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SPOTLIGHT

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The full licensing of replication origins in late G1 is normally enforced by the licensing checkpoint. In this issue, Matson et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201902143) show that this checkpoint is inoperative in cells exiting from G0, resulting in incomplete origin licensing and consequent replicative stress.

Whether a cell should commit to the cell division cycle and duplicate itself is one of the cell's most fundamental decisions. In many eukaryotic cells, this decision is made at the R point (restriction point), which flips transcriptional and posttranscriptional control circuits to activate the CDKs that drive progression into S phase and through the rest of the cell division cycle. Replicating the large amount of DNA in eukaryotic cells requires the controlled activation of hundreds or thousands of replication origins spread throughout the genome, so that no sections of DNA are left unreplicated and no section of DNA is replicated more than once. One key feature of this control is the regulated "licensing" of replication origins, which occurs exclusively during late mitosis and G1 (1). Origin licensing involves the stable loading of a pair of MCM2-7 hexamers onto replication origins; during S phase, in the presence of high CDK activity, these MCM2-7 hexamers are activated to form CMG (CDC45-MCM-GINS) replicative helicases that power replication fork progression (Fig. 1). This regulation ensures that no sections of DNA are replicated more than once in a single cell cycle; minichromosome maintenance complexes (MCMs) are removed from origins when replication forks initiate, and new MCM2-7 cannot be reloaded onto origins until progression through mitosis. This replication scheme means that the entire collection of origins that can potentially be used by a cell is established before S phase entry. When replication forks encounter problems in progressing along the DNA (such as DNA damage or tightly bound chromatin proteins), additional "dormant" origins can be activated to help complete DNA replication, but all these origins must have been already licensed before S phase entry (2). Cells must therefore anticipate any contingencies that might occur in S phase and license enough origins before they enter S phase.

Many eukaryotic cells possess a "licensing checkpoint" that ensures they only enter S phase once a sufficient number of origins have been licensed (4–7). In this issue, the study by Matson et al. sheds fascinating light on the licensing checkpoint and shows that, surprisingly, it does not operate efficiently in cultured cells entering S phase following serum starvation or contact inhibition.

Matson et al. show that, in cycling cells (cells that have recently divided in normal culture conditions), the licensing checkpoint operates like other cell cycle checkpoints (3). siRNA knockdown of the licensing protein CDT1 (required for loading MCM2-7 hexamers onto origins) delays CDK2 activation and progression through the R point until such time as normal MCM2-7 loading levels have been achieved. Disruption of normal R point control (through the overexpression of cyclin E or knockdown of p53) bypasses the checkpoint and allows CDT1-depleted cells to enter S phase with a reduced number of licensed origins.

In cells entering S phase from G0 (for example, after serum starvation and refeeding), the situation is very different (3). The researchers observe that knockdown of CDT1 causes no significant delay of S phase entry, so that cells enter S phase with low levels of DNA-bound MCM2-7. Knockout of p53 makes little difference to the effect, consistent with the idea that the licensing checkpoint is almost completely inactive in wild-type cells exiting from G0. Worse still, the lack of a licensing checkpoint in wild-type cells entering S phase from G0 has a clear consequence, as even in the absence of any perturbations, S phase cells have reduced levels of DNA-bound MCM2-7. Consistent with the idea that this reduces the number of dormant origins available to deal with normal endogenous replication problems, cells entering S phase from G0 show more signs of replication stress (as assessed via YH2AX foci and DNA-bound RPA) and are hypersensitive to gemcitabine and etoposide, drugs causing replication stress. This is also consistent with a recent report showing that cells re-entering the cell cycle after serum stimulation have increased replication stress–induced CDK2 suppression (8).

These results raise several profound questions about how genome stability is ensured in metazoan cells that may have to frequently exit and enter the cell division cycle. One obvious question is why, when re-entering the cell cycle, should cells bypass this important checkpoint with evident consequences for decreased genome integrity. It does not appear to be because there is insufficient time; in fact, Matson et al. observed that the period over which licensing occurs is actually longer upon G0 exit than it is in cycling cells (3). Of the essential four proteins required for origin licensing (ORC, CDC6, CDT1, and MCM2-7), neither CDT1 nor CDC6 activities appear limiting at G0 exit since normal levels of MCM2-7 loading are not achieved by overexpressing

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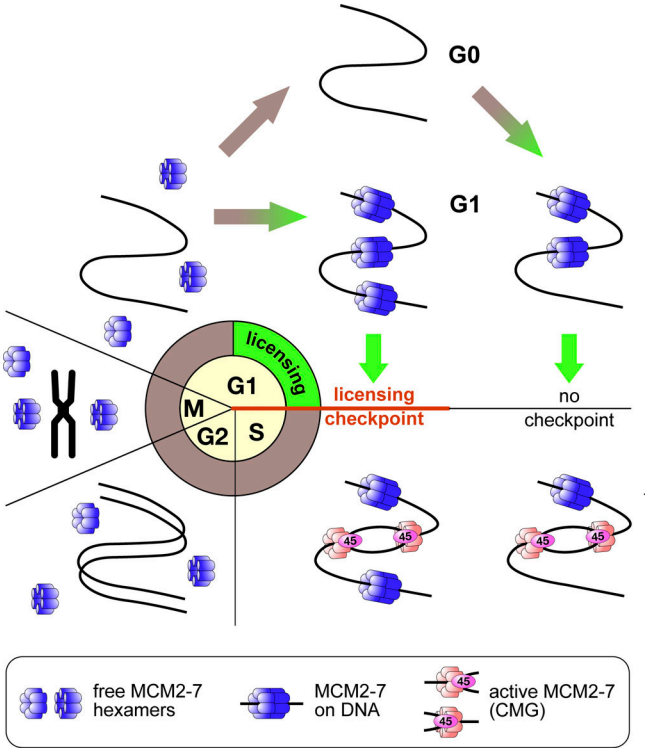

Figure 1. **Licensing in the cell division cycle and on passage through G0.** The small segment of chromosomal DNA that is shown encompasses three replication origins. On exit from mitosis (M), cells progress into G1 or into G0. In G1, the replication licensing system is activated (green), which causes MCM2-7 complexes (blue hexamers) to be loaded onto potential replication origins (i.e., origin licensing). The licensing system is turned off at the end of G1. Matson et al. (3) show that, in cycling cells, the licensing checkpoint is active and prevents cells from entering S phase if insufficient MCM2-7 have been loaded, but that in cells entering G1 from G0/quiescence, the licensing checkpoint is not active. During S phase, some MCM2-7 complexes are activated as helicases as origins fire (pink hexamers). MCM2-7 complexes are removed from replicated DNA, either during passive replication of unfired/dormant origins or at fork termination.

them. However, when G0 exit was delayed by a combination of p53 stabilization (which, among other things, delays CDK activation via induction of the $p21^{CIP1}$ CDK inhibitor) and expression of a stable version of CDC6 (allowing higher CDC6 levels in the absence of CDK activity), Matson et al. found that DNA-bound MCM2-7 were restored to the "normal" levels seen in cycling cells (3). Licensing can occur very rapidly in certain cell types, such as pluripotent stem cells (9). It is possible that there is some feature of the licensing that occurs on G0 exit that is intrinsically slow, maybe due to some feature of chromatin or chromosome structure at replication origins that differs between G0 and cycling cells. It is unclear what is different about the R point in cells exiting from G0 that means the licensing checkpoint is inactive.

The new results from Matson et al. (3) also raise a host of questions about how cells behave in real tissues in vivo and the extent to which this mirrors what is seen with tissue culture cells. The environment of cells in vivo, with a multitude of different short-range and long-range signals, is significantly more complex than the environment of cultured cells. This could mean that the way that cells transit between proliferating and nonproliferating states is profoundly different in vivo and in vitro. For example, we have recently shown that in the small intestinal epithelium, most proliferative $Lgr5^{+}$ stem cells express MCM2-7 proteins but are poised in an unlicensed G1-like state (10). It would be of great interest to apply the modern technology used by Matson et al. (3) to determine whether the same underlicensing that they observe in vitro occurs in similar circumstances in vivo.

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