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Review

The fork and the kinase: A DNA replication tale from a CHK1 perspective

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ABSTRACT

Replication fork progression is being continuously hampered by exogenously introduced and naturally occurring DNA lesions and other physical obstacles. Checkpoint kinase 1 (Chk1) is activated at replication forks that encounter damaged DNA. Subsequently, Chk1 inhibits the initiation of new replication factories and stimulates the firing of dormant origins (those in the vicinity of stalled forks). Chk1 also avoids fork collapse into DSBs (double strand breaks) and promotes fork elongation. At the molecular level, the current model considers stalled forks as the site of Chk1 activation and the nucleoplasm as the location where Chk1 phosphorylates target proteins. This model certainly serves to explain how Chk1 modulates origin firing, but how Chk1 controls the fate of stalled forks is less clear. Interestingly, recent reports demonstrating that Chk1 phosphorylates chromatin-bound proteins and even holds kinase-independent functions might shed light on how Chk1 contributes to the elongation of damaged DNA. Indeed, such findings have unveiled a puzzling connection between Chk1 and DNA lesion bypass, which might be central to promoting fork elongation and checkpoint attenuation. In summary, Chk1 is a multifaceted and versatile signaling factor that acts at ongoing forks and replication origins to determine the extent and quality of the cellular response to replication stress.

Contents

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1. Checkpoint signals during the S phase and the maintenance of genomic stability

Cell cycle checkpoints constitute key signaling networks that counteract the continuous threats that both internal and external factors pose to DNA. Checkpoints primary function is to inhibit cell cycle progression before entry into S phase (G1/S checkpoint), throughout S phase (S-phase checkpoint), before mitotic entry (G2/M checkpoint) or before entry into anaphase (mitotic spindle checkpoint) [1]. By controlling the start and/or progression of DNA replication, the S-phase checkpoint creates a time window to repair damaged DNA. In case of excessive or persistent DNA damage, checkpoint signals may also trigger apoptosis to avoid the propagation of aberrant genomes [2]. Therefore, checkpoint signaling contributes to the maintenance of genome integrity and avoids the development of diseases associated with genomic instability, such as cancer.

This review focuses on checkpoint kinase 1 (Chk1), a conserved serine/threonine protein kinase with a pivotal role in the S-phase checkpoint. Importantly, Chk1 regulates S phase progression not only after genotoxic stress, when DNA damage increases, but also during unperturbed replication (in the absence of exogenous damage). As we will discuss herein, different lines of evidence indicate that Chk1 regulates replication initiation [3–5], stabilizes replication forks [6,7] and promotes lesion bypass [8–10]. These Chk1-mediated mechanisms might prevent the collapse of ongoing forks and promote the proper resumption of DNA synthesis when the stalling signal is removed. Although not discussed in this review, Chk1 function exceeds the control of DNA synthesis. Particularly, solid evidence shows that Chk1 fulfills prominent roles in the G2/M and mitotic spindle checkpoints and in apoptotic signaling [11–14].

To analyze the contribution of Chk1 to DNA replication, we divided this review in five sections, including this one. The two following sections will concentrate on the molecular signals triggering Chk1 activation and modulating its localization; the subsequent one will focus on the function of Chk1 during DNA replication; and in the last section we will discuss how checkpoint signaling is attenuated, laying special emphasis on the molecular events that might allow forks "in check" to restart DNA replication.

2. DNA structures and protein networks upstream of Chk1 activation

In eukaryotic cells DNA replication starts at multiple sites called replication origins. Each origin initiates a pair of replication forks, each one moving bi-directionally away from the origin, so that DNA replication terminates when forks that initiated from adjacent origins converge. Each replication fork is associated with a replisome, a multi-component protein complex including the helicase, the polymerases and accessory factors such as the sliding clamp proliferating cell nuclear antigen (PCNA) and its loader the replication factor C (RFC). Importantly, each replication fork consists of a leading and a lagging strand, elongated by the replicative polymerases ε and δ, respectively. Synthesis of the leading strand is continuous, whereas that one of the lagging strand involves the elongation and subsequent ligation of primers (Okazaki fragments) (Fig. 1) [15]. Many other components are present at the replication fork, but because they are not the focus of this review, we refer our

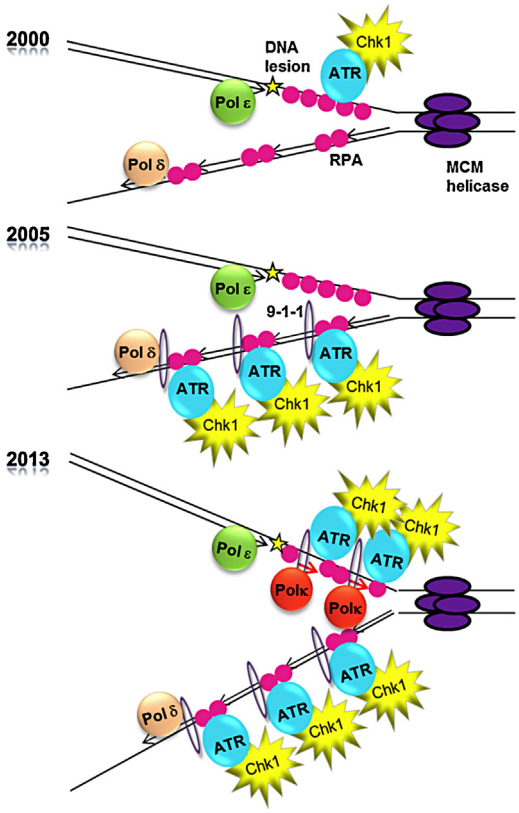

Fig. 1. Chk1 activation models over one decade. Year 2000: The uncoupling of the helicase and the replicative polymerase Pol ε generates long stretches of RPA–ssDNA, a pre-requisite to recruit and activate ATR. ATR then phosphorylates Chk1, resulting in Chk1 activation. Note that in this model only the leading strand is able to initiate checkpoint signaling. Year 2005: ATR activation is preceded by the loading of the 9-1-1 clamp onto the 5′ junction of an Okazaki fragment elongated by Pol δ. Note that in this model only the lagging strand is able to initiate checkpoint signaling. Year 2013: Current model for checkpoint activation (for simplification not all checkpoint components are shown). The TLS polymerase Pol κ elongates primers at the leading strand. Therefore, both strands contain primer–template junctions and thus equally allow the loading of the 9-1-1 clamp. Therefore, ATR-dependent Chk1 activation originates from the leading and lagging strands.