

Coupling vs. noise: The rise and fall of synchrony in the segmentation clock

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The sequential segmentation of the vertebrate embryo along its anterior-posterior axis into blocks of cells called somites is thought to be driven by the “segmentation clock” (Fig. 1A). This clock comprises a multi-cellular genetic network of oscillators located within the posterior presomitic mesoderm (PSM). Delta/Notch signaling has been proposed to couple these oscillators (Fig. 1B) such that they are spatio-temporally synchronized despite the presence of developmental noise. Desynchronization over developmental time could explain the zebrafish Delta/Notch mutant phenotypes in which only the first 6-10 anterior segments form correctly (Fig. 1C, first panel); alternatively, genetic differences in anterior and posterior patterning might be at work.

How this synchrony is established, and how its loss determines the position of segmentation defects in Delta/Notch mutants is unknown.

We employed techniques for quantitative perturbation of gene function to reduce Notch coupling in WT and *des*^{+/-} (*notch1a* heterozygote mutant), by applying (i) DAPT concentrations to reduce Notch activation, and (ii) antisense morpholino (MO) amounts to reduce translation of *notch1a* mRNA (Fig. 1B). We quantified the resulting organismal phenotypes with the Anterior Limit of Defects (ALD) (Fig. 1C), i.e., the number of the anterior-most defective segment. Both treatments gave consistent results: the ALD shifted posteriorly with lower treatment levels, and the difference between WT and *des*^{+/-} is consistent with a 0.5-fold difference in signaling (Fig. 1C,D).

We developed a physical theory that describes the dynamics of synchrony in the segmentation clock, which we considered as a population of mutually coupled phase-oscillators in the presence of noise; spatial aspects of cyclic gene wave patterns and properties of the Her-feedback oscillators were neglected. We obtained a relation between the ALD and the treatment level, n , (see equation inset Fig. 1D), which is in quantitative agreement with the experimental data (Fig. 1D). These results allowed us to quantify the clock’s noise, $2\sigma^2 \approx 0.8/\text{h}$, the WT Notch coupling strength, $\epsilon_{WT} \approx 4/\text{h}$, and the system’s robustness, $R \sim 5$, with robustness being the fold-reduction in Notch coupling just not leading to segmentation defects; furthermore a synchronization transition among these genetic oscillators is implicated. Hence, the Delta/Notch mutant phenotypes are now quantitatively understandable as a desynchronization process, where the desynchronization rate and consequently the segmentation defect position are set by the difference in noise and coupling strength.

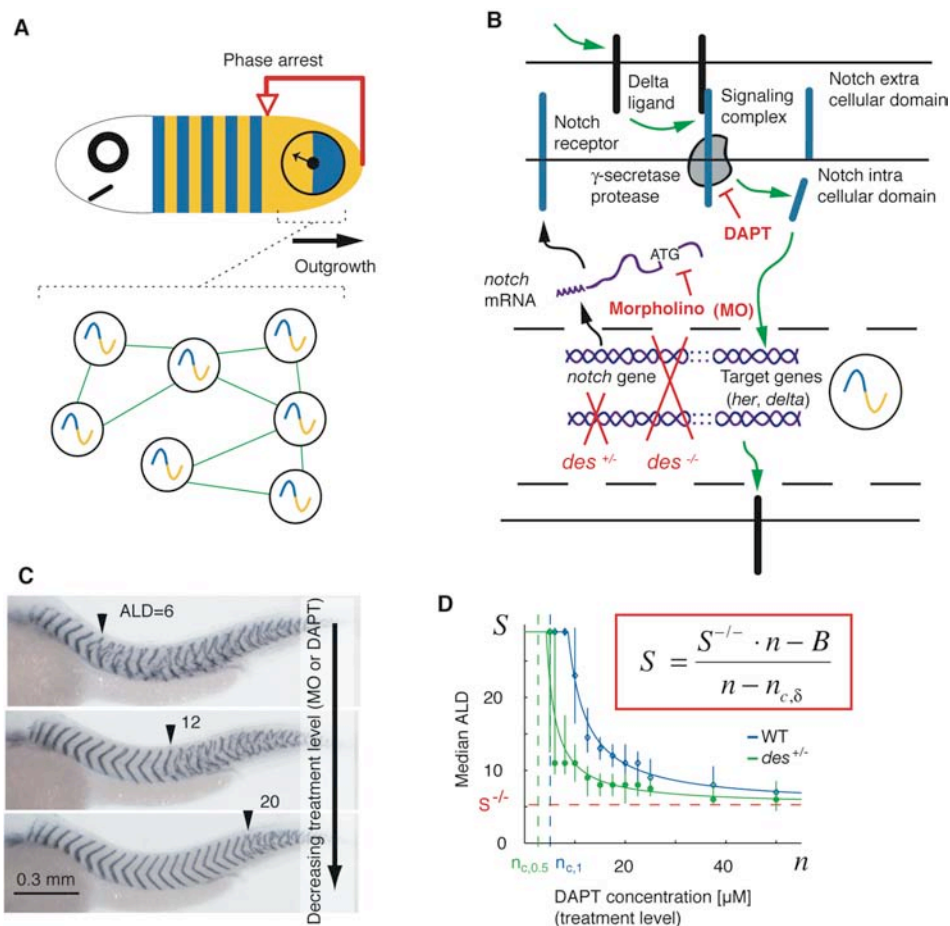


Figure 1: Quantitative understanding of the Delta/Notch mutant segmentation phenotype as desynchronization process by combining quantitative gene knockdown with a physical theory of coupled phase oscillators.

(A) Oscillation phase of the zebrafish segmentation clock in the outgrowing PSM is continuously imprinted at constant distance from the posterior end. Insufficient coherence among these coupled genetic oscillators within the PSM results in defective segment boundaries. (B) Production, activation, turn-over, and signaling of the Delta/Notch pathway; treatments used in this study to reduce or completely block inter-cellular Delta/Notch coupling (red): morpholino (MO), DAPT, mutants in *notch1a* (*des*^{+/-}, *des*^{-/-}). Coupling acts to decrease phase differences between oscillators, and opposes the phase randomizing effects of developmental noise. (C) Shifts in Anterior Limit of Defects of segment boundaries (ALD, arrowhead) due to decreasing treatment levels. (D) Functional representation of (C) (only DAPT results shown, MO being very similar). Inset: relation between ALD and treatment level, n , derived from physical theory. Solid lines: fit to this relation. Dashed lines: visual guidance marking saturating ALDs, $S_{ALD}^{-/-}$, and critical treatment levels, $n_{c,\delta}$, below which (theoretically) infinitely many correct segments can be formed.

Finally, we demonstrate two general mechanisms by which genetic oscillators can attain synchrony: (i) simultaneous gene induction during the initiation of the clock (Fig. 2A) and (ii) self-organized synchronization during rescue of the clock by increasing Notch coupling (Fig. 2B).

In conclusion, employing quantitative techniques for perturbation of gene function combined with a physical theory of coupled phase oscillators, we were able to quantitatively account for a collective, morphological process in a complex developmental system. Delta/Notch mutant zebrafish embryos would allow screening for compounds restoring Delta/Notch coupling (see rescue in Fig.2B), with potential therapeutic implications for human genetic mal-segmentation disorders.

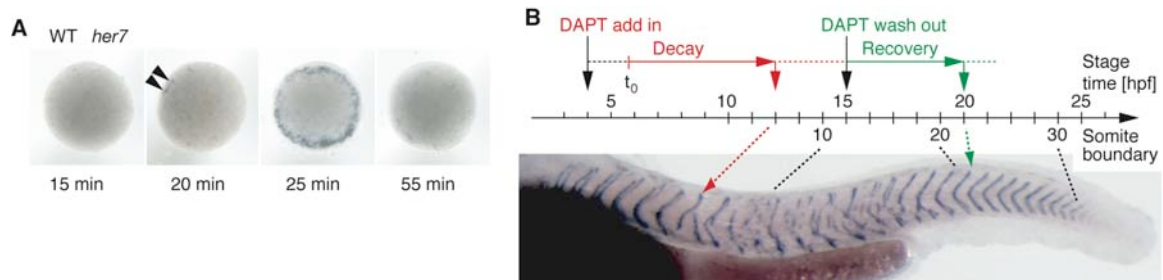


Figure 2. Two general mechanisms to attain synchrony among genetic oscillators: simultaneous gene induction and self-organized synchronization.

(A) First detection of the cyclic gene *her7* (arrowheads) in the presumptive mesoderm ring (animal pole view). Relative time starts at 5 hours post fertilization, when the zebrafish embryo is still spherical. 5 minutes after first detection complete ring of expression is observed, which is again absent after 30 minutes, corresponding to the natural period of the segmentation clock. Consequently, the genetic oscillators of the segmentation clock are induced synchronously, providing synchrony from the start. (B) DAPT pulse-chase experiment first reduces and then adds back the coupling among these genetic oscillators. The synchrony among these oscillators consequently first decays (ALD=6), but then is regained in a self-organized way (last defective segment ~21). The synchrony among oscillators is here indirectly read out by the segmentation defect positions, but monitoring cellular gene expression data (not shown here) leads to same conclusion.