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Review

Replisome components—Post-translational modifications and their effects

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ABSTRACT

The process of DNA replication is highly regulated, but at the same time very dynamic. Once S-phase is initiated and replication elongation is occurring, the cells are committed to complete replication in order to ensure genome stability and survival. Many pathways exist to resolve situations where normal replisome progression is not possible. It is becoming more and more evident that post-translational modifications of replisome components play a key role in regulating these pathways which ensure fork progression. Here we review the known modifications of the progressing replisome and how these modifications are thought to affect DNA replication in unperturbed and perturbed S-phases.

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1. Introduction

DNA replication is a process crucial for the life cycle of all eukaryotic cells, that must ensure that their genome is replicated faithfully, and only once per cell cycle. The initiation of DNA replication is tightly controlled by well understood mechanisms to prevent re-replication of an already replicated part of the genome (reviewed in [1]). Once replication is initiated the cells have to complete the process before mitosis in order to survive. Thus, the cells must be able to respond to problems during DNA elongation, if the replisomes encounter obstacles. These obstacles include DNA damage or replicative stress, which activate the S-phase checkpoint, protein mediated DNA replication barriers, difficult to replicate DNA (*e.g.* sequences that form secondary DNA structures), as well as replisomes arriving from the opposite direction [2,3]. To maintain genome stability different pathways have to be activated in response to different types of obstacles. Although very little is known about how this is achieved, more and more data indicate that post-translational modifications of

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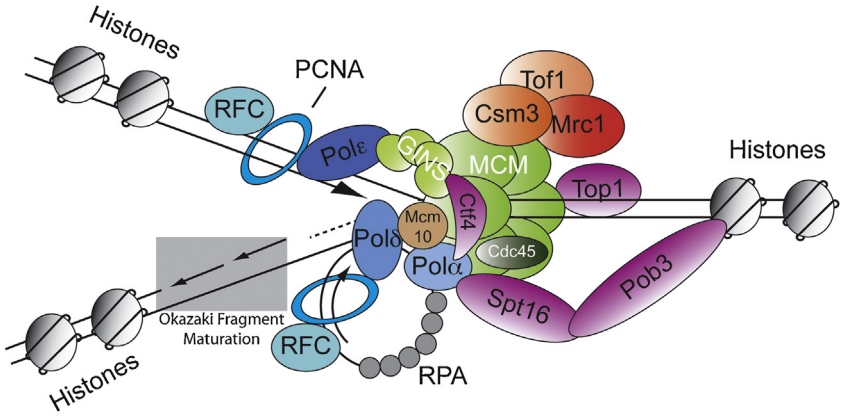

Fig. 1. Model of a processive replisome. Subunits of the following subcomplexes are drawn in different colours: helicase in green; polymerase in blue; extended replisome protection complex in red; factors in chromatin remodelling, cohesion and topoisomerases in purple; Mcm10 in brown and histones in grey.

replisome components play a key role. These modifications, which mainly include phosphorylation, SUMOylation and ubiquitination events, act to modify enzymatic activities, affect the stability of replisome factors, change the sub-cellular localization of factors or recruit new components to the replisome.

The exact composition of the active replisome is not known, but a large *Saccharomyces cerevisiae* sub-complex, the so-called replisome progression complex (RPC) has been purified [4]. In this chapter we will focus on posttranslational modifications of this core replisome complex and closely associated factors (Fig. 1).

2. Phosphorylation regulates the progression of the replisome

Stalling of the replisome due to inhibition of DNA polymerases by DNA damage or replicative stress (*e.g.* depletion of the nucleotide pool by hydroxy urea (HU) exposure) leads to the activation of the intra-S phase or the DNA damage checkpoint and subsequent delay of the activation of late origins and mitosis [1,2,5,6]. The sensing of DNA damage and checkpoint activation is a complex mechanism, which varies depending on the type of DNA damage and the cell cycle phase. In this section, we will focus on how the replisome participates in checkpoint signal transduction and how checkpoint signalling influences the progression of the replisome.

Two groups of large phosphoinositide 3′-kinase-like kinases (PIKKs) are responsible for the initial signalling. The ATR homologues Rad3 (*Schizosaccharomyces pombe*), Mec1 (*S. cerevisiae*) or ATR (metazoan) is recruited to ssDNA regions found at defective replication forks or processed DNA double-stranded breaks (DSBs). This requires the presence of the RPA protein complex that binds to naked ssDNA and the regulatory PIKK subunit Rad26 (*S. pombe*), Ddc2 (*S. cerevisiae*) or ATR-IP (metazoan). ATR is considered the principal kinase in checkpoint activation. The ATM homologues Tel1 (*S. pombe*, *S. cerevisiae*) and ATM (metazoan) are necessary for the initiation of checkpoint signalling at DNA DSBs.

The initial PIKK signal is locally amplified by so-called checkpoint mediator proteins *via* the recruitment of downstream effector kinases to the site of the DNA damage or the stalled fork. In eukaryotes, there are two families of effector kinases: (1) Cds1 (*S. pombe*), Rad53 (*S. cerevisiae*), CHK2 (metazoan) and (2) Chk1 (*S. pombe, S. cerevisiae*), CHK1 (metazoan). Once recruited to the DNA lesion or disabled fork, the effector kinase phosphorylates a range of downstream targets and triggers the checkpoint reaction. Interestingly, in metazoans CHK1 is the principal effector kinase and activated by ATR, whereas this role in yeast is fulfilled by the CHK2 homologues Cds1 (*S. pombe*) and Rad53 (*S. cerevisiae*).

The delaying of S-phase and stalling of replisomes through these pathways is vital for all eukaryotic cells to maintain genomic stability. For example, in *S. cerevisiae* a disruption of the S-phase checkpoint by a deletion of Rad53 leads to a significant increase of unreplicated regions and the appearance of replication intermediates associated with collapsed or replication incompetent forks [7,8].

2.1. Phosphorylation of the S-phase checkpoint mediator Mrc1 and the replisome protection complex

Studies in yeast have shown that the S-phase checkpoint mediator Mrc1 (*S. pombe*, *S. cerevisiae*)/CLASPIN (metazoan) is a component of the processive replisome and recruits the effector kinase to a stalled fork for efficient phosphorylation by the PIKK when the checkpoint is activated [2,4,9,10]. Here, we will describe this pathway and the involved factors.

In budding yeast, Mrc1 forms *in vitro* a heterotrimeric subcomplex (Fig. 1, red replisome subunits) with Tof1, the homologue of Swi1 (*S. pombe*)/TIMELESS (metazoan), and Csm3, the homologue of Swi3 (*S. pombe*)/TIPIN (metazoan) [11,12]. Interactions between the replicative helicase and all three proteins have been mapped [13]. Tof1 interacts with Mcm6, Csm3 with Mcm7 and Mrc1 with Mcm2 and Mcm3. Fission yeast Mrc1, Swi1 and Swi3 also form a heterotrimeric complex on DNA *in vitro* [14].

The main role of this heterotrimeric complex seems to be to stabilize replication forks following the exposure of cells to replicative stress or DNA damage and thus promote cell survival. Mrc1 is required to complete DNA replication after exposure to HU and to maintain normal fork progression rates, whereas Tof1 mediates fork stalling at some site-specific barriers [15–18]. Furthermore, the two factors act together to stabilize the interaction between the replicative polymerase and the replicative helicase when cells experience replicative stress [9]. Swi1 and Swi3 promote the survival of *S. pombe* cells after exposure to ultraviolet light (UV), HU and methyl methanesulfonate (MMS) treatment and prevent the formation of Holliday junctions at ssDNA [19–21].

The first evidence that S-phase checkpoint activation is one of the pathways in which this heterotrimeric complex acts to ensure cell survival comes from experiments on HU treated budding and fission yeast cells. These experiments show that loss of Mrc1 prevents the activation of Cds1/Rad53 (*S. pombe/S. cerevisiae*) and that Mrc1 hyper-phosphorylation is dependent on Rad3/Mec1 [22,23]. Moreover, Swi1 and Cds1 act in the same pathway to prevent fork collapse after HU treatment [19]. This mechanism has been studied in *S. pombe* in great detail. Fission yeast cells can only pass through mitosis without having completed replication when they can

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