

Segment boundary formation in *Drosophila* embryos

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**Camilla W. Larsen, Elizabeth Hirst, Cyrille Alexandre
and Jean Paul Vincent**

1. Introduction:

- Segment boundary formation:

=> subdivision of tissues into **distinct cell populations**

=> establishes sharply delimited domains of action for regulatory **control genes**

=> separation of cells into distinct **functional units**

=> in these functional units **controlled growth** and **patterning** can take place

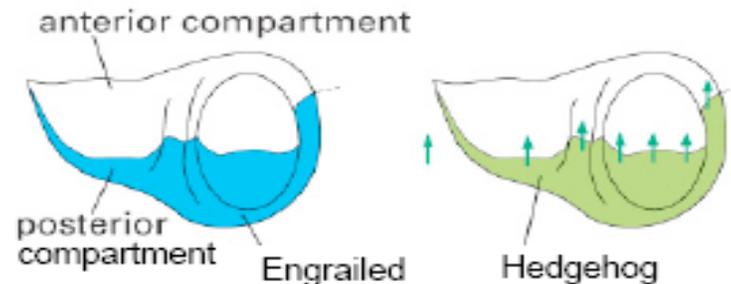
=> Example: AP-boundary in wing discs (discovered 30 years ago):

=> ***engrailed* (en)** expressed in **posterior compartment**

=> Engrailed activates expression of **Hedgehog** (paracrine factor)

=> signals across boundary and renders receiving cells **immiscible** with posterior cells (*dpp*-expression, *sal* and *omb*-activity)

=> maintenance of compartment boundary throughout lifetime by continuous *engrailed* expression



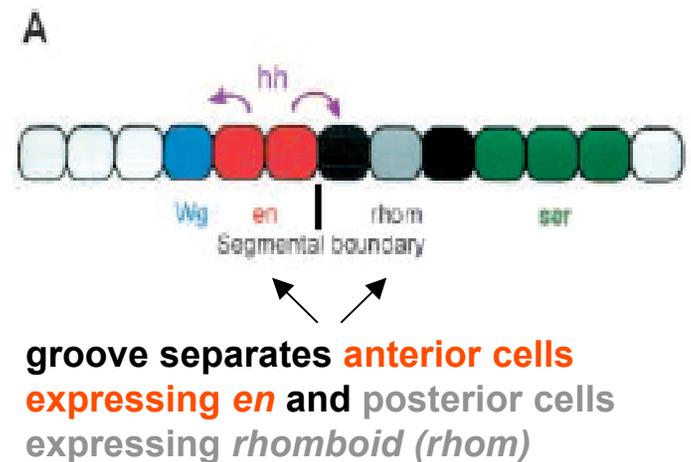
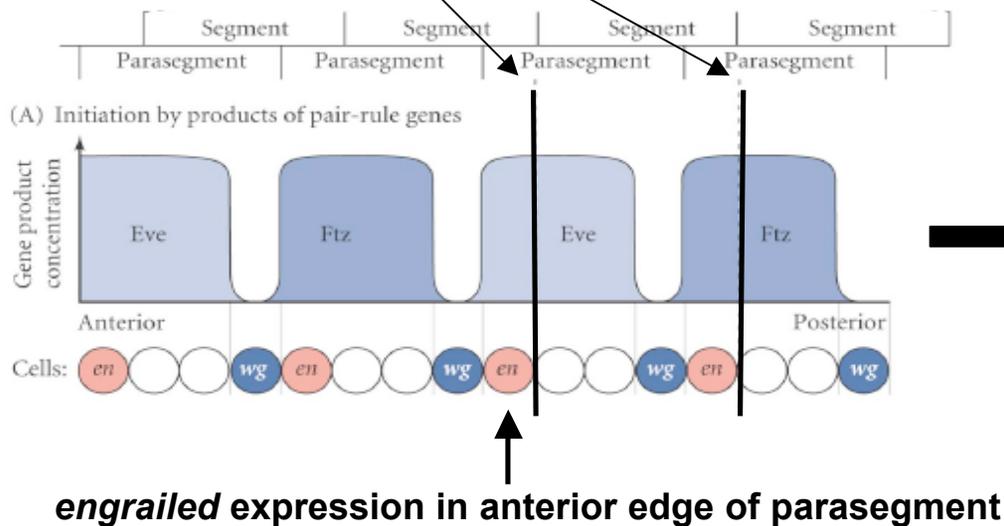
Drosophila embryo represents a system where boundary formation can be studied

=> **genetic** and **morphological** studies possible.

=> embryonic epidermis becomes divided into parasegments.

=> around stage 11: boundary on posterior edge of *en*-stripe forms = segment boundary.

=> **deep grooves in epithelium** mark edge of each segment.



2. Aims

=> to uncover the cell biological basis of segment boundary formation:

1. study of **morphological changes** that accompany segment boundary formation in *Drosophila* embryos
2. study of **genetic requirement** for segment formation in *Drosophila* embryos.

3. Results:

1. Morphological changes during segment formation

Method : Transmission electron microscopy

Visualization of embryonic epidermis: expression of fusion protein under control of *engrailed-Gal4*

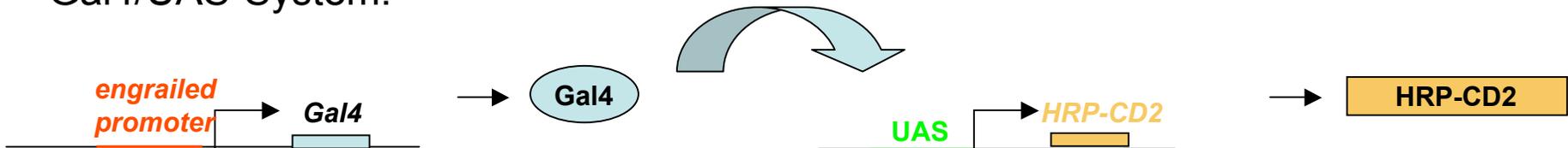
- Wg SP = Wingless signal-peptide

- CD2 = transmembrane molecule

- Horseradish peroxidase (HRP) = marker enzyme; triggers chemical reaction that results in generation of an electron-dense product.



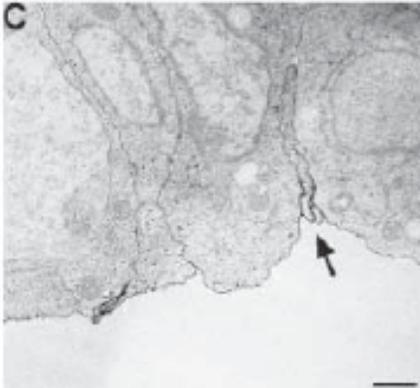
Gal4/UAS-System:



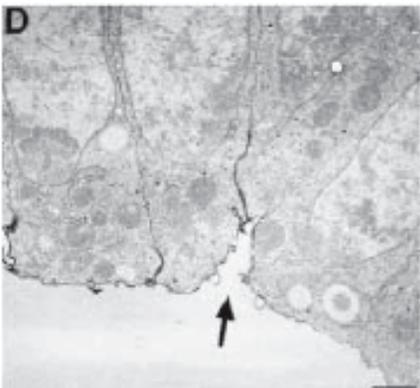
↪ HRP-CD2 produced in *en*-expressing cells; *en*-cells appear darker!

Groove-formation:

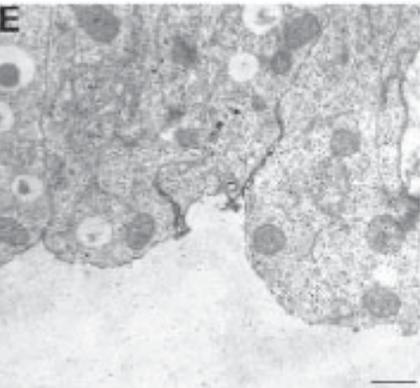
- view on horizontal sections of ventral epidermis, parasegment 9



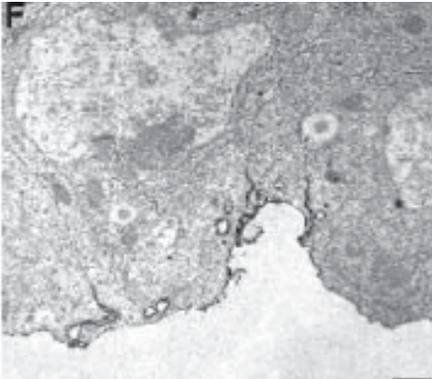
—→ a small dip appears between *en*-expressing and non-expressing cells shortly after germ band retraction.



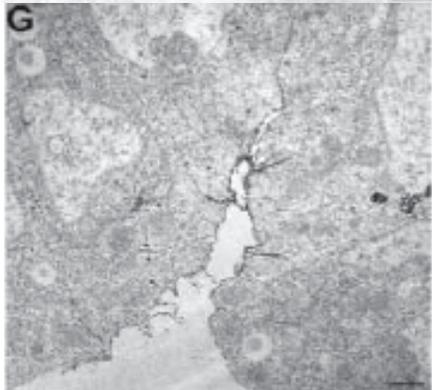
—→ cells loose apical contact and groove forms between them



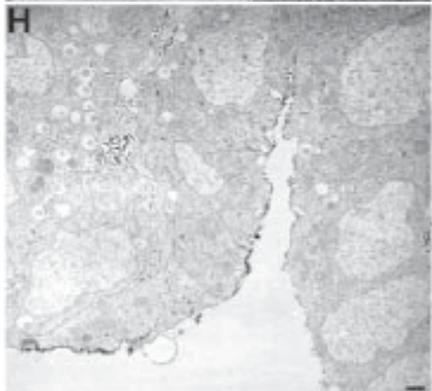
—→ Most posterior *en*-expressing cell constricts apically and moves inwards



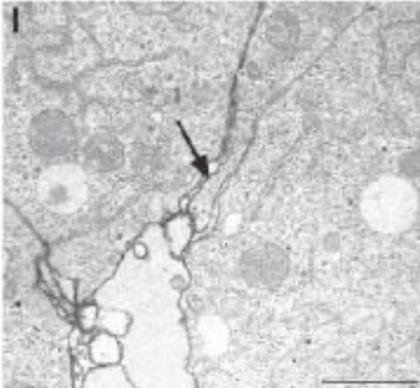
—▶ **this cell stays at bottom of groove, cell becomes bottle shaped** (sea urchins: invagination of ectoderm, amphibians: formation of dorsal blastopore lip)



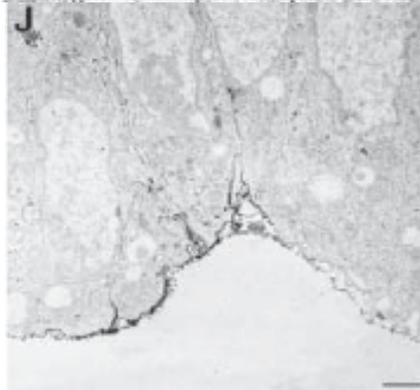
—▶ **more cells move in and groove is getting deeper**



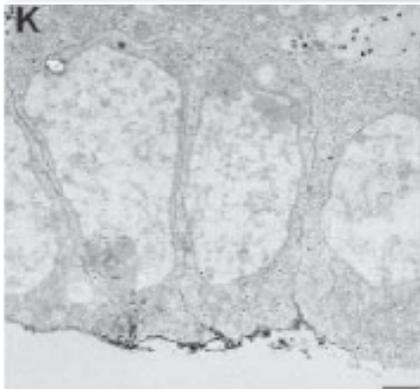
—▶ **groove is at its deepest**



—▶ cell stays at bottom of groove with apex constricted



—▶ at dorsal closure: groove regresses



—▶ groove disappeared (stage 15)

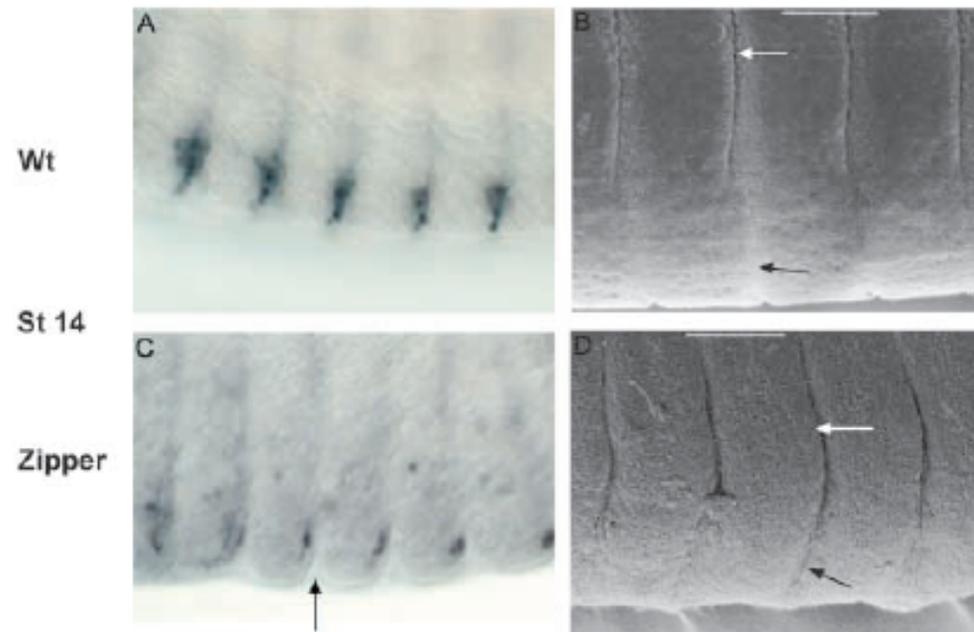
▶ groove formation involves specific **changes in cell contact** between cells forming the groove, **apical constriction** of most posterior *en*-expressing cells, and **Inward migration** of surrounding cells

Correlation between dorsal closure and disappearance of grooves

=> epidermis spreads dorsally to enclose whole embryo

=> need for additional surface area promotes groove regression

—> *zipper*-mutant: defective in dorsal closure



zipper-mutant: **grooves persist longer**
lateral grooves appear to be deeper

=> proof for correlation between dorsal closure and disappearance of grooves!

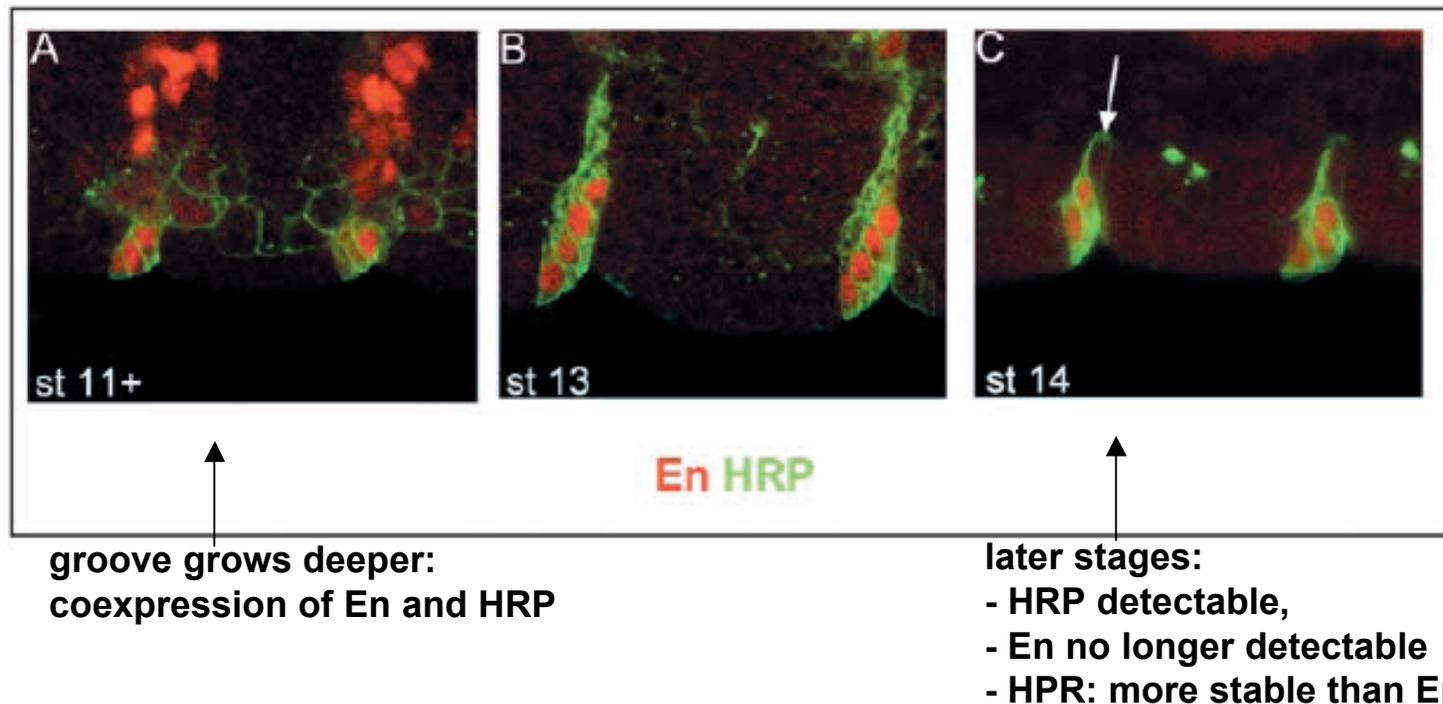
Results morphological studies:

- 1. Groove formation starts shortly after embryo retraction**
- 2. *engrailed*-expressing cell contracts apically, moves inwards and becomes bottle shaped**
- 3. surrounding cells move inwards, groove deepens**
- 4. Grooves regress at dorsal closure**

2. Genetic requirements for boundary formation

Posterior *en*-expressing cells cease to express *engrailed* in later stages

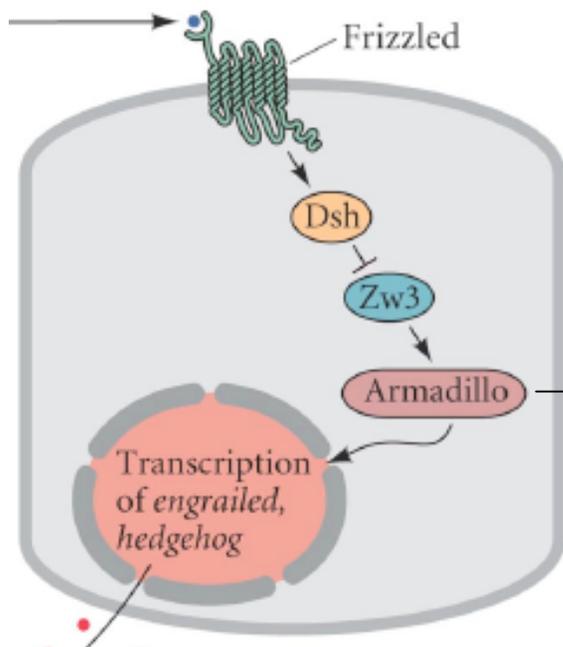
- as groove is deepest: *en*-expression ceases
- embryos expressing HRP-CD2 under control of *engrailed-Gal4* were stained for HRP (green) and Engrailed protein (red).



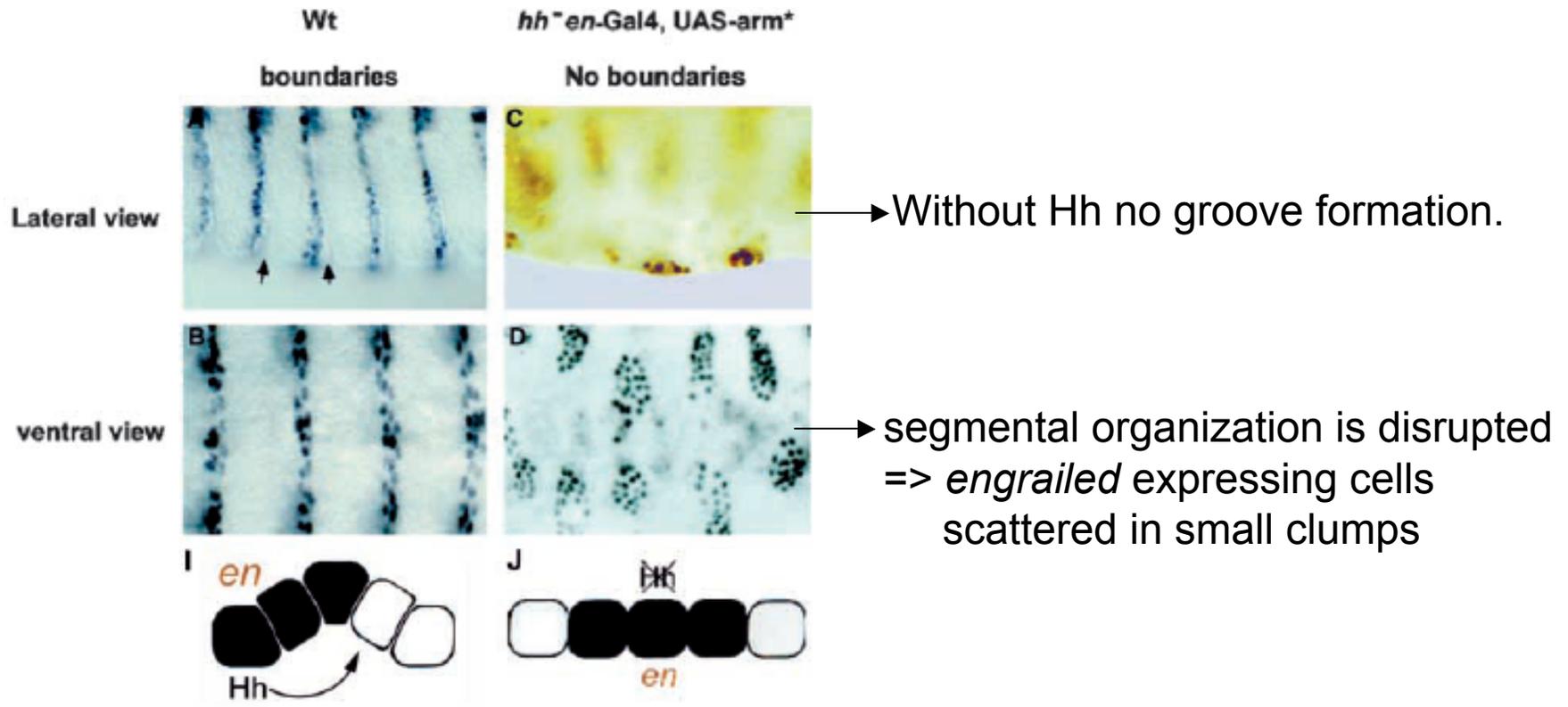
➔ most posterior *en*-cell undergoes morphological changes and after that ceases to express En.

Segment boundary formation requires Hedgehog signalling

- boundaries do not form in *engrailed* and *hedgehog* mutant embryos.
- expression of *engrailed* and *hedgehog* are interdependent.
- to investigate Hedgehog's contributions: **Hedgehog null-mutant**

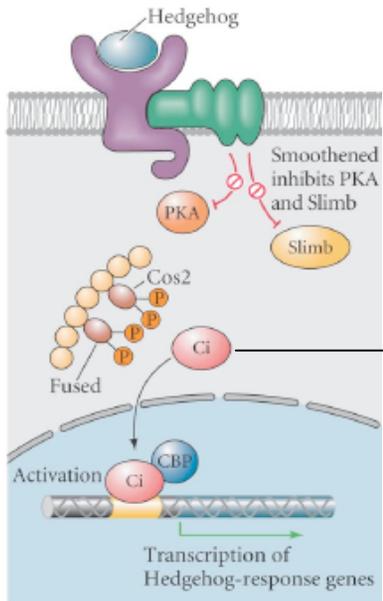


To maintain *engrailed*-expression: activated form of Armadillo was expressed under control of *engrailed-Gal4*.



=> Hedgehog signalling is required for segment boundary formation and maintenance segmental organization.

Which member of Hh signalling cascade is important for groove formation?

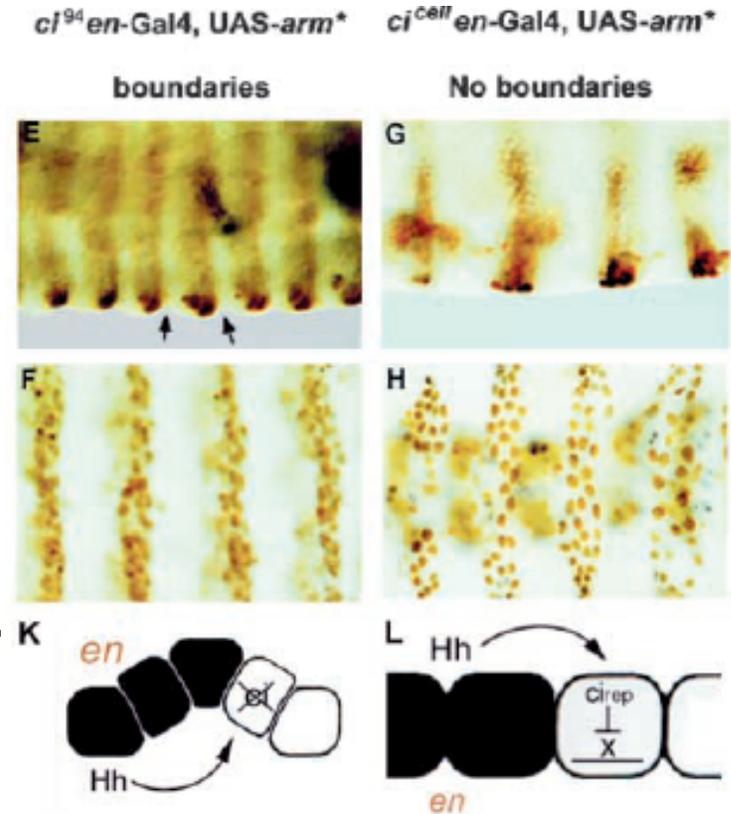


signalling by Hh is mediated by Cubitus interruptus (Ci)
Hh absent: Ci gets cleaved and acts as transcription repressor.
Hh present: Ci acts as a transcription activator

=> is Ci crucial for Hh signalling in groove formation?

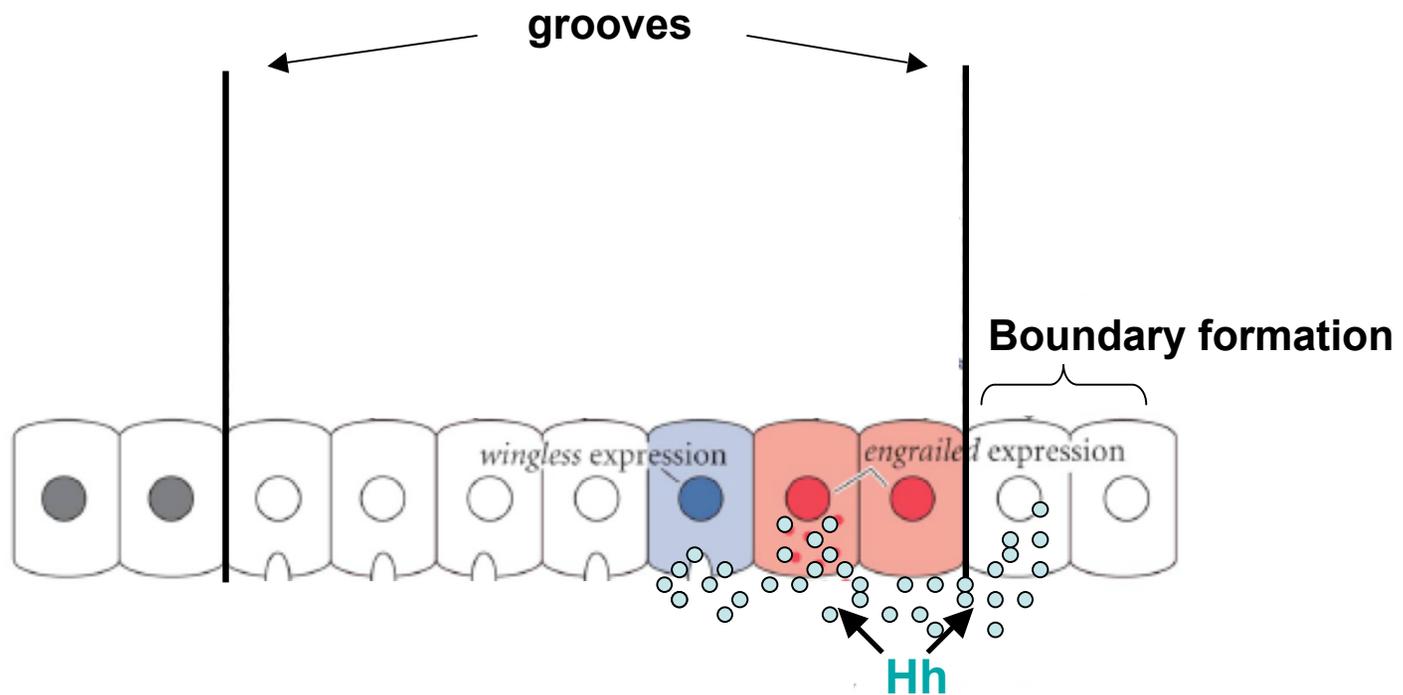
=> **embryos mutant for *ci***
allele1: no Ci produced
allele2: only repressor Ci produced

=> **target of Ci is required for boundary formation.**
In absence of Hh signalling expression of target is repressed.

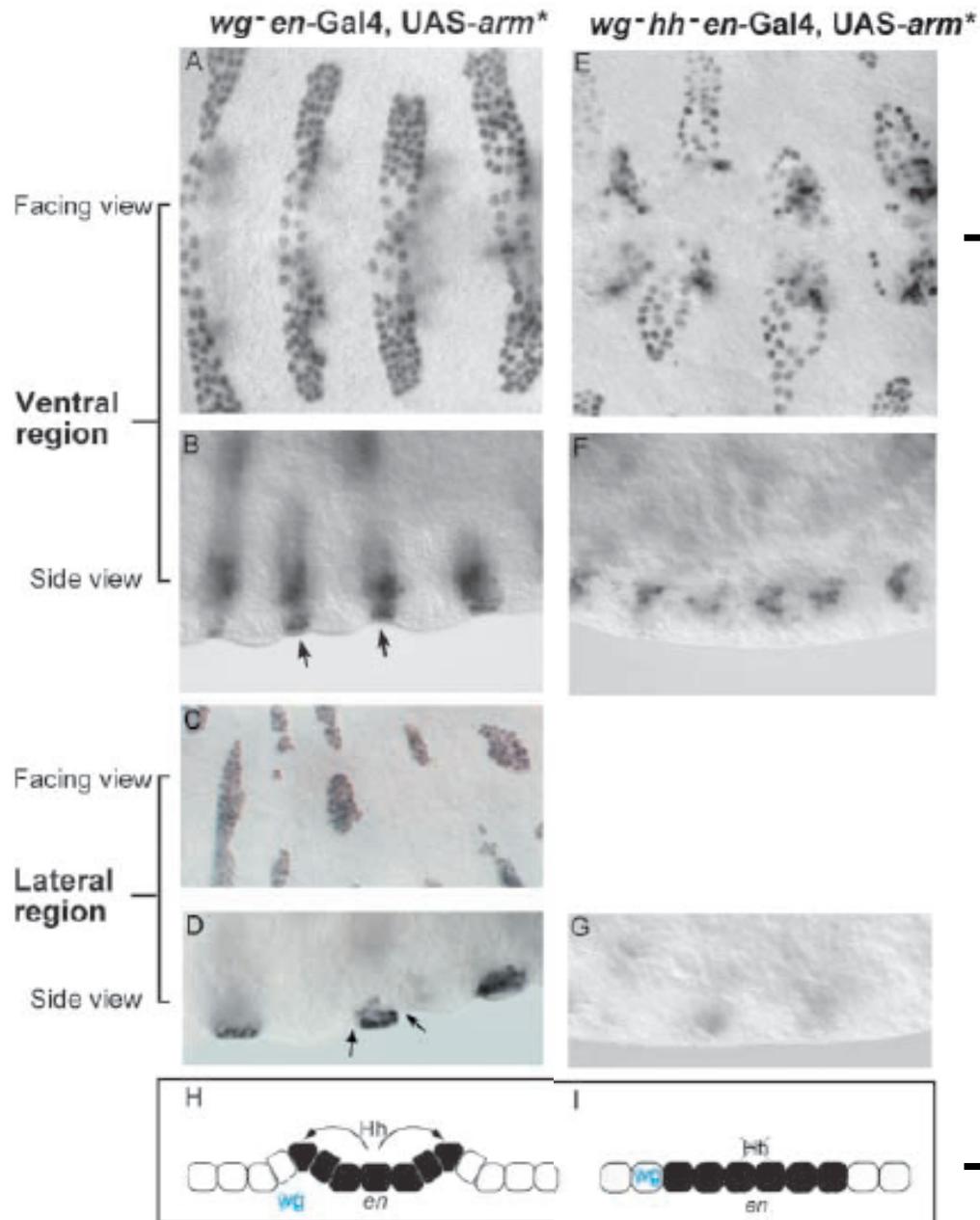


Wingless signalling inhibits segmental boundary formation

- Hh signals to cells located to the anterior and posterior of *engrailed*-expressing compartment
- segment boundaries only form at the posterior.
- => Wingless could prevent boundary formation there by interfering with Hh signalling



wingless-mutant: Will grooves duplicate?



→ groove formation due to Hh signalling

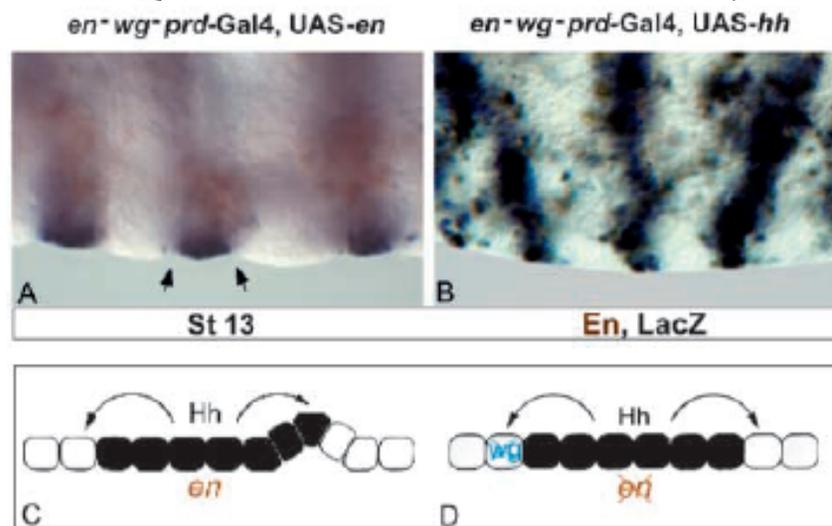
→ Wingless prohibits groove formation anterior of *engrailed*-expressing cells!

Engrailed is crucial for groove formation

rescue experiments:

exogenous *UAS/Gal4-en*-expression
in *en*-mutant embryo

exogenous *hh*-expression



=> Engrailed could contribute to segment boundary formation by regulating the expression of one or several effector genes

Hedgehog and Engrailed are continuously required in groove maintenance

- embryos transgenic for: ***wingless- engrailed- paired-Gal4 UAS-engrailed***

- ventral **grooves disappear prematurely** before stage 13

- lateral grooves are maintained until stage 14

- *paired-Gal4* driver: expression decays at stage 12 ventrally
expression decays at stage 14 laterally

=> expression of *engrailed* and *hedgehog* ceases

=> grooves disappear

=> these two genes could be continuously required throughout lifetime of groove

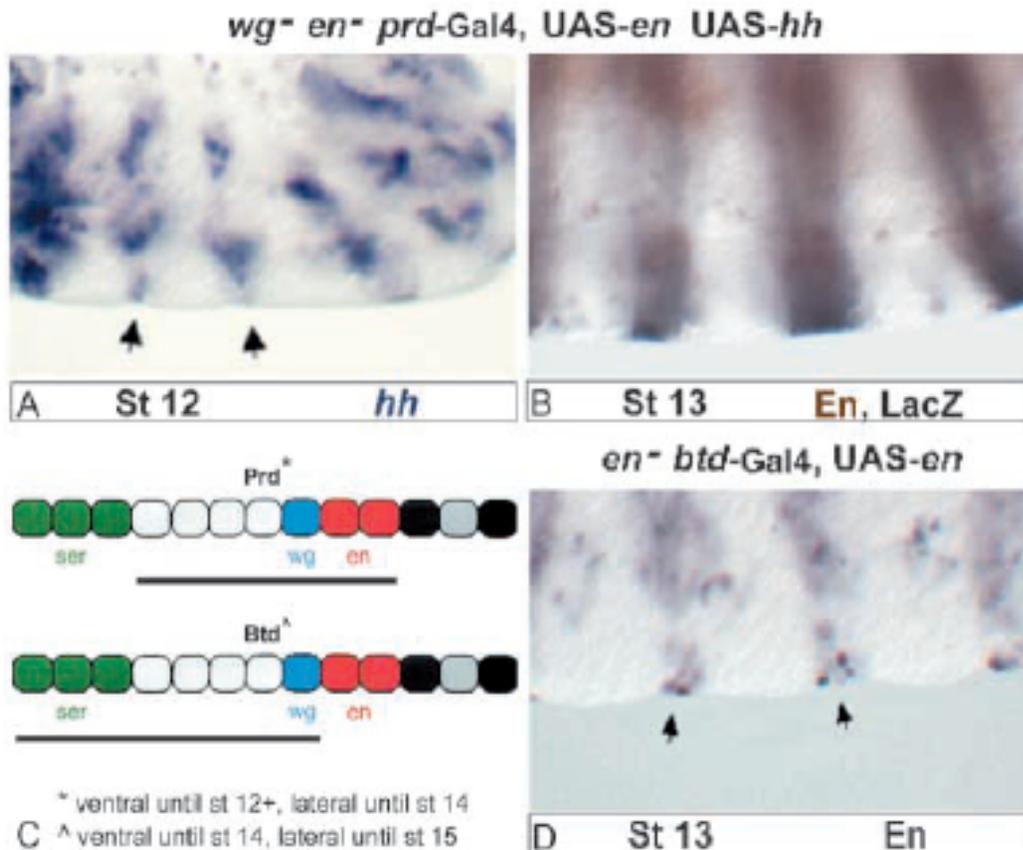
→ What will happen if *engrailed* expression is driven a different temporal pattern?

- *wingless- engrailed- paired-Gal4 UAS-engrailed*

- *wingless- engrailed- buttonhead-Gal4 UAS-engrailed*

=> *engrailed* is ventrally expressed **beyond stage 14**

=> grooves do **not disappear prematurely**



continuous expression of *engrailed* and *hedgehog* is required for groove maintenance

Results of genetic requirement studies:

- 1. Engrailed is crucial for groove formation and maintenance.**
- 2. Without Hedgehog signalling no groove formation and maintenance takes place.**
- 3. Wingless prohibits segment boundary formation anterior to *engrailed* expressing cell.**

4. Discussion

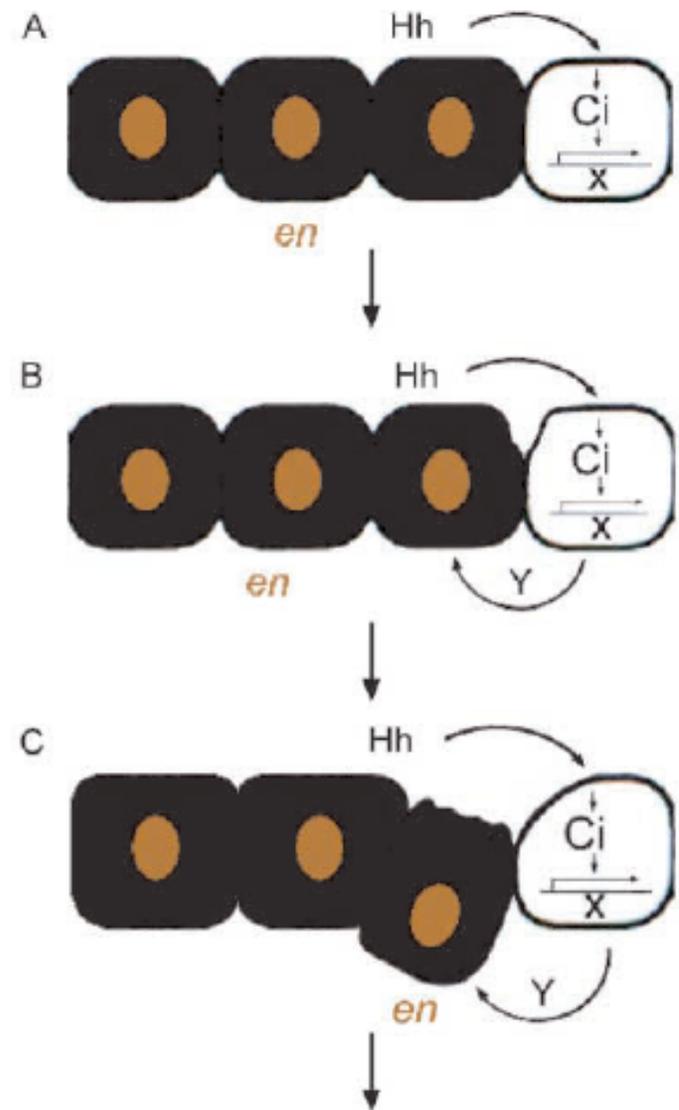
- Morphological changes that accompany groove formation were described
- Two key genetic requirements were identified:
 - 1. presence of Engrailed at anterior segment boundary**
 - 2. Hedgehog signalling at posterior segment boundary**

Morphological changes accompany segmental groove formation

1. Groove formation: initiated by Hedgehog signalling
Hh signalling prevents repression by Ci
=> expression of gene(s) x.

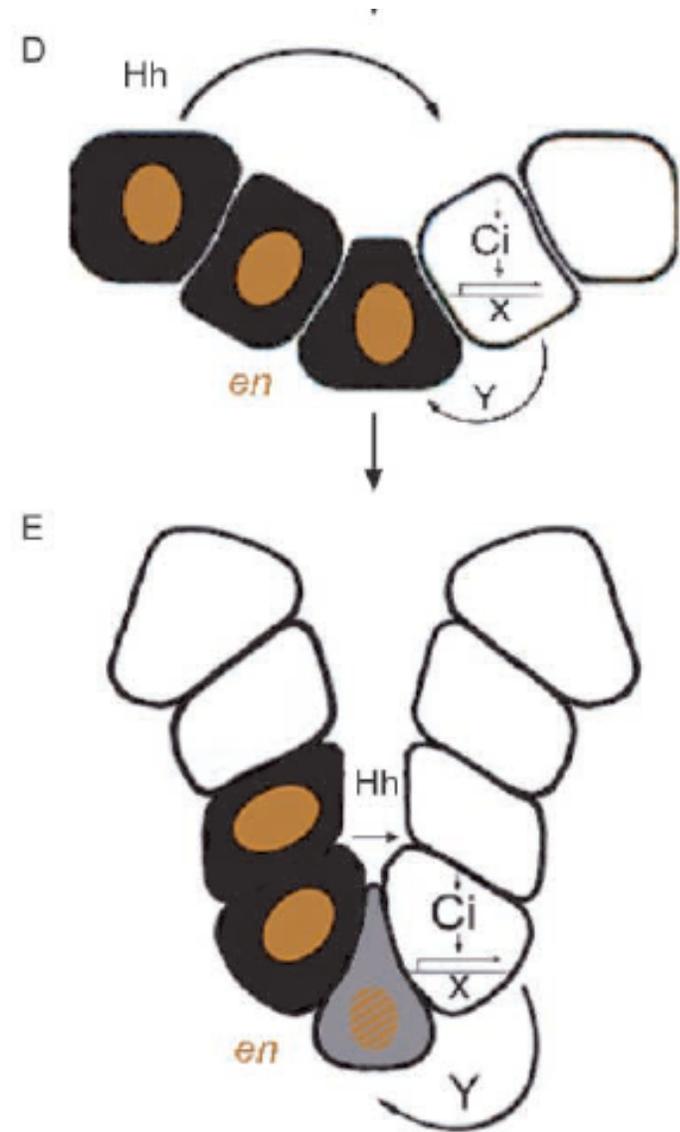
2. Groove founding cells loose contact on their apical side
An unknown signal (Y) feeds back on *engrailed*-
expressing cells.

3. Most posterior engrailed-expressing cell constricts
apically and moves inward.



4. Most posterior *engrailed*-expressing cell lies at bottom of forming groove.

5. Groove reaches deepest point. Most posterior *engrailed*-expressing cell acquires bottle shape. *engrailed*-expression is turned off.



Genetic requirements for groove formation

- **Engrailed and Hedgehog** have to be present
- ***engrailed*-expressing cell**: must receive a **signal** that induces the **morphological changes** (e.g. paracrine or contact dependant-signalling)
- **Activator form of Ci** is not required for boundary formation
=> boundary formation requires expression of a gene x that is repressed by Ci^{rep} but does not require Ci^{act} to be activated.
Hedgehog present: Ci no longer in repressor form => x expressed
- Segment boundaries only form **posterior to *engrailed*-expressing cell**
=> **Wingless represses boundary formation** at the anterior (disrupts Hh signalling)
- Engrailed target genes are still unknown