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Tethered Genes Get Checked during Replication

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Although events associated with replication stress have long formed the cornerstone of checkpoint activation, questions remain about how cells maintain the integrity of replicating genomes. Now, Bermejo et al. (2011) identify a mechanism directly linking checkpoint function to the relief of topological tension at nuclear pore tethered genes.

Nuclear pores, conserved molecular gates that punctuate the nuclear membrane, enable the regulated passage of RNAs and proteins across the nuclear envelope (Köhler and Hurt, 2010). In addition to this primary function, nuclear pores have assumed secondary functions, including the repair of damaged DNA or eroded telomeres, which are beneficial for genome integrity (Khadaroo et al., 2009; Nagai et al., 2008). In addition, sections of actively transcribed chromatin bind nuclear pores, facilitating export of the mRNAs from that region of the genome. However, the physical attachment of these genes to nuclear pores, a phenomenon broadly referred to as "gene gating" (Köhler and Hurt, 2010), might also create topological barriers that induce replication stress. In this issue of *Cell*, Bermejo et al. (2011) reveal that such a threat is real and also provide mechanistic insight into how cells exploit checkpoint signaling to deal with topological impediments during replication.

The study begins with a screen designed to solve the long-standing mystery of how checkpoints prevent stalled replication forks from collapsing into cytotoxic DNA double-strand breaks (DSBs). The authors exploit a well-characterized budding yeast system to identify new genes required for the checkpoint response. When yeast cells that are genetically compromised in their ability to respond to replication stress (in this case by mutations of Rad53, the key transducer of checkpoint signaling) are treated with low doses of hydroxyurea, a drug that generates replication stress, they accumulate reversed replication forks. Subsequently, these aberrant DNA structures are processed into bona fide DSBs. The authors applied these conditions to the yeast gene deletion library and selected mutants that rescued cell viability. To their surprise, they indentified components of THO and TREX2, complexes that are involved in tethering transcribed chromatin to nuclear pores and mRNA export, respectively (Köhler and Hurt, 2010). Suggesting that the attachment of transcribed chromatin to nuclear pores generates topological obstacles that stall advancing replication forks, Bermejo et al. (2011) find that specific mutations in *THO* and *TREX-2* also suppress fork reversal.

From the results of the screen, a hypothesis emerges: could one purpose of checkpoint signaling be to relieve topological strain associated with nuclear pore-coupled transcription (Figure 1)? In the next chapter of their investigation, the authors then perform a series of experiments that indeed link active transcription with replication stress. First, Bermejo and colleagues show that an inducible gene, which is attached to the nuclear periphery, compromises the viability of *rad53* mutants only when it is actively transcribed. They go on to show that these mutant cells can be rescued when an inducible DSB is inserted between the incoming fork and the actively transcribed gene. Providing further support that the checkpoint promotes active detachment of chromatin from the nuclear pore, Bermejo and coauthors show that Mlp1, a component of the nuclear pore complex implicated in gene tethering, is phosphorylated by the replication checkpoint machinery. Moreover, a phosphomimetic version of Mlp1 rescues replication-stressed *rad53* cells. Together, these data suggest that torsional stress accumulated at "gated genes" is subject to checkpoint surveillance.

The last chapter of the study concludes with a model, explaining that cells use replication checkpoint signaling to balance the costs and benefits associated with gene gating. The increased efficiency of cotranscriptional export of nascent mRNA comes at the cost of elevated torsional strain generated not only by the incoming replication fork, but also by the progression of the RNA polymerase (Figure 1). The checkpoint-mediated transient disruption of nuclear pore tethering opens up a window of opportunity wherein genomic loci, normally attached to the nuclear periphery, can complete replication due to the conditional relaxation of the region. This is important because, unlike transcription, which can occur reiteratively, each replication origin can fire only once during the cell cycle, and thus the collapse of a stalled replicon could cause heritable and/or irreversible damage to the genome.

The exciting work from Bermejo and colleagues opens new lines of investigation aimed at understanding how checkpoints protect the integrity of replicating genomes. Although the results provide important answers, they also raise a host of questions, including one highlighted by the authors themselves: how

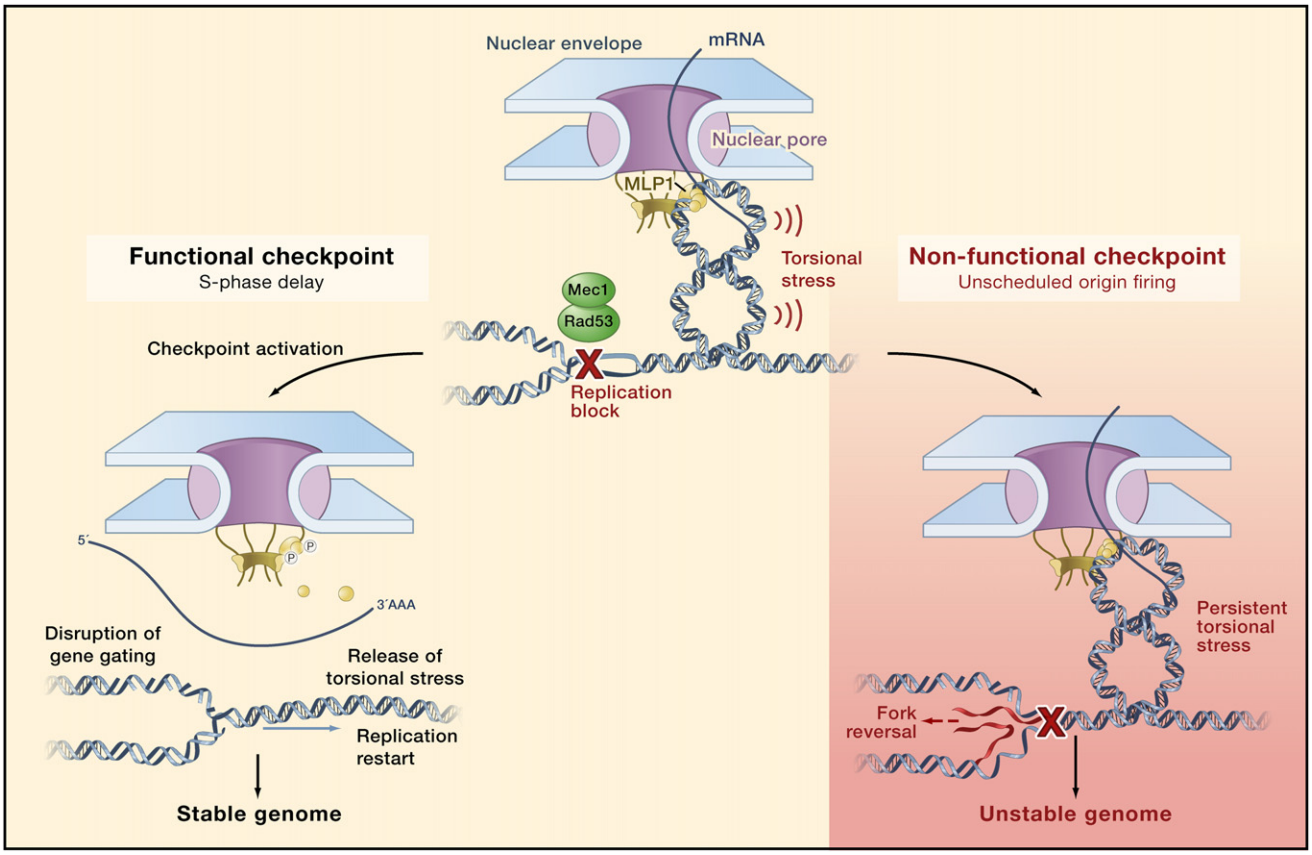

Figure 1. Replication Checkpoint Dismantles Gene Gating to Avoid Replication Fork Collapse
Physical attachment of transcribed genes to nuclear pores (gene gating) combined with torsional stress generated by the advancing replication fork activates checkpoint signaling, which is governed by the Mec1-Rad53 kinase cascade (top). The functional checkpoint (left) coordinates the major events that protect the integrity of the replicating genome. By slowing down S phase progression, checkpoint signaling gives cells the time that they need to relieve the suprahelical DNA torsion ahead of the stalled fork. Among the key targets of checkpoint kinases are subunits of the nuclear pore, including Mlp1, that tether transcribed chromatin to the nuclear envelope. Upon their phosphorylation, the gene gating machinery disassembles, releasing chromatin from nuclear pores and relieving its topological strain. These events allow resumption of replication fork progression. If the checkpoint does not function (right), replication stress, including unscheduled origin firing and persistence of torsional impediments at nuclear pores, ensues. As a result, replication forks reverse polarity and generate pathological structures, including cytotoxic DNA double-strand breaks. Such checkpoint malfunctions destabilize the genome.

do replication forks recognize that they are approaching an obstacle? Although we will have to wait for a definitive answer, Bermejo et al. (2011) suggest that the checkpoint machinery may actually sense mechanical vibrations that are generated by topological tension ahead of the fork. In support of this argument, the authors note that a number of checkpoint proteins, including the upstream activator of Rad53, Mec1, are enriched with structural features called HEAT repeats that function as elastic connectors capable of linking mechanical force and catalysis (Grinthal et al., 2010).

Another remaining question is how far away from the anchor point transcription-induced DNA torsion begins to affect replication. This is particularly pertinent for large mammalian nuclei in which the nascent transcripts derived from more centrally located genes may not always be physically coupled to nuclear pores. Although not all genes are closely tethered at the nuclear pore, other chromatin anchorage points, such as those formed by cotranscriptional engagement of spliceosomes, may create torsional strain whose transient dissolution by checkpoint signaling might be required for efficient replication. Intriguingly, the knockdown of splicing factors in mammalian cells generates spontaneous DNA breaks (Paulsen et al., 2009).

Structures beside nuclear pore tethers and spliceosomes are likely to prevent the free movement of DNA during replication. For example, recent evidence suggests that eukaryotic replicons are, in fact, stationary. Instead of moving away from the origin, the replication machinery stays put while the replicating DNA is actively spooled through (Kitamura et al., 2006). Likewise, recent results indicate that transcription of certain genes takes place in shared "transcription factories," which are thought to be immobile (Osborne et al., 2004). Therefore, it is likely that numerous structures and scenarios, beyond nuclear pores and gene gating, require checkpoint signaling to ensure efficient and accurate replication.

One other open issue is how the links between checkpoint signaling and gene gating relate to the dynamic properties of checkpoint kinases. Previous work in mammalian models has shown that

Chk2, the Rad53 ortholog, is highly mobile and spreads over the entire cell nucleus shortly after genotoxic stress (Lukas et al., 2003). This raises the question of whether checkpoint signaling modifies nuclear pores locally, at the specific stalled replication fork where torsional stress is encountered, or whether it detaches tethered genes throughout the nucleus.

The conclusions arising from the work by Bermejo and colleagues provide a framework to mechanistically decipher all of these issues and expand our knowledge of cellular responses to replication stress. Oncogenic deregulation of replication and transcription are intimately tied to replication stress (Halazonetis et al., 2008). Going forward, it will be important to consider the possibility that nuclear pore components, particularly those involved in tethering chromatin to the nuclear periphery, may be a source of replication stress in human diseases arising from the loss of genomic integrity, such as cancer.

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Nuclear Pore Structure: Warming up the Core

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Structural determination of the nuclear pore complex has been limited by the complexity and size of this cellular megalith. By taking advantage of exceptionally stable nucleoporins from the thermophilic fungus *Chaetomium thermophilum*, Amlacher et al. (2011) provide new insight into a core element of the nuclear pore scaffold.

Nuclear pore complexes (NPCs) are intricate biological machines that mediate all traffic between the nucleus and the cytoplasm in eukaryotic cells. NPCs are embedded in fusion pores between the inner and outer nuclear membranes and are composed of multiple copies of ~30 different proteins, termed nucleoporins (Nups) (Hetzer and Wente, 2009). NPC structure is likely conserved in all eukaryotes and exhibits an eight-fold rotational symmetry with additional filamentous extensions protruding from the nuclear and cytoplasmic facades (Figure 1). As one of the largest and most complex macromolecular assemblies, with an estimated mass of 40–60 MDa and ~500 individual polypeptide chains, the NPC has been a tough nut to crack. In this issue, the groups of Ed Hurt and Peer Bork reveal exciting new data on a central core element of the pore, using proteins from an unexpected thermophilic accomplice, the fungus *Chaetomium thermophilum* (Amlacher et al., 2011). Additionally, by reporting the full genome of this eukaryote the authors establish a new model organism for the structural analysis of large protein complexes.

Recent progress in the structural determination of the NPC has relied on the recognition of the modular nature of its building blocks: nucleoporins and their subcomplexes. The three broad classes of Nups include a small group of membrane-anchored proteins, a large group