

**Supplementary Figure 3: Some aspects of *prd* expression are not recovered by the simulation.**

This figure compares double FISH data of pair-rule gene expression with simulated transcript expression, as in Fig 3 and Supplementary Figure 2. (A) *prd* transcripts (magenta) are shown relative to *eve* (green), *odd* (blue) or *slp* (cyan) transcripts, at four different embryo ages (left) or simulation timepoints (right). Note that the figure presents a different set of developmental ages / simulated timepoints from those in Fig 3 and Supplementary Figure 3, in order to show cellularisation in greater temporal detail. At “early cellularisation”, the primary pair-rule genes are expressed but *prd* and *slp* are not; at “mid cellularisation”, *prd* is expressed but *slp* is not; at “late cellularisation”, *slp* is additionally expressed. While the simulated expression of *prd* is broadly appropriate early on (e.g. compare *prd* and *odd* expression at mid-cellularisation / T26), several aspects of *prd* expression are not recapitulated by the model. First, the changing phasing of *prd* posterior borders and *eve* anterior borders between mid-cellularisation and late cellularisation (see also Fig 5). Second, the splitting of the *prd* stripes at late cellularisation, slightly prior to the appearance of the secondary stripes of *odd* and *slp*. Third, the patterning of the *prd* “A” stripes (i.e. the narrow stripes formed from anterior portions of the early broad stripes, asterisks in the in situ images) and the consequent emergence of single-segment periodicity. (B) Uncropped views of the embryos shown in (A). Scale bars 100 µm.