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DEVELOPMENT

# Segmentation by sequestration

The sequential formation of the segments of the vertebral column is dictated by oscillations of gene expression that form a genetic clock in the posterior-most embryonic tissue early in development. In a recent study, Schröter *et al.* uncovered a novel mechanism by which periodic gene expression is achieved through a 'dimer cloud', from which formation of DNA-binding or non-DNA-binding dimers results in periodic gene regulation.

> "periodic gene expression is achieved through a 'dimer cloud', from which formation of...dimers results in periodic gene regulation"

The authors focused on zebrafish segmentation clock proteins from the Hairy- and enhancer-of-split-related (Hes or Her) protein family — these are basic helix–loop–helix (bHLH) transcriptional repressors. First, in co-immunoprecipitation experiments and in a microfluidic assay, they showed that all combinations of dimers can be formed between Her1, Hes6 and Her7. These proteins form a promiscuous 'dimer cloud'. However, only Her1 homodimers and Hes6–Her7 heterodimers are able to bind DNA. Importantly, these dimers bind the same regions of the cyclic genes *her1, her7* and delta C (*dlc*); thus, Her1 and Her6–Her7 dimers form two parallel feedback loops that act on the same sites. The dimer–gene interactions were also confirmed in a yeast one-hybrid assay.

Schröter *et al.* then analysed single mutants for *her1, hes6* and *her7*, which still allowed visualization of the RNA by hybridization. As each of three proteins studied is present in only one of the parallel feedback loops, it would be expected that all of the single-gene mutants would be competent for segmentation. This was the case for *her1* and *hes6* single mutants. However, in the posterior trunk of *her7* mutants, patterning and segmentation became defective in the tenth or eleventh segment, and this was accompanied by a decay in oscillations of *her1, her7* and *dlc* cyclic mRNA. This late onset of the phenotype suggested that the Her7–Hes6 heterodimer is not the only functional complex that contains these proteins. Double-mutant analysis showed that the *her7* phenotype could be alleviated by mutation of *hes6*, suggesting that the mutation phenotype of *her7* is caused by increased availability of *hes6*.

To analyse the regulation the clock period that determines the number and length of segments, the authors monitored single and double mutants using multiple-embryo time-lapse imaging of segment formation. Neither *her1* nor *her7* single-gene mutants have altered clock period. However, the authors' data indicate that Hes6 regulates the period of segmentation clock oscillations independently of its dimerization with Her7.

The authors then generated a mathematical model that accounted for all of the dimerization and DNA-binding capabilities of the proteins. Modelling the single mutants in this system revealed the dependence of cyclic mRNA oscillation period on Her1 and Her7 availability, as regulated by dimerization with Hes6. Thus, the authors predict from this model that formation of dimers from the 'dimer cloud' influences the relative stability of the proteins and that dimer formation is important for cyclic gene expression oscillations. They were able to confirm the oscillation of the constantly expressed Hes6 at the protein level *in vivo*.

Therefore, the authors show that a two-loop transcriptional circuit is regulated through the formation of dimers that do not bind DNA. The use of a pool of DNA-binding and non-DNA-binding dimers could be relevant to other clocks.

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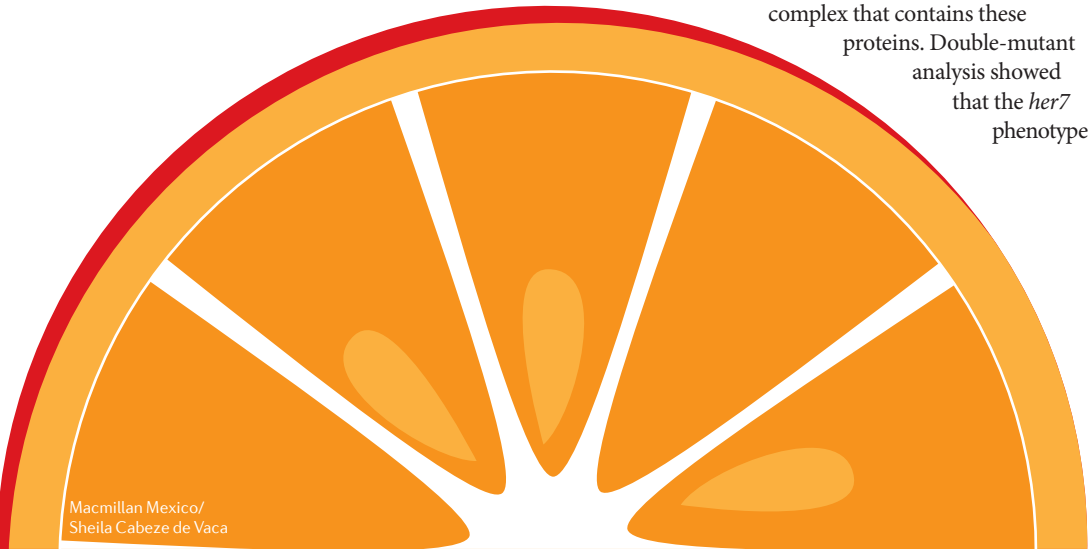

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**ORIGINAL RESEARCH PAPER** Schröter, C., *et al.* Topology and dynamics of the zebrafish segmentation clock core circuit. *PLoS Biol.* **10**, e1001364 (2012)