Ch3 L2.2

Gradients and segmentation in Drosophila early development

We start from considering a very old story that was worked out on the wonderful biology of early development in D. melanogaster.

why ?

Edward B. Lewis, Christiane Nüsslein-Volhard and **Eric F. Wieschaus** have received the Nobel Prize in Physiology and Medicine 1995 for this discovery.

(you may also see a Developmental Biology book here: https://www.ncbi.nlm.nih.gov/books/NBK10081/)

The principles of how Transcription Factors and Enhancers work that we have learnt in D. melanogaster

are essentially transferable to higher organisms

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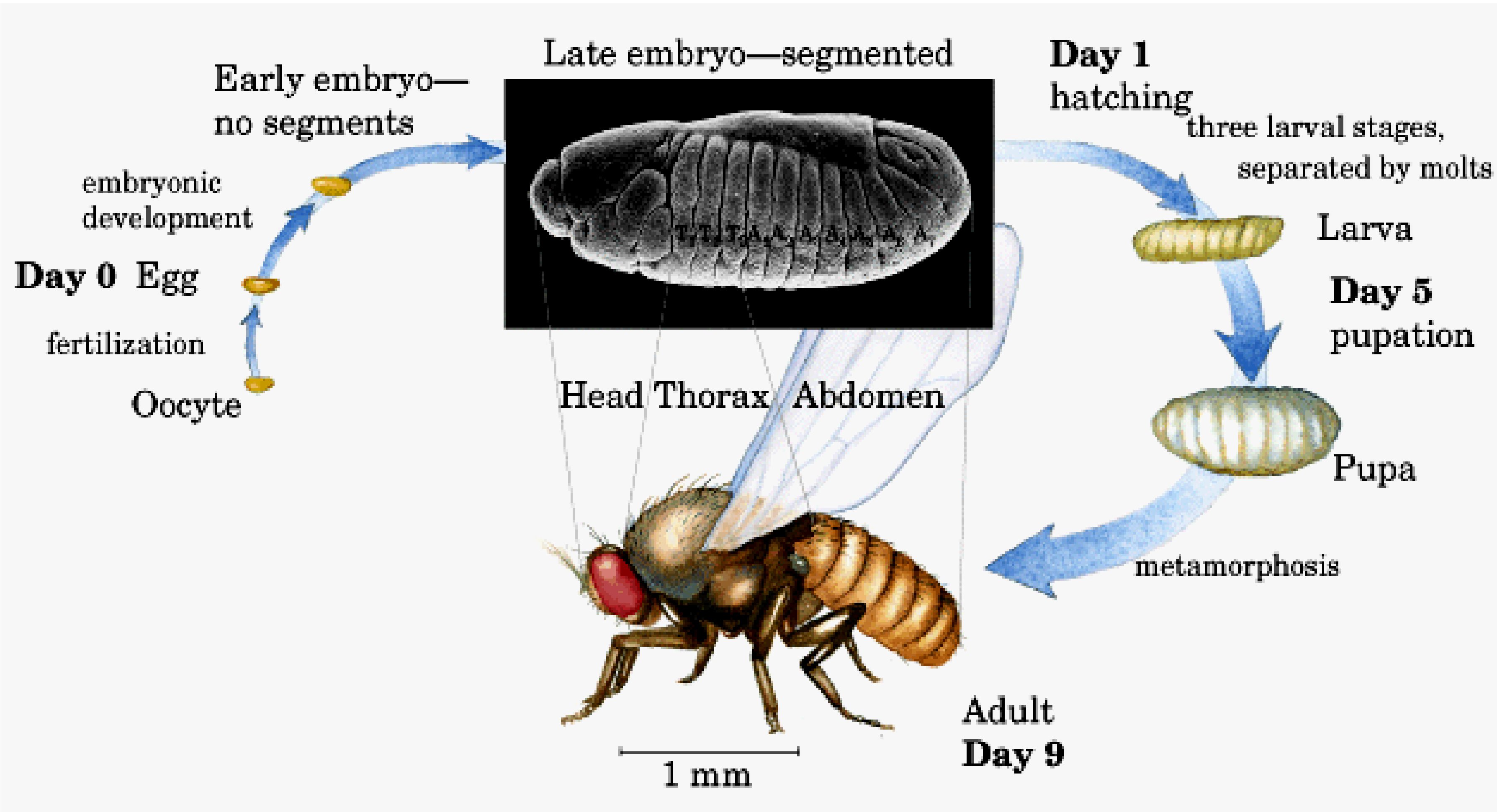
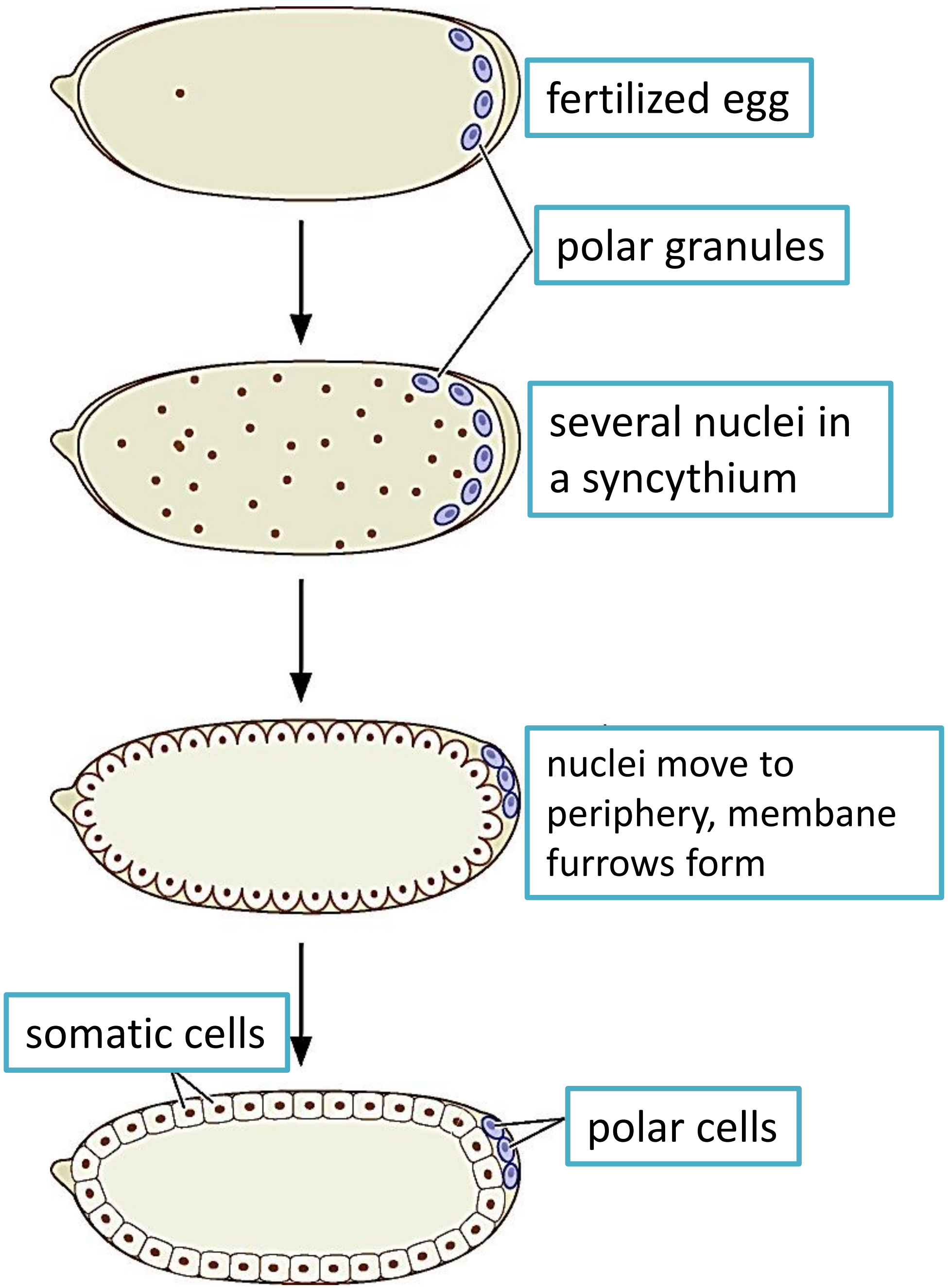
Dept of Molecular Biology

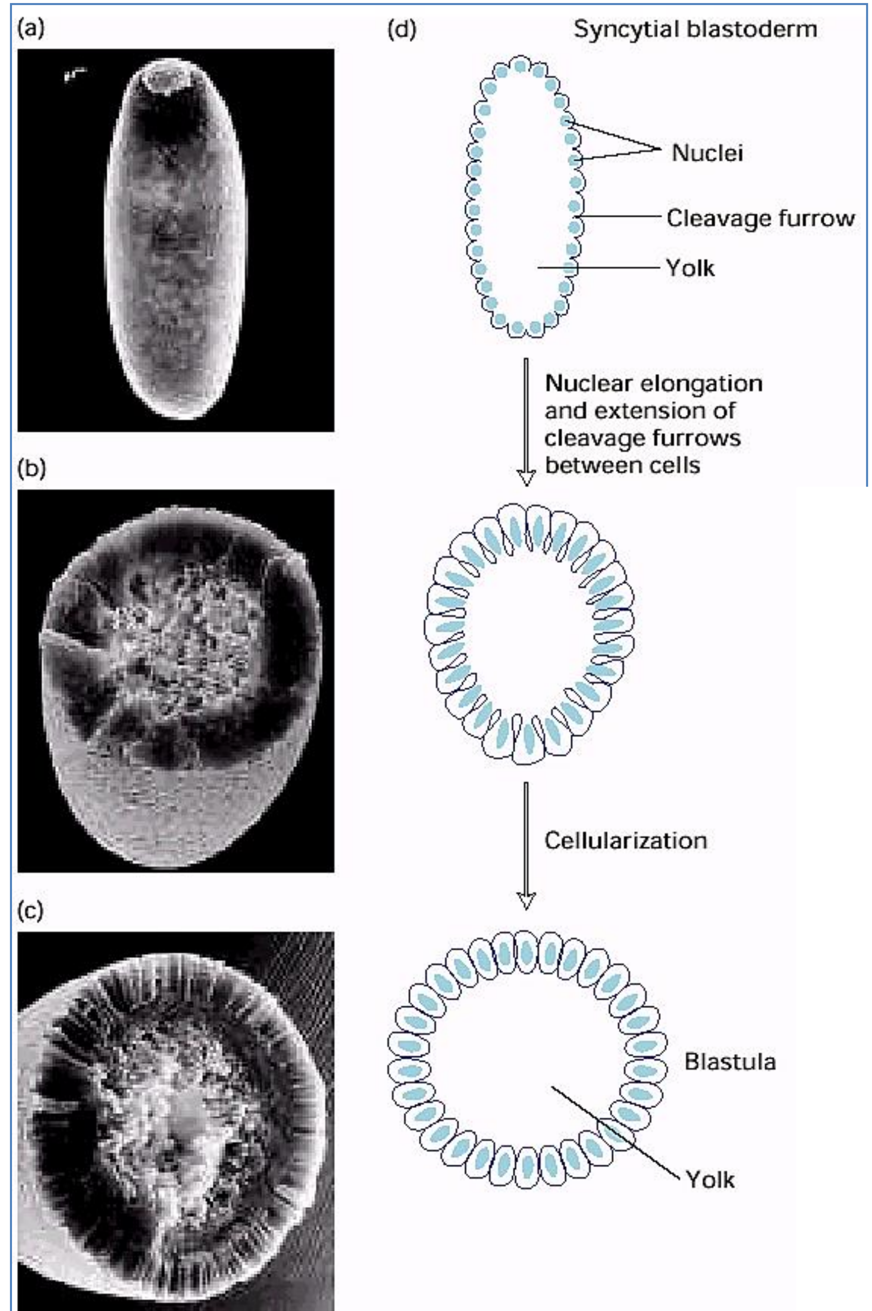
Mike Levine is also @ Princeton University now
Director of the Lewis-Sigler Institute for Integrative Genomics

but he moved from Berkeley (UC California Berkeley)

Princeton


Early embryo—
no segments
Late embryo—segmented
Day 1
hatching
three larval stages,
separated by molts
embryonic
development
Larva
Day 0 Egg
Day 5
pupation
fertilization
Head Thorax Abdomen
Oocyte
Pupa
metamorphosis
Adult
Day 9
1 mm


fertilized egg
polar granules
several nuclei in a syncythium
nuclei move to periphery, membane furrows form
somatic cells
polar cells


(a)
(b)
(c)
(d)
Syncytial blastoderm
Nuclei
Cleavage furrow
Yolk
Nuclear elongation and extension of cleavage furrows between cells
Cellularization
Blastula
Yolk

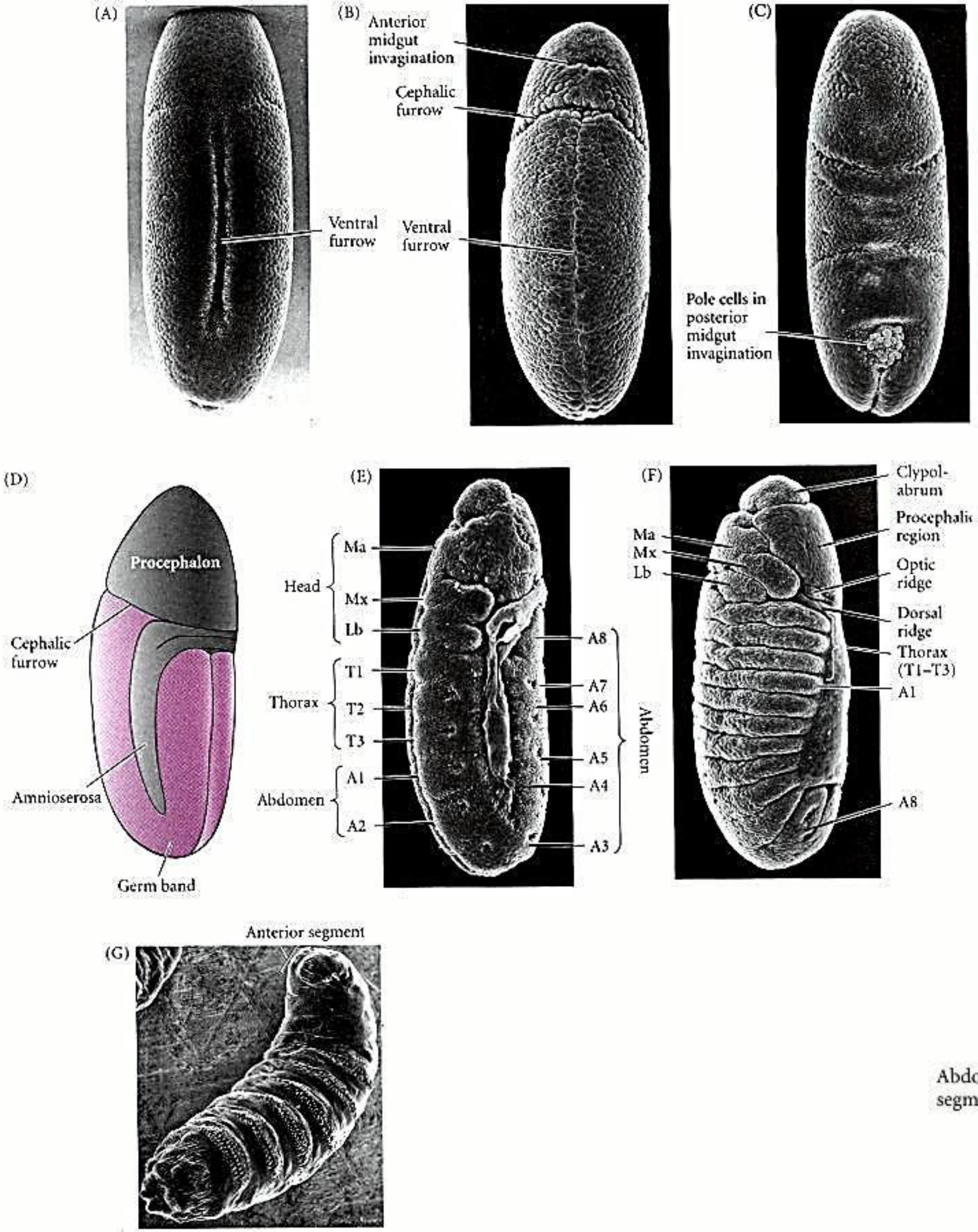
Superficial cleavage in a *Drosophila* embryo. The early divisions occur centrally. The numbers refer to the cell cycle. At the tenth cell cycle (512-nucleus stage 2 hours after fertilization), the pole cells form in the posterior, and the nuclei and their cytoplasmic islands ("energids") migrate to the periphery of the cell. This creates the syncytial blastoderm. After cycle 13, the oocyte membranes ingress between the nuclei to form the cellular blastoderm.

(*from: Gilbert SF. Developmental Biology 6th edition, 2000*)

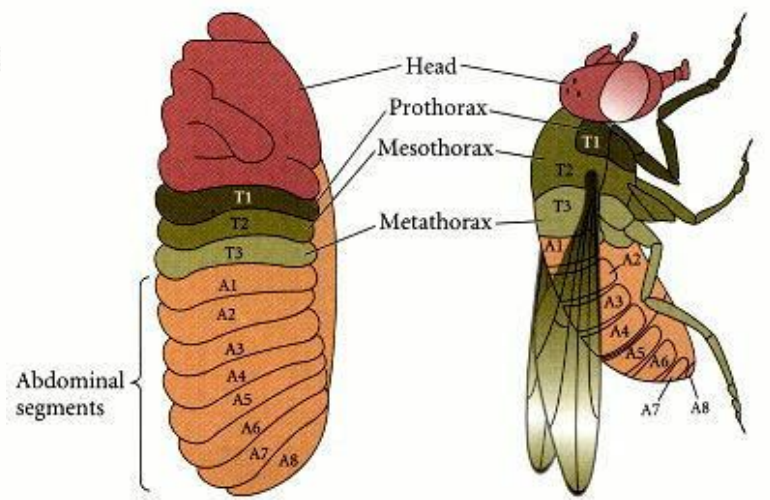
In *Drosophila,* the cellular blastoderm consists of approximately 6000 cells and is formed within 4 hours of fertilization.

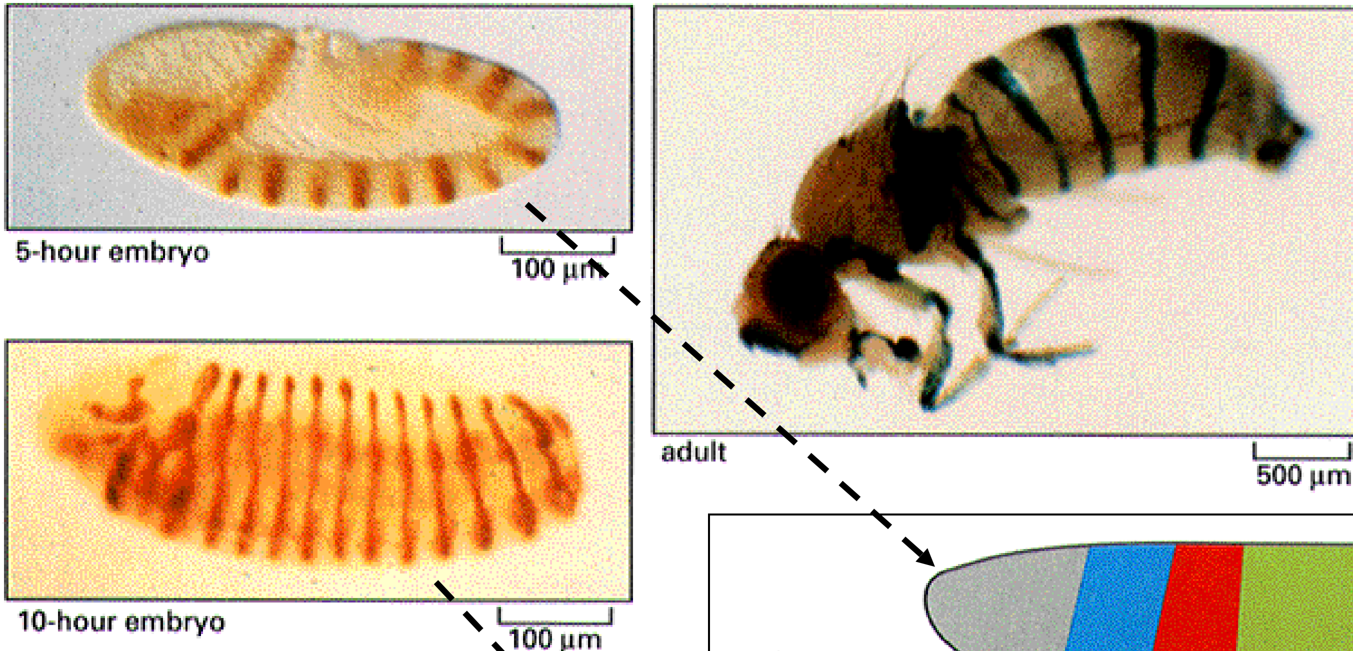
Transcription from the nuclei (which begins around the eleventh cycle) is greatly enhanced at cycle 14, when D. embryo forms cells (midblastula transition).

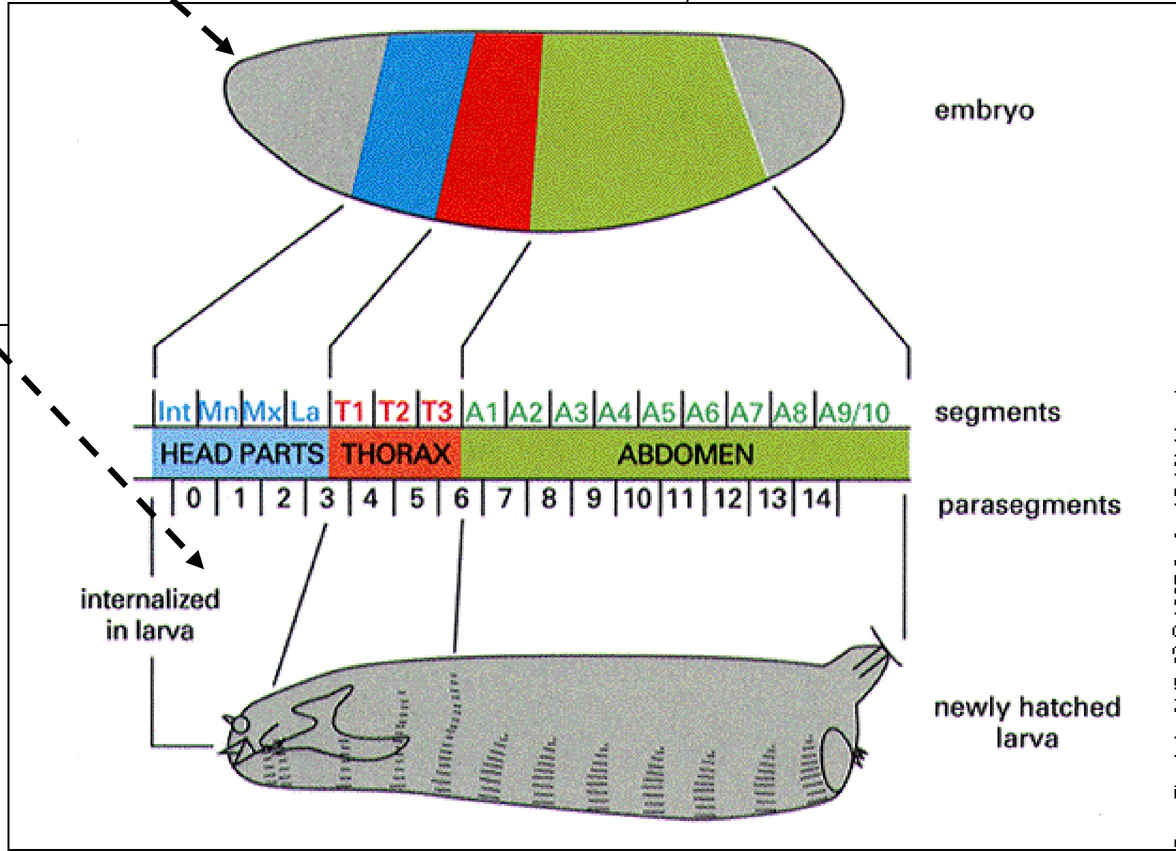
Eric F. Wieschaus
https://youtu.be/Ncxs21KEj0g



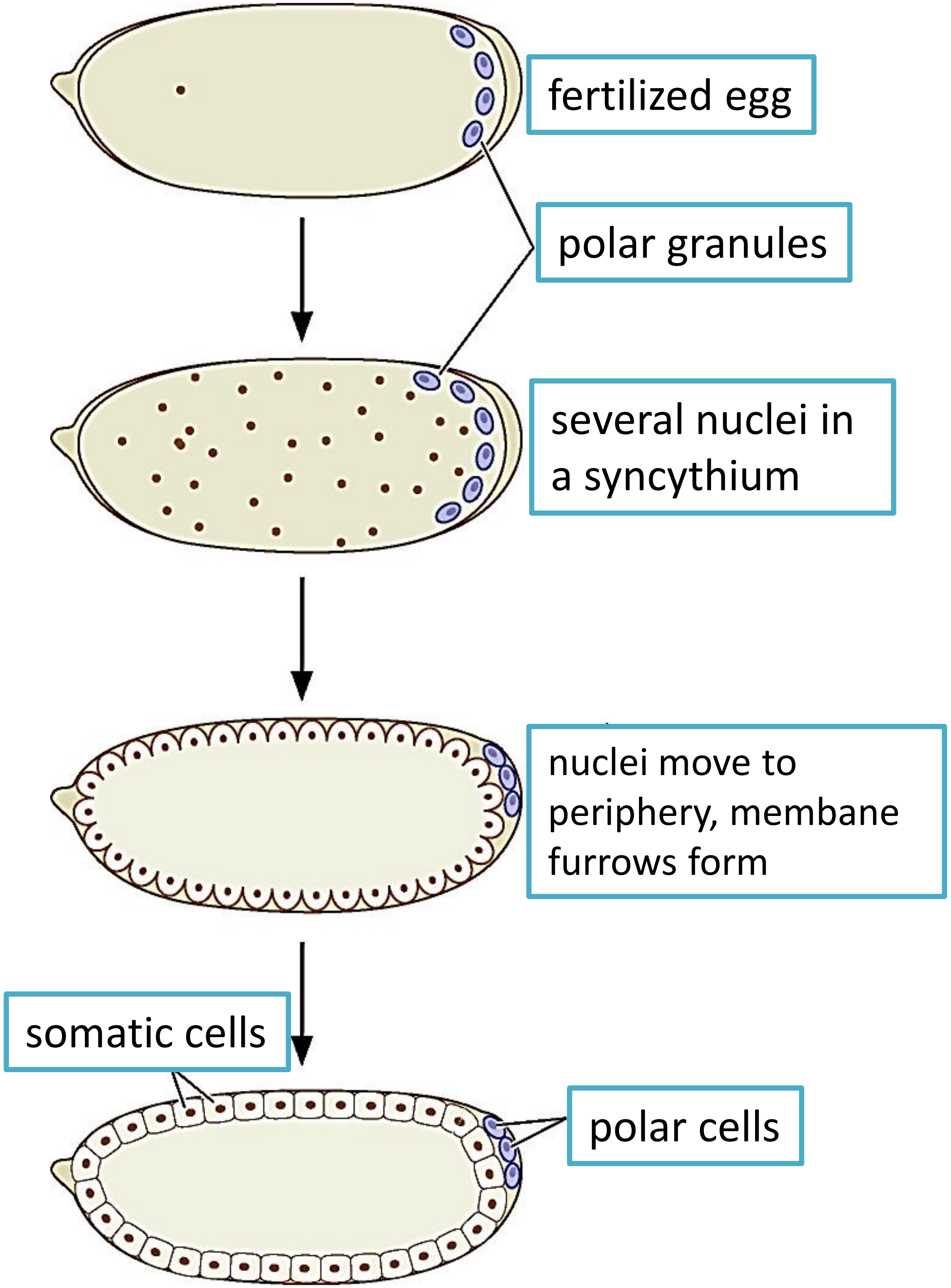
Gastrulation and body plan determination







embryo stained with Ab for Engrailed= homeoprotein back to early stages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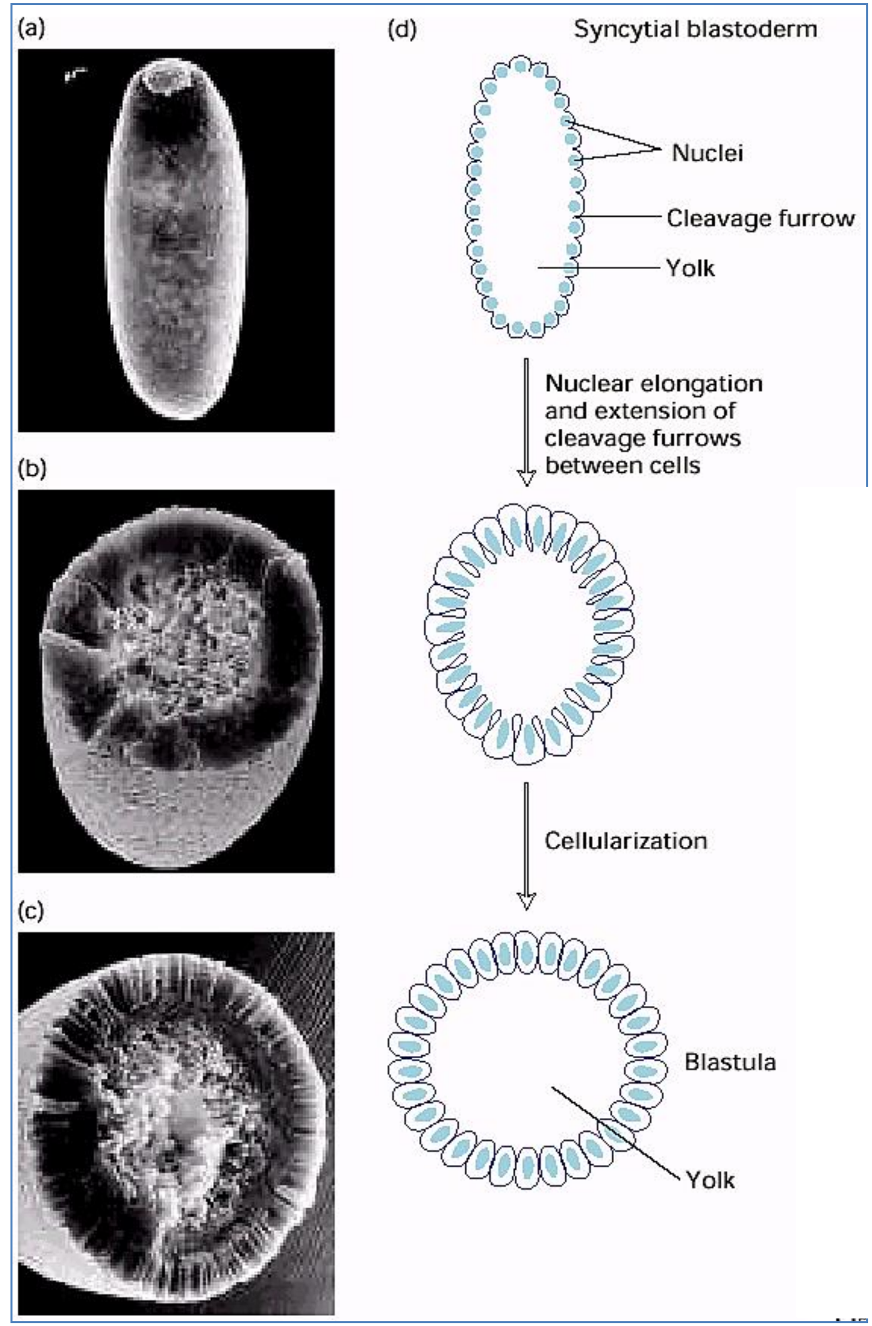
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Syncytial blastoderm
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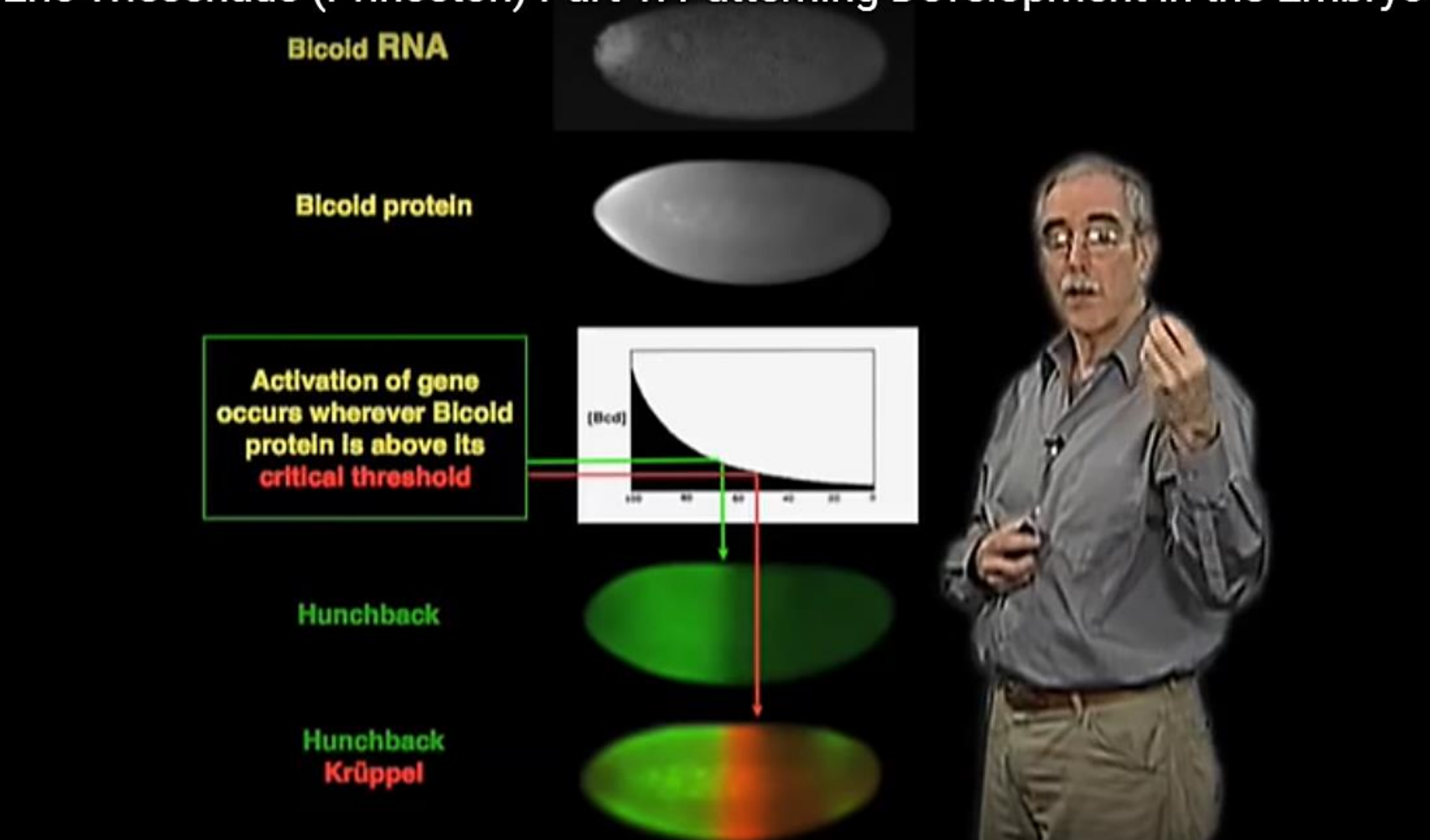
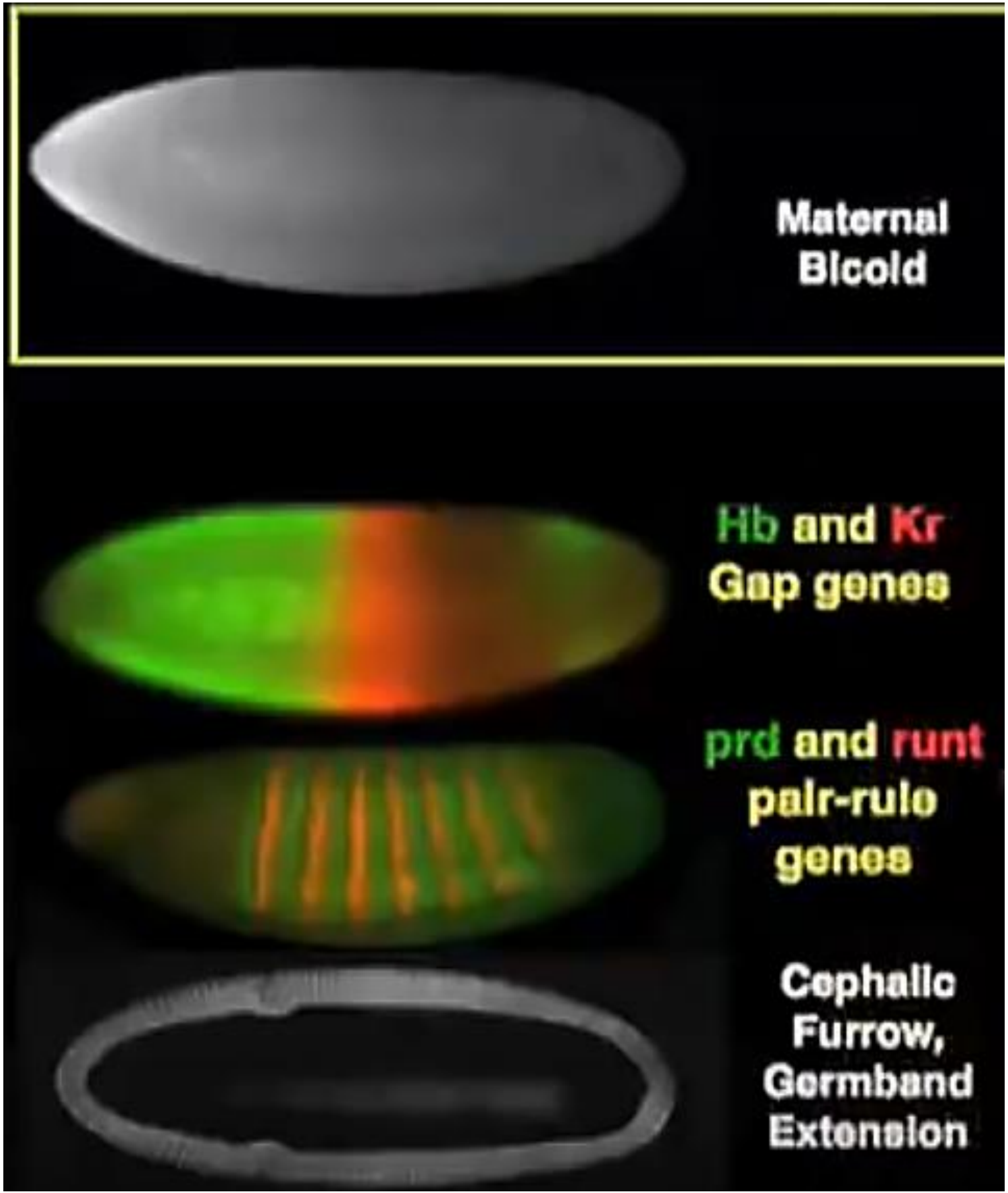
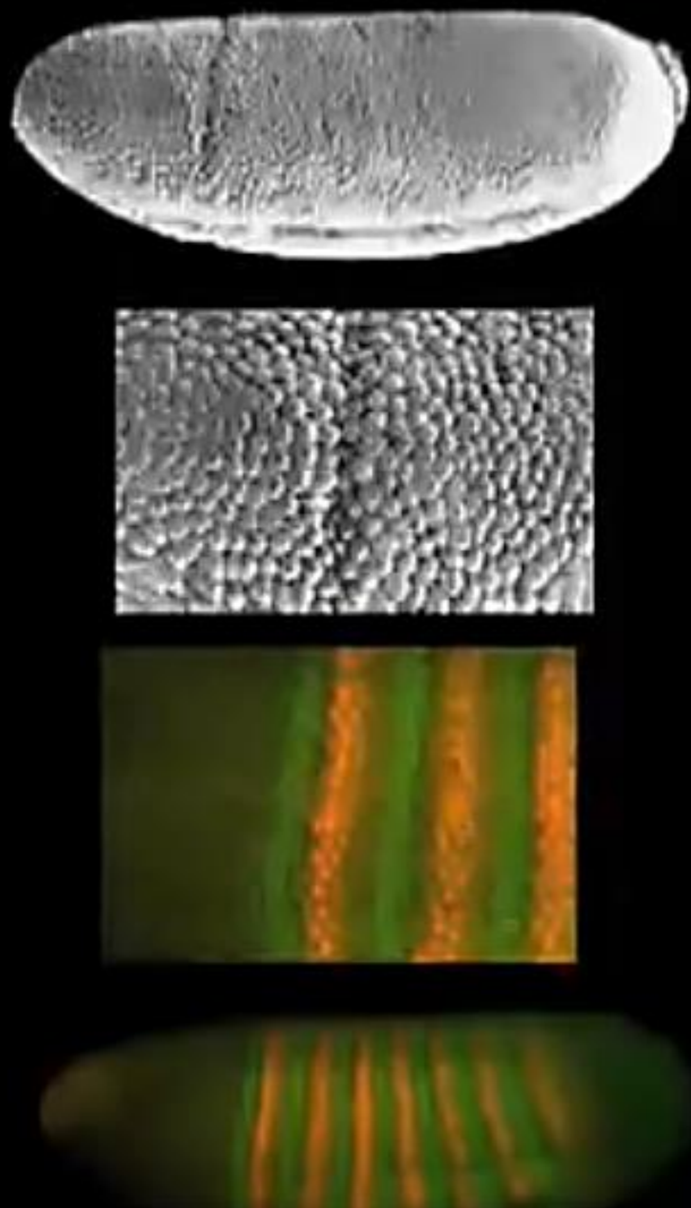
Eric Wieschaus (Princeton) Part 1: Patterning Development in the Embryo

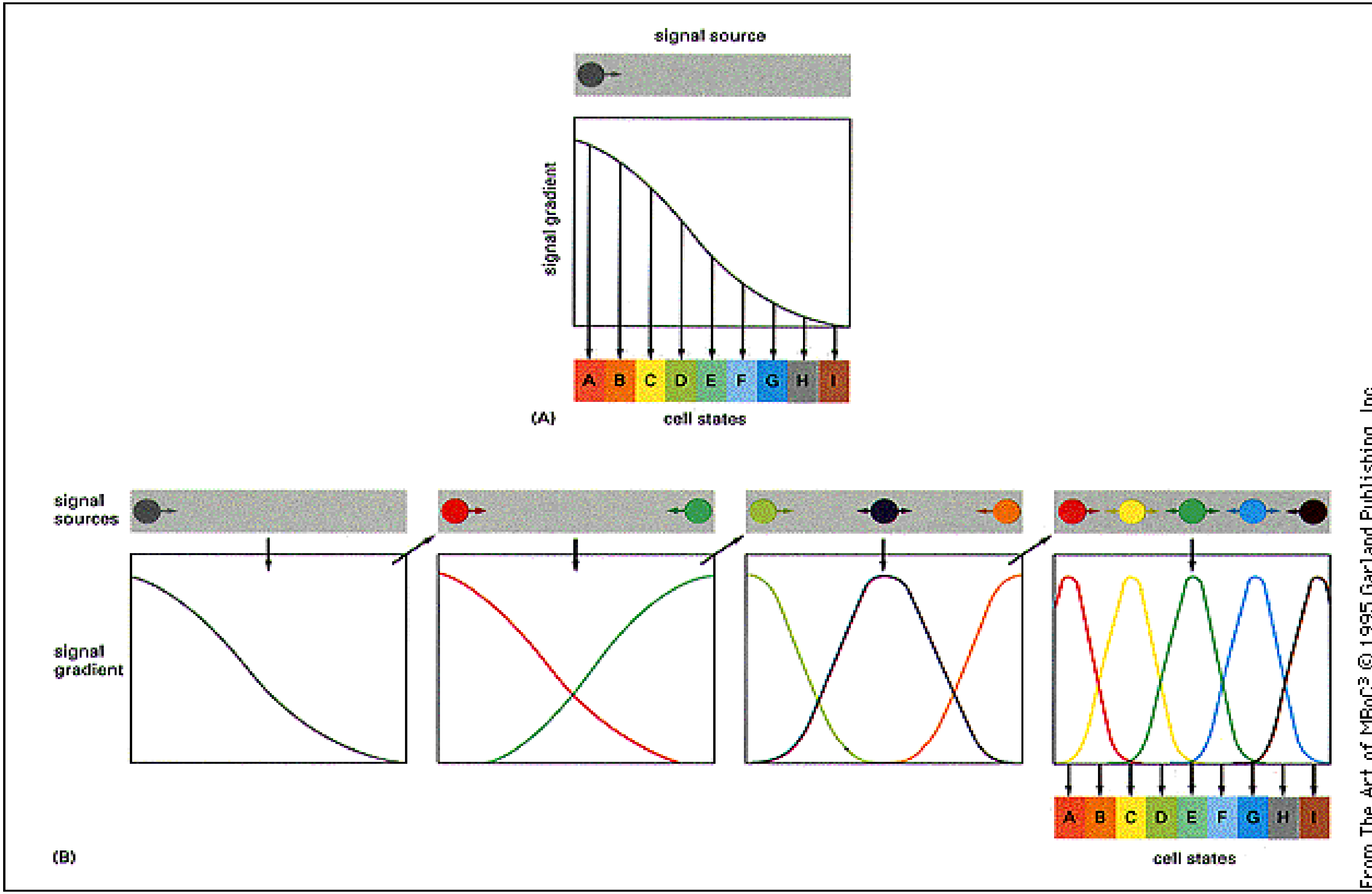




Maternal
Bicoid
Hb and Kr
Gap genes
prd and runt
pair-rule
genes
Cephalic
Furrow,
Germband
Extension

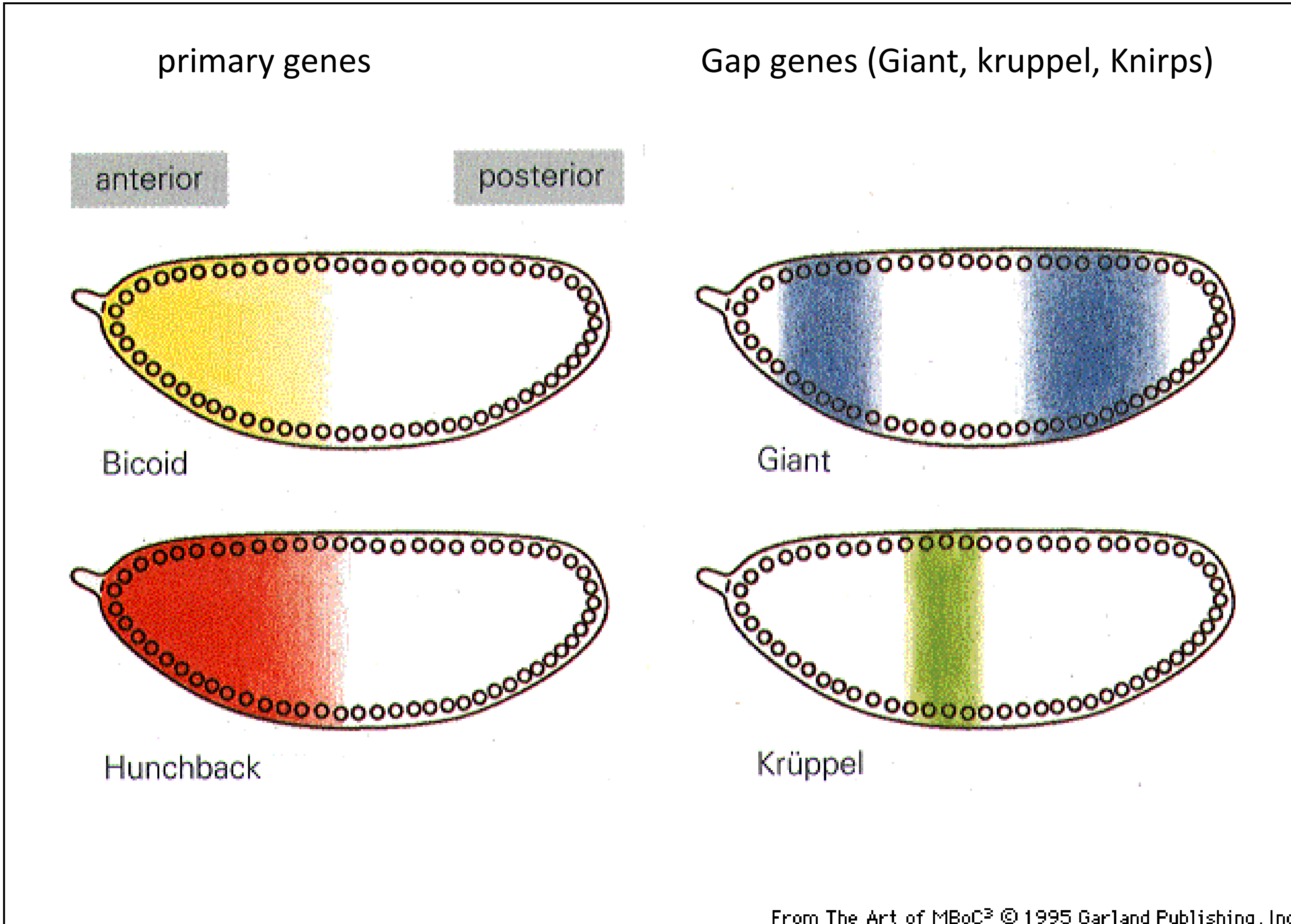
Patterns of cell behavior reflect underlying patterns of gene activity



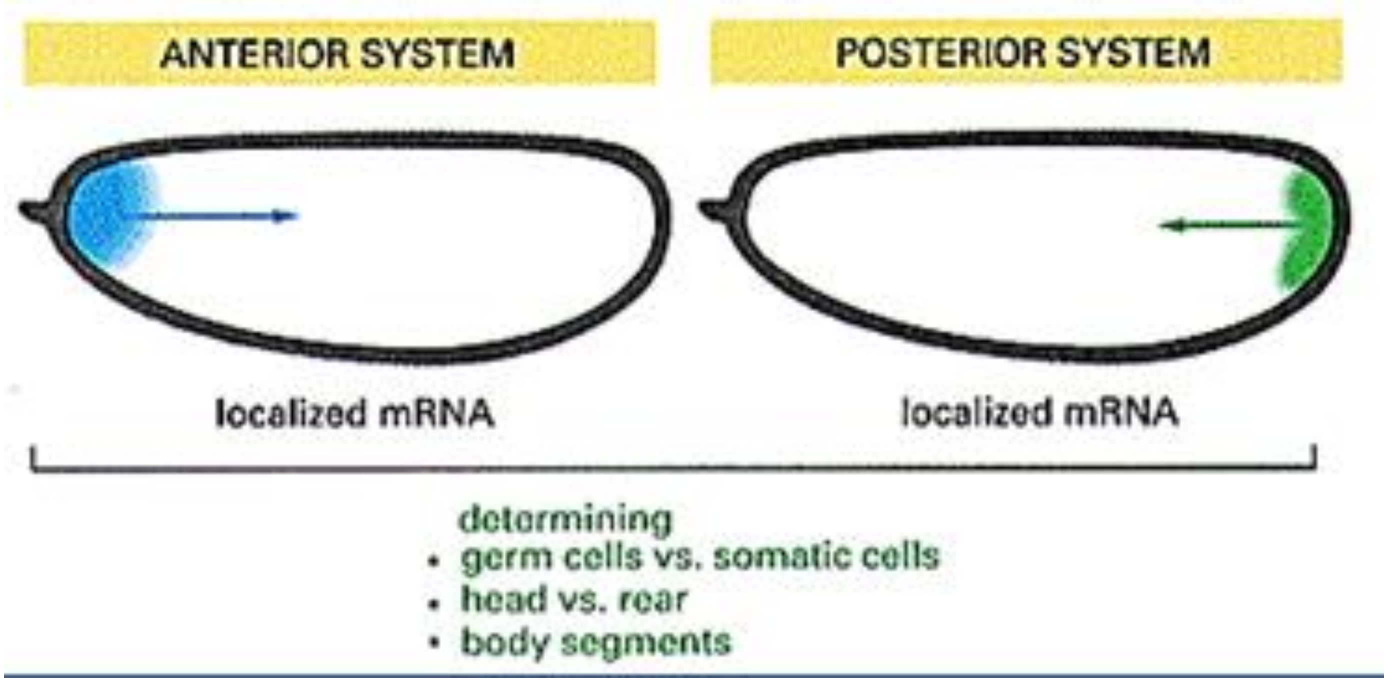


Risposte secondarie a gradienti primari possono generare segmentazione

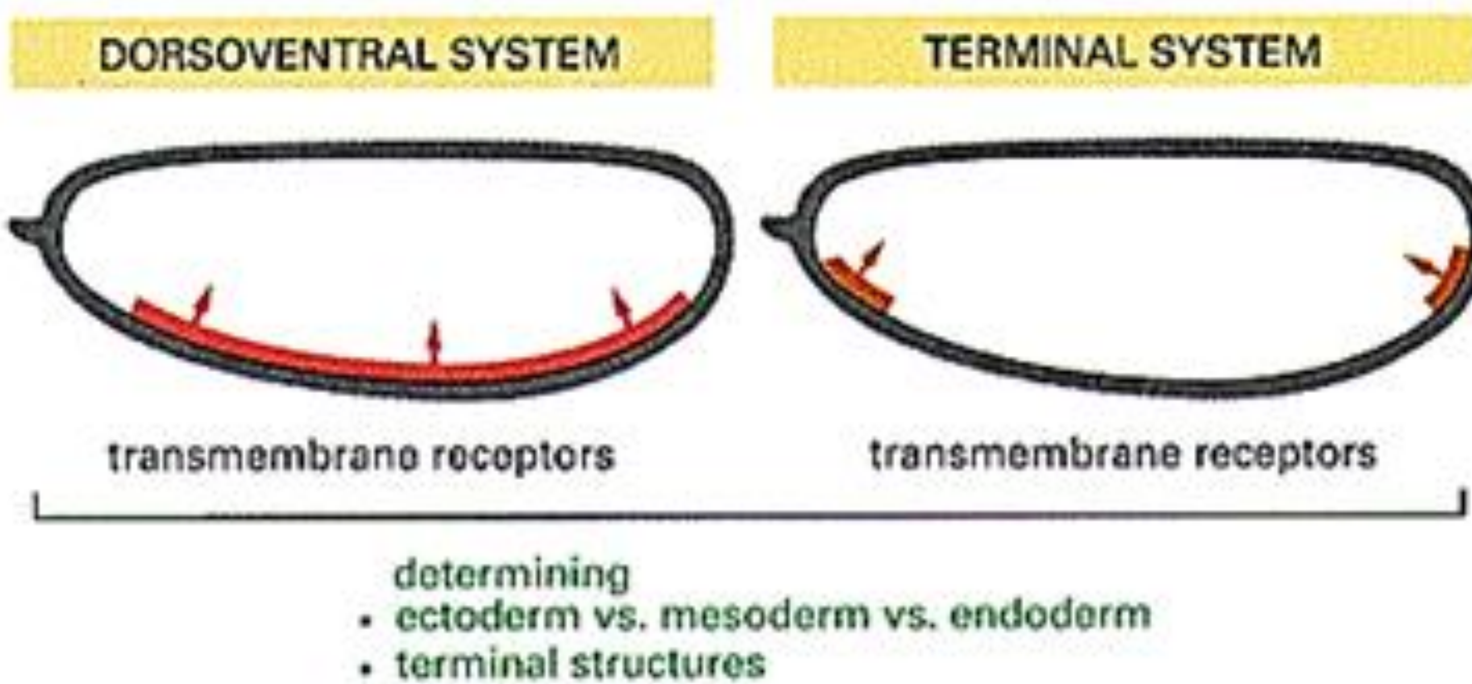
Similar gradients in head to tail direction



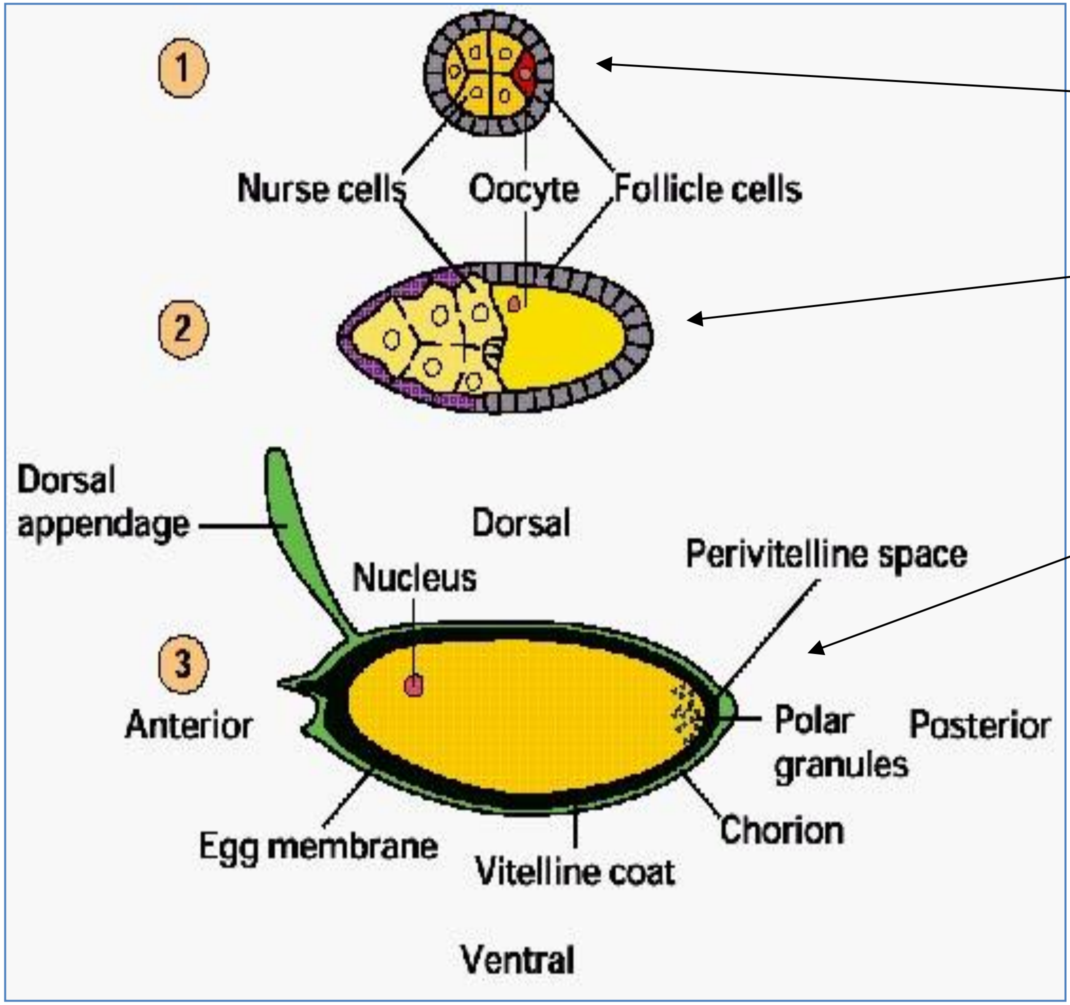
Where do these «signals» come from ?



Antero-posterior axis: maternal mRNAs


DORSOVENTRAL SYSTEM
TERMINAL SYSTEM
transmembrane receptors
transmembrane receptors
determining
• ectoderm vs. mesoderm vs. endoderm
• terminal structures

Where do «*morphogens*» come from ?



Each developing unit, or follicle, consists of a developing oocyte, nurse cells and a layer of somatic cells called follicle cells.

Stage 1: Early in oogenesis, the oocyte is about the same size as the neighboring nurse cells.

Stage 2: The nurse cells begin to synthesize mRNAs and proteins necessary for oocyte maturation, and the follicle cells begin to form the egg shell.

Stage 3: The mature egg is surrounded by the vitelline coat and chorion, which compose the egg shell. The nurse cells and follicle cells have been discarded, but some of the mRNAs synthesized by nurse cells, which become localized in discrete spatial domains of the oocyte, function in early patterning of the embryo.

Polar granules are distinct cytoplasmic structures located in the posterior region of the egg. This is the region in which germ cells arise.

This is the way primary morphogen gradients arise

Of course, if RNA, they direct synthesis of proteins, that will be in a concentration gradient more or less corresponding

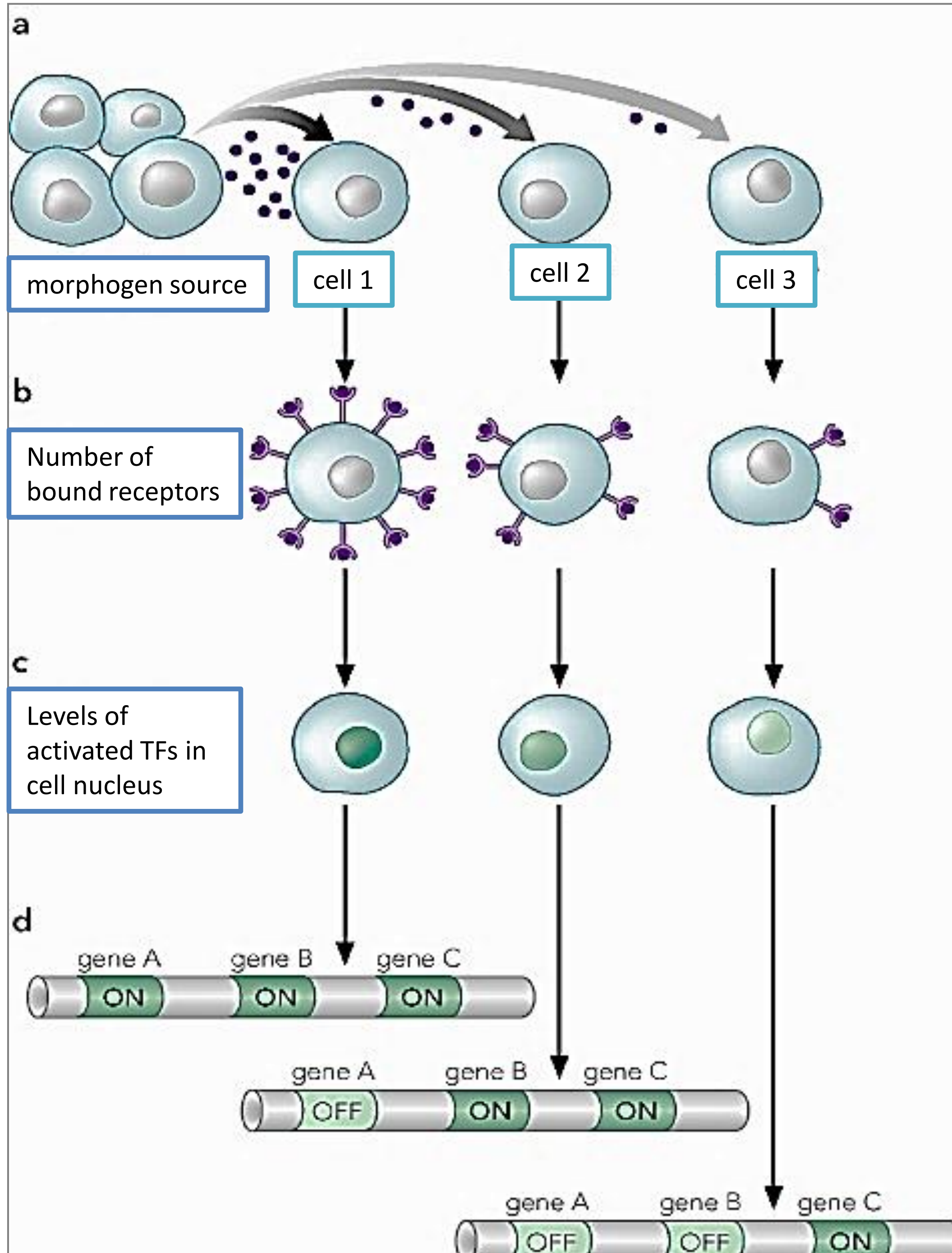
Why RNA does not diffuses away, since there are no cells at the early stage ?

These proteins, that are mainly Transcription Factors, bind to regulatory regions in secondary genes (mainly Enhancers)

There is a critical concentration of each TF at which binding to the Enhancer is **productive** (*stable enough to form compexes with coactivators, Mediator etc.*)

Same story for signally proteins.

The **dorso-ventral** axis has soluble signalling proteins as the primary morphogen



Lesson # 1:
Information in biological systems is **quantitative**

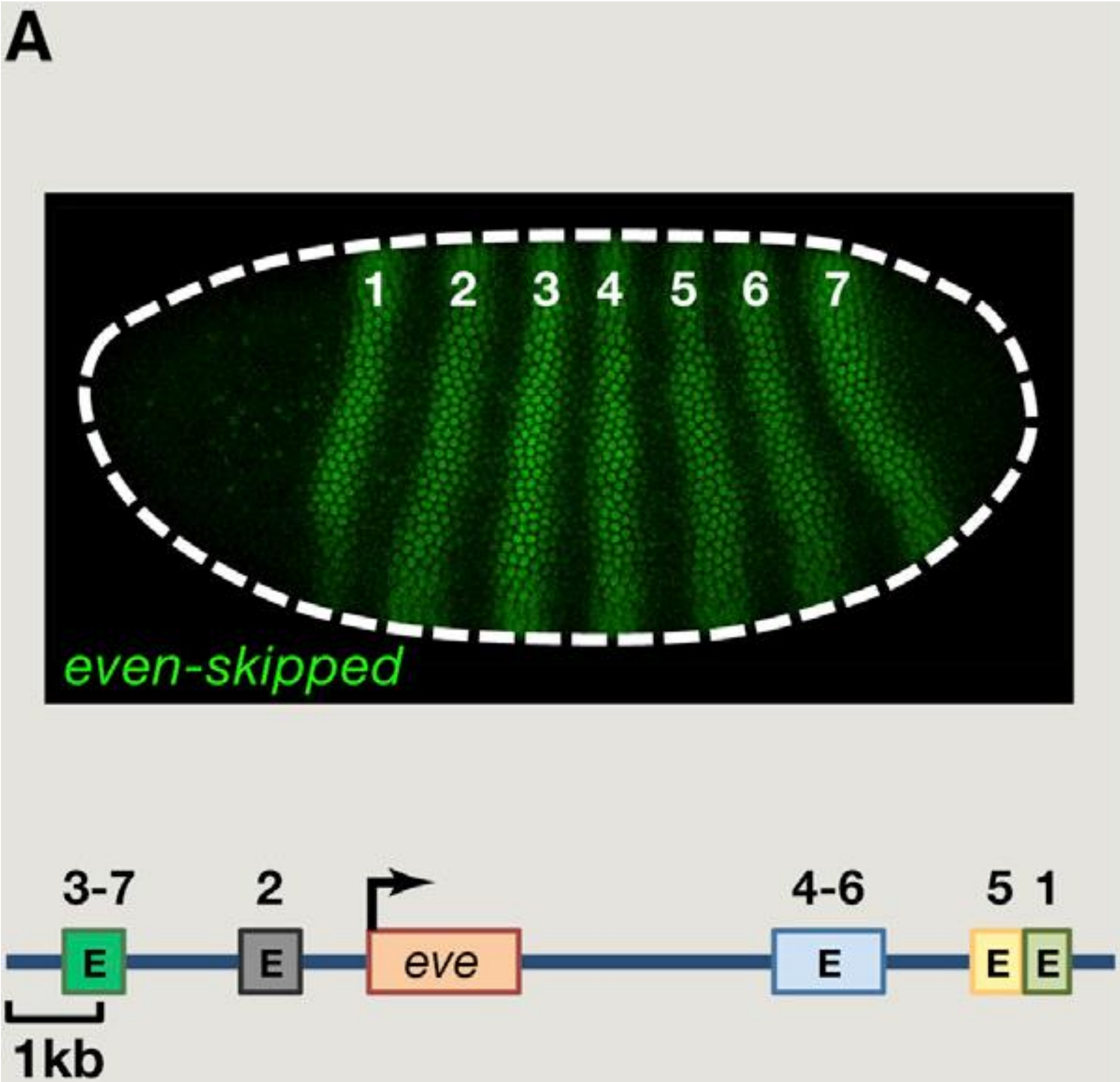
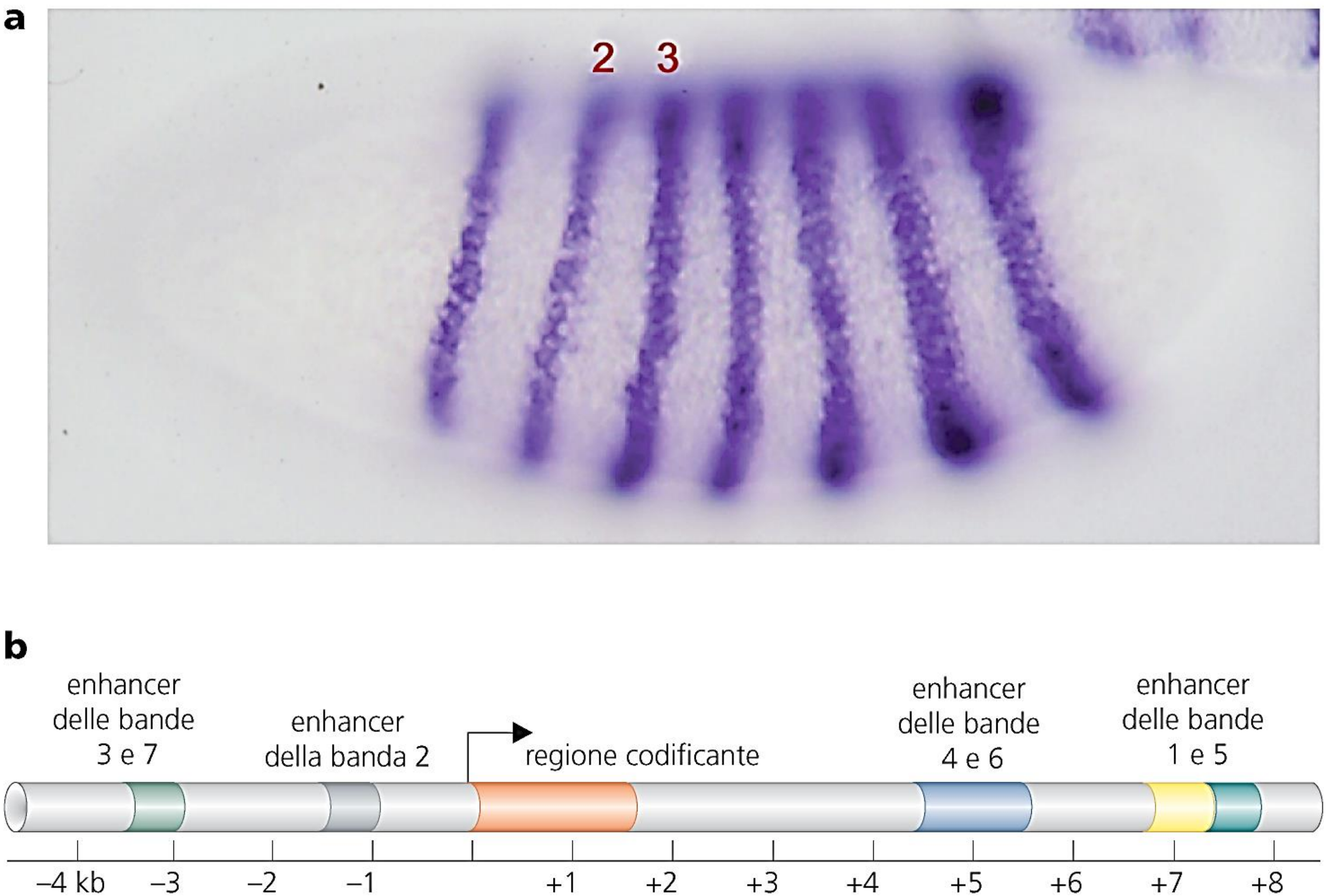


Figure 1. Organization of cis-Regulatory DNAs in Metazoan Genomes

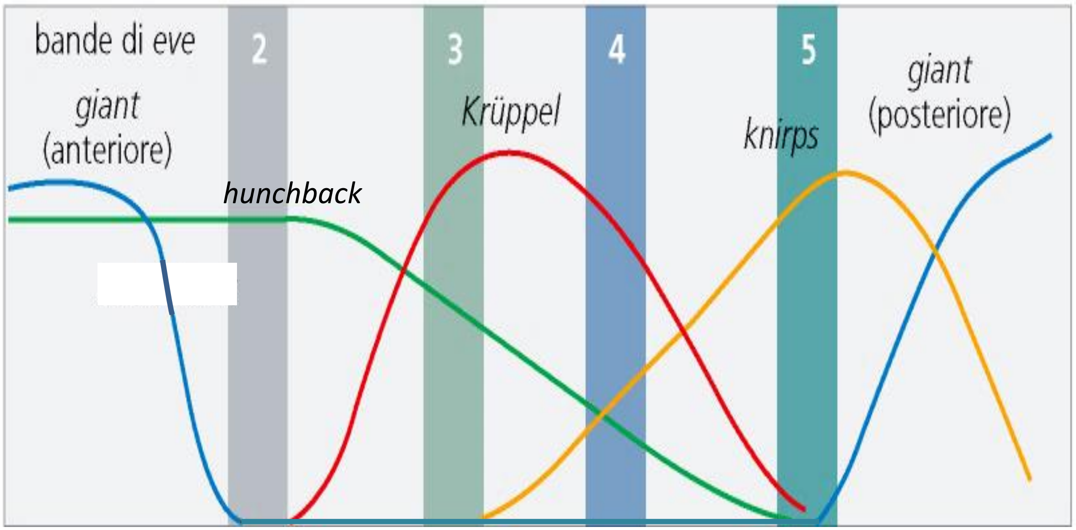
Metazoan genes are regulated by multiple enhancers.
(A) Organization of the even-skipped (eve) locus in the Drosophila genome. The eve gene is just 3 kb in length but is regulated by individual stripe enhancers (E) located in both 50 and 30 flanking regions. The eve stripe enhancers function in an additive fashion to produce seven stripes of gene expression in the early Drosophila embryo

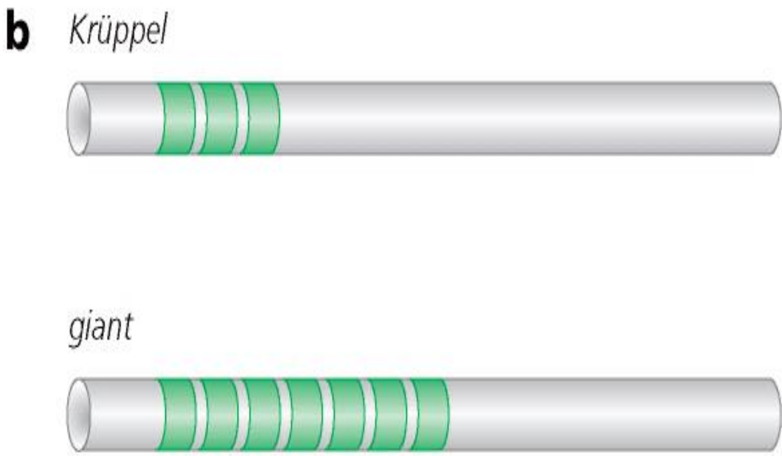
Levine et al., 2014

Eve (even-skipped) is the first “pair-rule” segmental gene: it has more than 12Kb essential regulatory sequences
Each enhancer is regulated by the exact combination of factors present in stripes

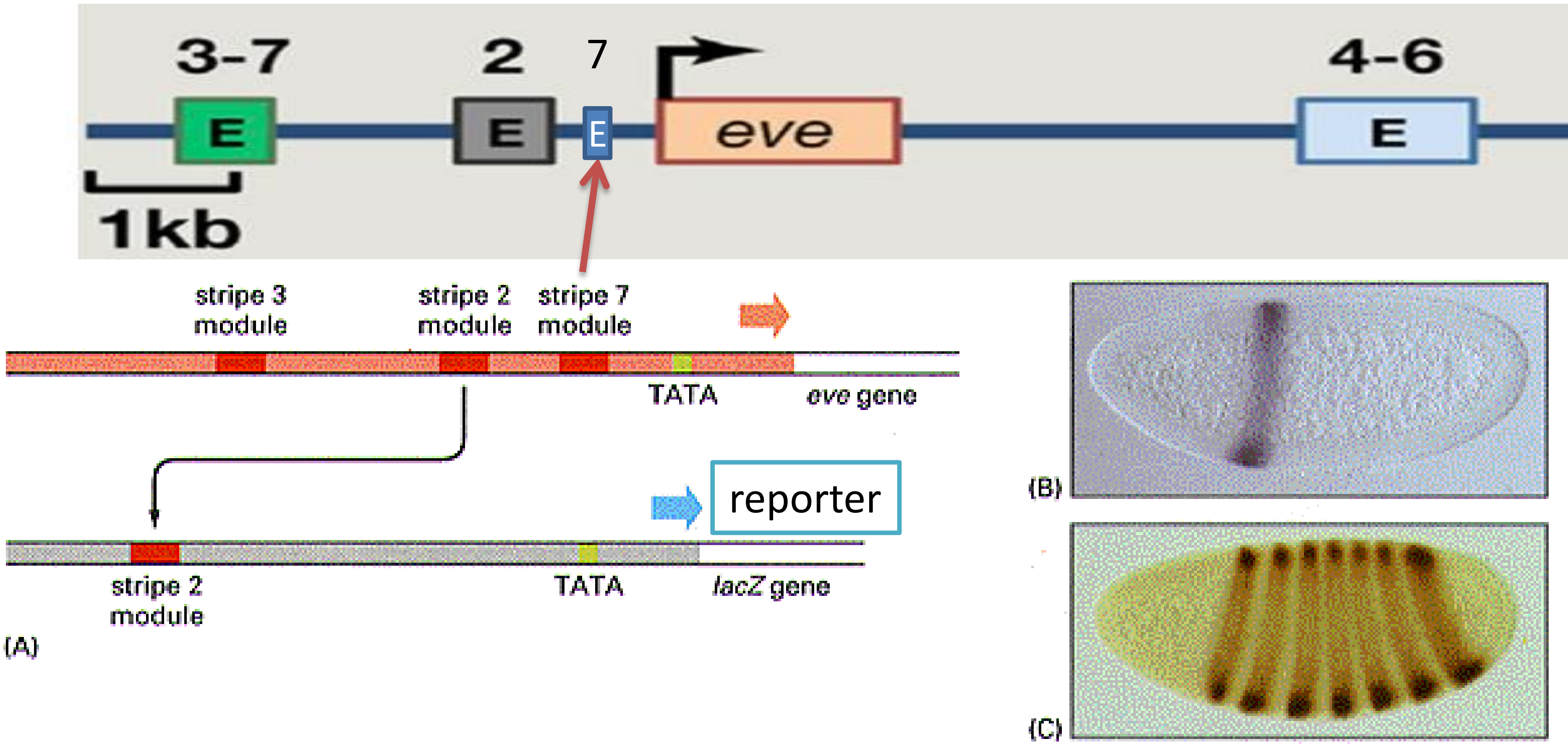


Hunchback is a **repressor**:
Kruppel enhancer has few sites, requires higher levels
Giant enhancer has more sites: lower levels are enough

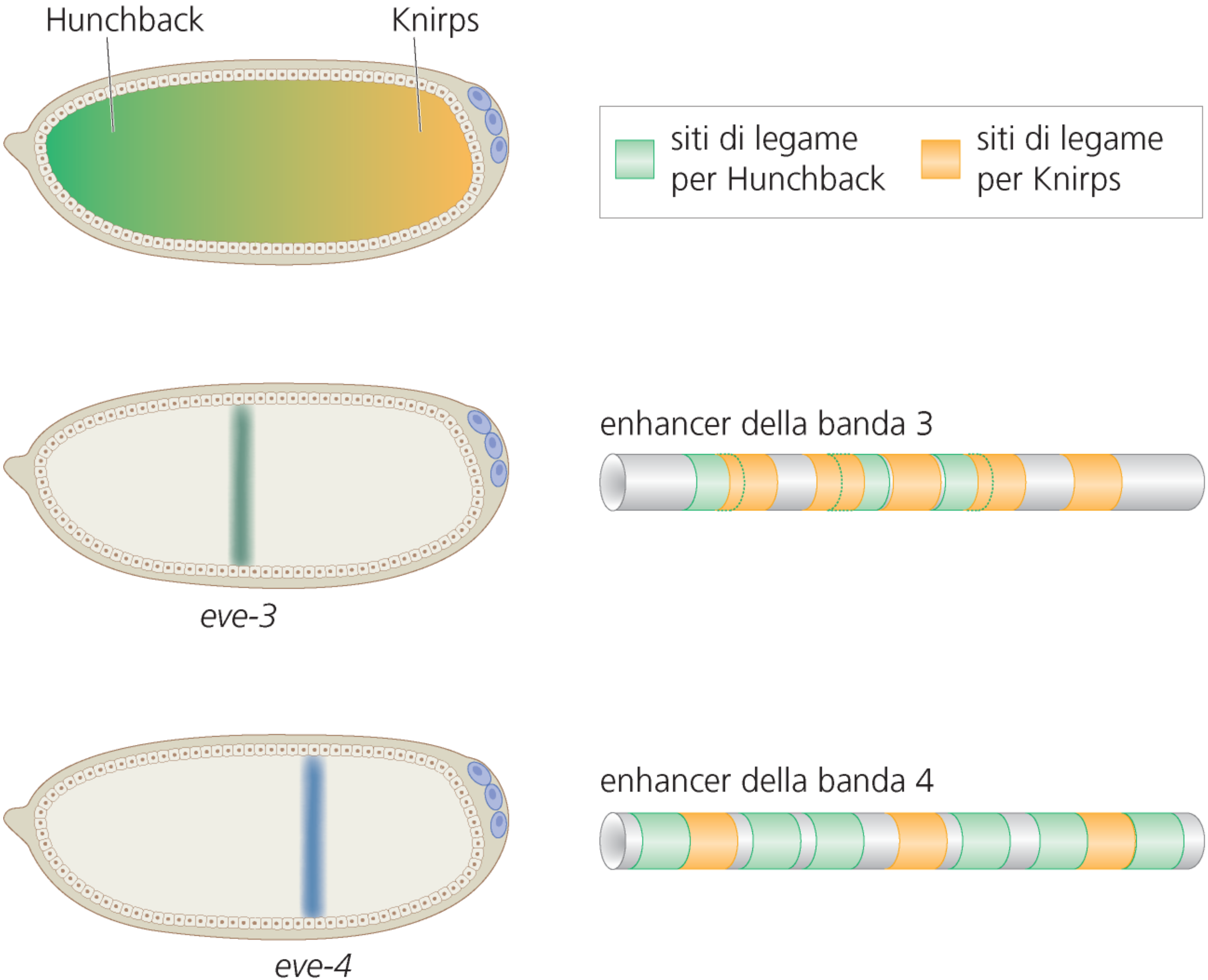


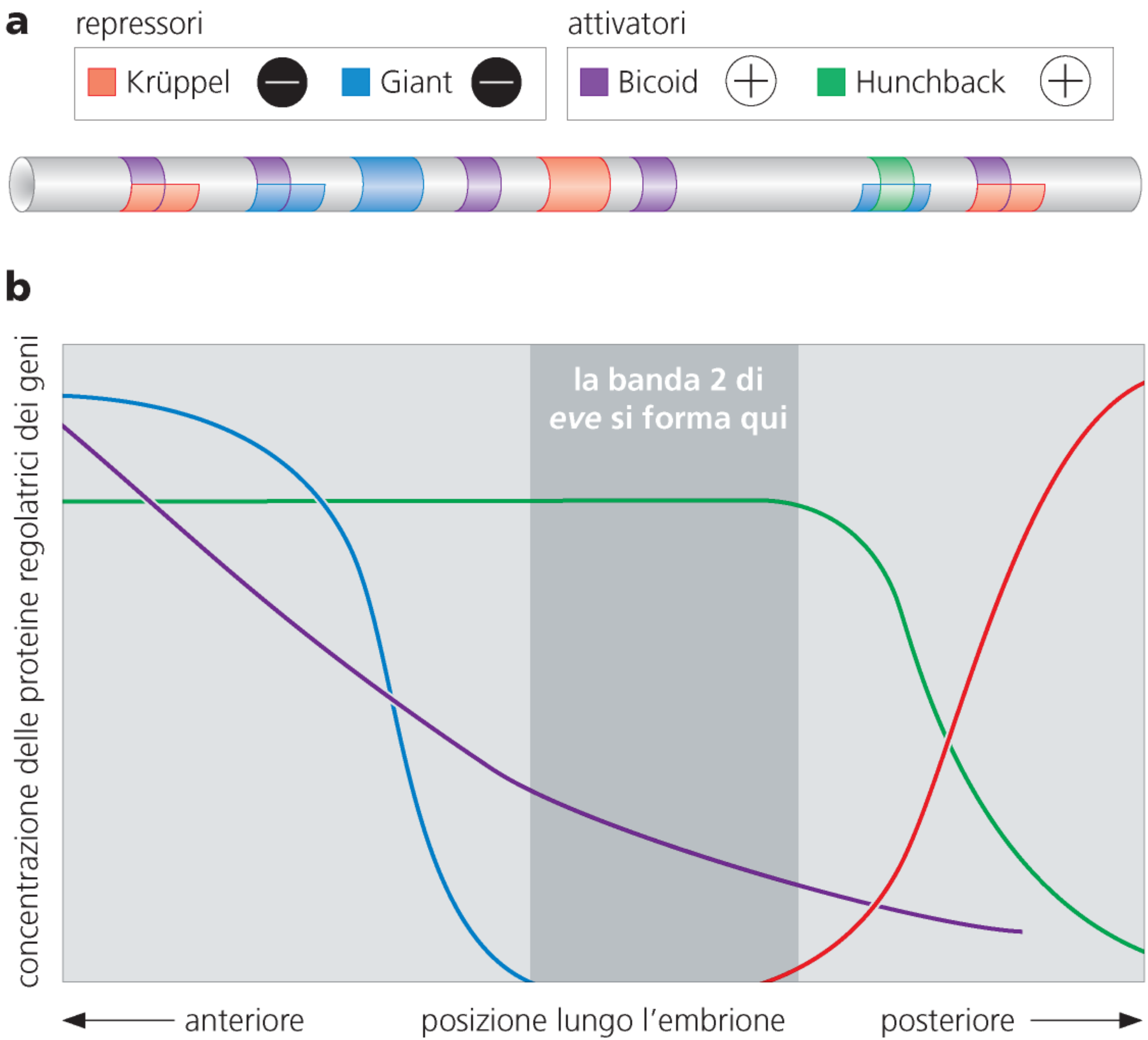


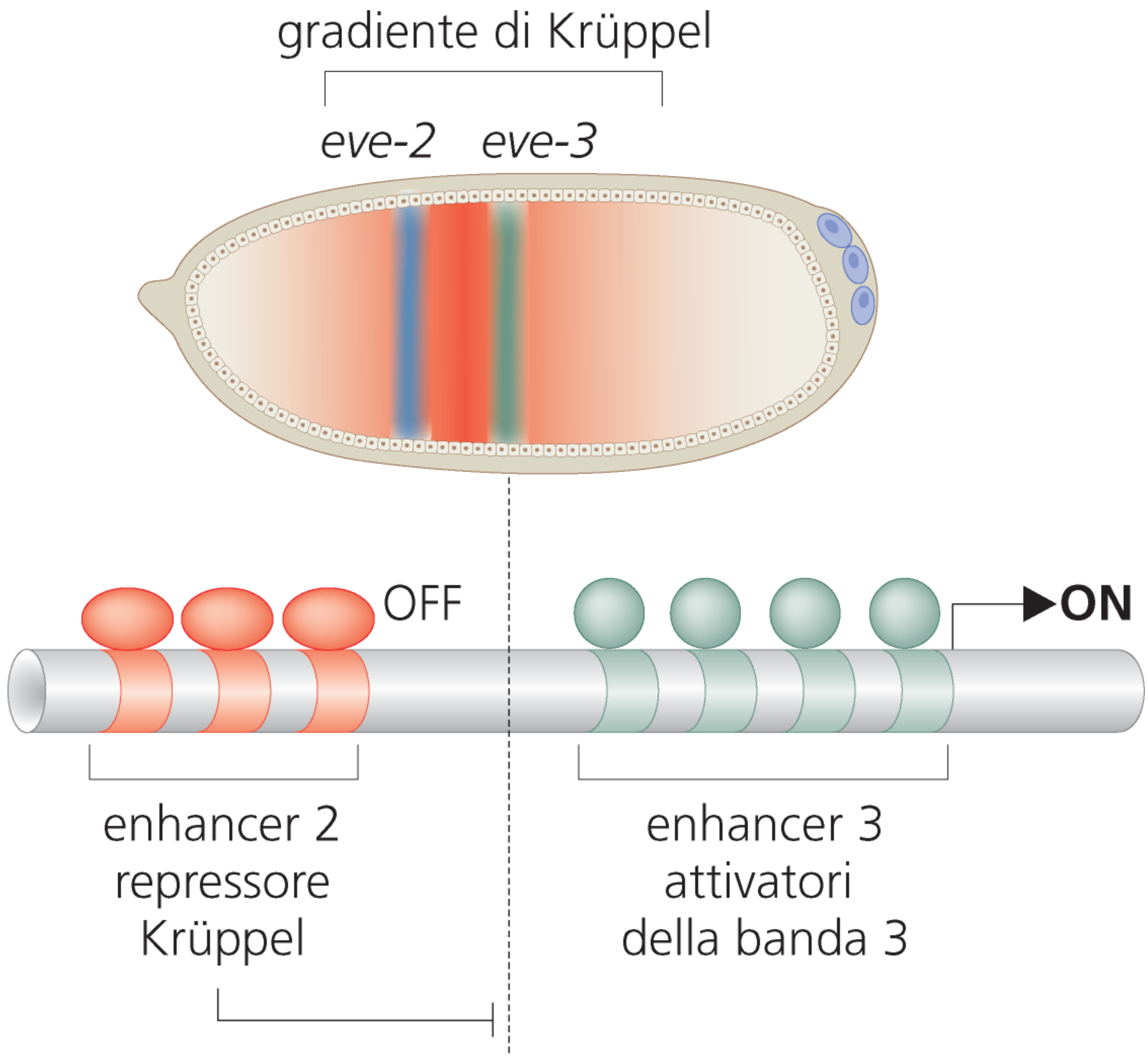
Lesson: the same TF shows different effects on different sites, based on its level of expression

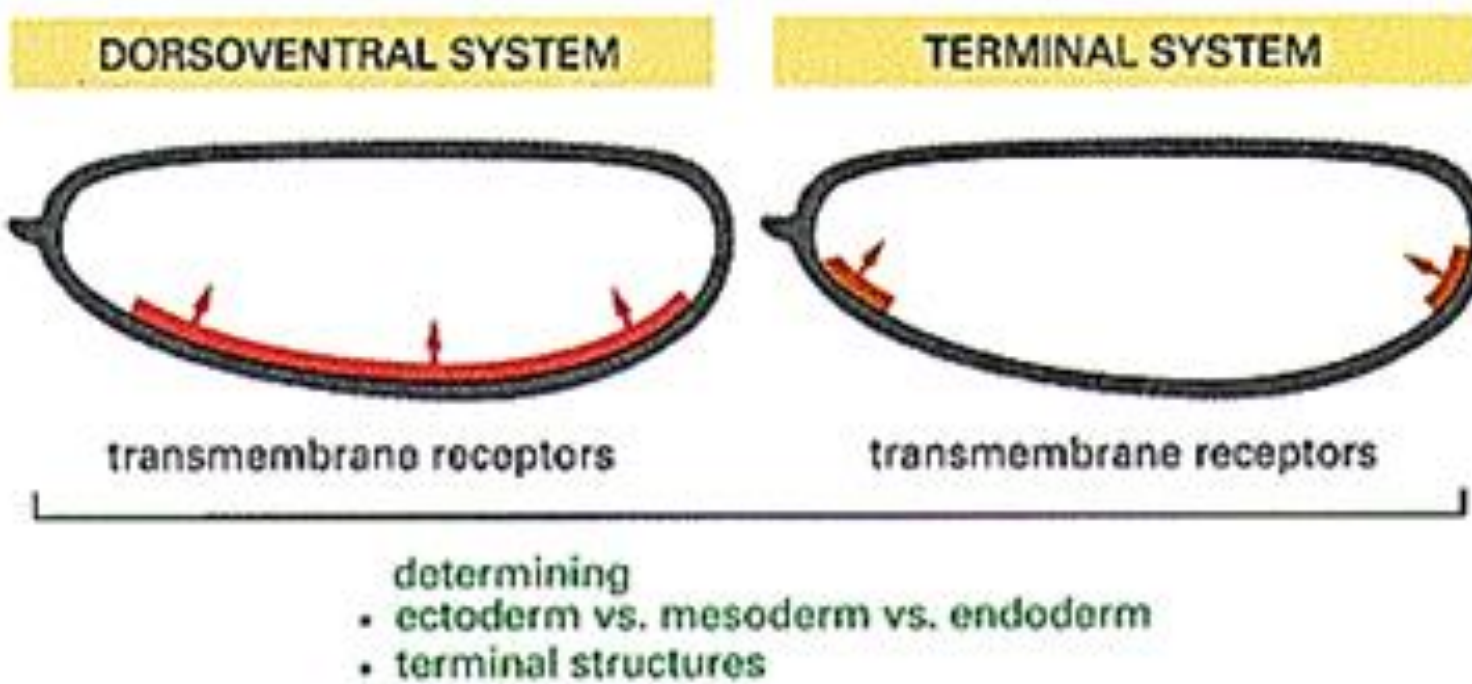


the art of MBoC3


Hunchback
Knirps
siti di legame per Hunchback
siti di legame per Knirps
enhancer della banda 3
eve-3
enhancer della banda 4
eve-4


a
repressori
Krüppel
Giant
attivatori
Bicoid
Hunchback
b
concentrazione delle proteine regolatrici dei geni
la banda 2 di
eve si forma qui
anteriore
posizione lungo l'embrione
posteriore


gradiente di Krüppel
eve-2
eve-3
OFF
ON
enhancer 2
repressore
Krüppel
enhancer 3
attivatori
della banda 3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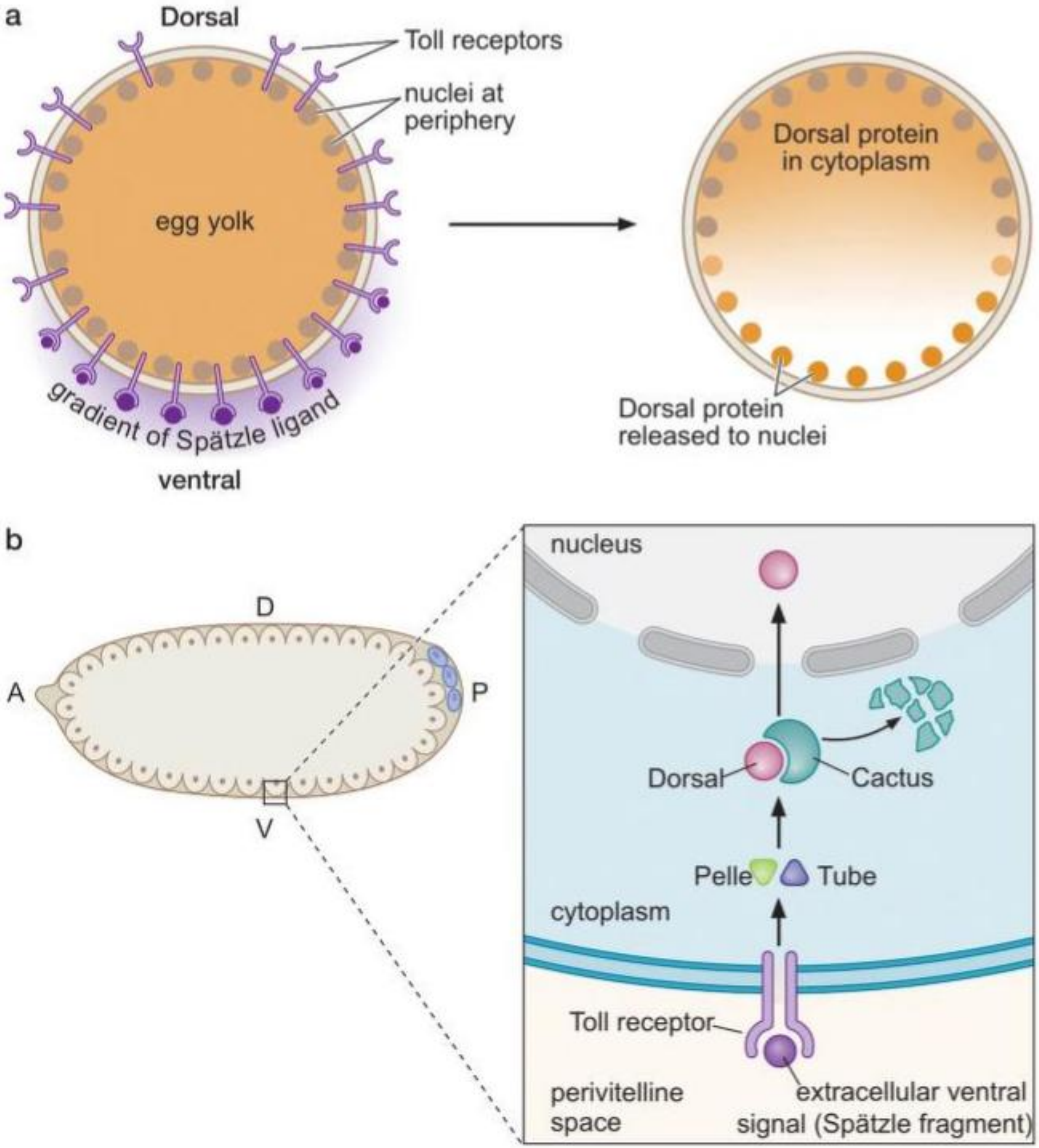
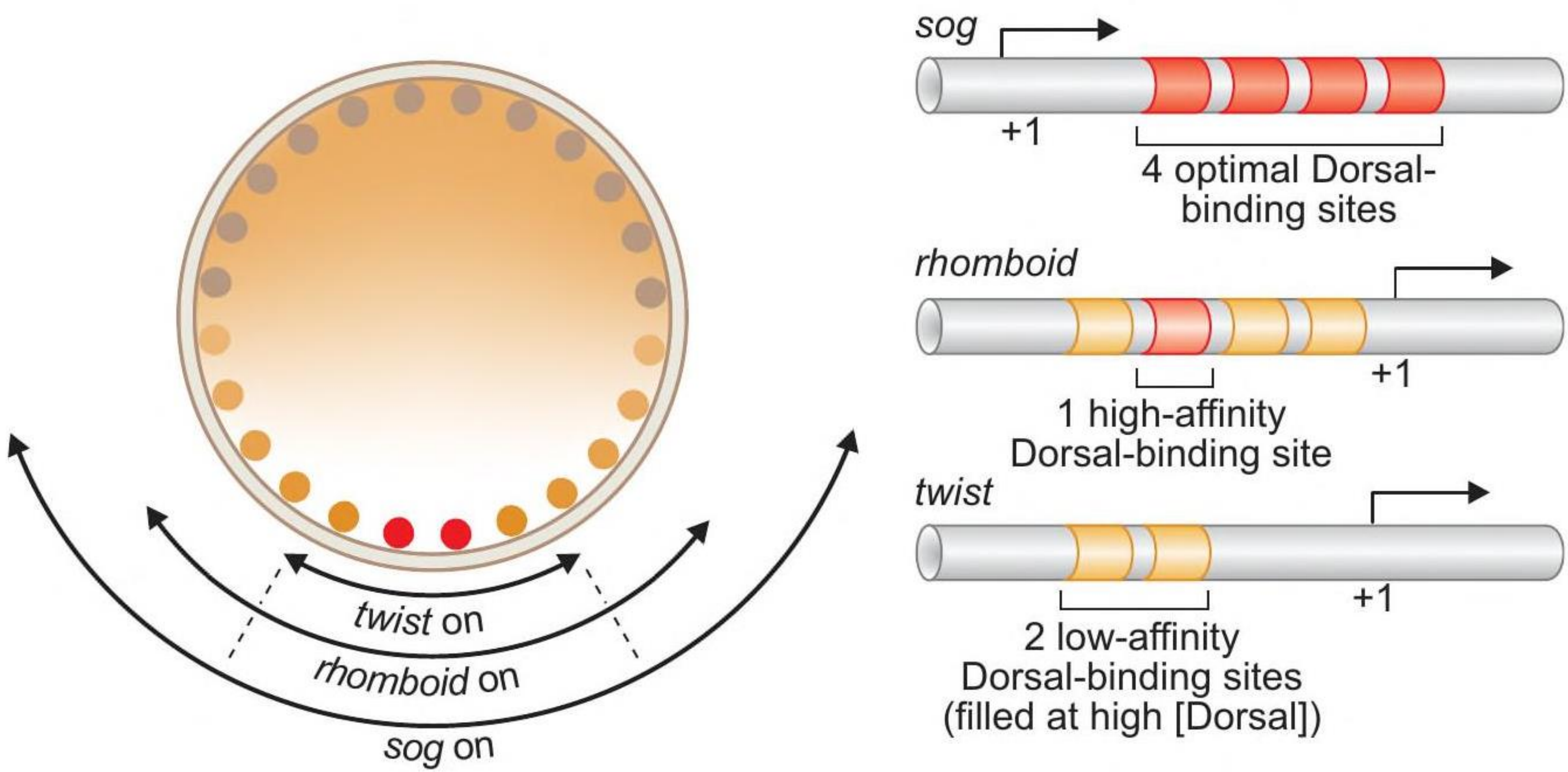
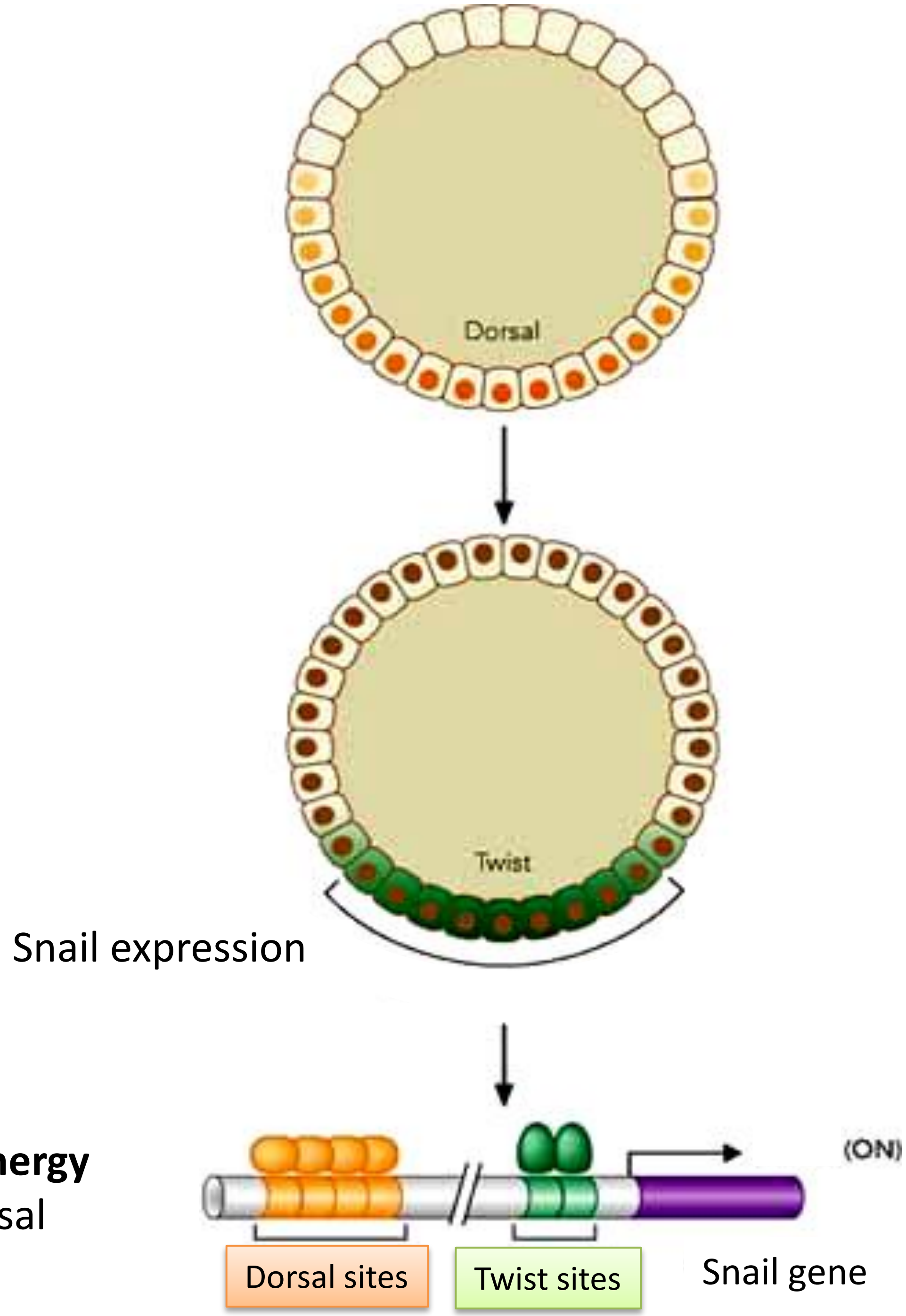


Dorsal is NFkB homologue



Twist 5' contains 2 low affinity sites for Dorsal (bound only were Dorsal is higher)
Rhomboid 5' enhancer cotains several sites: only one is high-affinity: it is on at high or intermediate levels of Dorsal.
Sog intronic enhancer contains 4 high-affinity dorsal sites: **on** in all cells where dorsal is present

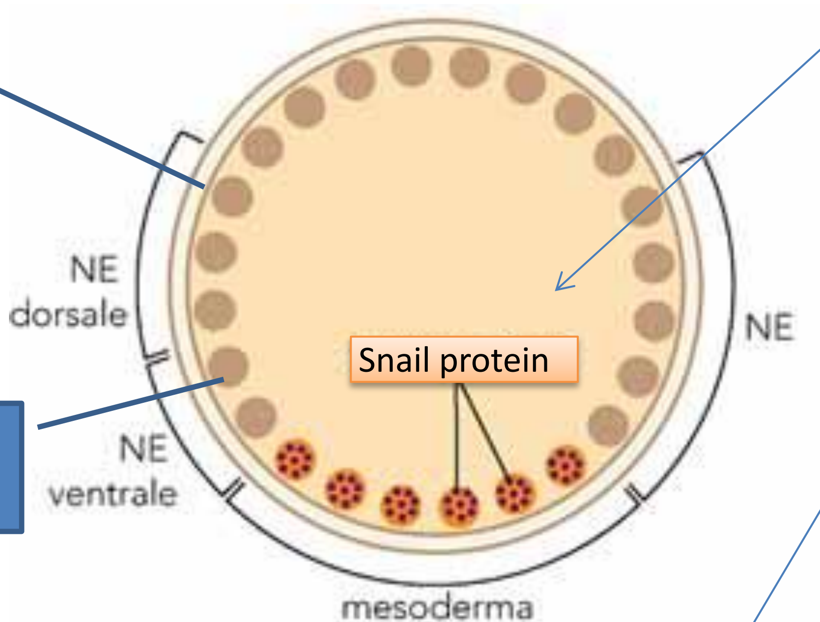


Snail is activated by **synergy** between Twist and dorsal

In ventral cells, twist + dorsal stimulate expression of the Snail repressor:

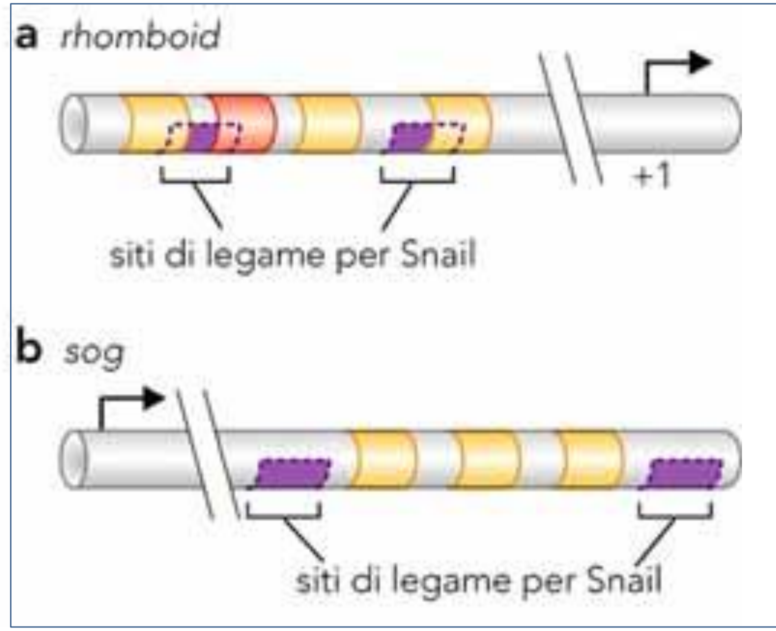
Snail is primary repressor of epithelial genes (e.g. E-cadherin)

Sog here



Snail protein

Romboid here



Snail expression in ventral cells limits expression of romboid and sog, making boundaries of expression sharply defined.

Lesson # 2
TFs act in a **combinatorial** fashion

On enhancers/PREs, TFs function follows these principles:

- combinatorial
- compositional
- cooperative

The principles of how Transcription Factors and Enhancers work that we have learnt in D. melanogaster

are essentially **transferable to higher organisms**

In Mammalian, we observe more TFs, more Enhancers per gene

higher level of complexity

(*indeed, mammalians are more complex than insects*)