



Heat shock produces periodic somitic disturbances in the zebrafish embryo

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Abstract

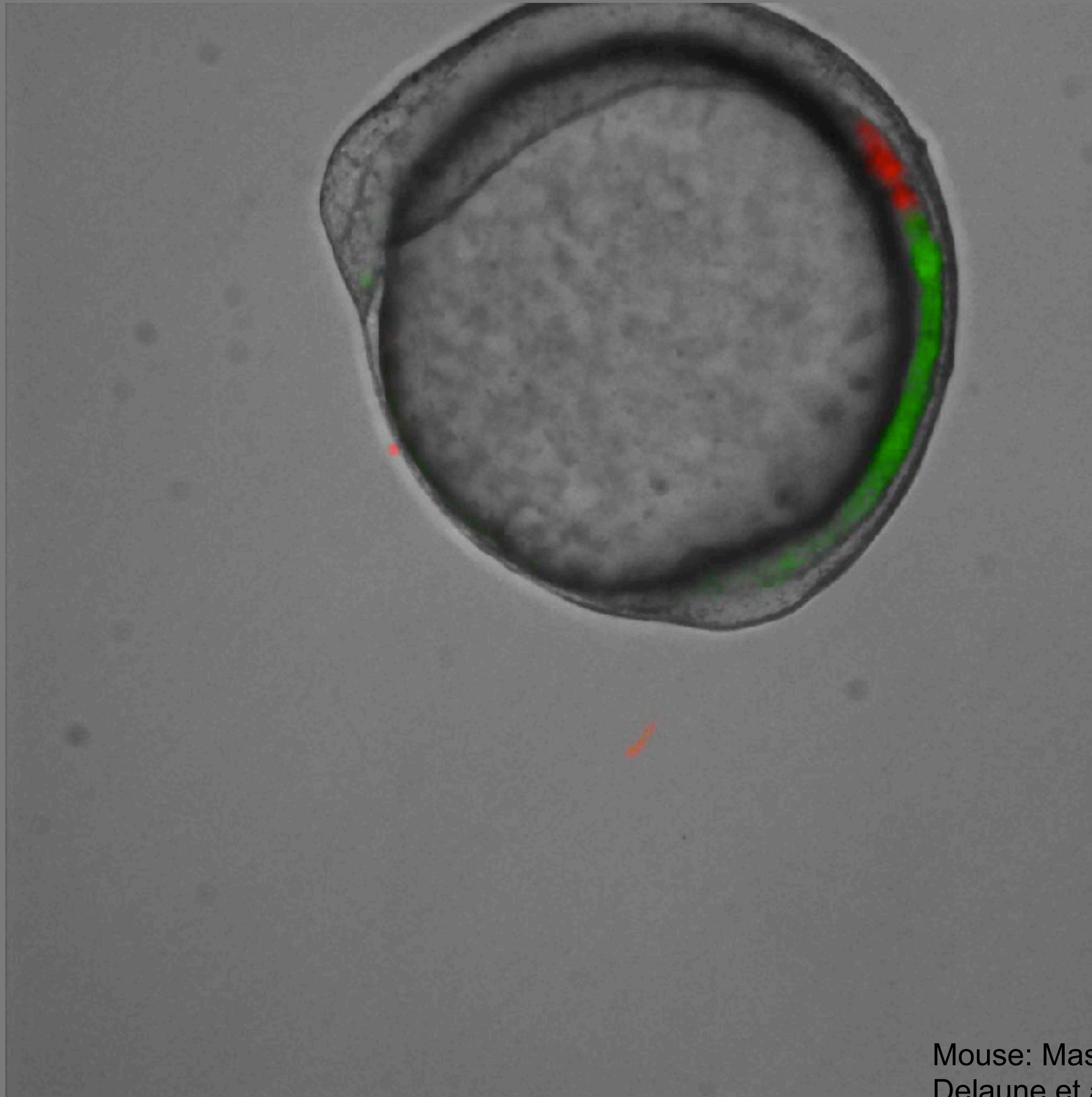
Environmental influences are known to produce segmental defects in a variety of organisms. In this paper we report upon segmental aberrations produced by brief heat shocks delivered to developing zebrafish embryos. The initial defects in the segmental pattern of somitic boundaries and motoneuron axon outgrowth were usually observed five somites caudal to the somite which was forming at the time of heat shock application. Segmental defects in zebrafish embryos exposed to a single heat shock treatment can occur in a periodic pattern similar to the multiple disturbances observed to occur in chick embryos. These data are discussed with regard to models involving cell cycle synchrony or ‘clock and wavefront’ schemes in the process of somitogenesis. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Zebrafish; Somitogenesis; Heat shock; Segmentation; Motoneurons

Body segments: added rhythmically and sequentially



A “segmentation clock” ticks in the Pre-Somitic Mesoderm



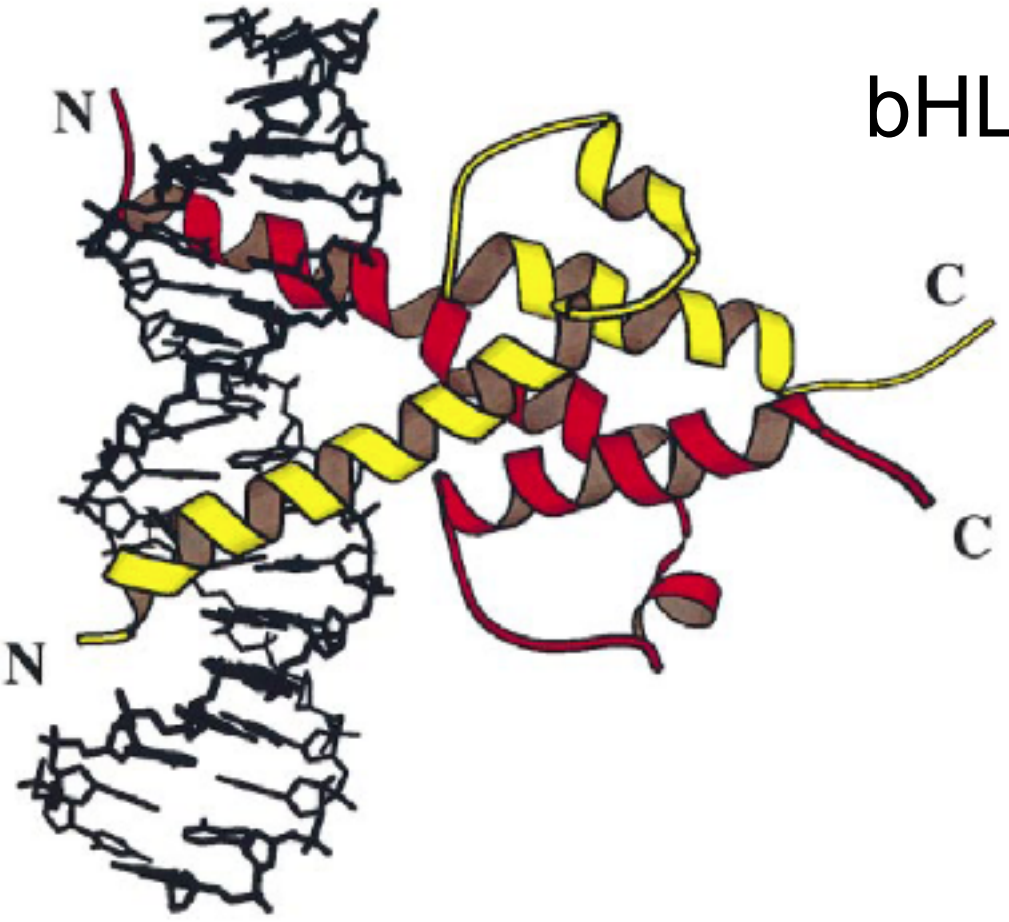
Palmeirim et al., 1997

cyclic reporter gene
her1-YFP/mespb-mKate2

Daniele Soroldoni

Mouse: Masamizu et al., 2006; Aulehla et al., 2008. Zebrafish:
Delaune et al., 2012; Soroldoni et al., 2014

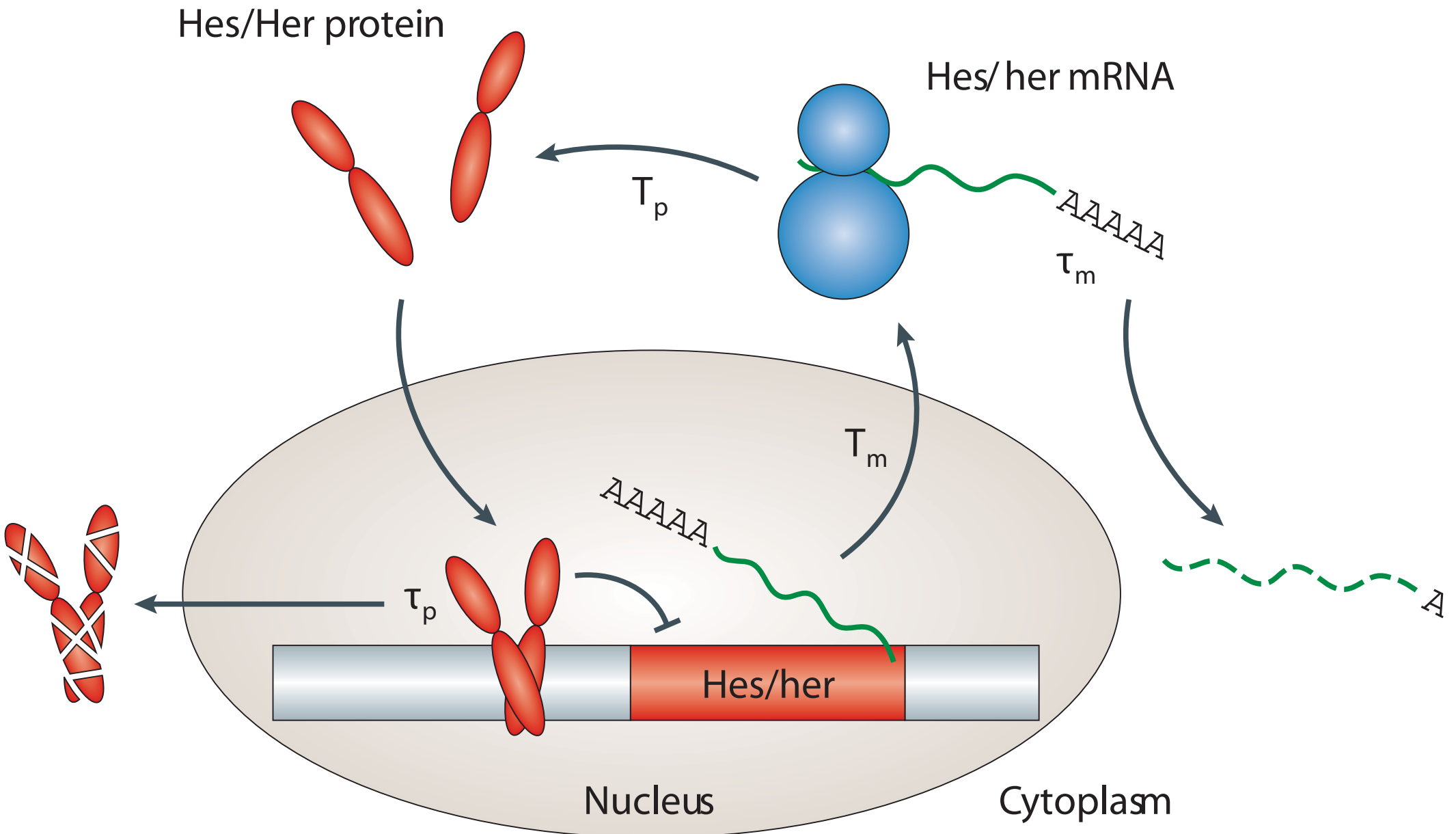
A model cellular oscillator - transcription translation cycle



Chick - *c-hairy1*

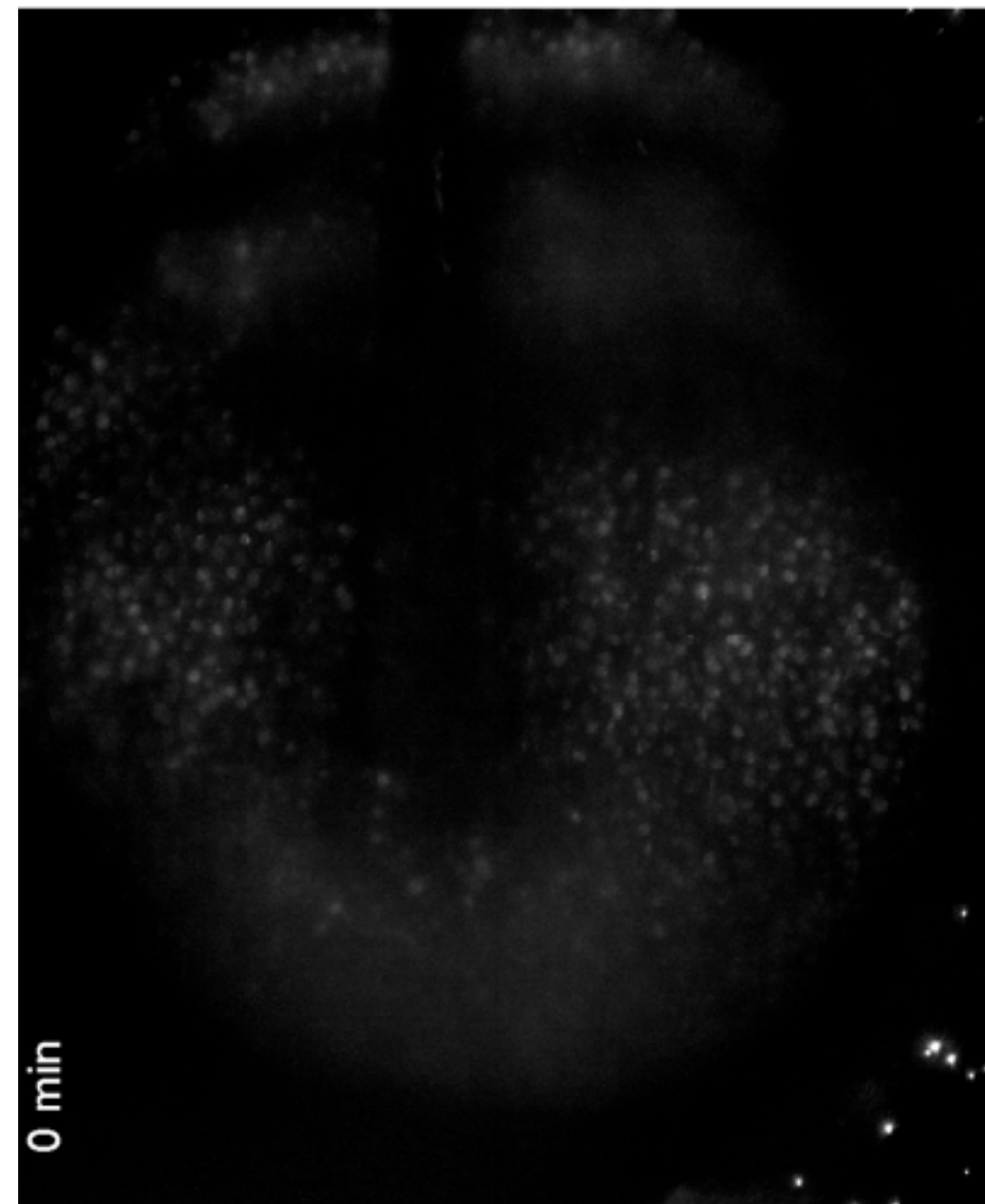
Mouse - *hes7*

Zebrafish - *her1*

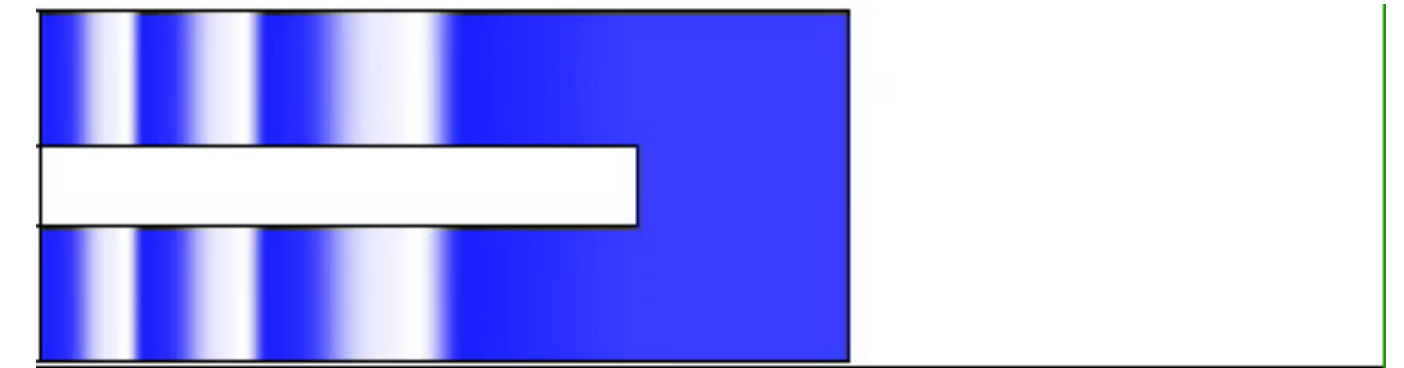


Shimizu et al., 1997; Oates and Ho, 2002; Lewis, 2003; Oates et al., 2009

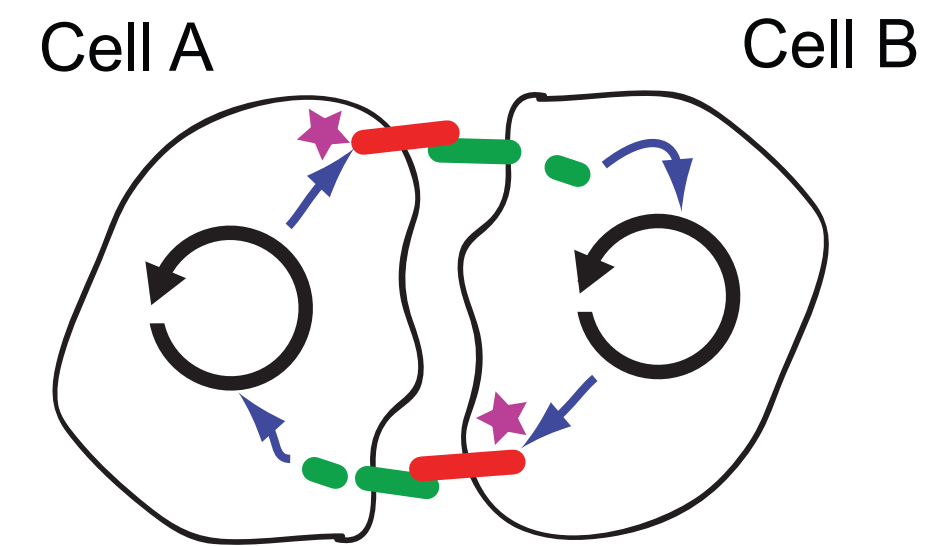
The segmentation clock is a tissue-level, rhythmic pattern generator



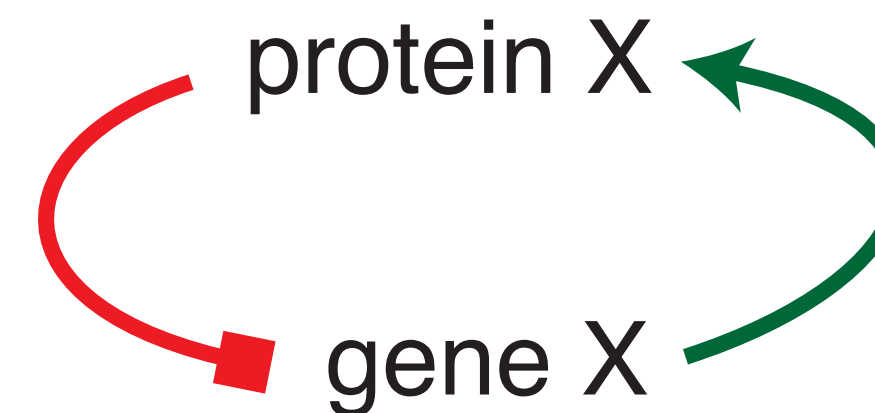
Tissue-level wave patterns



Local synchronization



Cellular oscillators





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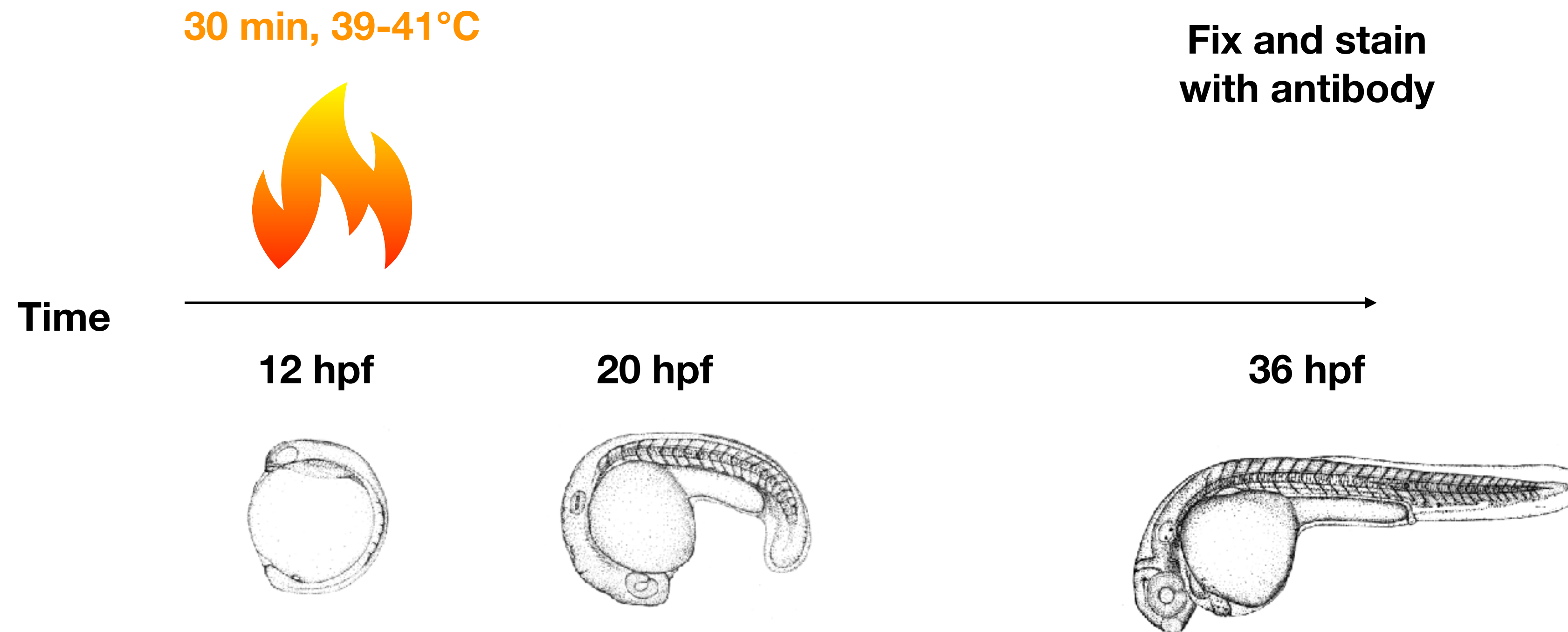
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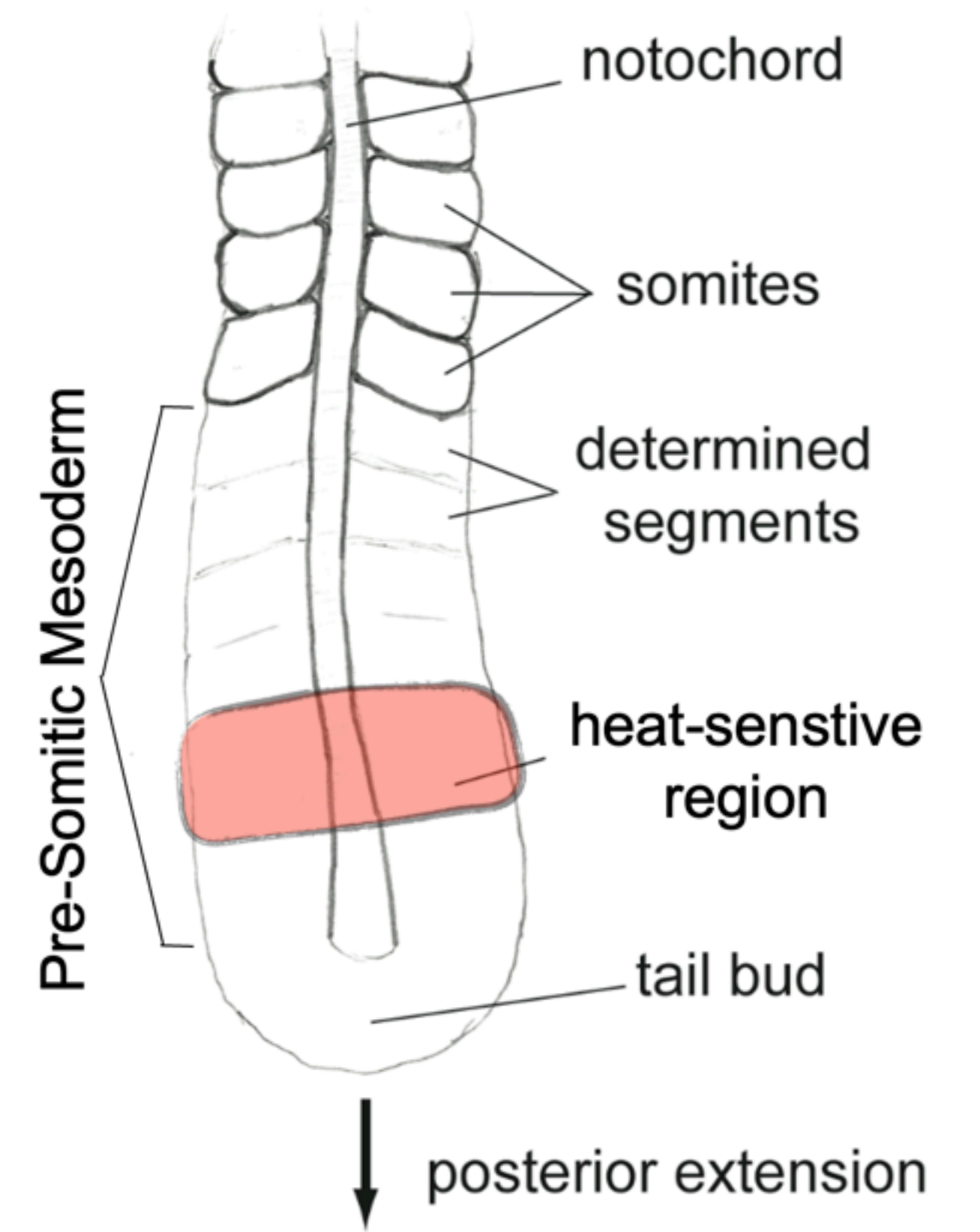
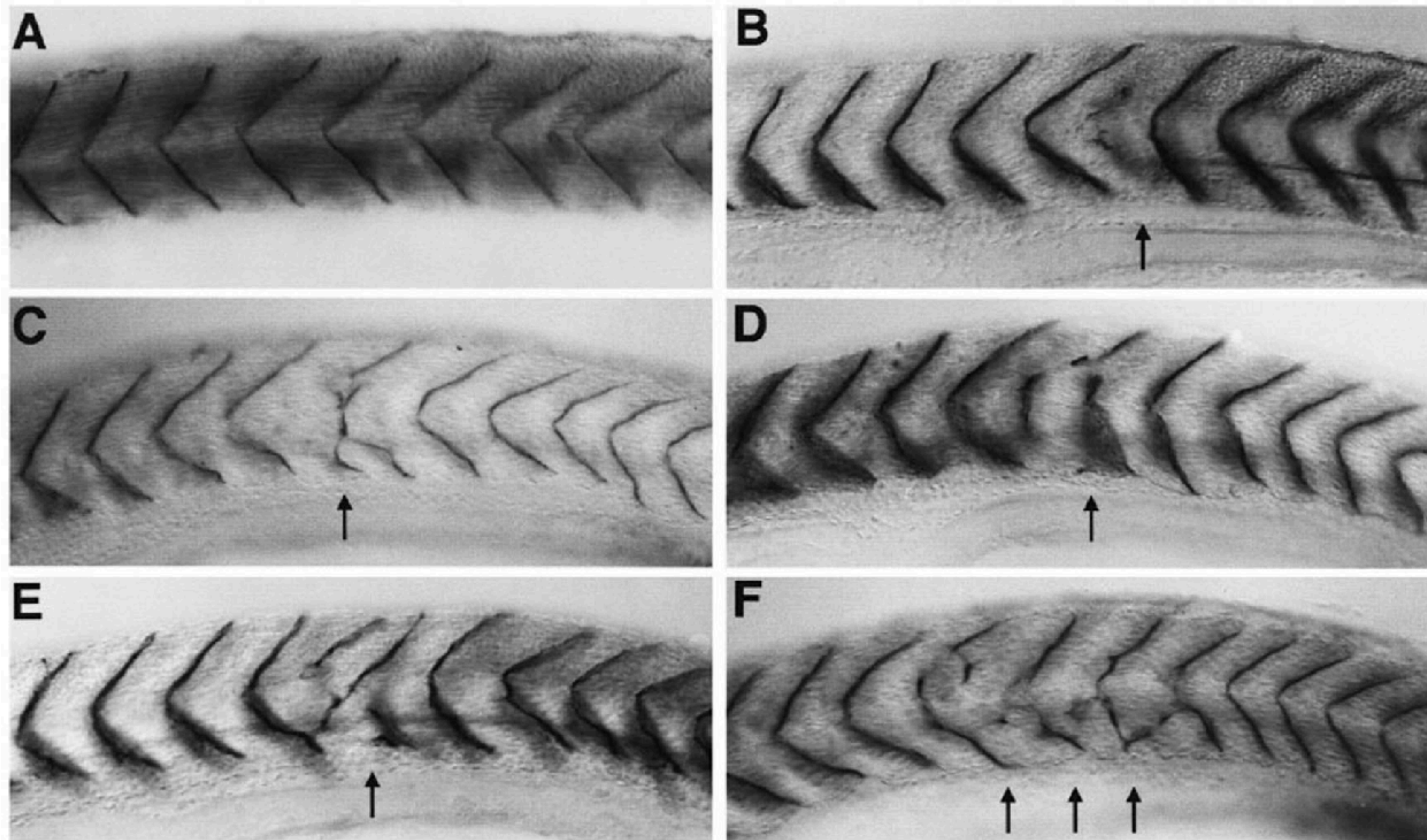
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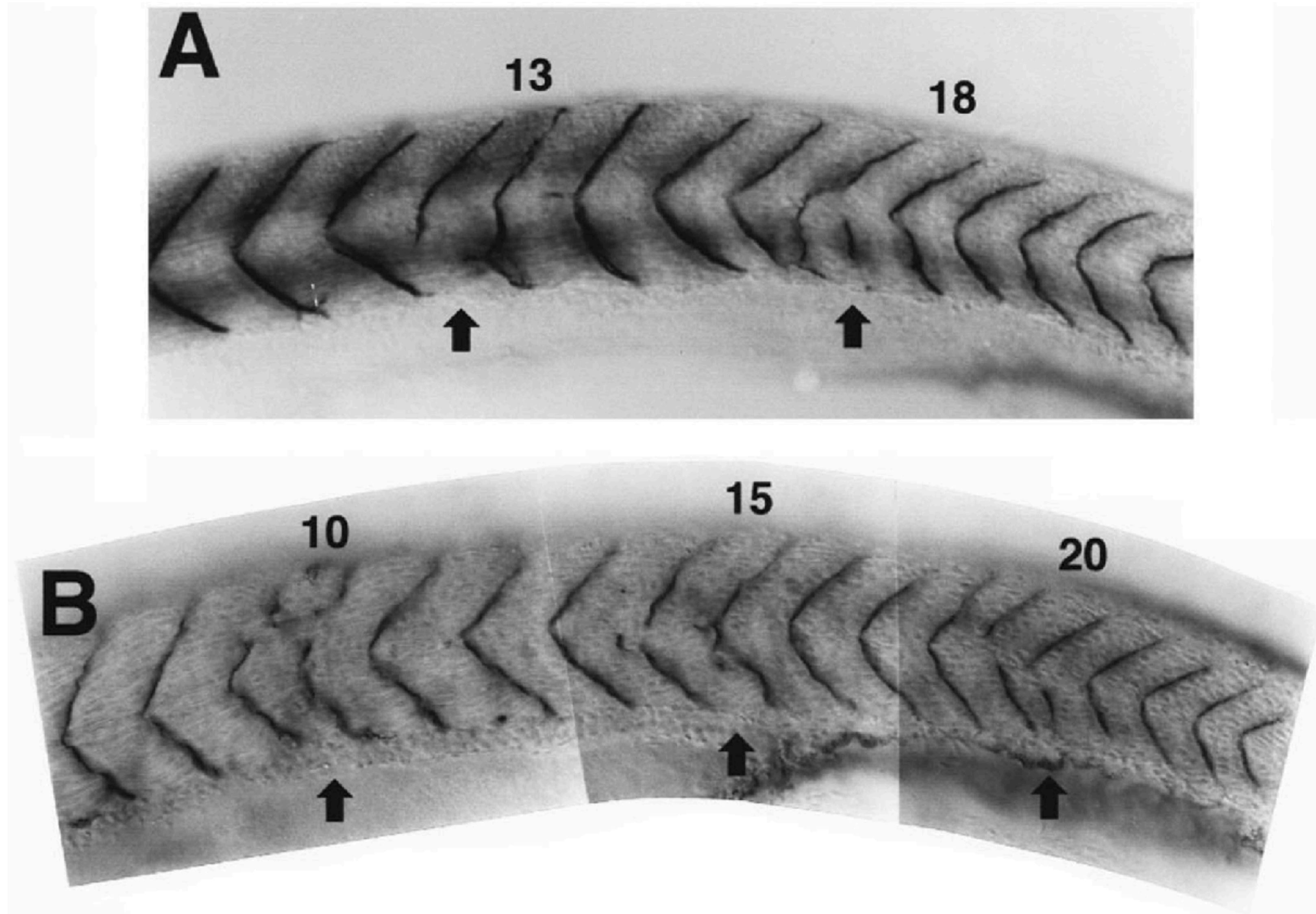
Experimental outline



The heat-shock effect

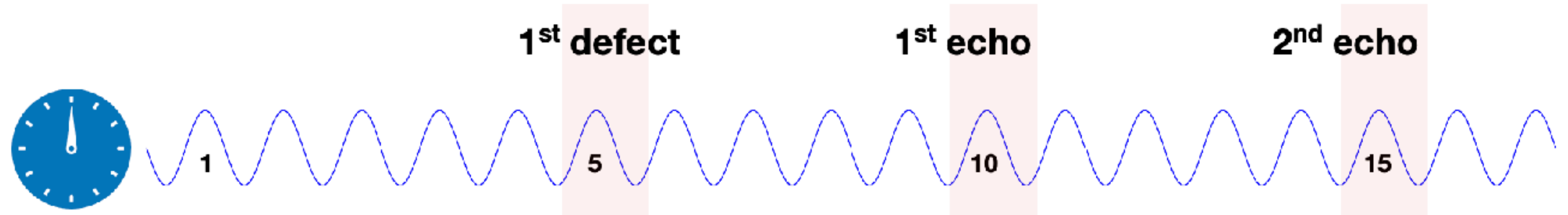


Ok, that's crazy

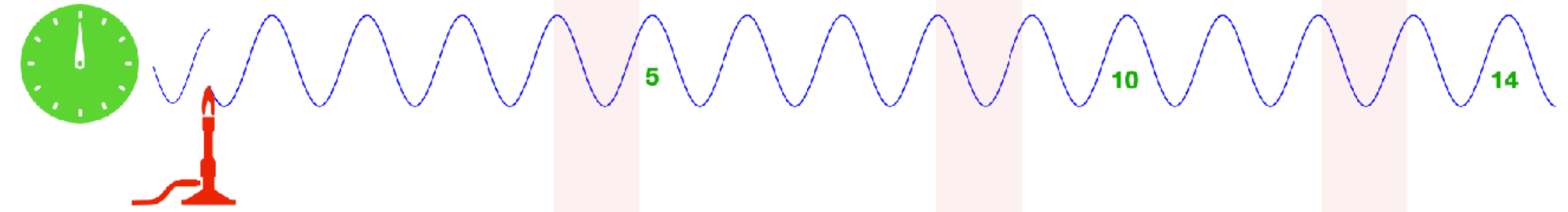


Models for the echo

Unperturbed
segmentation
rhythm



Interference from
internal
periodic process



Disruption by
external
periodic process



Disruption by
independent
processes



What's going on?

- Experiments done before cyclic genes were discovered in zebrafish
- We have some techniques in the lab
- Be precise in your question
- Be ambitious
- Think of the right controls
- Be realistic

Tools and approaches



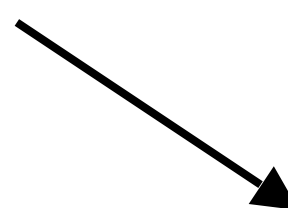
- **Microscopy** - measure period and morphology of somite formation
- **Transgenes** - measure period and pattern
 - Her1-YFP clock
 - Mesp_b - boundary cells,
 - DND - mature segment boundaries
- **Mutant** backgrounds - clock, synchronization
 - *her1;her7, deltaC ...*
- **Drugs** - signal gradients, synchronization
 - Inhibit Fgf, Notch, **Heat Shock Proteins** ...
 - Plus, whatever else you can think of -



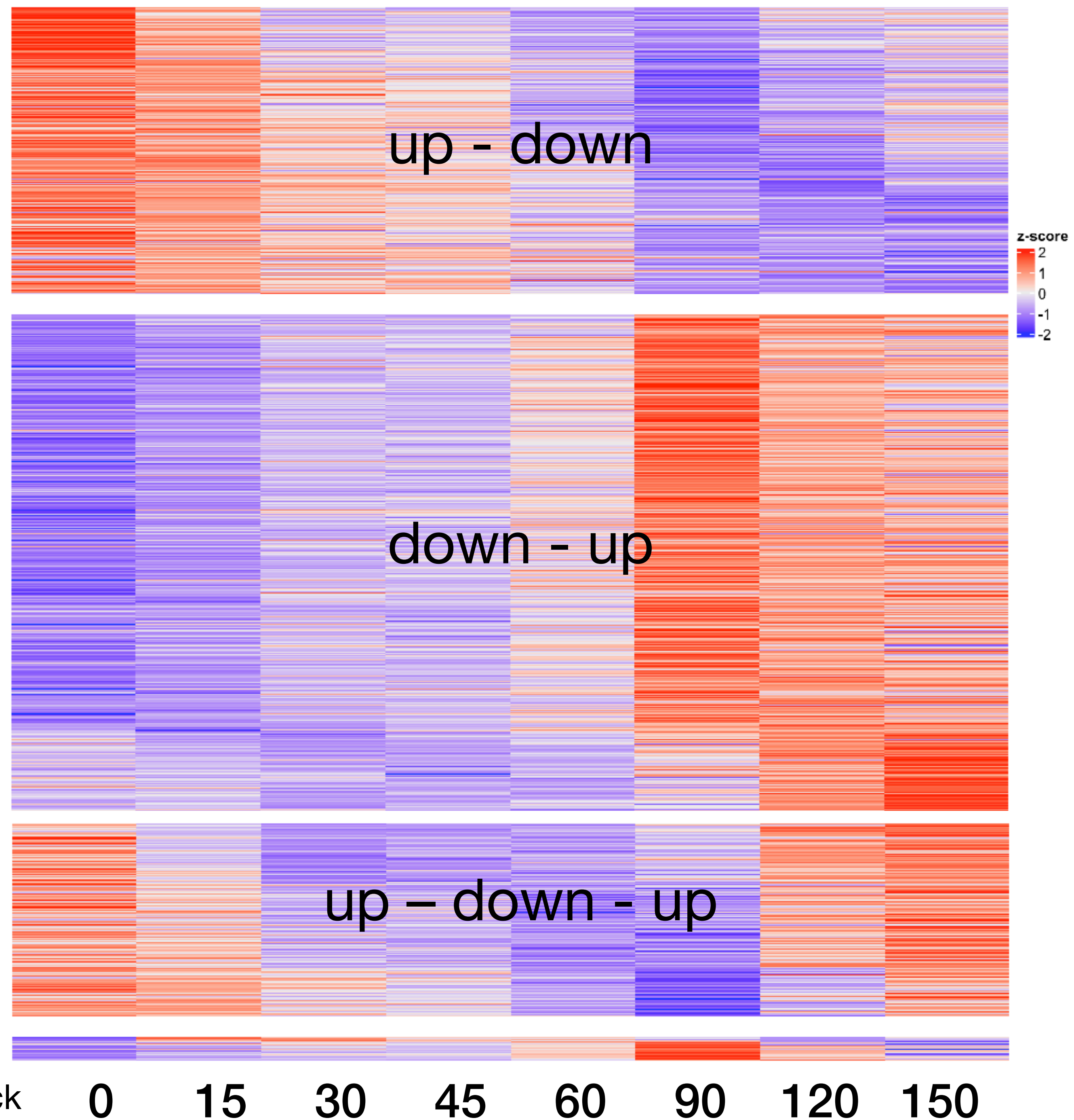
Differential gene expression time series after heat shock

- Bulk sequencing
- Dissected tail up to 3 latest somites
- 8 time points over 2.5 hours
- 10,897 genes read over entire timeseries
- 1971 genes affected by heat shock

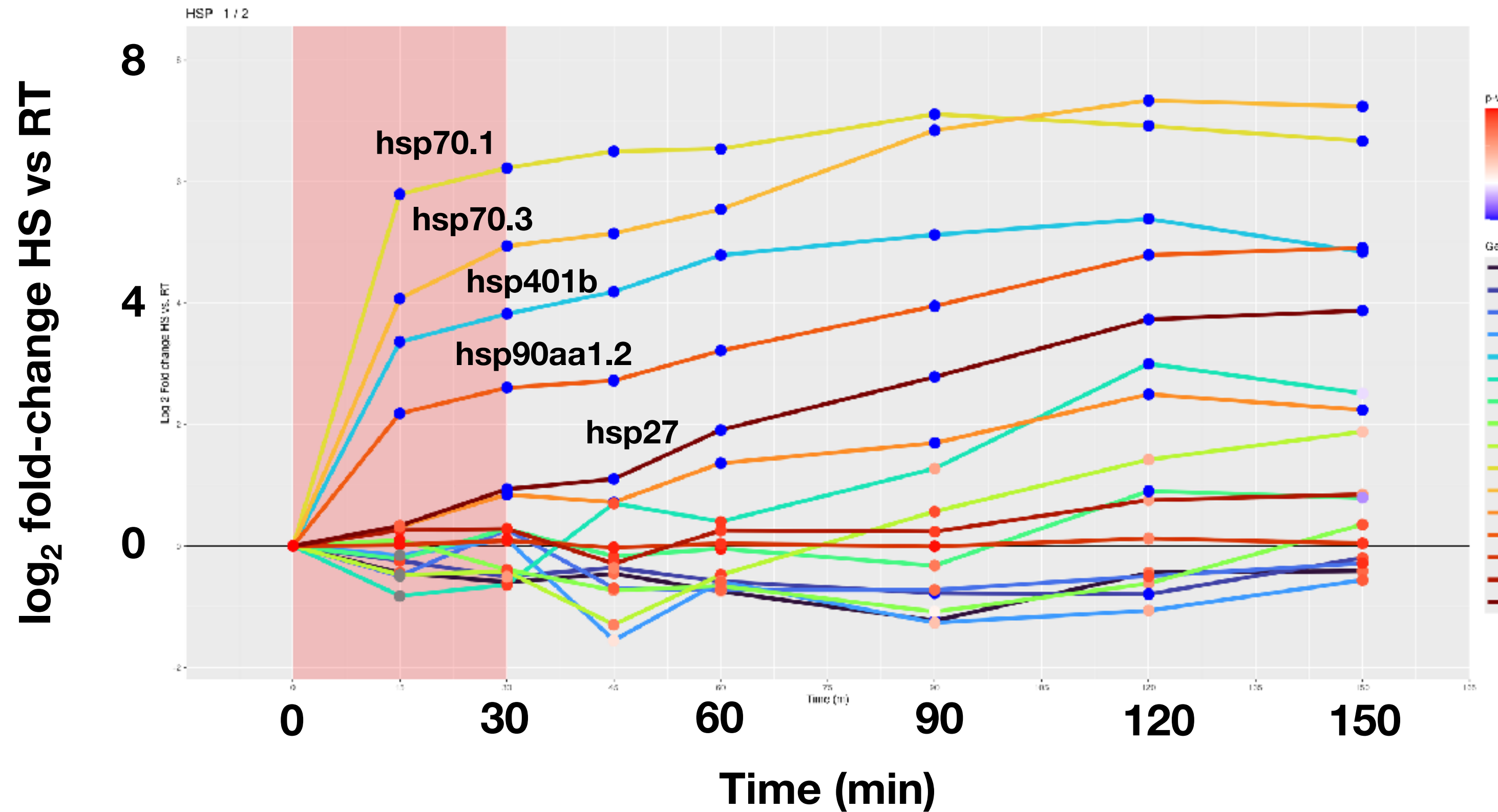
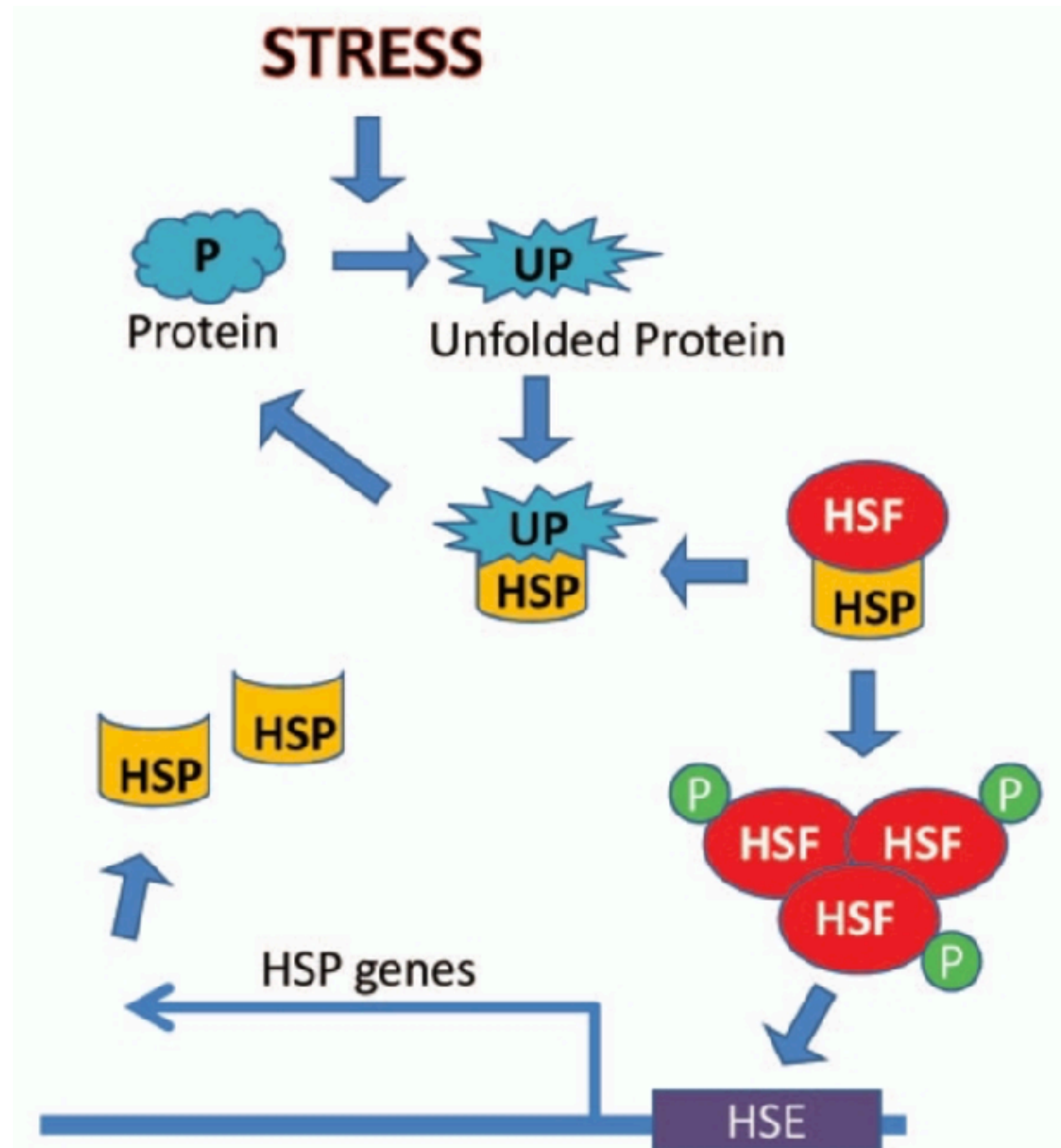
down – up - down



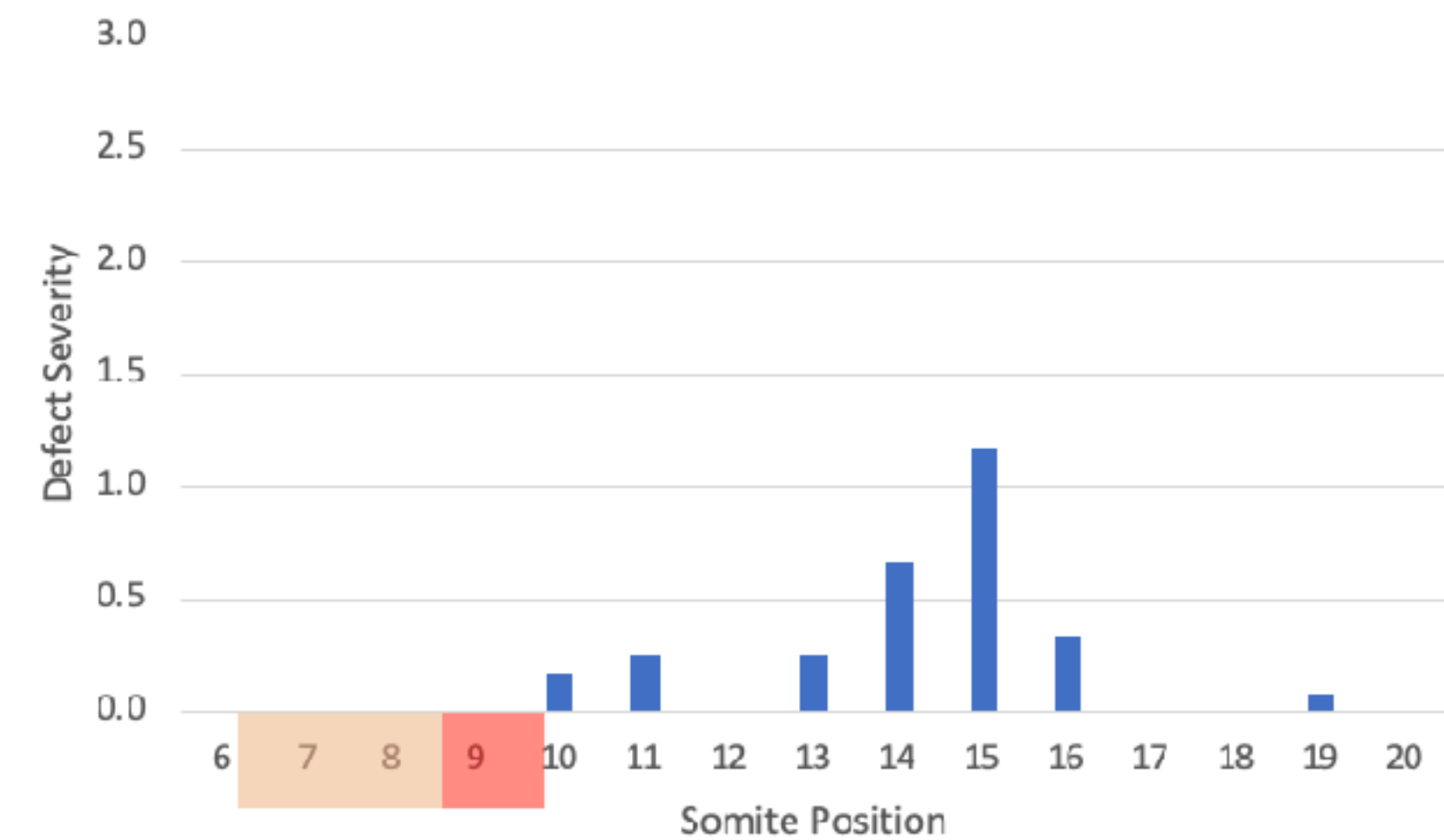
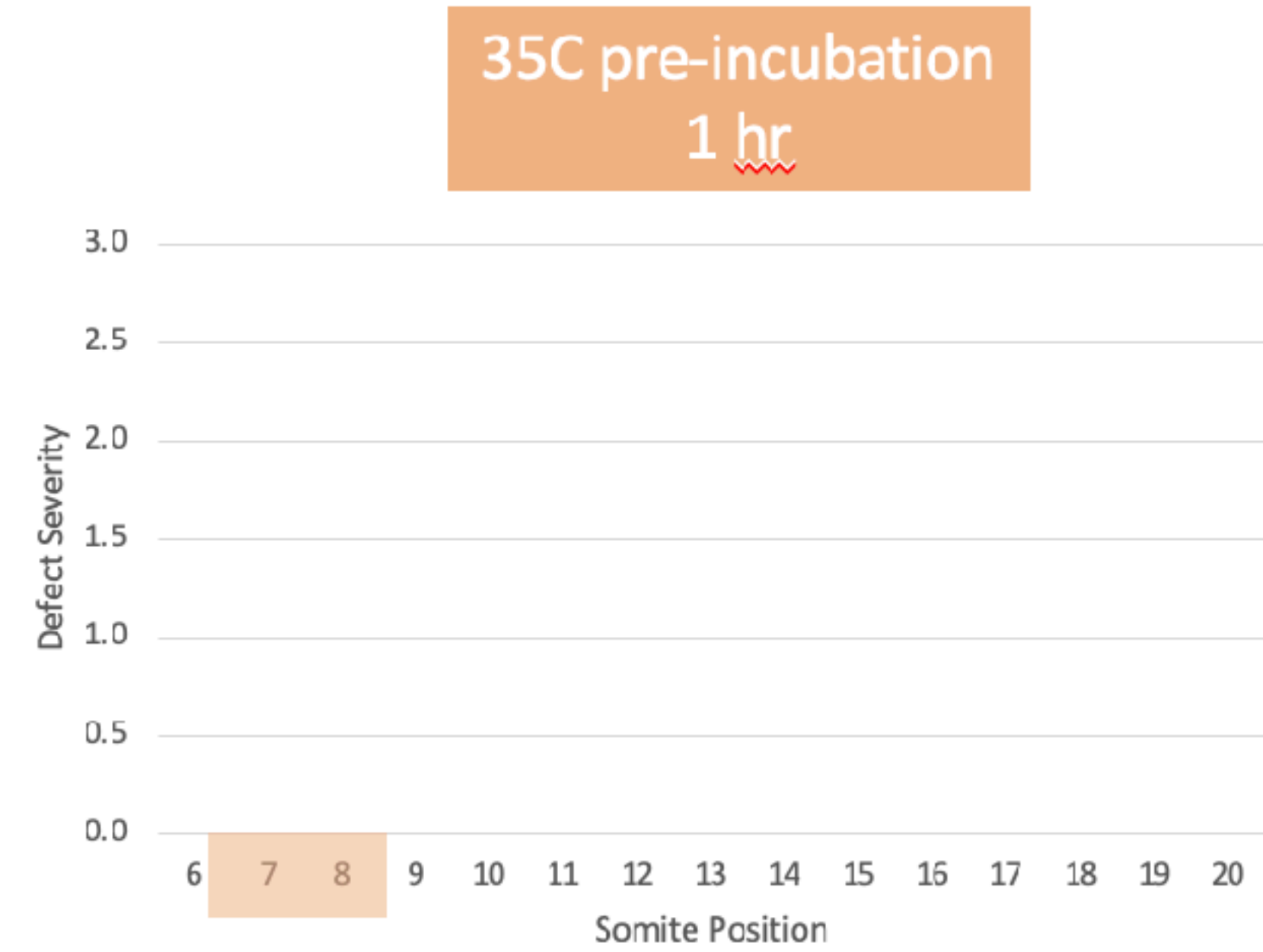
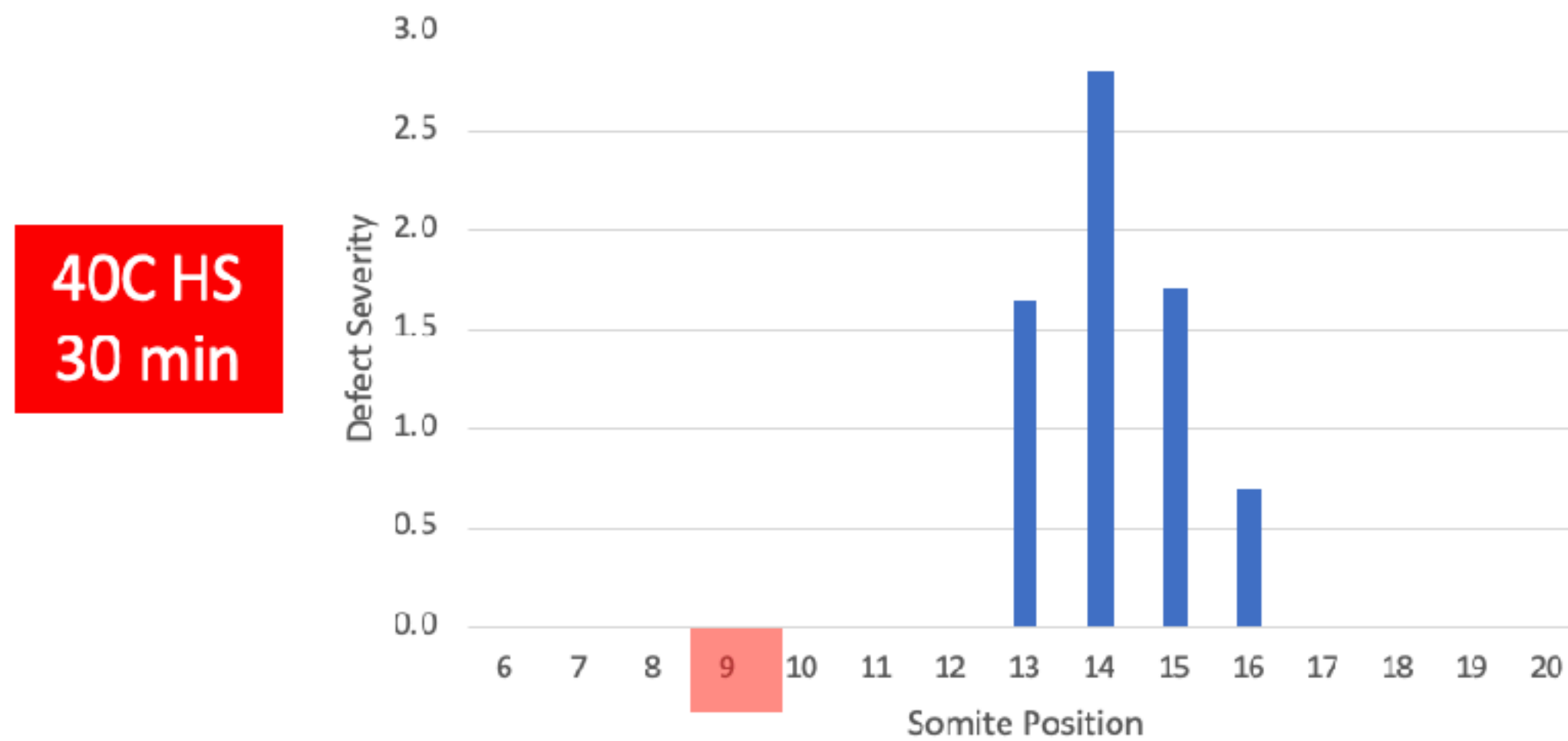
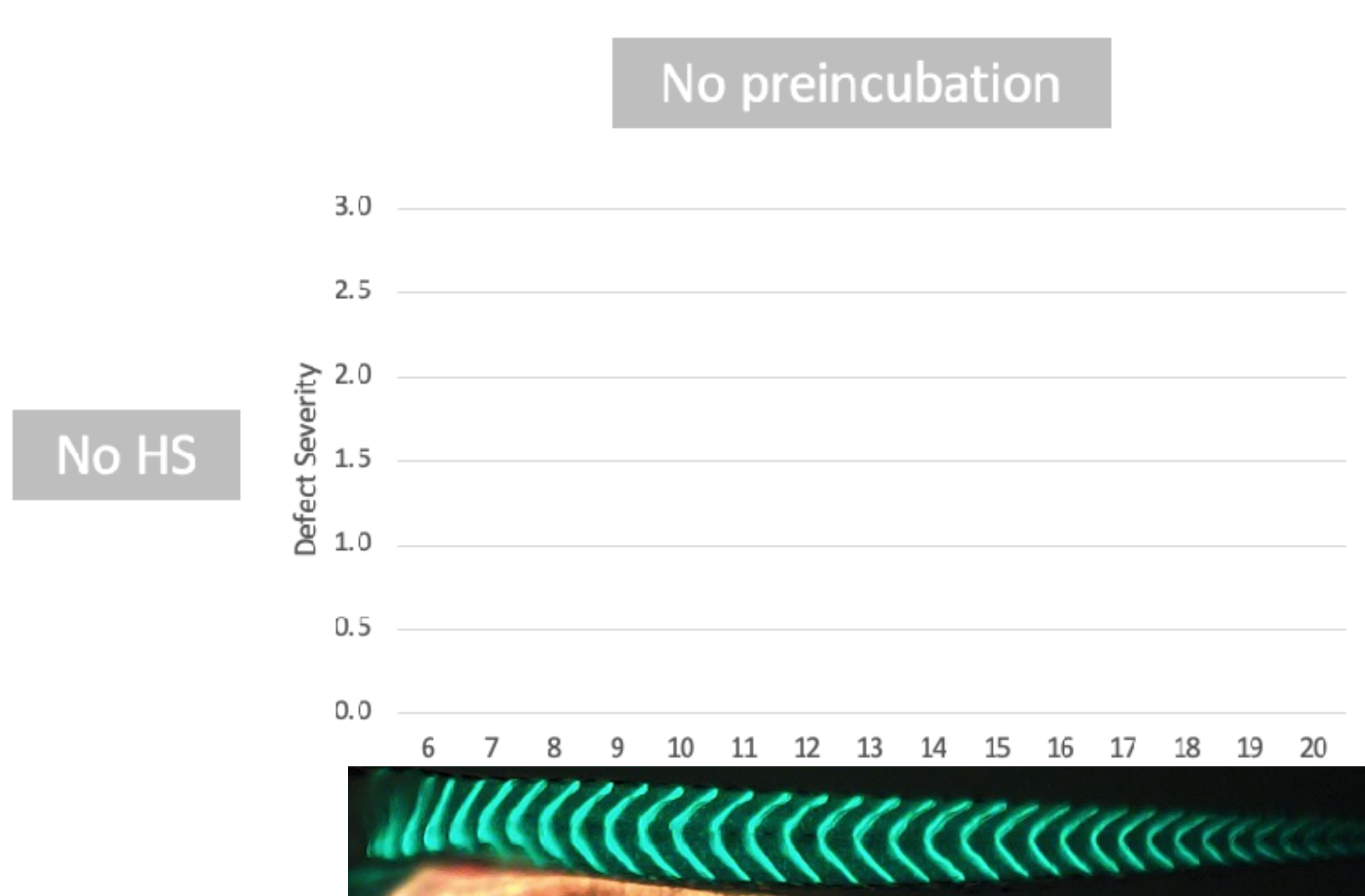
Minutes post heat-shock



Rapid induction of many heat shock genes



Heat-shock defects show *acclimatization*



Acclimatization requires **heat-shock proteins**

 No Drug

 KNK437
25 μ M

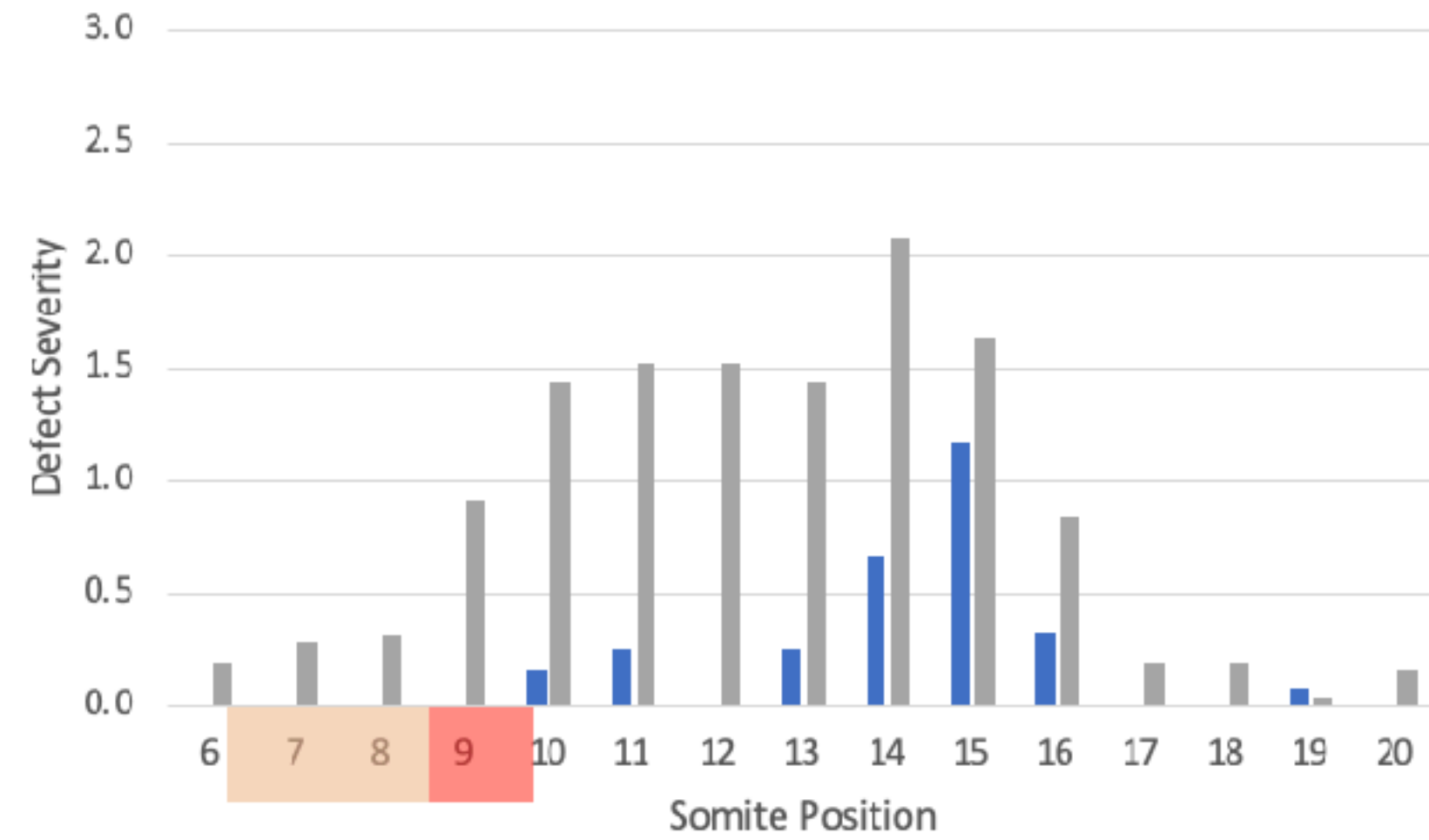
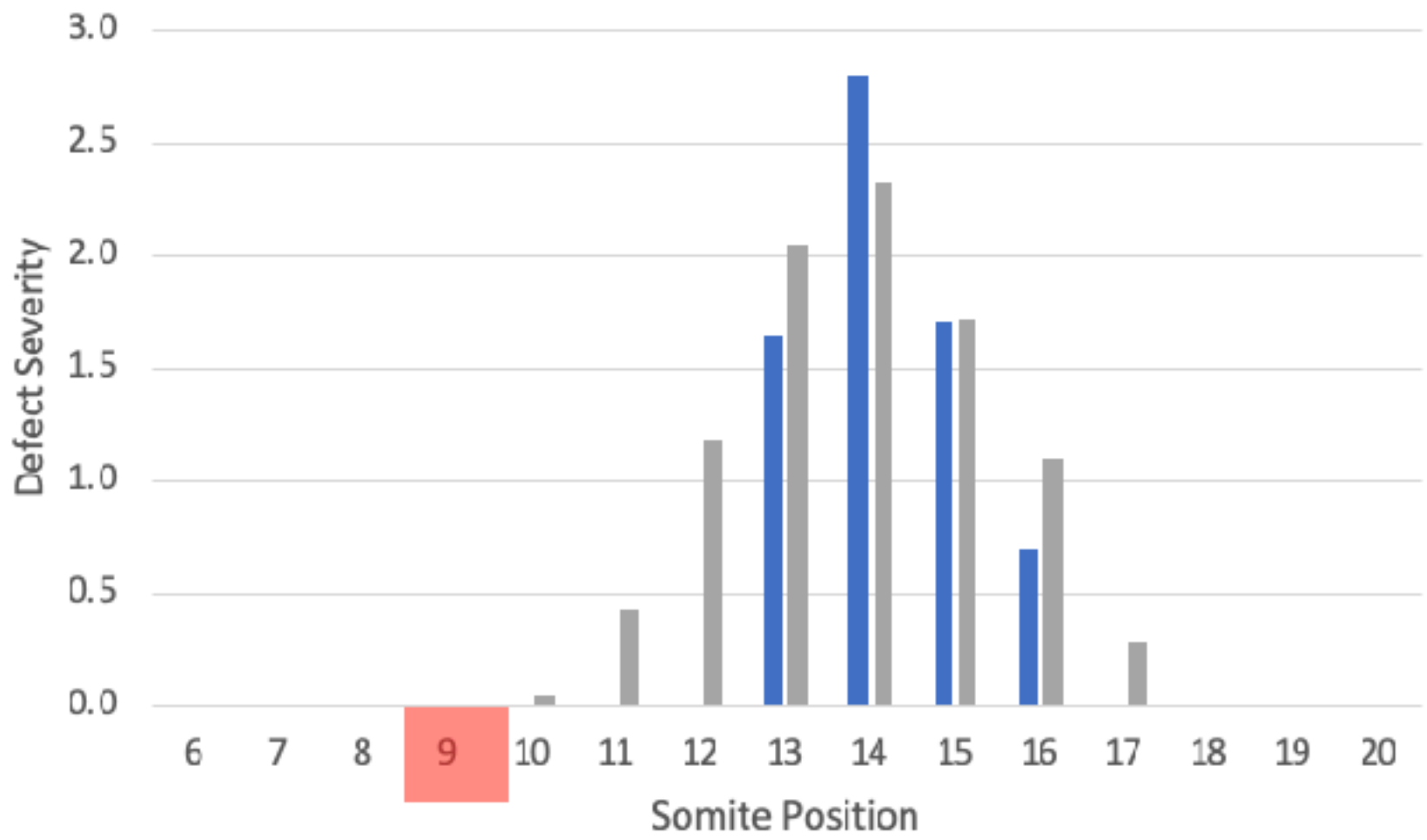
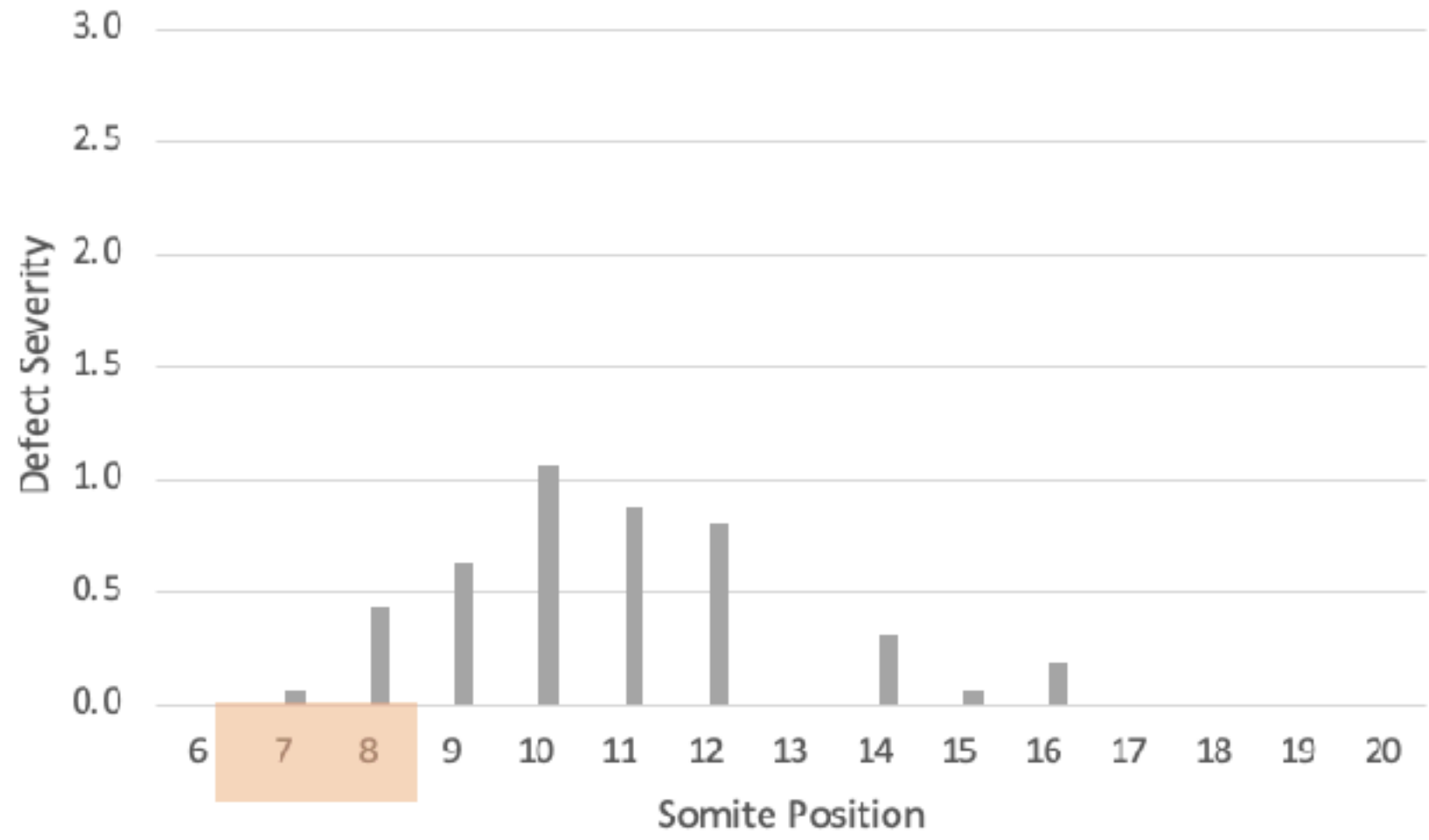
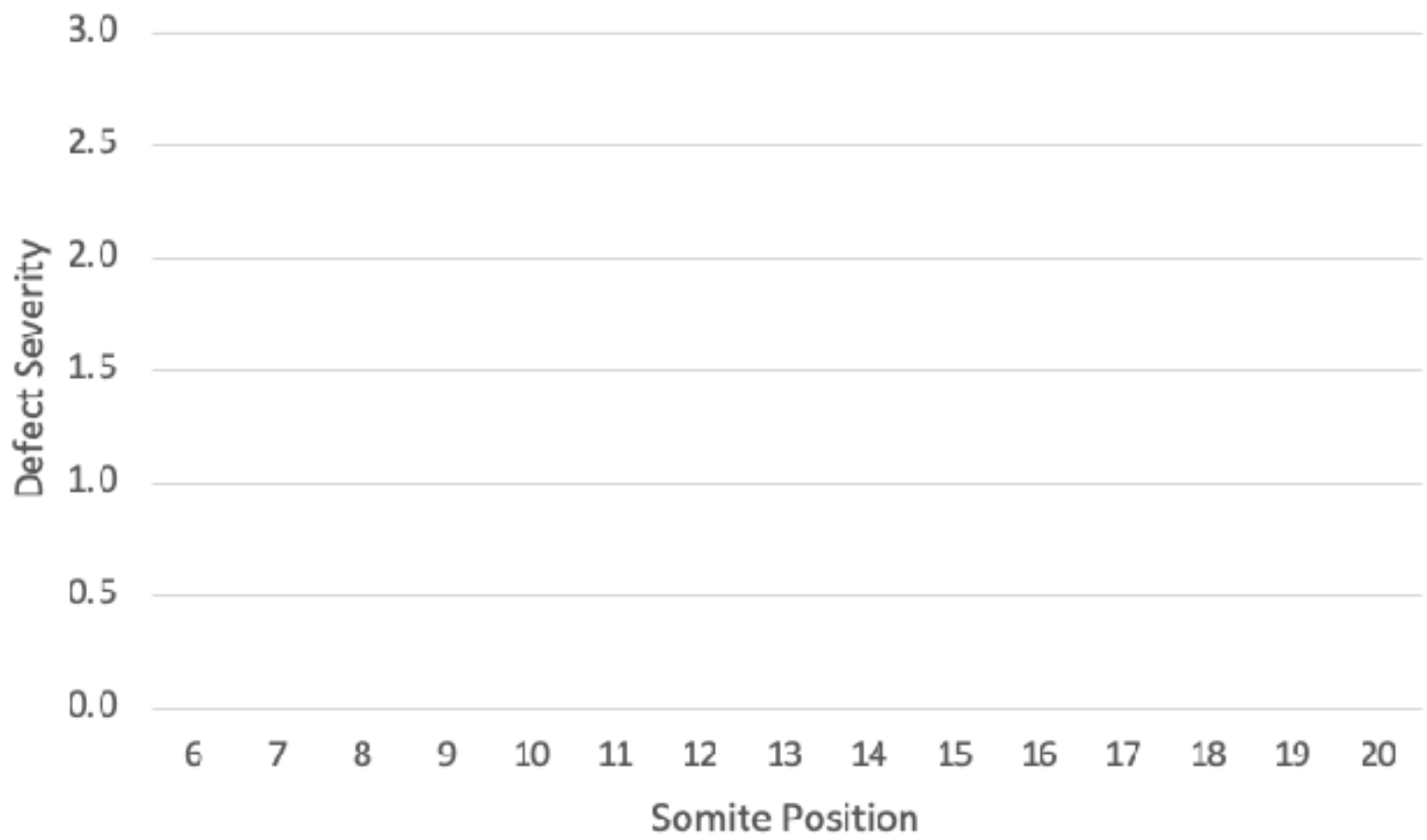
HSP105
HSP70H
HSP40

No preincubation

No HS

40C HS
30 min

35C pre-incubation
1 hr



~~Conclusions~~ *hints*

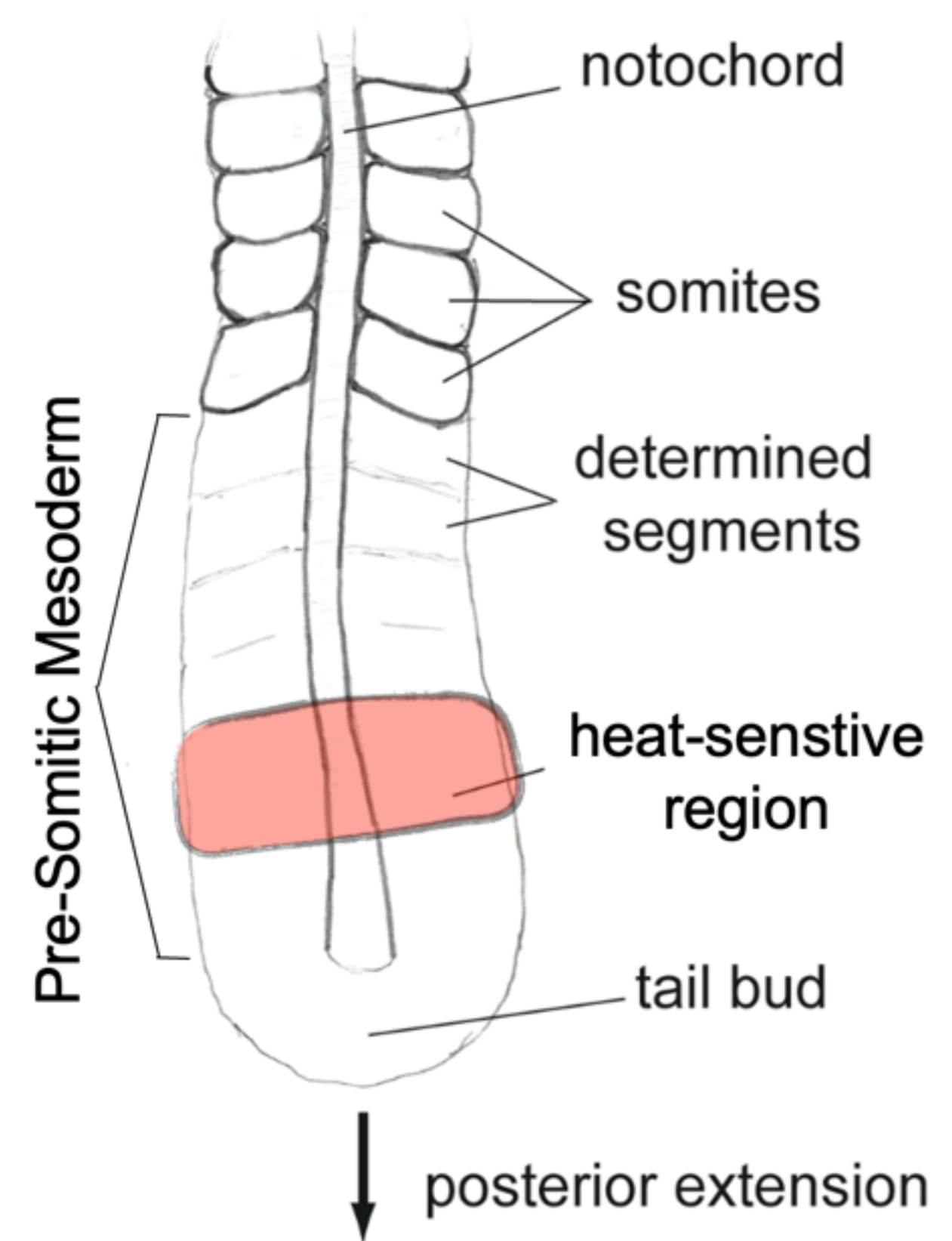
Heat-shock defects show *acclimatization*

Acclimatization requires **heat-shock proteins**

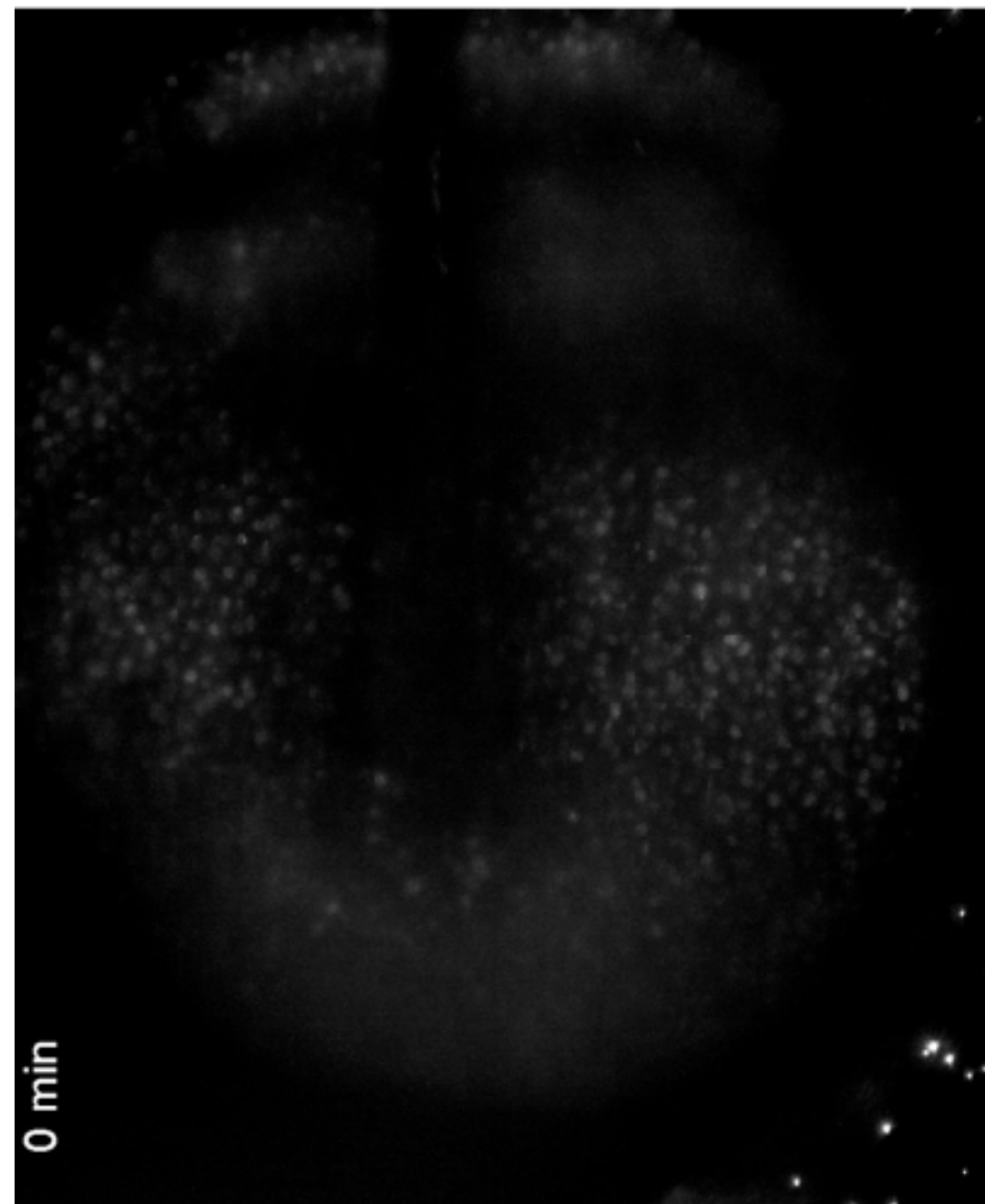
Hsp90a *not* involved
Hsp105, Hsp70, Hsp40 all candidates

Classical heat-shock effect **involves** HSPs

Heat-sensitive region is shaped by activity of HSPs

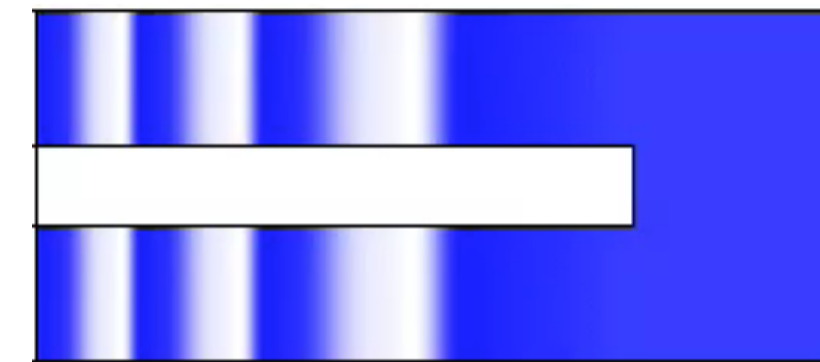


The segmentation clock is a tissue-level, rhythmic pattern generator - which tiers(s) are involved with the Heat Shock Effect?

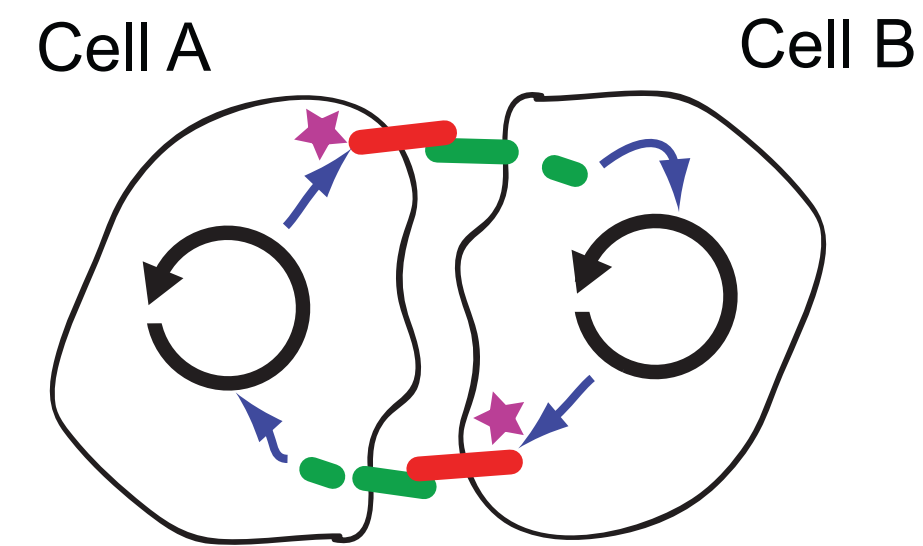


Sundar Naganathan

Tissue-level wave patterns



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