● COMMENTARY

Sir2 takes affirmative action to ensure equal opportunity in replication origin licensing

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In all eukaryotes, chromosomal DNA replication initiates at multiple sites distributed along chromosomes called origins of replication. The initiation of DNA synthesis is a two-step process involving the loading of the minichromosome maintenance (MCM) double hexamer on origins during the G_1 phase of the cell cycle and the activation of licensed origins during the S phase (1). This strict temporal separation of origin licensing from firing ensures that a given origin is activated once and only once per cell cycle, thereby preventing overreplication of the genome (Fig. 1A). To avoid underreplication, cells must also ensure that initiation events are evenly distributed along chromosomes. This is achieved by licensing many more origins than actually needed (2, 3). Consequently, only a fraction of potential initiation sites are activated during the S phase, the remaining ones serving as backup origins when fork progression is impeded (4, 5). Origin activation follows a defined replication timing program, in which open chromatin domains replicate early in S phase and heterochromatin replicates late (1). This sequential activation of replication origins allows a tight coordination between DNA synthesis and the production of histones and dNTPs. Moreover, the replication program can be interrupted when forks encounter DNA lesions and restarted once replication stress is relieved (6). The correct execution of the replication timing program is therefore essential to maintain the integrity of eukaryotic genomes.

Although the general rules governing origin firing are now well understood, the molecular mechanisms that determine the timing and efficiency of initiation at individual origins are still debated. In metazoan and in fission yeast, multiple MCM complexes can be loaded at a replication origin, increasing its probability to initiate DNA synthesis (7). In contrast, only one MCM double hexamer is loaded at origins in budding yeast and origin efficiency does not correlate with MCM levels (8). However, origin activation is modulated by epigenetic mechanisms involving the histone deacetylases Rpd3 and Sir2 (9, 10). Origin firing also depends on the ability of prereplication complexes to attract limiting

initiation factors (11–13). It is generally believed that these factors are recycled after initiation to activate additional origins, ensuring therefore a staggered activation of origins throughout the S phase. More recently, studies in budding yeast have added another layer of complexity to this regulation by showing that repeated elements containing replication origins, such as the ribosomal DNA (rDNA) array, compete with single-copy loci for limiting initiation factors (14). In the absence of Sir2, increased initiation at the 150 to 200 rDNA origins further increases the competition for limiting initiation factors and reduces initiation at unique loci (9, 14–16). As a consequence, $sir2\Delta$ cells reach G_2/M with replication gaps, leading to increased genomic instability (15).

In PNAS, Hoggard et al. (17) propose an alternative model for how the histone deacetylase Sir2 prevents genomic instability in budding yeast, through a mechanism that is independent of the derepression of rDNA origins. This model builds on the fact that replication origins are located within nucleosome-free regions to facilitate the loading of MCMs by the origin recognition complex (ORC), Cdc6, and Cdt1 (18, 19). It has been reported that nucleosomes interfere with MCM loading in $cdc6-4$ mutants, which have a reduced ability to assemble prereplication complexes (pre-RCs) (20). In the absence of Sir2, higher levels of H4K16 acetylation would increase the mobility of origin-proximal nucleosomes (Fig. 1A), facilitating MCM loading and cell growth (20, 21). However, why does this increased licensing activity rescuing growth defects in $cdc6-4$ mutants lead to more genomic instability in a wild-type context? To answer this question, the authors measured the chromatin levels of Mcm2, Sir3, and H4K16ac in wild-type and $sir2\Delta$ cells by chromatin immunoprecipitation and next-generation sequencing. They also monitored the consequences on the kinetics of DNA replication using a label-free approach called SortSeq. In $sir2\Delta$ cells, they observed a higher level of both H4K16ac and Mcm2 at early origins relative to late origins, indicating that Sir2 dampens pre-RC assembly at early origins. They
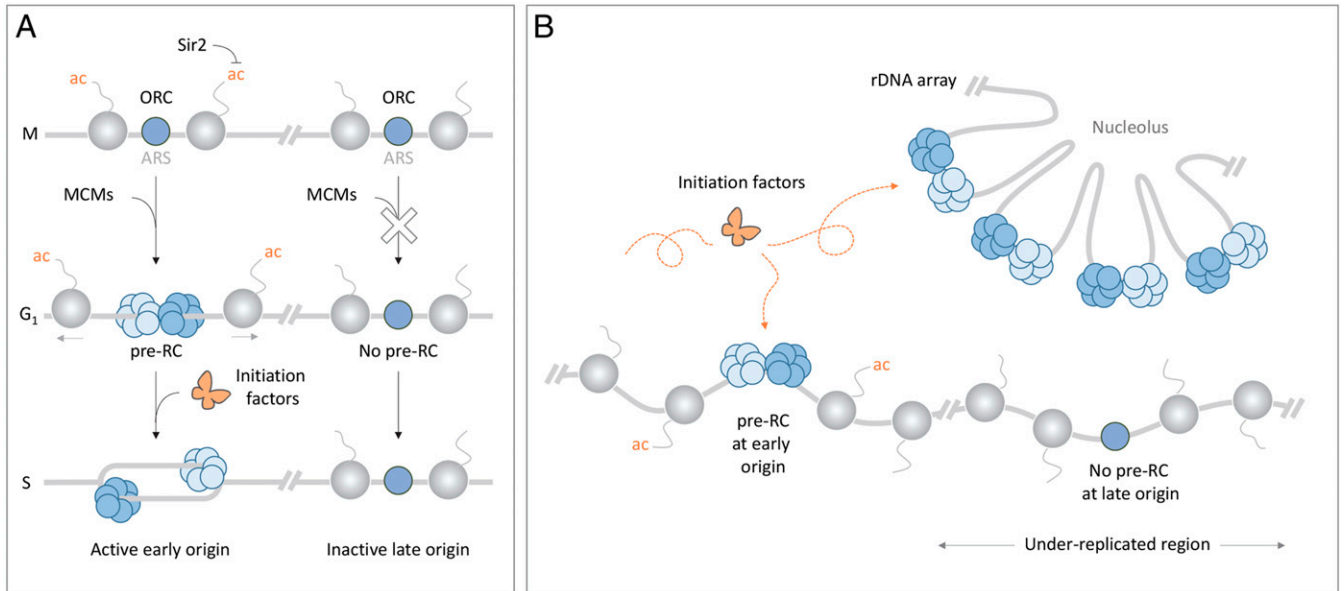

Fig. 1. The histone deacetylase Sir2 dampens the activity of early origins to favor an equal distribution of initiation events throughout the budding yeast genome. (*A*) The activation of replication origins in budding yeast depends on the binding of the origin recognition complex (ORC) to autonomous replicating sequences (ARS) and the loading of Mcm2-7 double hexamers in a Cdc6- and Cdt1-dependent manner to form the prereplication complex (pre-RC). Although ORC is bound to origins throughout the cell cycle, pre-RCs can only be assembled in G_1. Cyclin-dependent and Dbf4-dependent kinases (CDK and DDK) trigger origin firing upon entry into S phase. Since origin activation depends on a set of limiting initiation factors, initiation events occur sequentially throughout the length of the S phase. The licensing of replication origins occurs in the context of chromatin. Nucleosomes flanking early origins (but not late origins) are acetylated on H4K16, which increases their mobility and facilitates MCM loading. By deacetylating these nucleosomes, Sir2 would reduce the advantage of early origins over late origins for pre-RC loading, ensuring therefore a more even distribution of licensed origins throughout the genome (17). (*B*) In the absence of Sir2, suboptimal pre-RC assembly at late origins increases the risk of underreplication in late-replicating regions and the persistence of replication gaps (17). Since *sir2Δ* cells show an increased activation of ribosomal DNA (rDNA) origins, the reduced activation of late origins could also be due to a competition for limiting initiation factors (14, 15). Replication defects observed in *sir2Δ* mutants could therefore be due to a combination of origin licensing and activation defects, even though the relative contribution of both mechanisms remains to be established. Note that late origins refer here to euchromatic late origins and not to telomeric origins, which are directly repressed by Sir2.

also found that late origins were less efficiently activated, which is consistent with earlier studies (14) and explains the persistence of replication gaps at the end of S phase (Fig. 1*B*). This reduced activation of late origins in *sir2Δ* mutants was not suppressed in strains bearing a single-nucleotide polymorphism in rDNA origin, weakening its activity (22). Altogether, these data indicate that Sir2 does not only act through the rDNA to regulate the activation of single-copy origins but also acts directly at the level of individual origins. These results also argue for a model in which Sir2 promotes the licensing of late origins by reducing the ability of early origins to load MCMs, contributing therefore to a continuum of origin activation throughout the S phase.

This provocative model raises a number of questions and opens directions for future research. The first question concerns the relative contribution of Sir2's distinct functions in origin licensing (17) and in origin activation through an rDNA-mediated process (14, 15). To rule out a contribution of the rDNA in the replication defects observed in *sir2Δ* mutant cells, Hoggard et al. monitored the activity of late origins in a strain bearing weak rDNA origins and found that reduced rDNA replication did not restore initiation at late origins, confirming thereby that Sir2 acts directly at origins to execute its function. Although this conclusion is valid, this experiment does not formally rule out the possibility that inactive rDNA origins could sequester initiation factors and therefore impede initiation at late origins. This view is supported by a recent study from the Bedalov laboratory (15) showing that weakening rDNA replication suppresses replication gaps in the same strain. It is therefore likely that Sir2 acts both directly and indirectly to modulate euchromatic origin activation (Fig. 1*B*). This is reminiscent of Rpd3, a histone

deacetylase involved in the repression of late origins firing that acts both locally and through an rDNA-mediated mechanism (10, 14).

Another interesting question concerns the mechanism by which Sir2 reduces the rate of initiation at early origins relative to late origins. It has been proposed that origin-proximal nucleosomes interfere with pre-RC assembly, unless if they are acetylated on H4K16 and can be pushed aside to accommodate the loading of the MCM double hexamer (20). Although this model is plausible, other mechanisms could be envisaged. For instance, it has been recently reported that Sir2 represses origin activation at the rDNA by silencing noncoding transcription through rDNA origins. Indeed, increased transcription in *sir2Δ* cells displaces MCMs to a nucleosome-free region, increasing the probability of origin firing (16). Since pervasive transcription is a widespread process, similar mechanisms could also operate at single-copy early origins. However, recent evidence indicates that pervasive transcription has rather a negative effect on single-copy origins by inactivating pre-RCs (23, 24). Transcriptionally inactivated origins can be repetitively licensed in G_1 to reestablish their functionality after transcription (25). It is therefore tempting to speculate that Sir2 promotes genome integrity by reshuffling the distribution of pre-RCs in the genome in a transcription-dependent manner. This would ensure that the most favored early origins are not systematically used, to the detriment of late origins located in a less favored environment.

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