CELL CYCLE

An unlikely pairing

Previous studies have indicated that the origin recognition complex (ORC) has roles beyond the formation of pre-replication complexes (pre-RCs) in early G1 phase. Indeed, Shimada and Gasser now report that the ORC functions during sister-chromatid pairing in mitotic yeast cells.

Using an inducible depletion construct of genomic *orc2*, which encodes a component of the ORC, the authors found that *Orc2* depletion does not impair initiation of replication at pre-formed pre-RCs, but that progression through mitosis was delayed. This G2–M-phase delay could reflect the activation of a mitotic checkpoint. Rad53 kinase, which is induced by the DNA-damage checkpoint, was transiently activated in *Orc2*-depleted cells in late S–G2 phase.

However, elimination of Rad53 activation accelerated the G2–M transition only slightly, which indicated that another checkpoint might be activated. This was shown to be the spindle-assembly checkpoint.

Shimada and Gasser hypothesized that *Orc2* depletion might impair sister-chromatid cohesion, which in turn activates the spindle-assembly checkpoint. Indeed, chromatid pairing was defective in *Orc2*-depleted cells, even though cohesin was stable and properly associated with chromatin. Genetic studies showed that the *Orc2*-related defects were additive to those caused by the improper loading of cohesin on chromatin. Also, the cohesion defects could be reversed by reinducing *Orc2* in G2-phase cells, which does not work

Budding yeast cells going through mitosis. DNA is stained in blue, spindle microtubules in green, nucleoli in red and telomeres in yellow (by FISH). Image courtesy of S. Gasser, Friedrich Miescher Institute, Basel, Switzerland.

for cohesin. Last, the introduction of a large array of ORC-binding sites in cohesin-deficient cells suppressed local sister-chromatid separation, enabling cohesin-independent sister-chromatid cohesion. Together, these data suggest two separate mechanisms for sister-chromatid cohesion — one mediated by cohesin and one by ORC.

The crucial question that remains is: how does ORC mediate sister-chromatid pairing? The authors propose a model in which ORC promotes sister-chromatid cohesion by forming a bridge between sister chromatids that is independent of the cohesin ring. Whether other ORC-binding factors have a role in this process is not yet known.

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“The crucial question that remains is: how does ORC mediate sister-chromatid pairing?”

ORIGINAL RESEARCH PAPER Shimada, K. & Gasser, S. M. The origin recognition complex functions in sister-chromatid cohesion in *Saccharomyces cerevisiae*. *Cell* **128**, 85–99 (2007)

FURTHER READING Hirano, T. At the heart of the chromosome: SMC proteins in action. *Nature Rev. Mol. Cell Biol.* **7**, 311–322 (2006)

WEB SITE

Susan Gasser's laboratory: http://www.fmi.ch/html/research/research_groups/epigenetics/Susan_Gasser/Susan_Gasser.html

DEVELOPMENT

Reaching one's range

Secreted morphogens form concentration gradients that provide positional information during development; therefore, morphogen concentration and distribution must be tightly regulated. But how is the range and shape of these gradients controlled? González-Gaitán and colleagues now show that morphogens with different gradient ranges have different mechanisms and kinetics of spreading through the epithelium.

The authors compared two morphogens, Decapentaplegic (Dpp) and Wingless (Wg), which both spread non-directionally in the *Drosophila melanogaster* wing but with different spreading characteristics; Dpp forms a longer range gradient than Wg. To investigate how the gradient range is controlled, they used fluorescence recovery after photobleaching (FRAP) of a functional green fluorescent

protein (GFP)–Dpp, or GFP–Wg, fusion construct and measured four important kinetic parameters that govern morphogen spreading: the production rate, the effective diffusion coefficient, the immobile fraction and the degradation rate.

The values of the four kinetic parameters differed for Dpp and Wg. For example, the degradation rate of Wg was approximately five times higher than that of Dpp, which could account for the shorter range of the Wg gradient. Different values were also observed for the immobile fraction of these molecules — 62% of the Dpp molecules were immobile, whereas the Wg pool was almost fully mobile at 25°C.

Last, the authors analysed the role of endocytosis on gradient formation by using a thermosensitive dynamin-mutant *shibire^{ts1}* to look at the acute

effects of blocking endocytosis in a FRAP experiment. Endocytosis was required not only for Dpp degradation, but also for Dpp movement. By contrast, Wg transport and degradation were independent of dynamin-mediated endocytosis.

The authors concluded that “different morphogen gradients can be generated by independent fine-tuning of the four important kinetic parameters ... and that different morphogens may use different mechanisms of transport and cellular machineries to achieve the formation of morphogen gradients.” Further analysis is now required to measure the kinetics of other morphogens, such as Hedgehog, and to investigate whether the mechanisms of morphogen transport are conserved among different species.

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ORIGINAL RESEARCH PAPER Kicheva, A. et al. Kinetics of morphogen gradient formation. *Science* **315**, 521–525 (2007)

FURTHER READING González-Gaitán, M. Signal dispersal and transduction through the endocytic pathway. *Nature Rev. Mol. Cell Biol.* **4**, 213–224 (2003)

Morphogen FRAP: wing disc showing GFP–Dpp expressed in the endogenous source with a stripe of bleached fluorescence adjacent to the source of production. Image courtesy of Marcos González-Gaitán, Department of Biochemistry, University of Geneva, Geneva, Switzerland.