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An intrinsic checkpoint model for regulation of replication origins

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Abstract

A recent paper by Alvino et al., (MCB 2007; 27:6396) challenges the standard model of the DNA replication checkpoint. Their work shows that the checkpoint does not simply prevent late origins from firing in the presence of the nucleotide synthesis inhibitor hydroxyurea; instead it delays origin firing to maintain the regular order of origin firing relative to the now much slower rate of fork elongation. To explain these results, this perspective proposes a model in which the timing of origin firing is intrinsically coupled to the rate of fork elongation by the fact that late origins can only fire after early forks have terminated and released some essential replisome factor. This coupling fails in a checkpoint mutant background because stalled forks disassemble and release replisome factors prematurely, allowing for unregulated origin firing.

Keywords

DNA replication; checkpoint; Rad53; replication origin; hydroxyurea; replication arrest

For several decades after the discovery that radiation damage causes cell cycle arrest, it was widely assumed that the arrest was an intrinsic result of cellular damage—that damage mechanically prevents cell division or DNA replication and therefore the cell cycle could not continue until the damage is repaired. That paradigm was shattered by the work of Weinert and Hartwell who showed that the cell cycle delay of budding yeast in response to DNA damage was an active regulatory response that could be bypassed by mutation.[1] They coined the term 'checkpoint' for such a pathway and introduced 'relief of dependency' as a way to test if a cell cycle arrest is due to a checkpoint.[2] The relief of dependency test is a simple one: if you have a treatment that leads to a cell cycle arrest and you can find a condition, such as a mutation or drug, that bypasses the arrest caused by the treatment, then you can call the arrest a checkpoint. The term 'checkpoint' is used much more broadly than the original Weinert and Hartwell definition, but it still generally conjures up the image of a signaling pathway that recognizes DNA damage or some other problem and inhibits cell cycle progression by a mechanism independent of the problem.

One well-studied example is the budding yeast replication checkpoint, which prevents mitosis when replication is blocked, for instance by nucleotide depletion caused by the ribonucleotide reductase inhibitor hydroxyurea (HU). The well-supported dogma in the field is that the checkpoint recognizes the stalled replication forks and activates the Rad53 checkpoint kinase, which phosphorylates downstream targets to stabilize the stalled forks and block mitosis.[3] Rad53 is also believed to prevent the firing of subsequent replication origins, an apparently reasonable precaution if replication is blocked.

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The later part of the model, that Rad53 inhibits late-origin firing, is called into question by a recent paper from the Brewer and Raghuraman labs.[4] In this paper, they use a concentration of HU that reduces nucleotide synthesis to a rate that greatly slows, but does not completely block, DNA synthesis. They show that late origins are not specifically inhibited; rather the entire progression of S phase is delayed in proportion to the reduction in replication fork elongation. Therefore, late origins fire, but not for many hours, long after the end of the time courses originally used to monitor their firing. At one level, it is a semantic difference whether late origins are blocked or just delayed. But, the paper presents two observations that have important mechanistic implications. The first is that all origins are delayed in HU, not just late ones. The second is that the delays in origin firing are closely linked to the extent of replication inhibition, so that the overall kinetics of replication is slowed, but the timing of origin firing relative to the extent of replication is maintained. Neither of these observations is consistent with the current standard checkpoint model.

Alvino et al. put forward a modified checkpoint model to account for their results.[4] They propose that Rad53 is activated by the single-stranded DNA that accumulates in front of stalled replication forks, as in the standard model. However, in their model, Rad53 does not completely inhibit subsequent origin firing; instead it reduces the rate of origin firing by downregulating the activity of the Cdc7/Dbf4 replication kinase. Furthermore, the downregulation of Cdc7/Dbf4 does not disrupt the relative timing of origin firing, so the order in which origins fire is maintained.

Although this model explains the data, it requires a mechanism to keep the timing of origin firing in sync with fork progression. In particular, the checkpoint needs to be able to measure the extent of replication slowing and reduce the rate of origin firing by just the same amount. If the relative delay in origin firing was greater than the inhibition of elongation, fewer origins would fire because they would be passively replicated by forks from neighboring earlier origins. Conversely, if the delay in origin firing was less than the inhibition of elongation, more origins would fire because fewer would be passively replicated. However, the striking result is that replication profiles with and without HU are super-imposable. How the checkpoint would synchronize the origin firing rate to match the extent of replication inhibition it is unclear.

An alternative model harkens back to the old ideas of intrinsic cell cycle arrest. What if, instead of an active checkpoint that delays origin firing in HU-treated cells, origin firing was directly inhibited by the reduction in elongation? Such a mechanism could act if replication fork initiation requires a limiting factor that is part of the elongating fork—a polymerase, for instance. If there was a limiting amount of polymerase, later forks could initiate only once polymerases were released by the termination of earlier forks. If origin firing depends on polymerase recycling, late origin firing would be intrinsically dependent on fork elongation. Similar models have been proposed to explain the regulation of origin firing efficiency in unperturbed S phase.[5,12] Here, polymerase recycling would ensure that the firing of late origins would be delayed whenever elongation was slowed, independent of the canonical replication checkpoint mechanism. Such a mechanism might be called an intrinsic checkpoint because its mechanism is an intrinsic biochemical coupling of one cell cycle process to another and yet, as explained below, it meets the ‘relief-of-dependency’ test because it is bypassed by mutation of Rad53. The intrinsic checkpoint model avoids the problem described above by naturally synchronizing elongation rate and origin timing. If new origins cannot fire until old forks have disassembled, then the kinetics of origin firing are intrinsically linked to the kinetics of replication fork progression.

The obvious problem with such an intrinsic checkpoint model is that mutation of *rad53* disrupts the checkpoint and causes origins to fire early in the presence of HU. This result led to the

model of checkpoint-dependent inhibition of origin firing in the first place. However, it is possible that Rad53 exerts its influence on origin firing indirectly through its regulation of fork stability. Rad53 is known to be required for the stable stalling of forks in HU.[6] If polymerase recycling is required for new origin firing, the Rad53-dependent sequestration of fork proteins in stably stalled forks would indirectly prevent origin firing in HU. In the absence of Rad53, HU-stalled forks disassemble.[7] Such disassembly would release fork proteins, making them available to initiate new forks and thus lead to the observed origin firing in HU-treated *rad53* mutant cells. In this model, Rad53 does not inhibit origin firing per se, but the loss of Rad53 does allow origins to continue to fire in HU, producing the observed Rad53-dependent inhibition of origin firing.

This intrinsic checkpoint model for the regulation of origin firing in response to HU runs counter to the prevailing notion of the replication checkpoint as a standard signal transduction pathway, but it explains the available data using known or plausible replication mechanisms. Moreover, it makes testable predictions. For one, other treatments that slow fork progression, such as MMS or sub-lethal doses of aphidicolin, should show similar elongation-correlated delays in origin-firing kinetics. In addition, slowing replication in a way that does not activate the replication checkpoint should also delay origin firing. Another prediction is that, since forks are thought to stall and disassemble in HU-treated *rad53*Δ cells more quickly than they would normally terminate in untreated wild-type cells, more origins should fire sooner in HU-treated *rad53*Δ. Such an increase of origin firing has been reported, although the direct comparison between HU-treated *rad53*Δ and untreated wild-type cells has not been made.[8,9] Lastly, the intrinsic checkpoint model does not easily explain the phenotype of the *mec1-100* allele, which appears to allow late origins to fire without compromising fork stability.[10] The model could be reconciled with the *mec1-100* results if these cells have a hybrid phenotype, with intermediate fork instability leading to intermediate firing of late origins. This possibility is consistent with a report of partial destabilization of stalled polymerases in *mec1-100* cells.[11] Whether the model is supported by future studies remains to be seen. Nonetheless, it provides an alternative explanation for the intriguing results of Alvino et al.,[4] and revives an old-fashioned way of thinking about checkpoint control.

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