CELL CYCLE

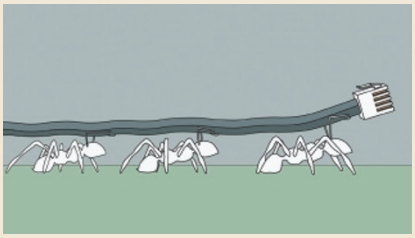
A supporting role?

In vertebrates, different complexes of cyclins and cyclin-dependent kinases (Cdks) trigger S phase and mitosis. In *Xenopus* egg extracts, Cdk2–cyclin E can promote entry into S phase, but not mitosis — the reverse is true for Cdk1–cyclin B. What is responsible for this specificity? In yeast, a single B-type cyclin can be made to promote entry into both S phase and mitosis. So, can Cdk1–cyclin B be made to support DNA replication in vertebrates?

To answer these questions, Tim Hunt and colleagues removed two possible reasons for the inability of Cdk1–cyclin B to support replication — its switch-like response to increasing cyclin B levels and its predominantly cytoplasmic localization.

Cyclin E only supports replication in the nucleus, and cyclin B1 is kept out of the nucleus by a nuclear export signal (NES). So, the authors created several fusion proteins that they introduced into *Xenopus* egg extracts in which cyclin E was depleted and replication reduced to 10–15% of control levels. They found that glutathione-*S*-transferase (GST)–cyclin B1ΔN120 (no NES) did localize, albeit slowly, to the nucleus, but couldn't support replication. By contrast, GST–cyclin E–B1 (no NES, but has the nuclear localization signal (NLS) of cyclin E) not only localized to the nucleus but supported replication too, as did GST–cyclin B1ΔN120 plus the NLS of the SV40 large T antigen. This indicated that an NLS is enough for cyclin B1 to support replication.

Hunt and colleagues then looked at the timing of replication and mitosis in the supplemented extracts, as the concentrations of GST–cyclin E–B1 that restored replication overlapped with those that initiated mitosis. Most replication was restored by 200 nM GST–cyclin E–B1 but a higher/lower concentration was ineffective. At this concentration, replication ceased after ~1 h in time with entry into mitosis. By adding a high concentration of the catalytic domain of the Cdc25B phosphatase (an activator of the Cdk1–cyclin B kinase activity) to overcome the inhibitory effects of the Wee1 and Myt1



kinases on Cdk1, the authors reduced the concentration of cyclin E–B1 needed to restore replication to 80 nM — a concentration that did not trigger mitosis. Cdc25B also allowed GST–cyclin B1ΔN120 to support replication, but not GST–cyclin B1ΔN82, which retains its NES.

So, Cdk1–cyclin B1 can support replication if it is localized in the nucleus. And the fact that there is a block on such a supporting role suggests "…that access to substrates in time and space plays a critical role in determining the function of particular Cdk–cyclin complexes".

Natalie Wilson

References and links

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TRANSCRIPTION

Organ grinder not monkey?

SpoOA, a transcriptional activator protein in *Bacillus subtilis*, is a well-established central player in sporulation. Now, a new study, published in *Genes and Development*, provides compelling evidence that SpoOA, rather than simply controlling the initiation of sporulation, is predominantly a cell-specific transcriptional regulator.

B. subtilis is a well-characterized model system for differentiation and programmed cell-specific gene expression. During sporulation in *Bacillus*, two cell-types form: the mother cell and the forespore, which is destined to become a spore. Without the SpoOA response-regulator, sporulation doesn't occur.

A complex phosphorelay integrates metabolic, environmental and cell-cycle cues to activate SpoOA. Phosphorylated SpoOA activates the transcription of sporulation genes, which tips the balance so that sporulation occurs. So, as in eukaryotes, phosphorylation of key proteins regulates cell-cycle progression.

However, Fujita and Losick noticed that an operon in the mother cell controlled by SpoOA was transcribed after sporulation had initiated, so they investigated whether SpoOA controls gene expression following sporulation initiation. Surprisingly, SpoOA was shown to be active throughout sporulation. Furthermore, it was active in a cell-specific fashion. SpoOA accumulated in the mother cell, and overexpression of a constitutively active SpoOA mutant in the forespore drastically reduced sporulation efficiency. Finally, expression, in the mother cell, of a truncated form of SpoOA, which competed with native SpoOA for phosphorylation, reduced sporulation efficiency. So, the location and activity of SpoOA is crucially important for development. Viewed in this light, SpoOA is analogous to the related transcription factor CtrA in *Caulobacter crescentus*. Like SpoOA, CtrA is a master regulator that becomes a cell-specific transcription factor during the cell cycle of *Caulobacter*. CtrA activates or represses the expression of one-quarter of the *Caulobacter* cell-cycle-regulated genes, integrating DNA replication, morphogenesis and cell division. *Caulobacter* divides to produce two cell types: a stalked cell and a swarmer cell, which is a dispersal cell that swims until conditions allow renewed cell division.

CtrA binds to, and silences, the origin of replication in swarmer cells — initiation of chromosome replication depends on temporally controlled proteolysis of CtrA in the stalked cell. Why is SpoOA activity restricted to one cell type? When the wall is synthesized between mother cell and forespore, two-thirds of the forespore chromosome remains in the mother cell. As the excluded chromosome is pumped into the forespore, certain genes are asymmetrically expressed. So, asymmetric expression of phosphorelay genes might result in SpoOA phosphorylation only in the mother cell. This could ultimately decide the fate of SpoOA, through targeted proteolysis of unphosphorylated SpoOA in the forespore. Comparisons between the parallel systems in *Caulobacter* and *Bacillus* will undoubtedly push forward our understanding of programmed cell-specific gene expression.

Susan Jones, Associate Editor,
Nature Reviews Microbiology

References and links

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