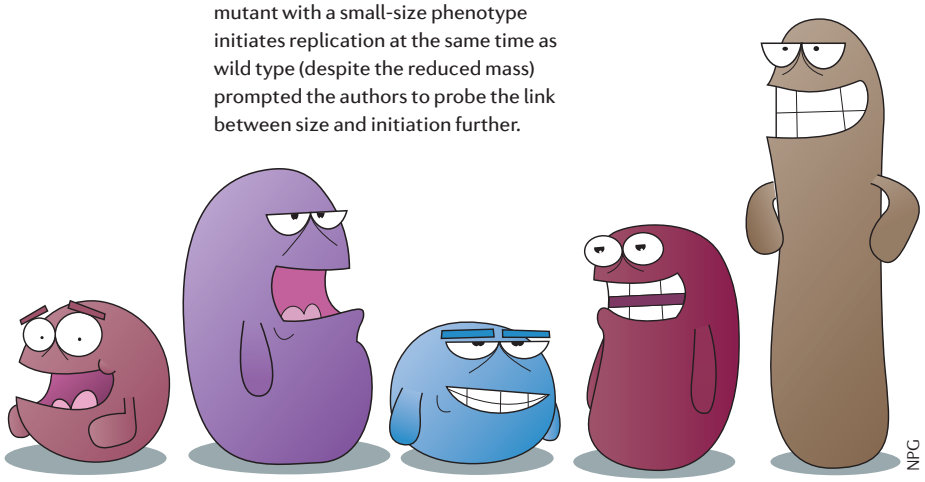
CELLULAR MICROBIOLOGY

Replication comes in all sizes

Cells must integrate their growth, DNA replication and cell division cycles to ensure faithful inheritance of their genetic material. It was previously thought that, in bacteria, the growth-dependent accumulation of active DnaA provided a universal mechanism to couple growth with DNA replication initiation. However, as Levin and colleagues now show, there are at least two distinct mechanisms for controlling the timing of initiation.

The paradigm for control of DNA replication initiation comes from *Escherichia coli*, in which active DnaA (a highly conserved ATPase that triggers initiation in all bacterial species studied) accumulates as the cell grows until, at a particular cell size, there is sufficient active protein to unwind DNA at the origin and provide access for the replication machinery. However, the observation that a *Bacillus subtilis* mutant with a small-size phenotype initiates replication at the same time as wild type (despite the reduced mass) prompted the authors to probe the link between size and initiation further.

Using the small *B. subtilis* mutant and two small *E. coli* mutants, which had roughly the same overall generation time as their wild-type counterparts, the authors found that the *E. coli* mutants delayed initiation of DNA replication until they grew to the size at which wild-type cells initiated replication. By contrast, the *B. subtilis* mutant initiated replication at the same time in the cell cycle as the wild type, so mutant cells were smaller than wild-type cells at initiation. Importantly, the overall DnaA concentration in the three mutants matched that in the respective wild-type cells, meaning that a reduced total DnaA amount in the mutants (~30% lower than wild-type levels) correlated with a delay in DNA replication initiation in *E. coli* but not in *B. subtilis*.



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According to the *E. coli* model, smaller cells (with lower levels of active DnaA) should have fewer replication origins and replication forks than wild-type cells, and this was indeed the case for the small *E. coli* mutants. Moreover, increasing the amount of DnaA in these *E. coli* cells rescued the initiation timing and origin number phenotype. By contrast, the small *B. subtilis* mutant had the same number of origins and forks as wild-type cells, despite the 30% reduction in total DnaA.

These results indicate that the accumulation of available active DnaA to the crucial level is growth dependent in *E. coli*, so that cells initiate DNA replication at a specific size, but cell cycle dependent in *B. subtilis*, so that cells initiate at a set time in the cycle. The two species are known to use different positive and negative regulatory methods to alter the active/inactive DnaA ratio and DnaA access to the origin throughout the division cycle. A more complete appreciation of these regulatory methods and their integration with growth and/or the cell cycle will lead to a better mechanistic understanding for both branches of replication initiation control.

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ORIGINAL RESEARCH PAPER Hill, N. S. *et al.* Cell size and the initiation of DNA replication in bacteria. *PLoS Genet.* **8**, e1002549 (2012)
FURTHER READING Weart, R. B. *et al.* A metabolic sensor governing cell size in bacteria. *Cell* **130**, 335–347 (2007) | Katayama, T. *et al.* Regulation of the replication cycle: conserved and diverse regulatory systems for DnaA and *oriC*. *Nature Rev. Microbiol.* **8**, 163–170 (2010)