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Right time, right place—DNA damage and DNA replication checkpoints collectively safeguard S phase

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The DNA replication checkpoint (DRC) and the DNA damage checkpoint (DDC) are two closely linked signaling cascades that adjust S phase to the presence of DNA lesions and other replication impediments. Two recent studies published in *The EMBO Journal* shed new light on their relationship in budding yeast, collectively showing that the two pathways—while sharing several factors—differ in the location and kinetics of their activation, suggesting that they constitute different branches of an integrated cellular response to impaired DNA replication.

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See also: **J Bacal *et al*** (November 2018) and **N García-Rodríguez *et al*** (May 2018)

Genome stability is threatened during S phase, when DNA lesions and other impediments to DNA replication impair the faithful duplication of the genetic information. Therefore, DNA replication is safeguarded by two separable, but related mechanisms termed DNA replication checkpoint and intra-S phase DNA damage checkpoint (Branzei & Foiani, 2009). These checkpoint pathways represent conserved phosphorylation-based signaling cascades, which trigger both local and cell-wide responses to the presence of DNA damage and replication perturbation. Interestingly, DRC and DDC share several essential components, such as the sensor kinase (Mec1-Ddc2, budding yeast homologs of mammalian ATR-ATRIP) and the effector kinase (Rad53 in budding yeast), but are defined by the mutually exclusive involvement of specific mediator proteins, yeast Mrc1 (DRC) and Rad9 (DDC).

Since the discovery of Mrc1 and Rad9, the relationship between DRC and DDC has been intensely studied (summarized in Branzei & Foiani, 2009) leading to a picture that appears contradictory at first glance. On the one hand, DRC and DDC are thought to react to different signals, stalled replication forks, and DNA lesions, respectively, with checkpoint responses adjusted to these different forms of replication impediments (Branzei & Foiani, 2009). On the other hand, both pathways seem to act redundantly, given that cells deleted for *MRC1* and *RAD9* show synergistic phenotypes.

Through series of elegant experiments, two recent studies by the Ulrich and Pasero laboratories (Bacal *et al*, 2018; García-Rodríguez *et al*, 2018) now uncover important differences and similarities between the two checkpoint pathways. Overall, a picture emerges whereby DRC and DDC act at distinct locations and with distinct timing but trigger a highly similar downstream response. This suggests that both pathways should be viewed as two cooperating branches of an integrated cellular response to safeguard genome stability during S phase.

It is well established that the presence of DNA lesions in S phase triggers Rad9-dependent activation of the DDC, whereas replication fork stalling by nucleotide depletion triggers Mrc1-dependent activation of the DRC (Pellicioli *et al*, 1999; Alcasabas *et al*, 2001). Thus, one interpretation could be that different means of fork stalling may lead to distinct types of DNA structures, which in turn are differentially recognized by the two checkpoint pathways. However, activation of Mec1-Ddc2, the essential upstream sensor kinase in both pathways, depends solely on RPA-mediated recognition of single-stranded DNA (ssDNA; Zou & Elledge, 2003), making a qualitative difference between the DRC and DDC signals less likely.

García-Rodríguez *et al* (2018) now shed new light on this problem by showing that both checkpoint pathways are activated at different locations with respect to replication forks (Fig 1A). While the Mrc1-dependent DRC is activated directly at replication forks (Katou *et al*, 2003), they find that the Rad9-dependent DDC instead reacts to gaps on nascent DNA behind replication forks, exposing ssDNA on the template strand (García-Rodríguez *et al*, 2018). Previous electron microscopy (Lopes *et al*, 2006) and genetics work (Callegari & Kelly, 2006; Daigaku *et al*, 2010; Karras & Jentsch, 2010) had collectively suggested the existence of postreplicative gaps, which could form in nascent DNA at sites of UV- or MMS-induced DNA lesions through a mechanism that involves re-priming of DNA replication downstream of the stalling lesion. Two key findings of the Ulrich study are that (i) such gaps in newly replicated DNA are extended by the nuclease Exo1, and (ii) that Exo1-dependent nucleolytic processing is required for efficient activation of the DDC (García-Rodríguez *et al*, 2018). This implies that DRC and DDC both react to the formation of ssDNA, but at

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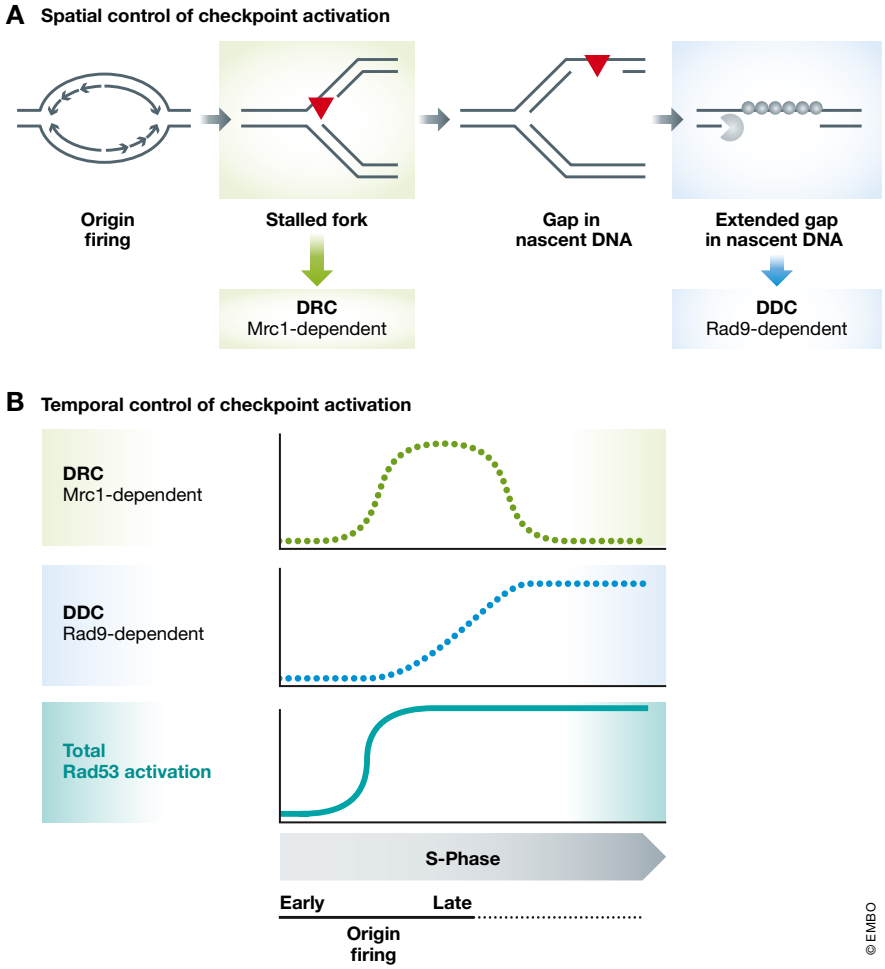

Figure 1. Spatial and temporal aspects of DRC and DDC activation in S-phase.
(A) Spatial separation of DRC and DDC activation. In the presence of fork-stalling DNA lesions (red triangle), the DRC may sense the presence of stalled forks, while DDC activation involves nucleolytic extension of gaps in nascent DNA. Signals recognized by DRC and DDC involve ssDNA coated with RPA (gray circles). (B) Temporal separation of DRC and DDC activation upon the presence of DNA lesions in S phase. In the presence of DNA damage, Mrc1-dependent Rad53 activation (DRC) is fast but transient, while Rad9-dependent Rad53 activation (DDC) is slower and occurs only after late origins have fired. The transient nature of DRC activation suggests that DRC signals mature to become DDC signals.

different locations: while the DRC may signal directly from a stalled replication fork, the DDC appears to become activated at processed, postreplicative gaps.

This idea that DDC activation depends on DNA processing, while DRC activation does not, concurs with a key finding of Bacal *et al* (2018), namely that the two checkpoint pathways show distinct activation kinetics (Fig 1B). Specifically, even upon treatment with MMS, which generates a largely Rad9-dependent response (Alcasabas *et al*, 2001), they observe faster activation of the DRC than the DDC. In this case, however, DRC activation occurs only transiently, suggesting that MMS-treated cells may transition from a DRC-dependent to a DDC-dependent response (Bacal *et al*, 2018).

In essence, budding yeast DRC and DDC both lead to the activation of the same checkpoint effector kinase, Rad53 (Pellicioli *et al*, 1999; Alcasabas *et al*, 2001), which in turn triggers the known S-phase-specific checkpoint responses including stabilization of (stalled) replication forks and inhibition of late origin firing (Branzei & Foiani, 2009). Activation of either pathway should therefore be sufficient for stabilization of forks and inhibition of late origin firing. Notably, Bacal *et al* (2018) find that, although the intra-S phase DDC does not inhibit late origin firing, this is simply due to the slower DDC activation in relation to the DRC. Forcing DDC activation prior to S-phase entry still inhibits late origin firing, consistent with DRC and DDC being equally capable of controlling late origin firing (Bacal *et al*, 2018).

Furthermore, García-Rodríguez *et al* (2018) provide new insight into the essential but still poorly understood mechanism by which Rad53 stabilizes replication intermediates: In a potential feedback control mechanism, Exo1 (and likely other nucleases) that initially promotes DDC activation by extending the ssDNA signal is inhibited by activated Rad53, mechanistically extending earlier observations (Morin *et al*, 2008; Segurado & Diffley, 2008). Finally, a potential third checkpoint function to safeguard DNA replication emerges from the Pasero study. They show that activation of the DDC triggers active slowing down of replication forks (Bacal *et al*, 2018), a phenomenon that appears to be conserved in mammalian cells upon replication stress (Somyajit *et al*, 2017). What the benefit of such replication slowdown could be and whether the yeast DRC might also induce a similar effect are questions that need to be tested in future research.

Taken together, the newly discovered temporal and spatial differences in activation of DRC and intra-S phase DDC offer an intriguing explanation for the dichotomy of the two checkpoint pathways. They also raise questions about the molecular nature of the signals recognized by both pathways. In case of the DDC, previous work has demonstrated the importance of ssDNA (and ssDNA/dsDNA junctions) as key signal mediating recruitment of the sensor kinase Mec1-Ddc2, the checkpoint mediators 9-1-1, Dpb11 and Rad9, and ultimately Rad53 (Zou & Elledge, 2003; Finn *et al*, 2011). The DRC signal is much less understood, but could potentially involve branched DNA structures at stalled forks. While such qualitative differences between the DRC and DDC signals may exist, it should also be considered that both pathways may simply have quantitatively different signal thresholds, such as the amount of exposed ssDNA. This concept could potentially explain why DRC signals can apparently be easily converted into DDC signals, allowing cooperation of the two checkpoint pathways.

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