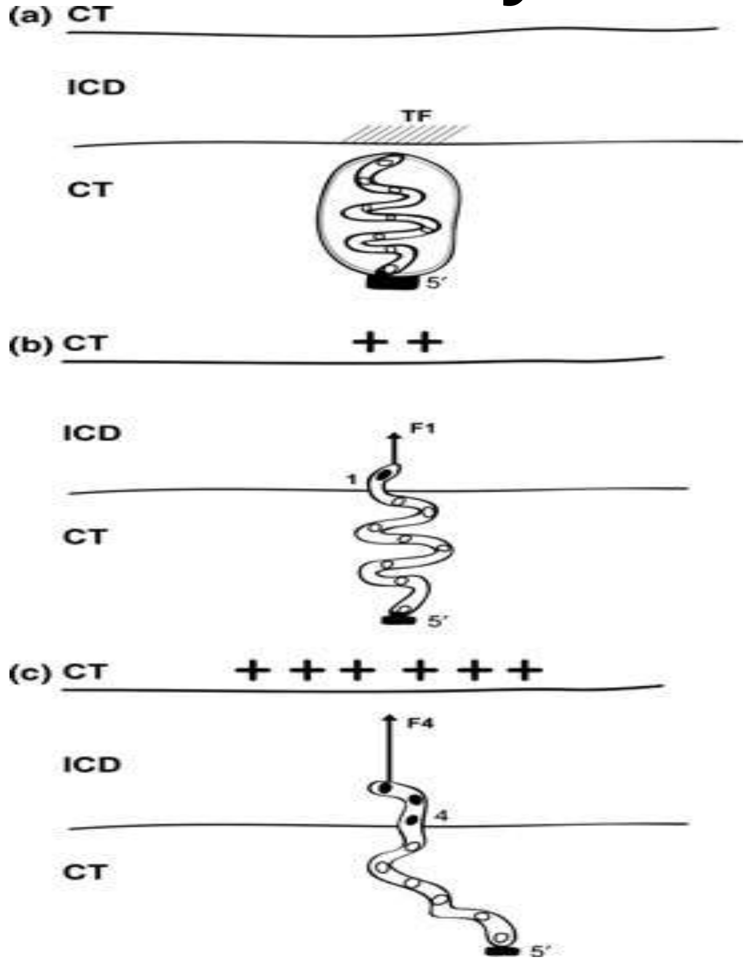
- The produced molecules are transported and deposited inside the nucleus

- Examples:1. SMAD2 (Gurdon *et al*. 2002)
- 2. DSH from Wnt transduction (Itoh 2005)

Morphogen gradients: FGF, SHH Concentr. threshold- time intervals

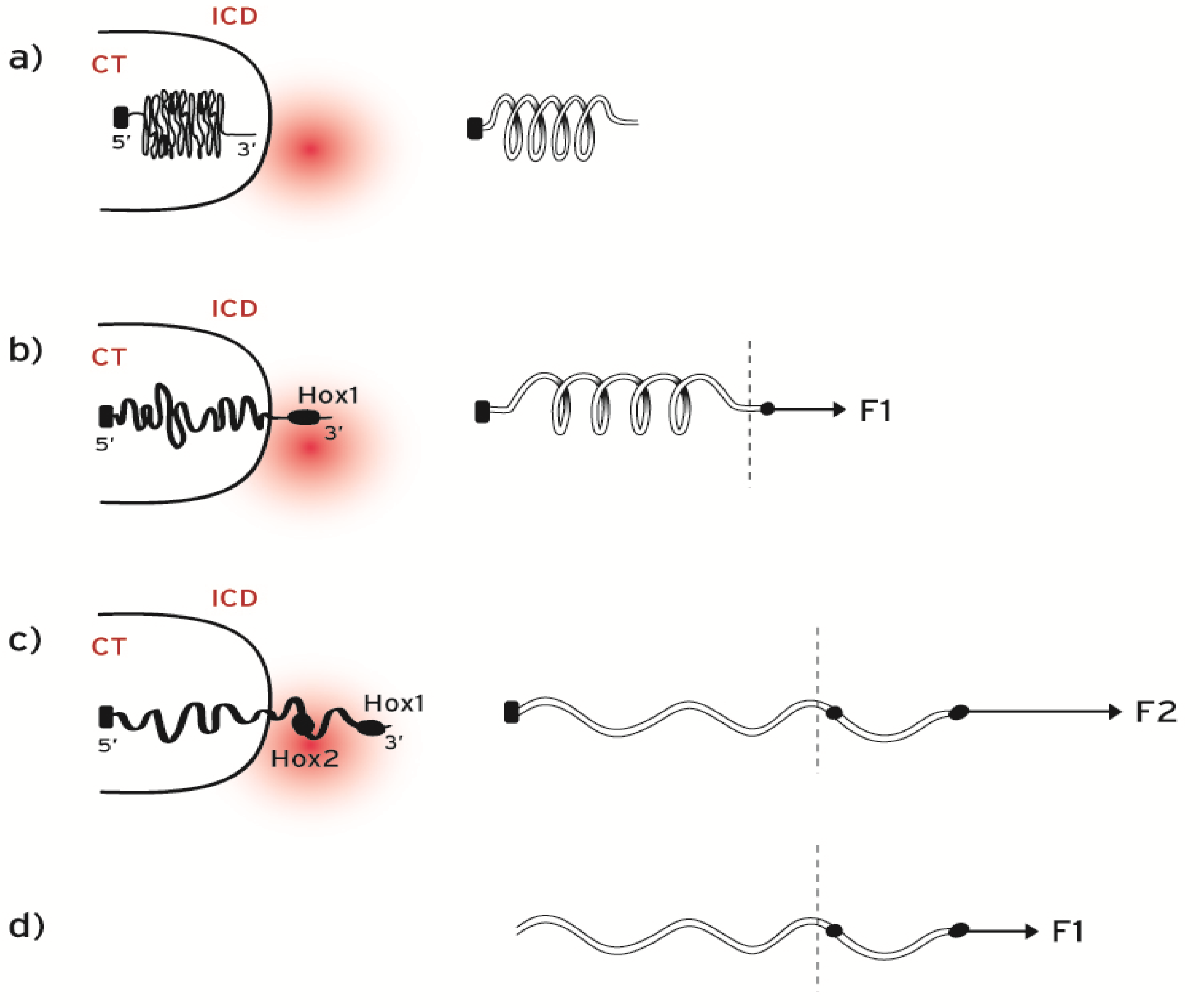


Inactive cluster inside CT. Genes under **F** move sequentially in the transcription factory domain (TF)

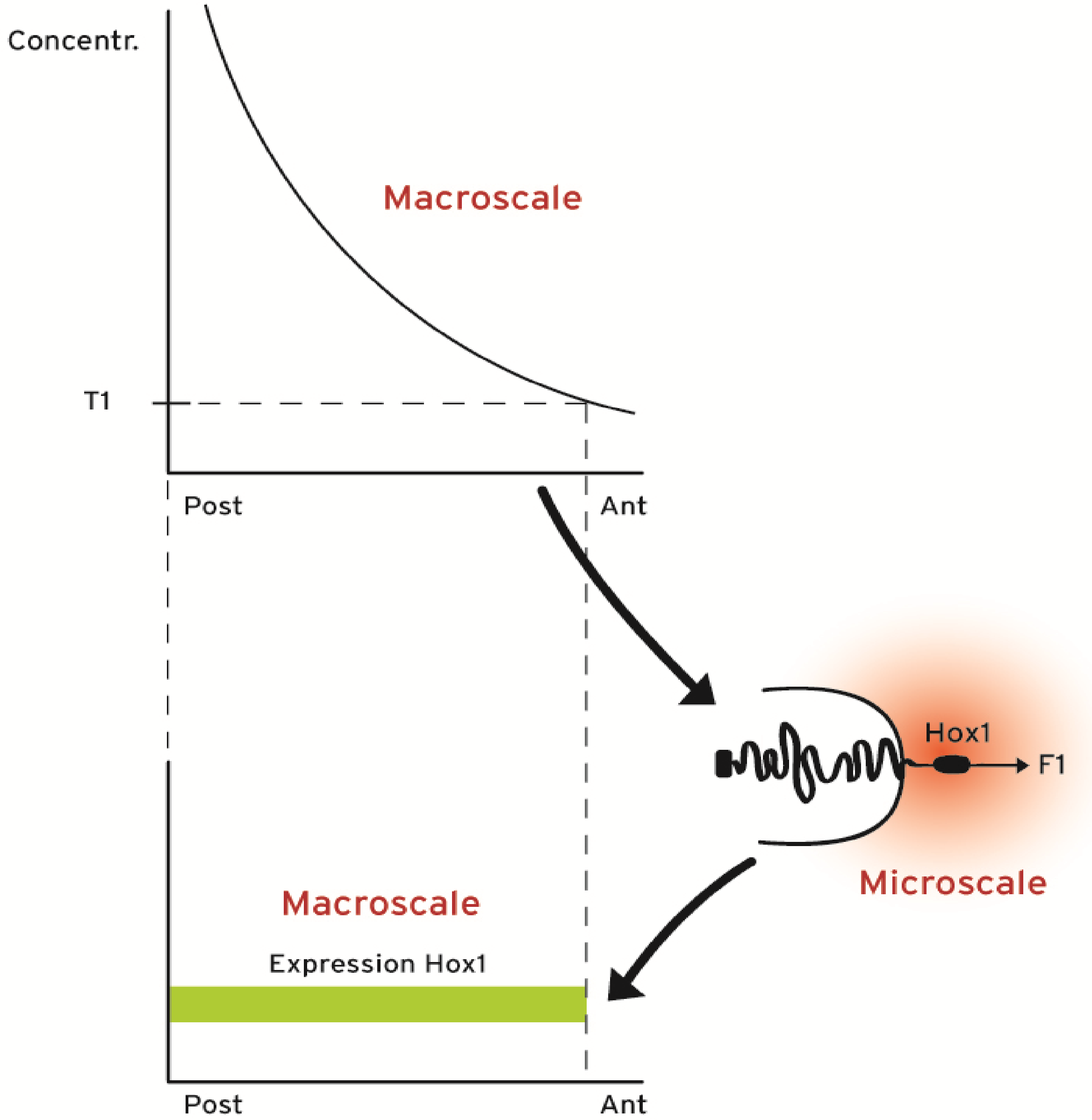


Mechanical analogue

- HOX cluster behave like an elastic spring fixed at the 5’ end and loose at the 3’ end.
- Spring expansion is proportional to force.
- Force is proportional to negative charge N.
- Force is proportional to positive charge P.
- Negative charges are almost evenly distributed in the cluster.



Mechanical analogue



Macroscale ⟶ Microscale ⟶ Macroscale

DNA moves
NOT the RNApolymerase

Traditional view: RNA polymerase diffuses in nucleoplasm to bind to DNA.

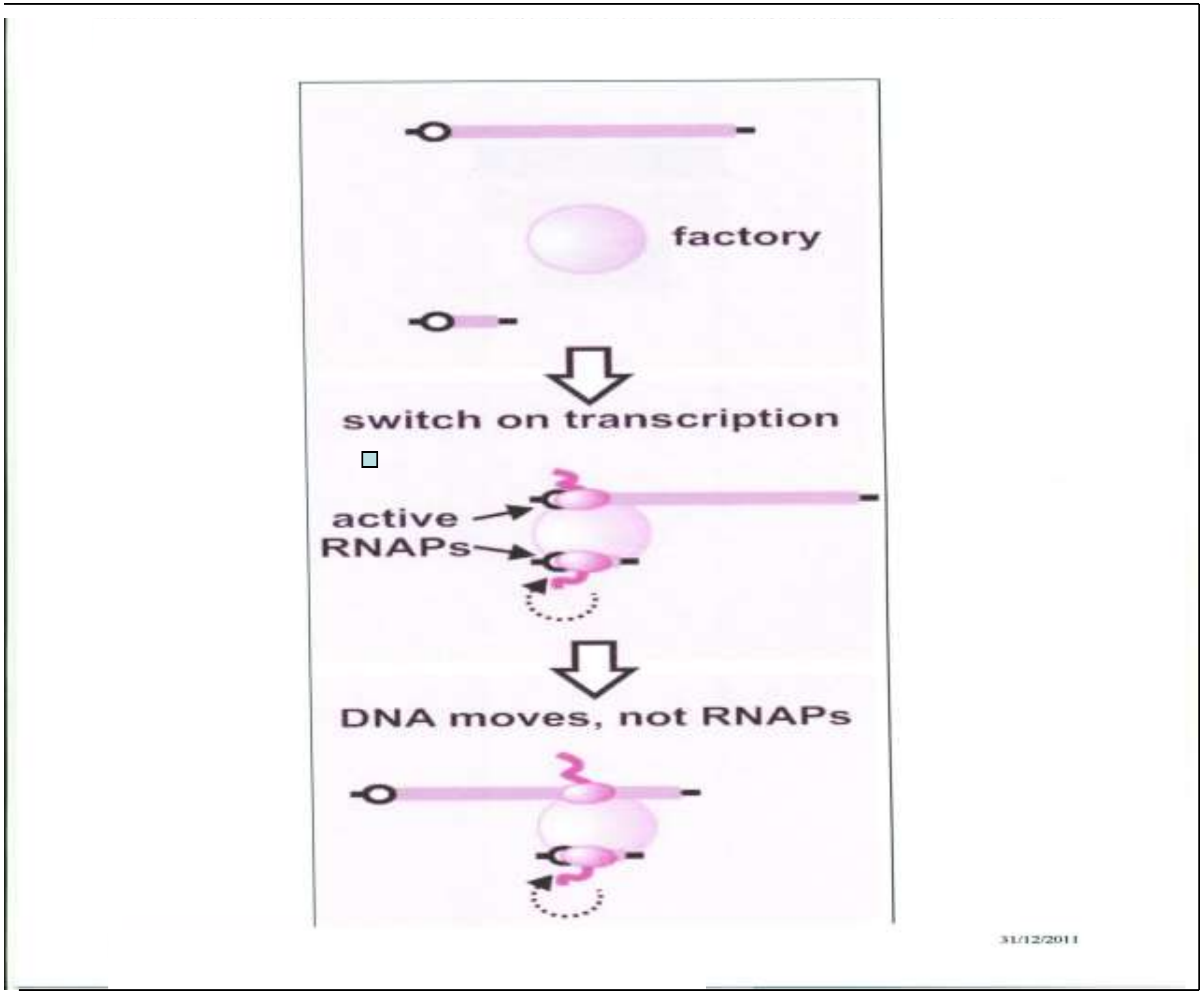
P.R. Cook *et al*. (Oxford) observe

that Polymarases are immobilized in Transcription Factories (TF).

DNA moves toward polymerase and

transciption starts.

DNA movement

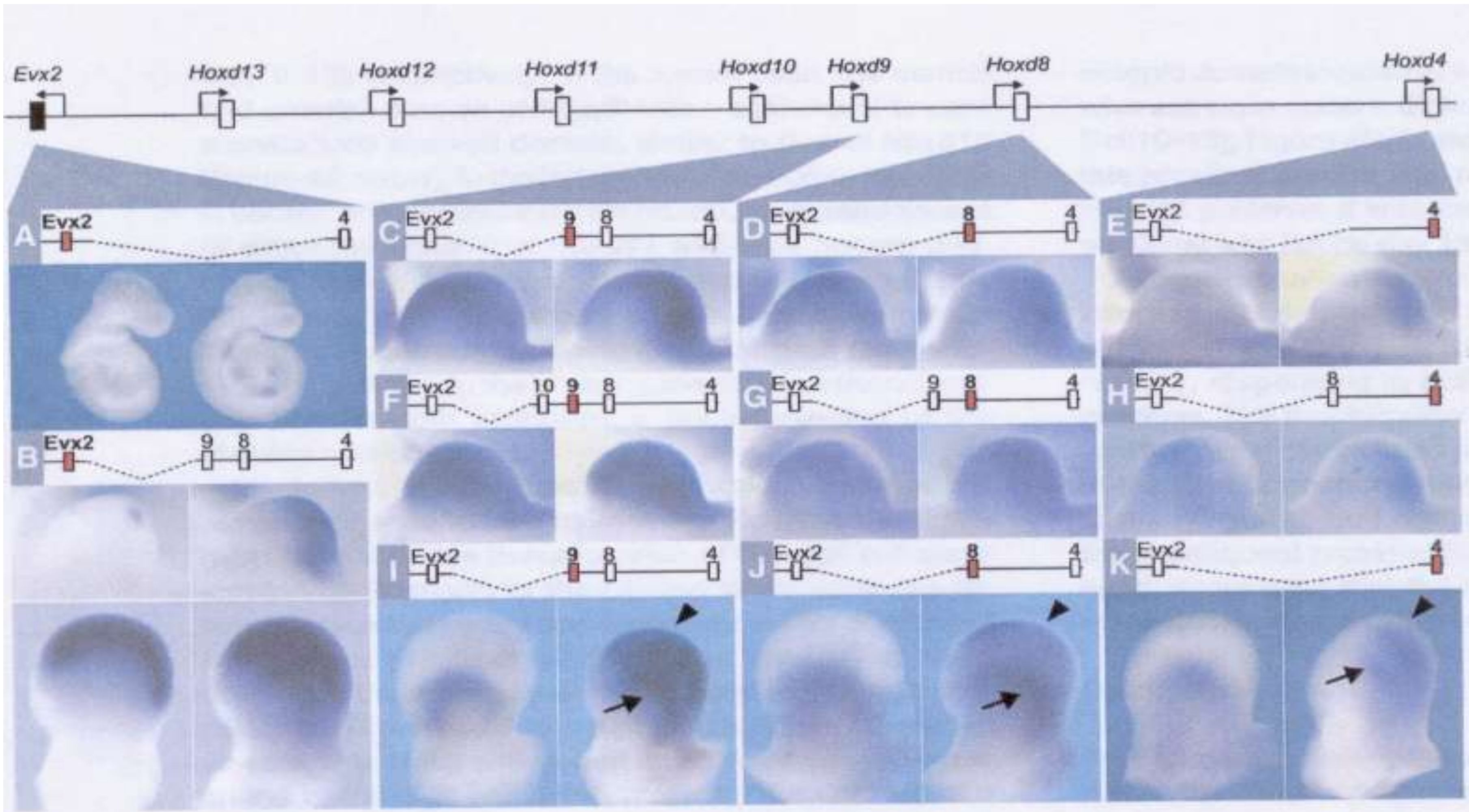


Genetic engineering experiments (Duboule's Lab)

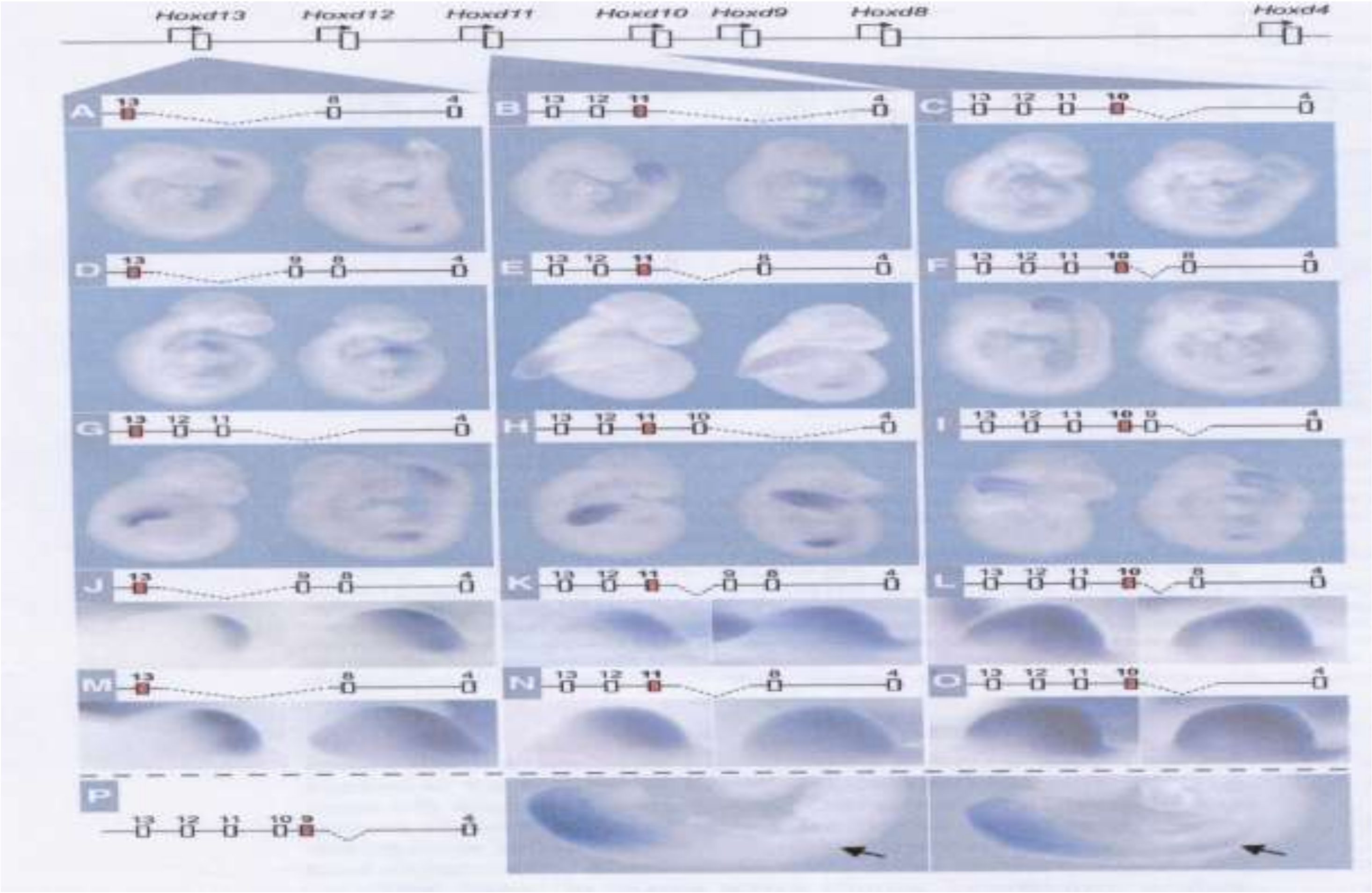
Transgenic mice with modified Hoxd cluster (TAMERE- targeted meitoic recombination)

- Hox gene deletions
- Hox gene duplications
- Hox gene transpositions Hoxb1→Hoxd
- Centromeric inversions

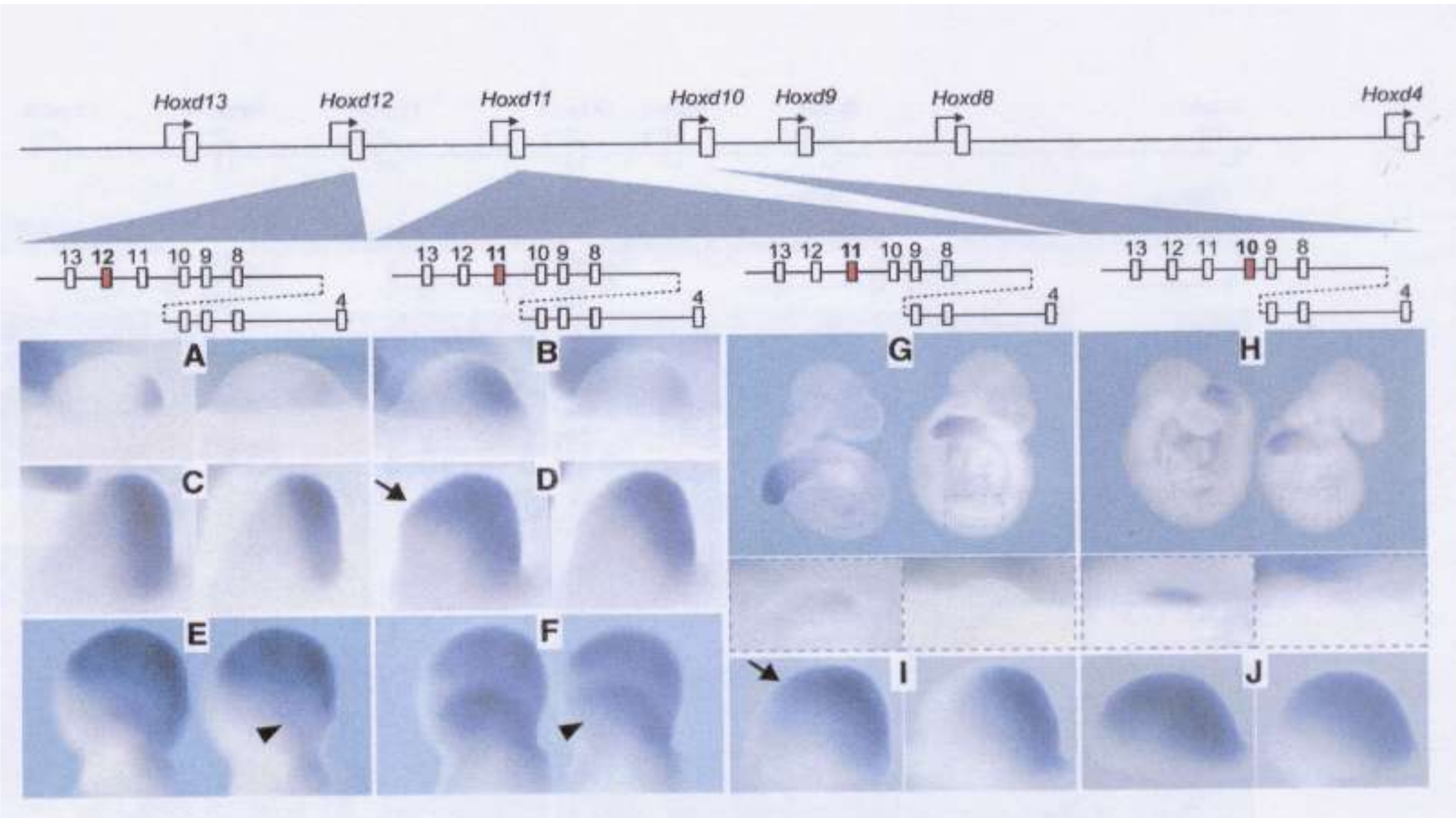
Posterior Hoxd deletions (C,F) Posteriorization



Anterior Hoxd deletions (M,N) Anteriorization- Premature expression

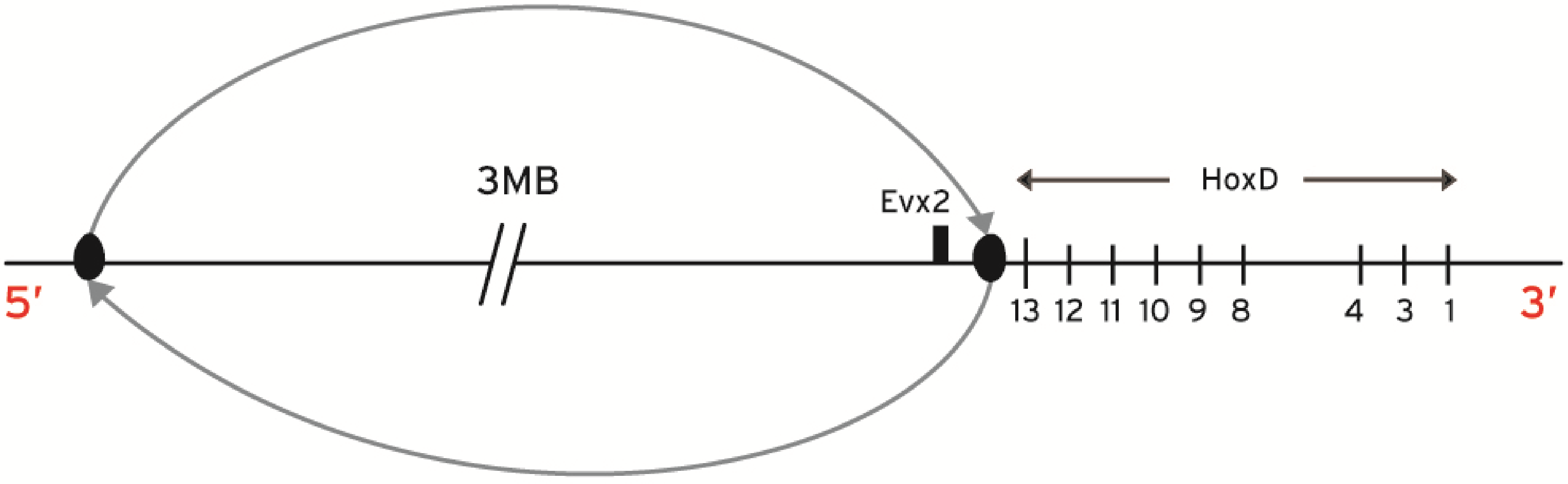


Anterior Hoxd duplications (C,D);(I,J)



Gene transpositions (Bickmore-Duboule *et al*. (2008) *J. Cell Sci.*

- *Hoxb1* is transposed at the 5’ end of
- *Hoxd* cluster.
- At st.9.5 of the *wt* limb bud, the cluster is decondensed but not extruded in ICD.
- In the *Hoxb1-LacZ* transgenic embryo the *Hoxd* region loops out in ICD- in agreement with the biophysical model
- (N increases $\rightarrow$ F increases).



Centromeric inversion

Large region (3 Mb) posterior to Hoxd13 is inverted.

Inversion includes Evx2.

- Premature Hoxd13 activation
- Anteriorization-

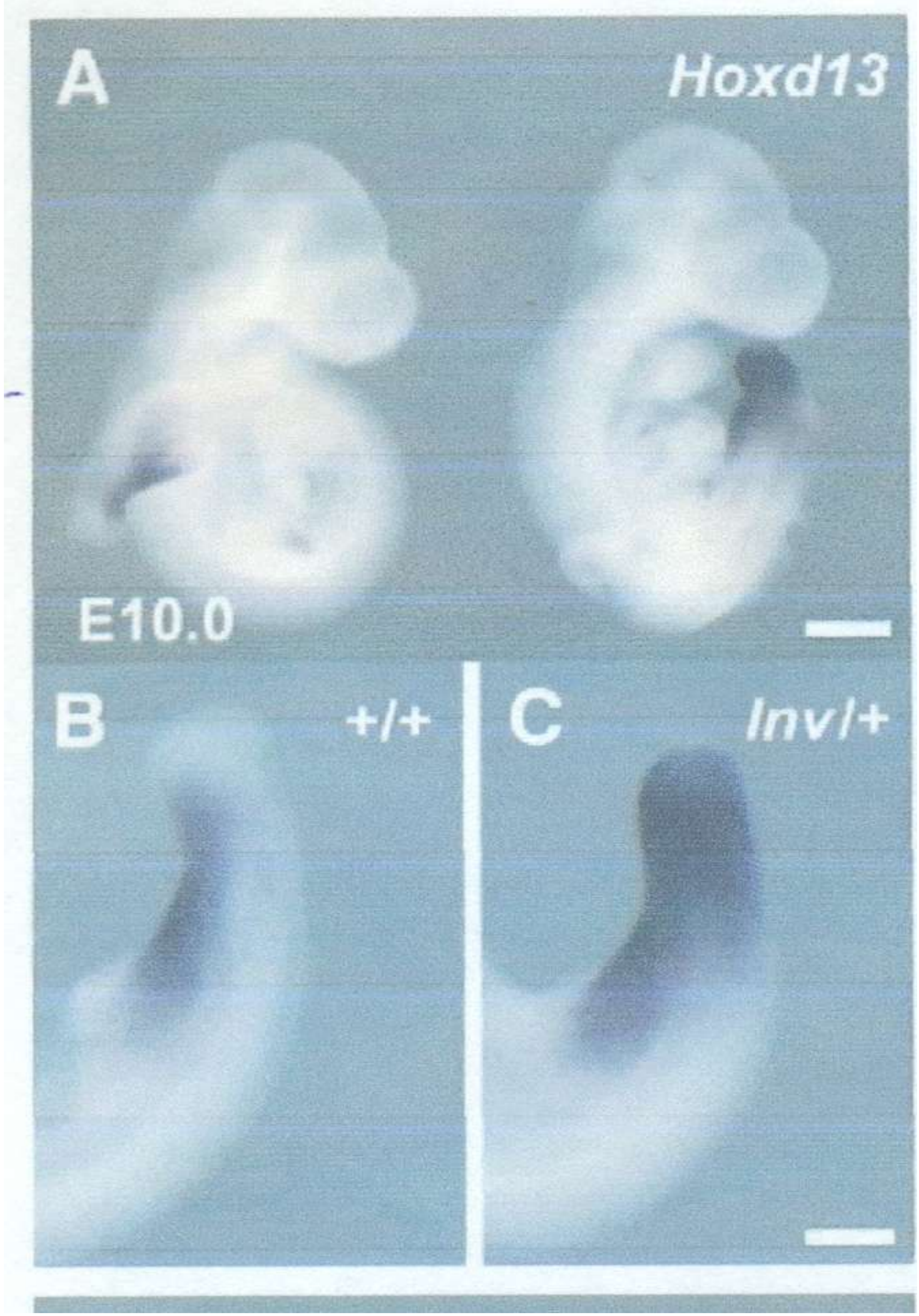
(up-regulation)

(A)

Whole-mount embryo

(B,C)

Hoxd13 expression in Caudal end



Divergent interpretations at early stages

1) TW M: 'Landscape effect' (l.e.) inhibits Hoxd expressions. When l.e. is removed expressions expand prematurely.

2) B M: Cut-off of fixed end of elastic spring. The spring is loose and shifts with smaller force → premature anteriorization.

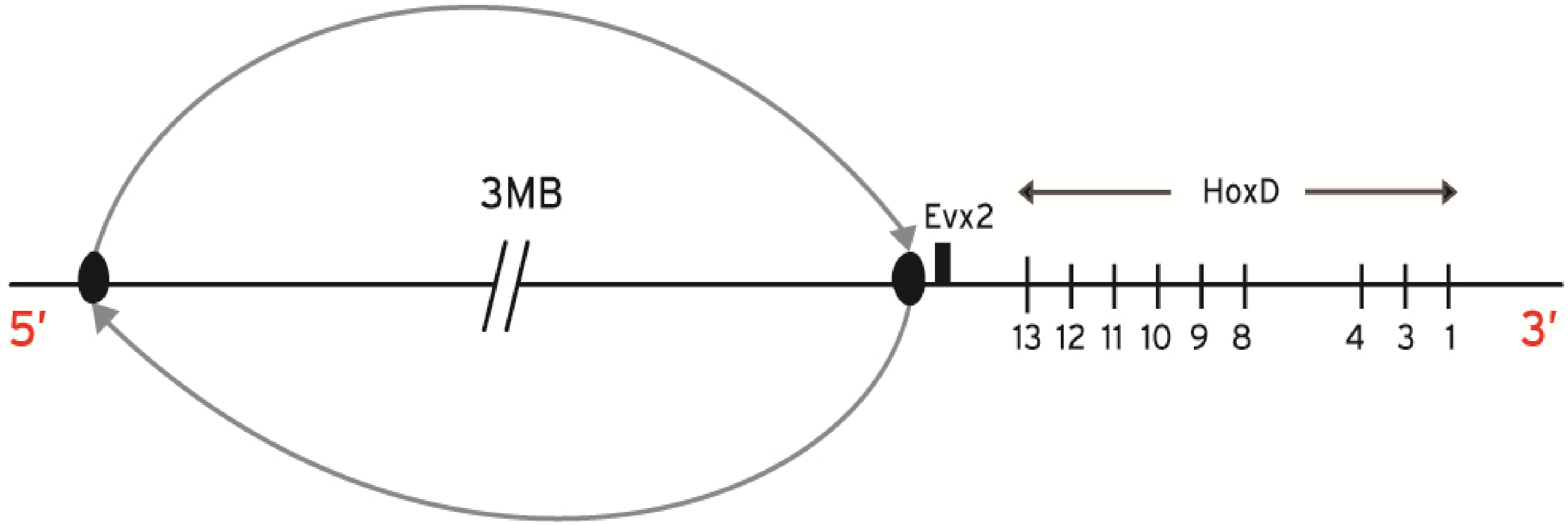


Figure 5

Proposed experiment:
Centromeric inversion-
posterior to Evx2

Model predictions for the early stages

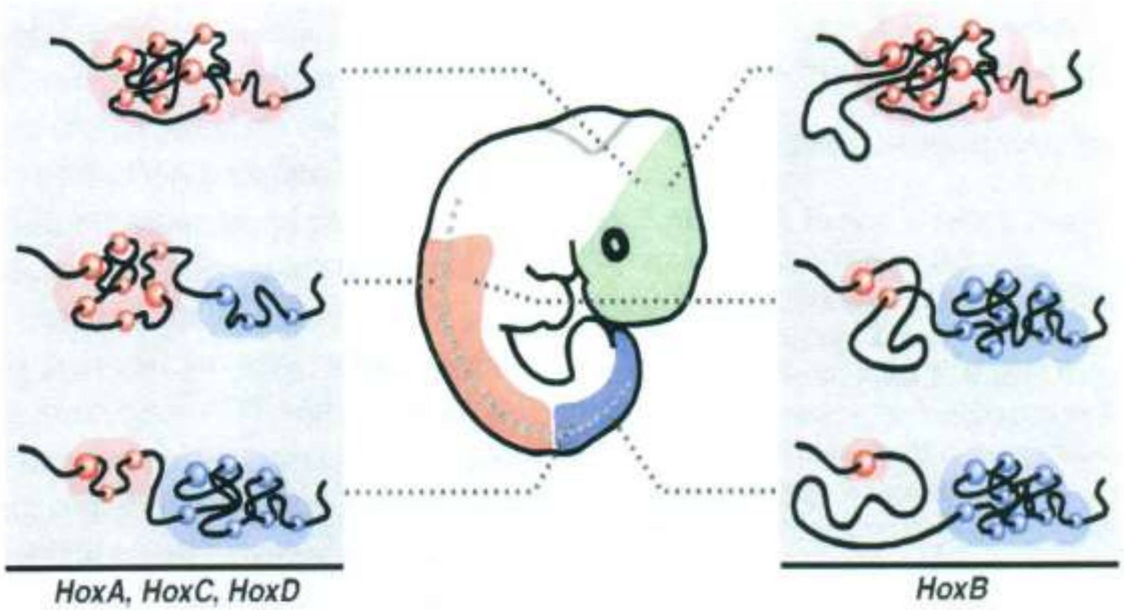
- 1) TW M: Hoxd expressions expand prematurely at early stages.

- B M : Hoxd expressions do not differ from the *wild type* expressions.

Comparison of the two models

1. BM takes into account multiscale nature of collinearity- TW M does not.
2. BM can explain quantitative collinearity- TW M does not.
3. Several experimental results are explained by BM but remain unexplained by TW M.

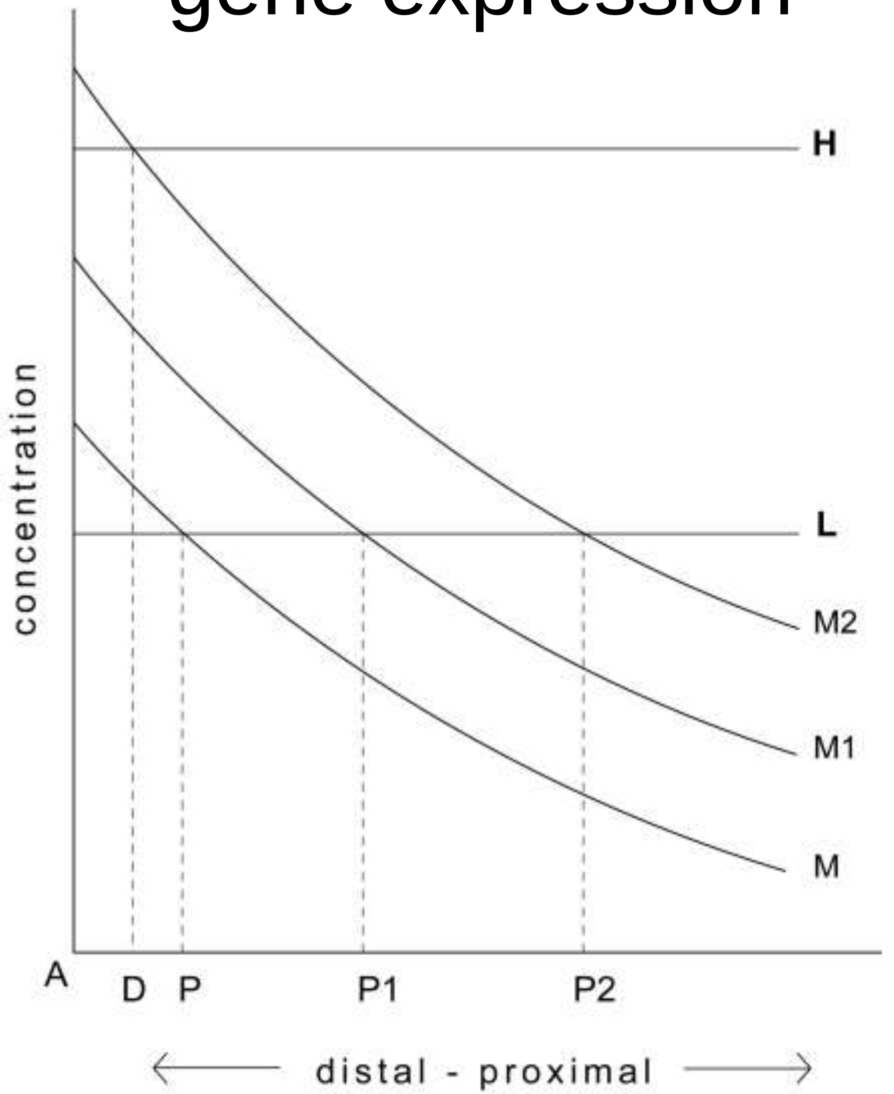
A new Duboule model



“Genes move stepwise from an inactive compartment to a transcriptionally active domain”

Noordermeer *et al*. Science **334,** 222 (2011)

Gradients: upper and lower thresholds for gene expression



Modification of ‘P’ in F=N*P

Macroscale: Implantation of a FGF4 bead causes increase of morphogen gradient. Around the bead the morphogen exceeds the upper threshold for Hoxa13.

Microscale: P increases→ F increases Hoxa13 moves beyond TF.

Entanglement of macro- and micro- scales

Another manifestation of multiscale nature of HOX collinearity

1. Macroscale range between morphogen thresholds
2. Microscale domain of transcription factory

A bead soaked in FGF4 is implanted posteriorily into st. 21 wing bud.
After 6 hours Hoxa13 expression is switched off around the bead.