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The checkpoint response to replication stress

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ABSTRACT

Genome instability is a hallmark of cancer cells, and defective DNA replication, repair and recombination have been linked to its etiology. Increasing evidence suggests that proteins influencing S-phase processes such as replication fork movement and stability, repair events and replication completion, have significant roles in maintaining genome stability. DNA damage and replication stress activate a signal transduction cascade, often referred to as the checkpoint response. A central goal of the replication checkpoint is to maintain the integrity of the replication forks while facilitating replication completion and DNA repair and coordinating these events with cell cycle transitions. Progression through the cell cycle in spite of defective or incomplete DNA synthesis or unrepaired DNA lesions may result in broken chromosomes, genome aberrations, and an accumulation of mutations. In this review we discuss the multiple roles of the replication checkpoint during replication and in response to replication stress, as well as the enzymatic activities that cooperate with the checkpoint pathway to promote fork resumption and repair of DNA lesions thereby contributing to genome integrity.

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1. Checkpoint function, genome integrity and cancer

Inadequate responses to replication stress or defects in DNA repair underlie many forms of cancer [1,2]. Multiple significant links between checkpoint proteins and genome stability have been identified in human and other mammalian organisms, as well as in various model systems [3,4]. The checkpoint cascade, also often referred to as the DNA-damage response, is highly conserved in eukaryotic organisms. Although initially most of the research was conducted in budding and fission yeast [5], the past years of research have shown that homologs exist for all the components discovered in yeast, although the pathway is more elaborated in mammals [6]. Besides its role in regulating cell-cycle transitions, the checkpoint pathway has profound roles in responding to replication stress and mediates essentially all responses to DNA damage (Fig. 1).

Collapsed forks or fragile zones are prone to lead to chromosome rearrangements or translocations and a large number of studies document on the effect of checkpoint mutations on genome-wide and site-specific stability [1,3,4,7]. It is also known that chromosomal instability leading to different types of chromosome rearrangements as well as chromosome loss plays an important role in cancer development [2]. In line with these reports, checkpoint mutations are often found in cancer, and many human genetic syndromes that lead to cancer predisposition are caused by mutations in genes that protect the genome integrity during chromosome replication.

This linkage between replication and cancer underscores the importance of understanding how cells cope with aberrant replication forks. In the following sections we discuss on the molecular mechanisms employed by replication checkpoints to stabilize the replication forks and to assist and coordinate different damage-tolerance mechanisms that contribute to repair and chromosome integrity (Fig. 1).

2. The replication checkpoint cascade

The replication checkpoint is a sensor-response system activated by impeded replication forks or other types of lesions that occur in S-phase, and is crucial for stabilizing replication forks and fragile sites [8,9]. There are different types of independent molecular complexes that sense and signal different types of damage, of which the RPA-coated single stranded (ss) DNA is a central player, although not always responsible or sufficient to activate the replication checkpoint [6,10]. Other checkpoint factors, such as TopB1, the Mre11-Rad50-Xrs2 (MRX) complex in yeast (the Mre11-Rad50-Nbs1 (MRN) complex in mammals), and the 9-1-1 complex have also been implicated in the activation of the replication checkpoint or the recruitment of sensor kinases to stalled forks [6,11] (Fig. 2).

Of the checkpoint proteins, we have learned most about the ATM and ATR, Tel1 and Mec1 in yeast, respectively. Tel1/ATM responds mainly to double strand breaks (DSBs) whereas Mec1/ATR is activated by ssDNA and stalled forks [11,12]. The Mec1/ATR path-

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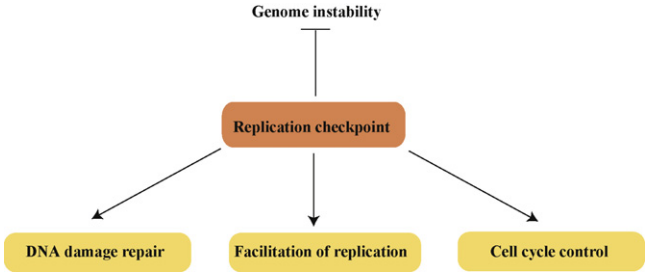

Fig. 1. General scheme of the checkpoint responses to replication stress and the primary impact of these functions on genomic stability.

way is the keystone of the replication checkpoint and a simplified view of this transduction cascade is shown in Fig. 2.

The stalled replication forks most often expose ssDNA, generated by the uncoupling between leading and lagging strand polymerases or by the action of the MCM replicative helicases that may continue the unwinding ahead of the stalled replication fork [13,14]. Once formed, the ssDNA is bound by RPA, and the ssDNA–RPA complex plays two critical roles in recruiting independently Mec1-Ddc2 (ATR-ATRIP) and the clamp loader Rad24 (Rad17 in mammals) (Fig. 2). Physical interactions between RPA and checkpoint proteins have been demonstrated and shown to be required for checkpoint activation and tolerance to replication stress [15]. Rad24, which has similarity to the large subunit of replication factor C (RFC), Rfc1, interacts with the four smaller subunits of RFC, and this RFC-like clamp loader complex is responsible for loading the PCNA-related Rad17-Mec3-Ddc1 in yeast or the 9-1-1 complex (Rad9-Rad1-Hus1) in mammals onto DNA [16]. Although the order of the subsequent events is not entirely clear, it is believed that co-localization of Mec1/ATR and of the 9-1-1 complex allows interaction between these proteins at damage sites and subsequent phosphorylation of the 9-1-1 complex by ATR. Another important player in ATR activation appears to be Dpb11 in yeast and its functional ortholog TopBP1 in human. Dpb11/TopBP1 interacts directly with Mec1/ATR,

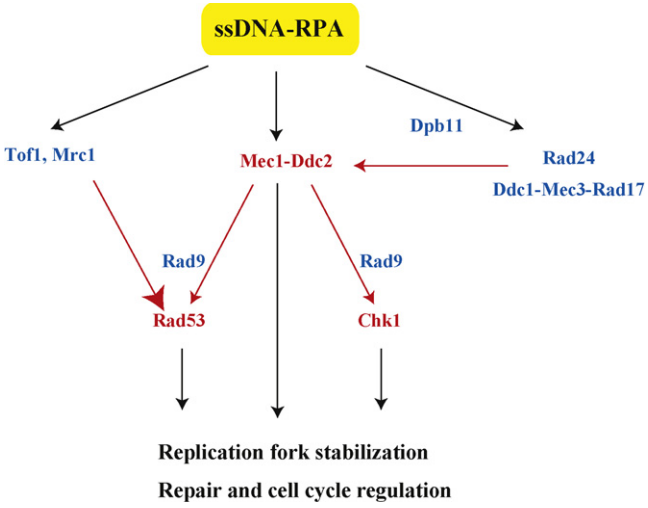

Fig. 2. Schematic representation of the mechanisms that lead to the activation of the replication checkpoint in S-phase. Single stranded DNA coated with RPA triggers the recruitment of Mec1-Ddc2 together with the one of Rad24 and of the checkpoint clamp composed of Rad17-Mec3-Ddc1. Mrc1, Tof1, and Dpb11 are also associated with replication forks. Interaction and phosphorylation events occurring between Rad17-Mec3-Ddc1, Dpb11 and Mec1-Ddc2 lead to Mec1 activation. Subsequently, Mrc1 and Rad9 function as adaptors to promote Rad53 hyperphosphorylation to elicit a cell cycle response. Mec1 and Rad9 also promote Chk1 phosphorylation. Mec1, together with Rad53 and to some extent also Chk1, mediate the response to replication stress by promoting fork stabilization, fork processing and DNA repair events.

binds the 9-1-1 complex and is critical for Mec1/ATR activation [17–19]. Following this step, additional Mec1/ATR substrates called mediators are recruited and play an important role in amplifying the checkpoint signal throughout the cell. In mammalian cells this pathway is quite elaborated and several aspects are not well understood. Here we will mainly focus on the checkpoint signal amplification and the mediators in budding yeast (Fig. 2). The best-characterized mediator in *S. cerevisiae* is Rad9, which functions as an adaptor between Mec1 and the Rad53 checkpoint kinase [20]. Rad53 (Chk2 in mammals although its functional ortholog is Chk1) plays pivotal roles in response to replication stress, and its phosphorylation is essential for checkpoint control [21,22]. Rad53 is phosphorylated in a Mec1-dependent fashion in response to both DNA damage and incomplete replication. Rad9 functions predominantly in the G1/S and the G2/M transitions of the DNA damage checkpoints, and only to some extent in response to replication fork arrest [23] (Fig. 2). Rad9 is recruited to DSBs and hyperphosphorylated in a Mec1 and Tel1-dependent manner. It is proposed that Rad9 catalyzes the activation of Rad53 by acting as a scaffold to promote Rad53 autophosphorylation [24–26] (Fig. 2). In response to replication stress, however, amplification of the replication checkpoint signal depends on Mrc1 [23] (Fig. 2). Mec1 phosphorylates Mrc1 on multiple sites and mutation of these sites in the *mrc1*^{AQ} mutant suppresses Rad53 hyperphosphorylation in response to replication stress [27]. However, experiments have so far failed to demonstrate a physical interaction between Mrc1 and Rad53 and thus the molecular mechanism through which Mrc1 activates Rad53 has yet to be characterized. The mammalian counterpart of Mrc1, Claspin, interacts with the effector kinase Chk1 (the functional ortholog of Rad53) and is essential for its hyperphosphorylation [28–30]. The molecular mechanism of how the checkpoint signal is amplified in mammalian cells is complex, and it appears that different signals trigger the formation of alternative Claspin–Chk1 complexes [6,10,30,31].

In response to replication fork arrest caused by deoxynucleoside triphosphate (dNTP) depletion with hydroxyurea (HU), Mrc1 carries out the adaptor function between Mec1 and Rad53 [23] (Fig. 2). However, in the absence of Mrc1, the checkpoint mediator Rad9 can partially substitute for Mrc1 and promote Rad53 hyperphosphorylation, although likely leading to the formation of different phosphoisoforms having distinct molecular functions [23,30]. Following autophosphorylation, Rad53 is released from the Rad9 (and likely the Mrc1) complex, leading to an amplification of the checkpoint signal [21,24]. Mec1 activates not only Rad53, but together with Rad9, another checkpoint kinase, Chk1 [7,22]. Chk1 mediates, in cooperation with Rad53, the G2/M checkpoint arrest [7], but recent reports have shown that Chk1 may also influence the stabilization of replication forks in the absence of Rad53 [32] (Fig. 2).

Before discussing the mechanism through which the replication checkpoint acts to stabilize replication forks, we like to note that a number of different situations causing lesions or replication stress do not lead to formation of ssDNA and do not activate the replication checkpoint. Examples include interstrand crosslinks and camptothecin (CPT) treatment as well as fork pausing at natural pause sites such as the rDNA replication fork barrier [10,14]. In such situations, the response to the stress factor or the repair of the initial lesions occurs in a checkpoint-independent fashion or requires additional proteins to activate the replication checkpoint.

3. Causes of fork stalling and general mechanisms through which the replication checkpoint responds to replication stress

Replication fork progression is often impeded by exogenous or endogenous DNA damage [14]. In addition to lesion-induced replication fork stalling, the replisome pauses or slows down signif-

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