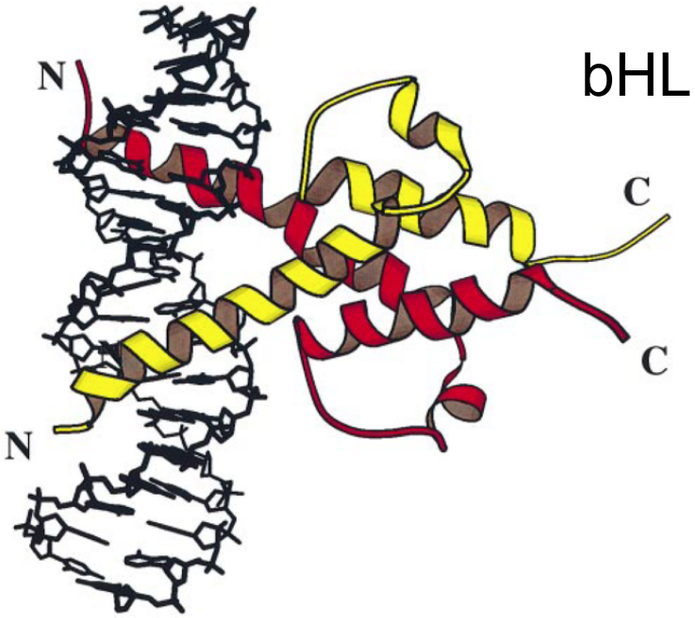
Body segments: added rhythmically and sequentially

Schröter et al., 2008 *Developmental Dynamics*

A model cellular oscillator - *transcription translation cycle*

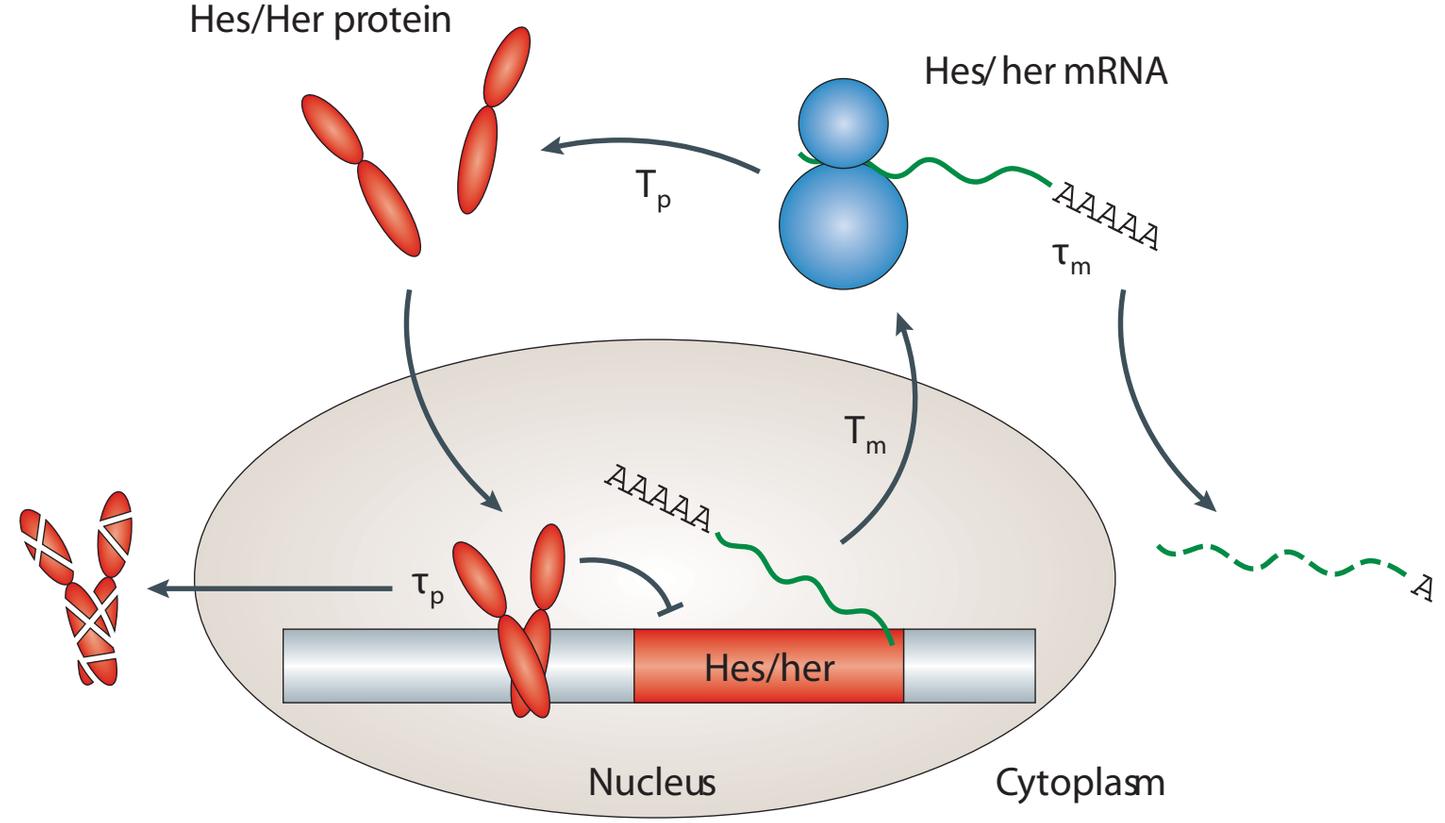


bHLH - DNA crystal

Chick - *c-hairy1*

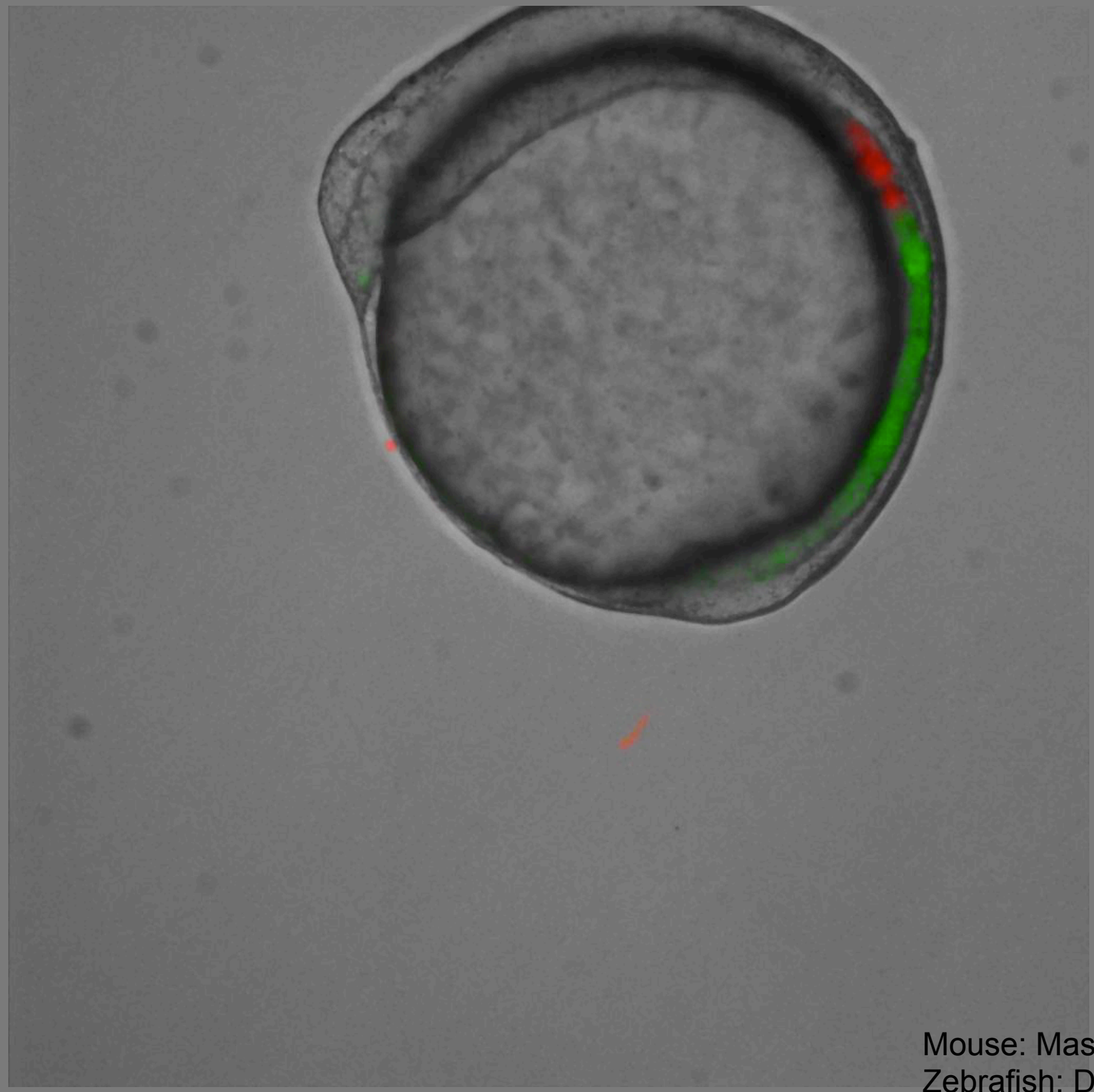
Mouse - *hes7*

Zebrafish - *her1*



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

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



Palmeirim et al., 1997

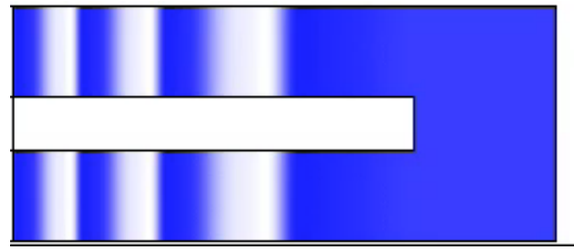
cyclic reporter gene
her1-YFP/*mespb*-mKate2

Daniele Soroldoni

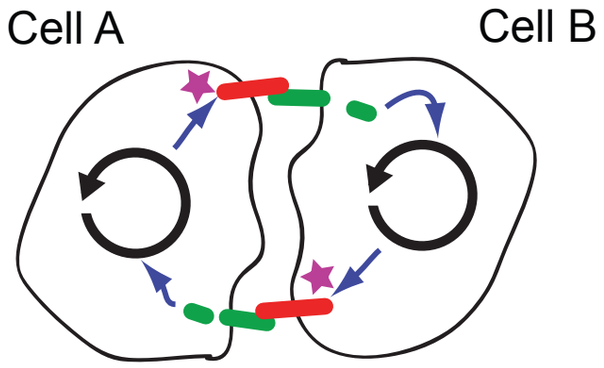
Mouse: Masamizu et al., 2006; Aulehla et al., 2008.
Zebrafish: Delaune et al., 2012; Soroldoni et al., 2014

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

Tissue-level wave patterns



Local synchronization



Cellular oscillators

Oates et al., 2009, 2012

ELSEVIER

Mechanisms of Development 85 (1999) 27–34

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.

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

Roy et al., 1999

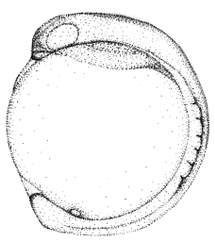
Experimental outline

30 min, 39-41°C

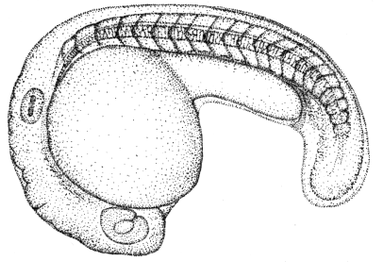
Fix and stain
with antibody

Time

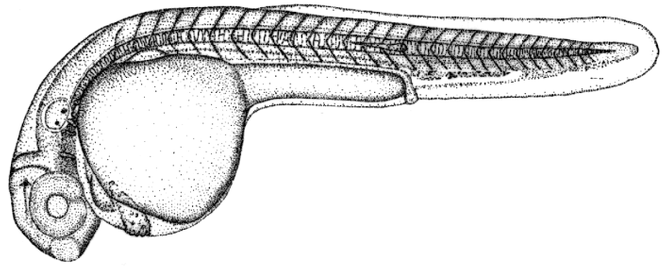
12 hpf



20 hpf

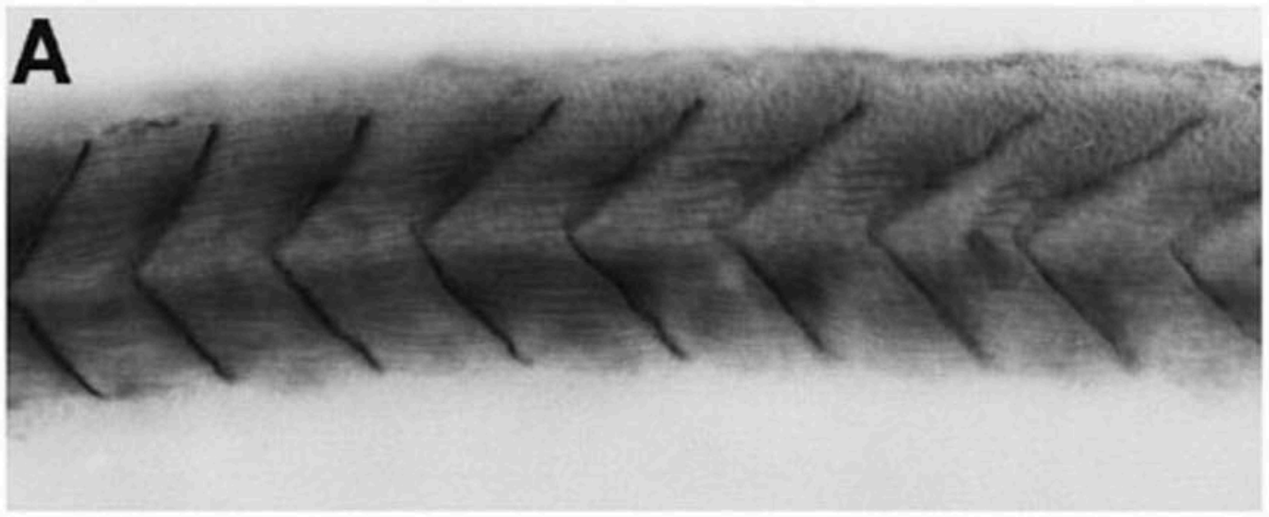


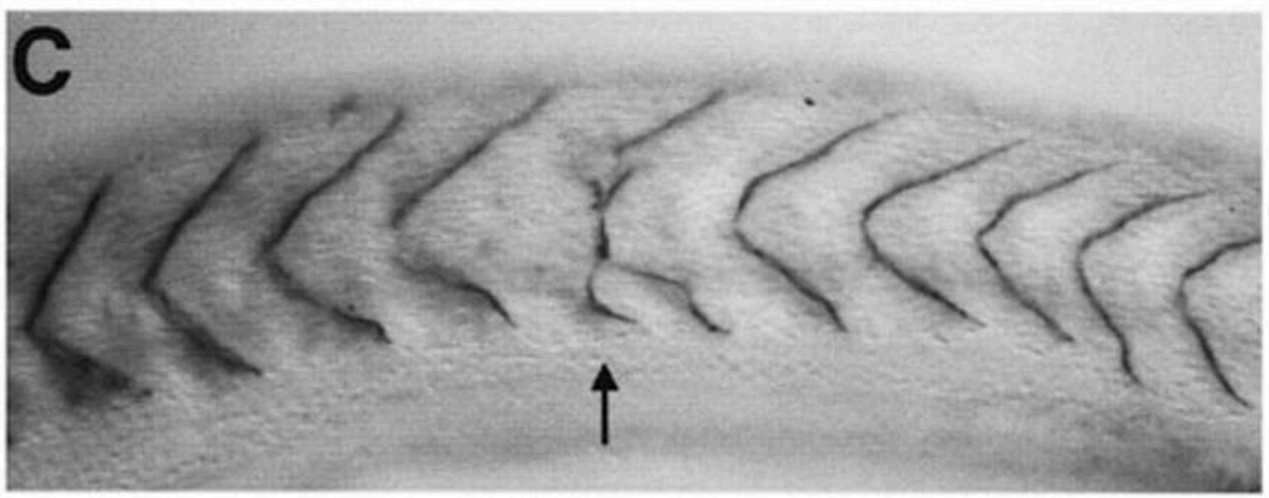
36 hpf

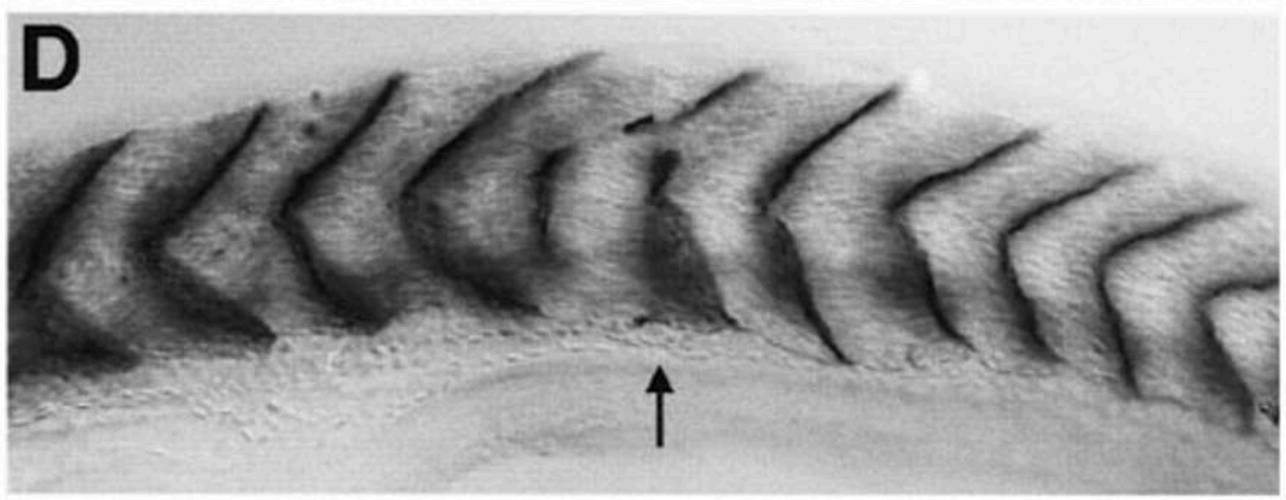


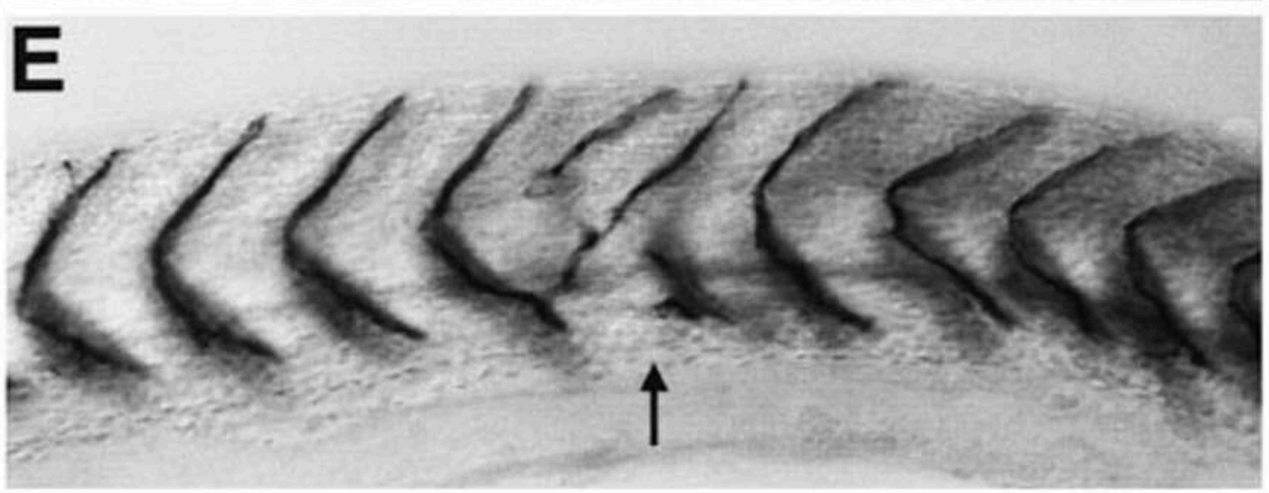
Roy et al., 1999

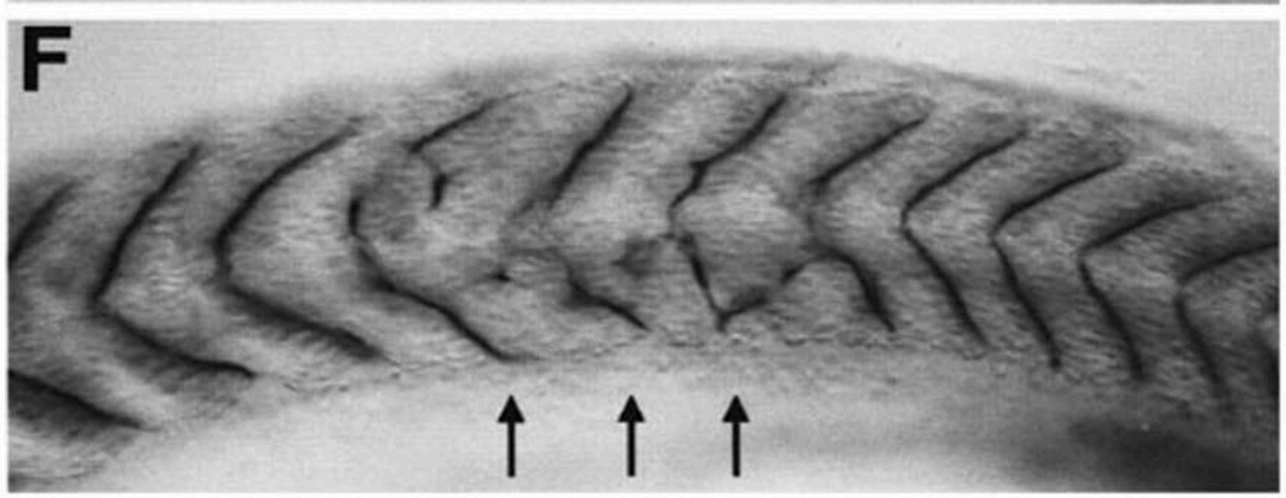
The heat-shock effect





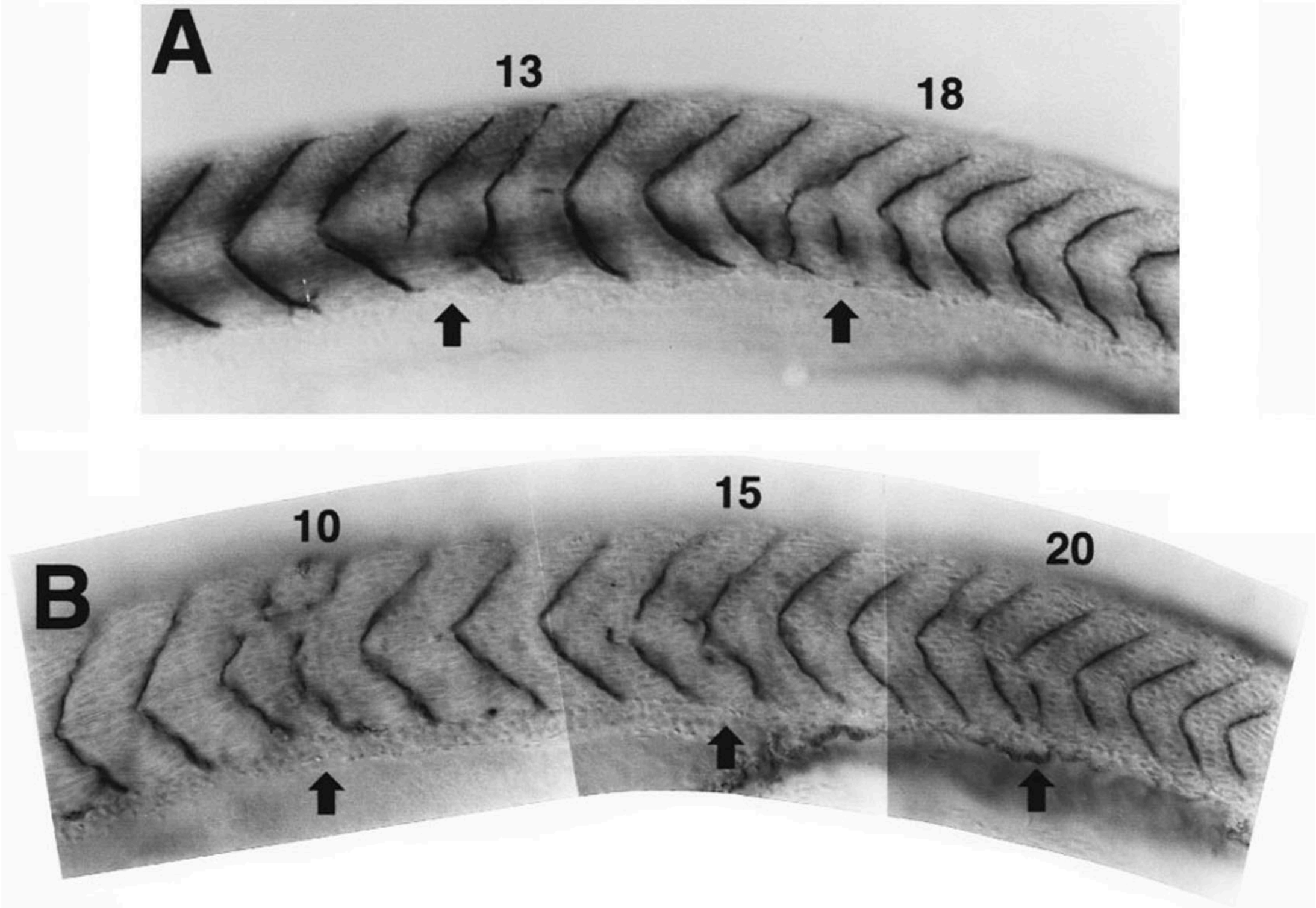




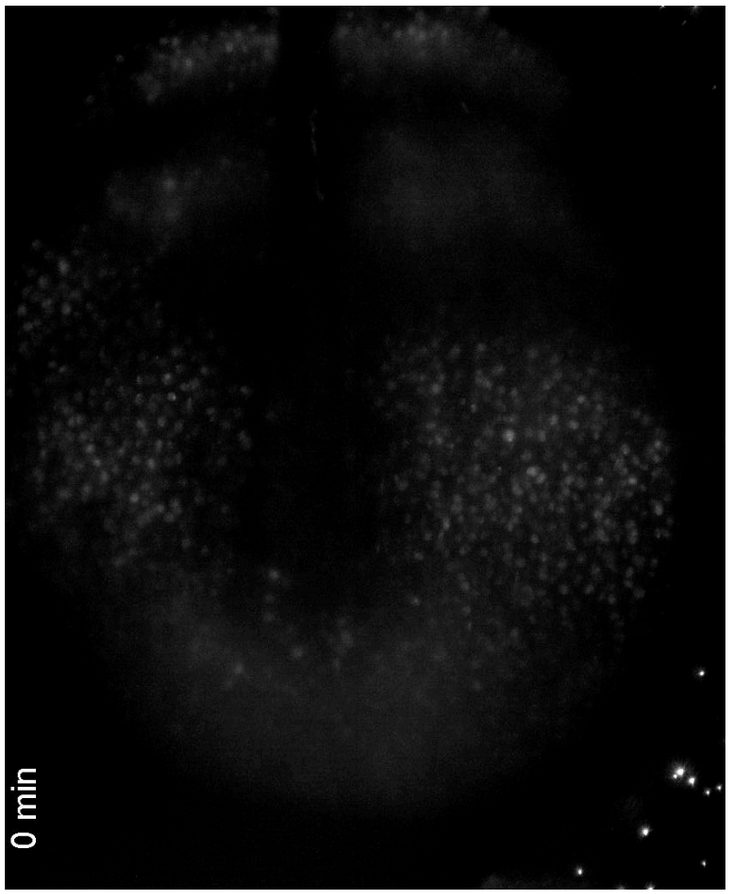


Roy et al., 1999

Ok, that's crazy

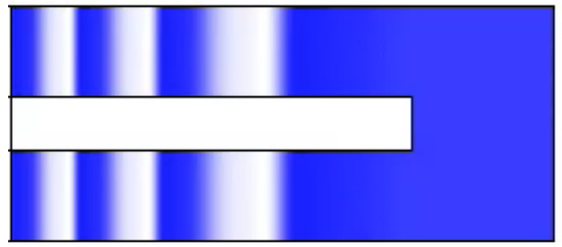


The segmentation clock is a tissue-level, rhythmic pattern generator

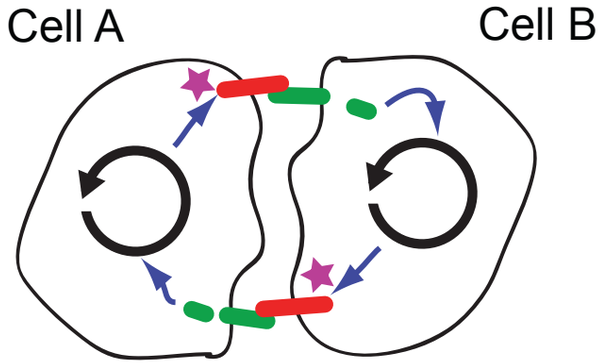


Sundar Naganathan

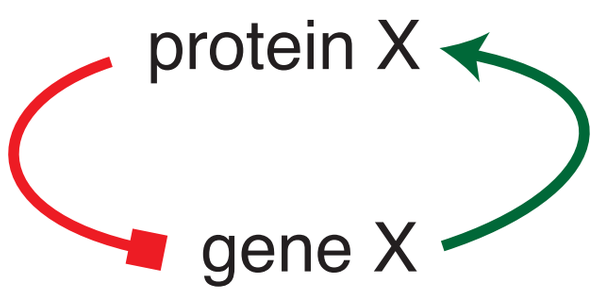
Tissue-level wave patterns



Local synchronization



Cellular oscillators



Oates et al., 2009, 2012

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

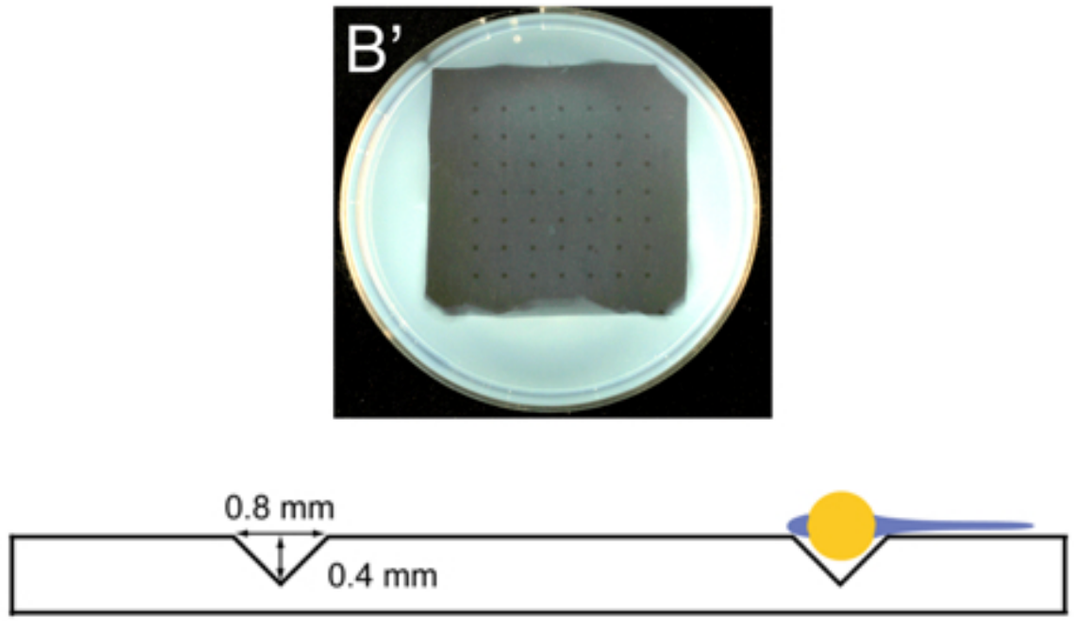
Tools and approaches

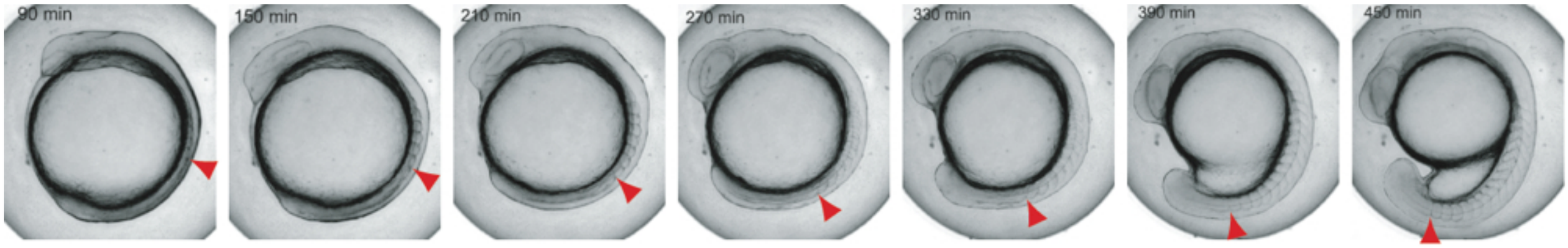
- Time lapse microscopy - measure period and morphology
  - Somite formation
- Clock transgenes - measure period and pattern
  - Her1-YFP (plus, mespb - boundary cells, DND - mature segment boundaries)
- Mutant backgrounds - clock, synchronization
  - *her1;her7*, *deltaC …*
- Drugs - signal gradients, synchronization
  - Inhibit Fgf, Notch …

  - Plus, whatever else you can think of -
  - How could you deliver the heatshock?
  - Single cells versus the tissue?

Measuring *Segmentation period* in the zebrafish

Schröter et al., 2008;
Herrgen et al., 2009

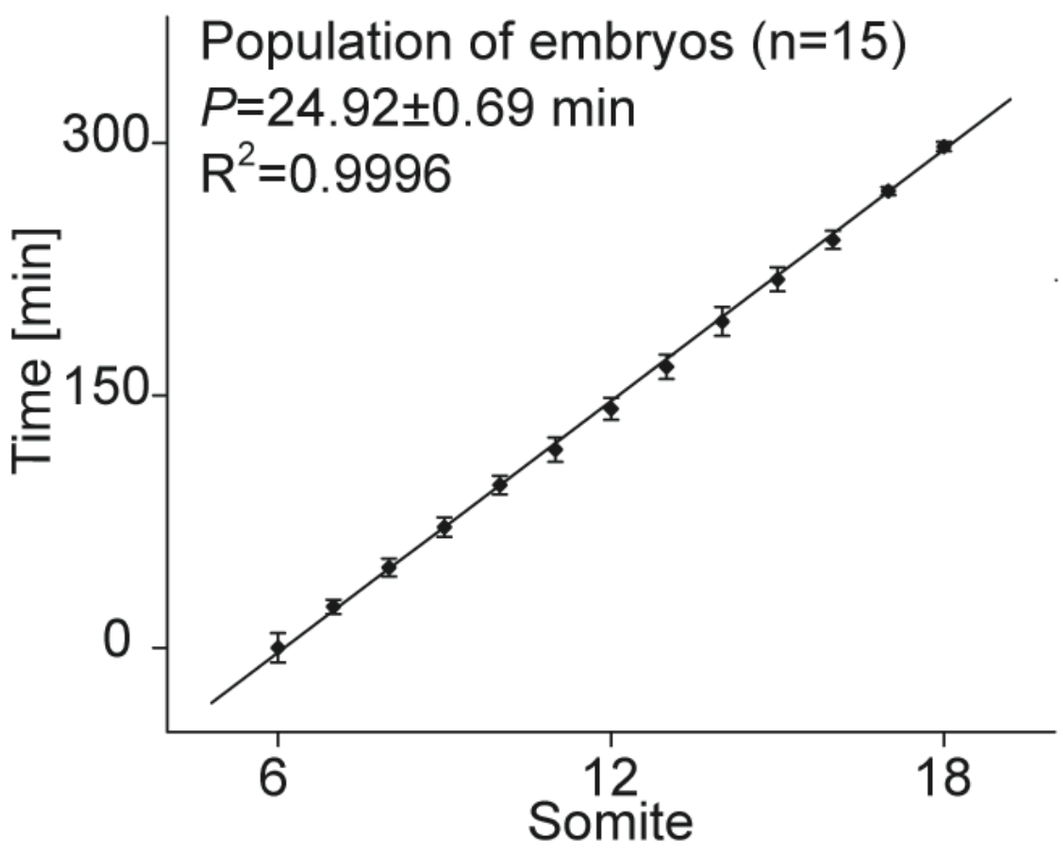


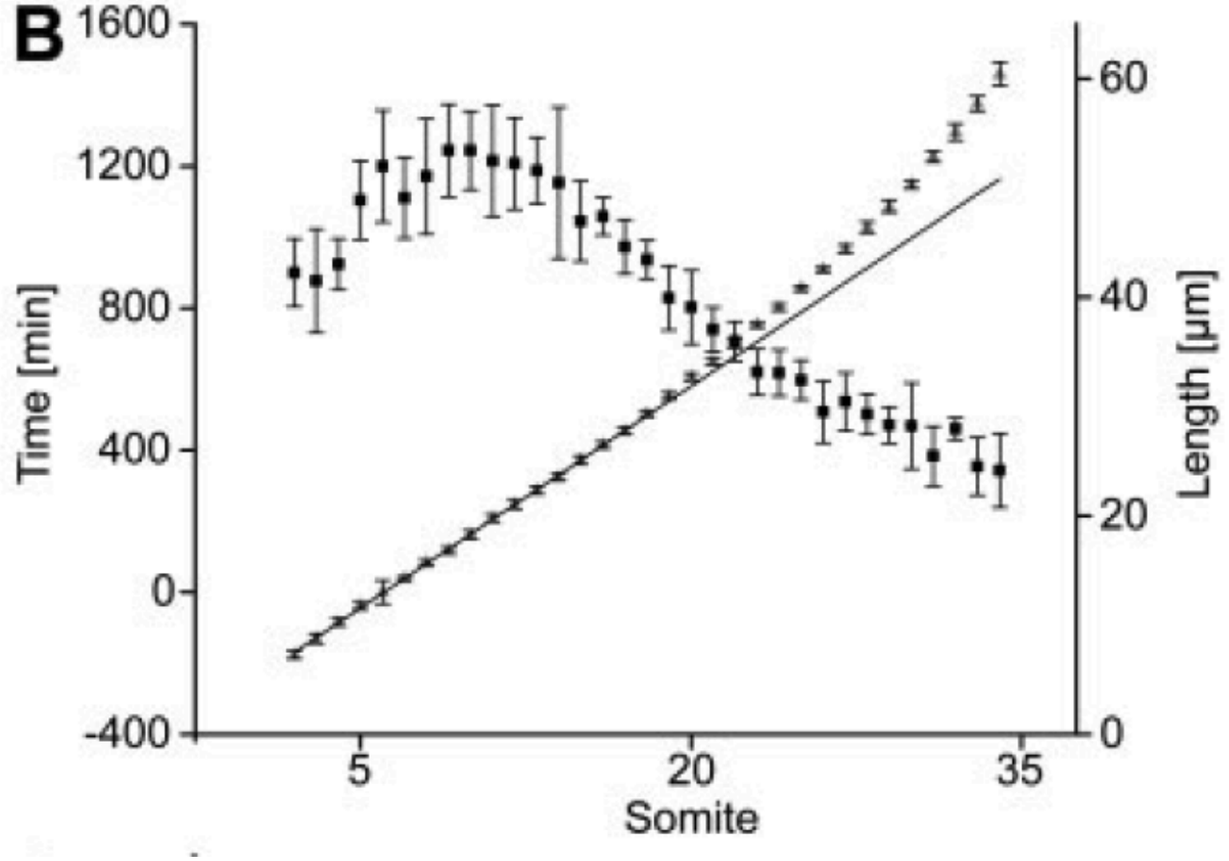


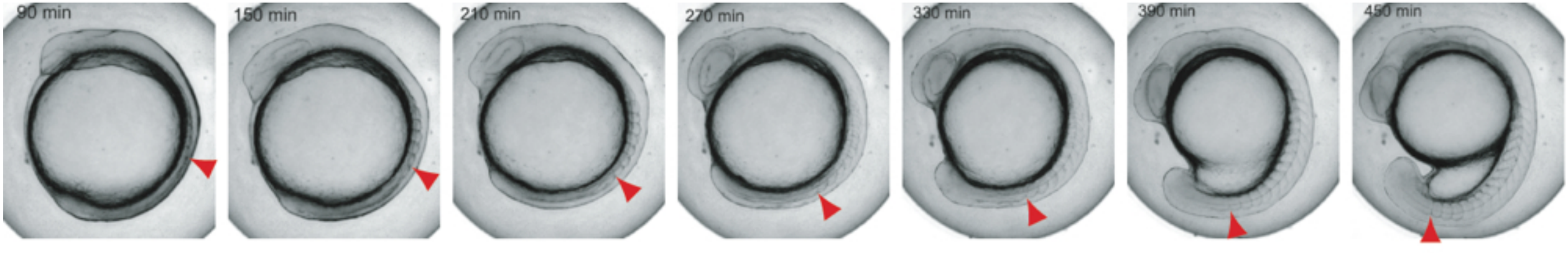
time →

Schröter, C., Herrgen, L., Cardona, A., Brouhard, G. J., Feldman, B., & Oates, A. C. (2008). Dynamics of zebrafish somitogenesis. *Developmental Dynamics*, *237*(3), 545–553. http://doi.org/10.1002/dvdy.21458

Segmentation period and length





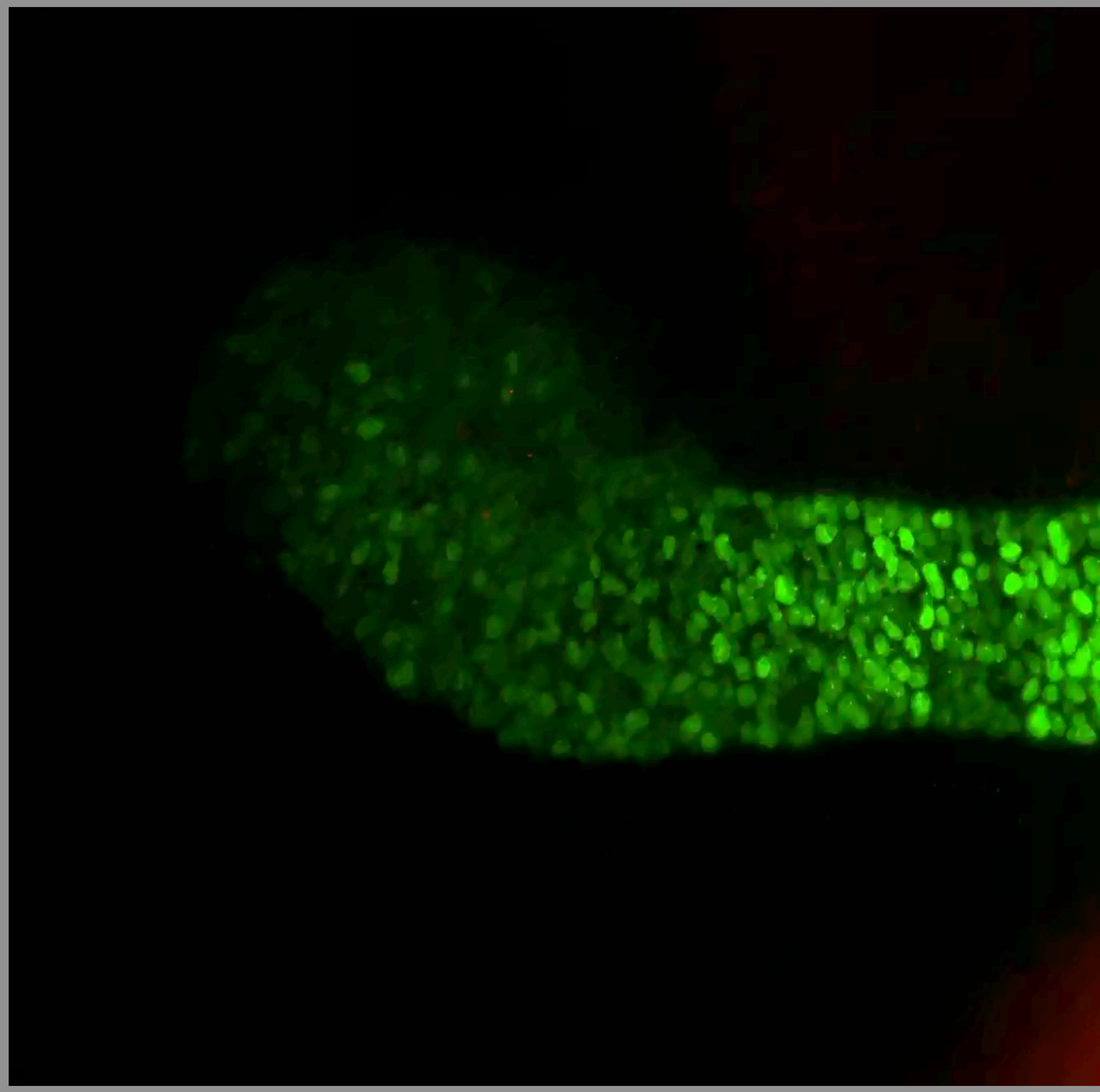


time →

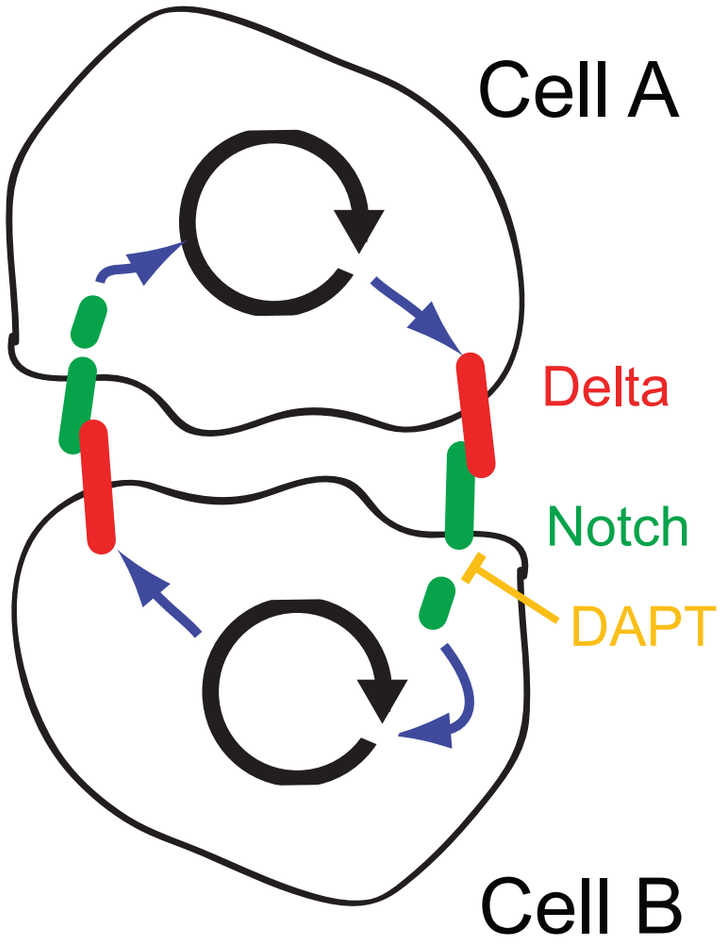
Live imaging - cool, but tricky and low throughput

Whole Embryo Time-lapse

SPIM

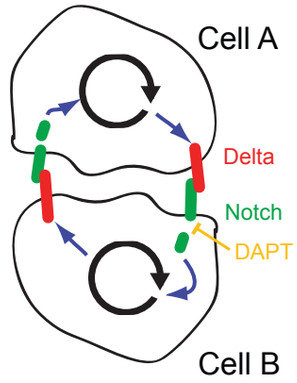


In situ hybridisation of endogenous cyclic genes - easy and high throughput

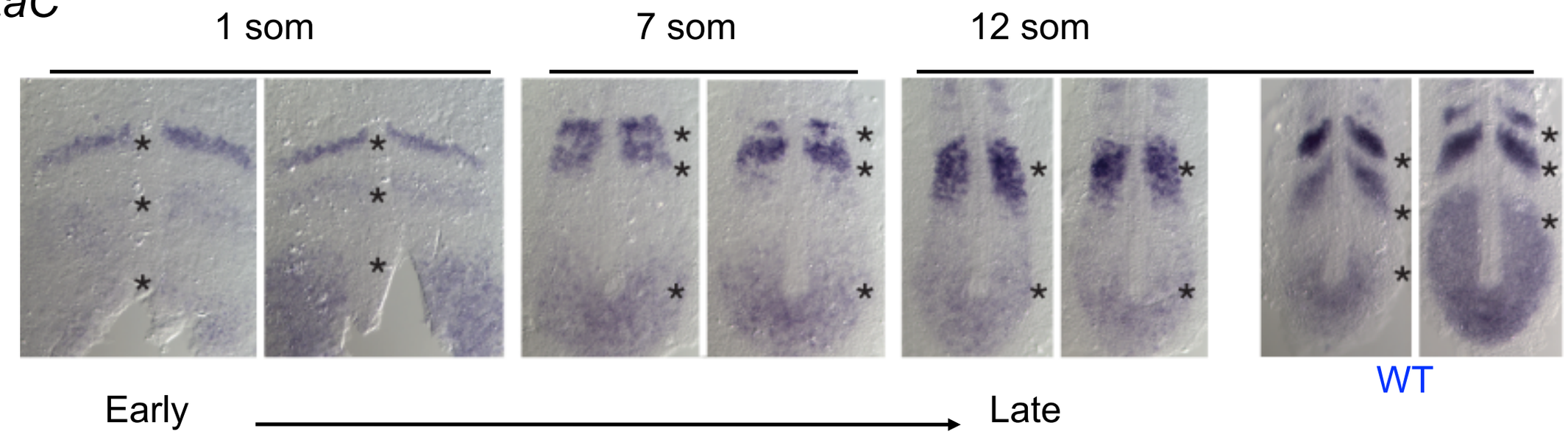


Jiang et al., 2000
Lewis, 2003
Horikawa et al., 2006
Riedel-Kruse et al., 2007
Ozbudak et al., 2008
Delaune et al., 2012

Blocking Notch disrupts segmentation and wave pattern



deltaC



Riedel-Kruse et al., *Science* 2007