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RESEARCH HIGHLIGHT

Plk1: unexpected roles in DNA replication

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FOR20, a conserved centrosomal protein, is essential for Plk1 to localize to the centrosome during the S phase and regulate DNA replication. The absence of either Plk1 or FOR20 can stall the cell cycle by a previously unknown intra-S phase centrosomal checkpoint.

The accurate segregation of chromosomes in mitosis is essential to warrant genetic stability in eukaryotic cells. Malfunction of this precisely regulated process can lead to aneuploidy, a major cause of cancer. The activities of several mitotic kinases, notably cyclin-dependent kinase 1 (Cdk1), Aurora kinases and polo-like kinase 1 (Plk1), are crucial for error-free chromosome segregation. From prophase to metaphase, Plk1 localizes to centrosomes and kinetochores and regulates different aspects of spindle assembly including bipolar spindle formation [1]. A key function of Plk1 at the centrosomes is to facilitate γ-tubulin recruitment and centrosome maturation, separation, and microtubule nucleation during late prophase and prometaphase. Thus, interfering with Plk1 function in human cells leads to a prominent prometaphase/metaphase-like arrest, which is dependent on the activation of the spindle assembly checkpoint [2]. Considering these aspects, it is not surprising that 25 years after the discovery of polo in *Drosophila melanogaster* functional studies on the mammalian orthologues of polo have been focusing almost exclusively on the mitotic role of Plk1. Remarkably, Plk1 has become a promising therapeutic target for cancer treatment [3]. Only recently, the role of Plk1 during the S phase is attracting considerable attention.

Daughter chromosomes are generated as a result of DNA replication during the S phase of the cell cycle. Although, it has been known that Plk1 is expressed during the S phase as well, albeit at lower levels as compared to the G2 and M phases [1], only recently, Liu and co-workers have suggested a novel role of Plk1 in facilitating DNA replication under stress [4]. Plk1 and its homologues in other eukaryotic organisms have now been demonstrated to be able to interact with, phosphorylate and regulate a number of targets that are involved in the formation of the pre-replicative complex (pre-RC) and DNA replication during the G1 and S phases, respectively. These targets include, Orc2, which is a component of the origin recognition complex (ORC) and interacts with Orc3, a core subcomplex within the machinery of the pre-RC; minichromosome maintenance 2-7 (Mcm2-7), which form the MCM complex to unwind the DNA and facilitate the movement of the replication fork; Dbf4, which couples with Cdc7 to phosphorylate MCM for its release from the DNA template following its successful replication; the histone acetyl-transferase binding to the origin recognition complex 1 (Hbo1), which regulates the recruitment of Mcm2 and 6 to the MCM at the pre-RC [4, 5]. The formation of the pre-RC during the G1 phase essentially licences the cell for DNA replication while its disassembly at the onset of the G2 phase ensures that the DNA is replicated only once per cell cycle during the S phase [6]. As Plk1 can regulate key members involved in both the formation and the release of the pre-RC from DNA, it is likely that this key kinase plays a role in replication licensing as well. While novel functions of Plk1 in DNA replication during the S phase continues to be identified, existing data have conclusively established that Plk1 plays a critical role in regulating DNA replication in cells.

It has been already reported that the centrosomes are the seats for the majority of Plk1-regulated processes during mitosis [7]. However, recently, it has been established that during the G1/S phase, Plk1 mainly localizes to the nucleus of cancer cells but not of normal cells and its depletion reduces the centrosome amplification in hydroxyurea-treated U2OS cells [8, 9]. Shen *et al*. [10] provide one of the first evidences relating the recruitment of Plk1 to the centrosome to S phase progression. Their work focuses on a newly identified and highly conserved centrosome localized protein called FOR20 (FOP-related protein of 20 KDa), which has been known for its role in the formation of primary cilium [11]. However, it is now evident that FOR20 is also essential for S phase progression as RNAi-mediated knockdown of FOR20 resulted in cellular defects leading to the arrest of these cells in the S phase, which was completely rescued by the ectopic expression of exogenous FOR20 (Figure 1). Interestingly, while FOR20 depletion appears to stall the cells in the S phase, this may be due to the failure of Plk1 to localize to the centrosome in the absence of FOR20 during the S phase. It was demonstrated by the authors that the mere expression of Plk1 was not sufficient to promote S phase progression in the absence of FOR20. Expression of Plk1 mutants that lacked the ability

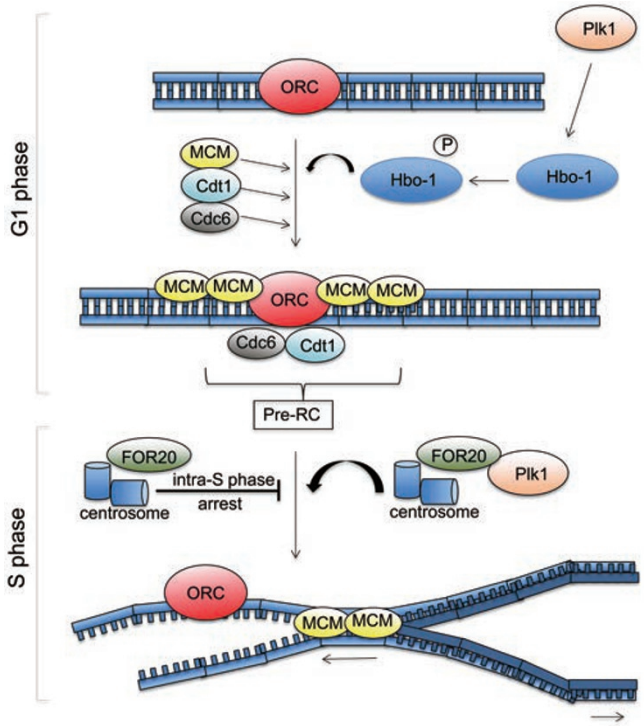

Figure 1 ORC binds to the replication origin on the un-replicated DNA strand. During the G1 phase of the cell cycle, the initiation factor Cdc6 and DNA replication factor Cdt1 as well as the MCM complex are recruited to origins where ORC binds to form the pre-replicative complex (pre-RC). The histone acetyltransferase binding to the origin recognition complex 1 (Hbo1) protein is phosphorylated by Plk1 at Ser57, which then regulates the recruitment of Mcm2 and 6 to the MCM complex at the pre-RC. For the DNA replication to occur during the S phase, the recruitment of Plk1 to the centrosomes via FOR20 is vital. In the absence of Plk1, the cells get arrested by an intra-S phase checkpoint and DNA replication stops completely.

to localize to the centrosome in cells depleted of endogenous Plk1 also led to S phase arrest, even in the presence of FOR20. In both cases, the cells could be rescued from this arrest by the expression of either Plk1 mutant that can bind to the centrosome irrespective of the absence of FOR20; or wild-type Plk1 and Plk1 mutant that could bind to the centrosome in the presence of FOR20. Thus, the authors have demonstrated a novel intra-S phase DNA replication licensing function of Plk1 through its FOR20-mediated recruitment to the centrosome during the S phase. Failure to secure this licence triggers a previously unknown centrosomal checkpoint for DNA replication during the S phase of the cell cycle. This finding corroborates with earlier reports showing that Plk1 depletion leads to the activation of the DNA damage checkpoint at the G1/S phase, causing reduced DNA synthesis, slower cell cycle progression and ultimately apoptosis [12]. Quite intriguingly, this article suggests that the kinase activity of Plk1 as well as its PBD are not essential for its role in regulating DNA replication and S phase progression as a kinase inactive Plk1 mutant could efficiently rescue S phase arrest in the absence of endogenous Plk1. While it still needs to be investigated how Plk1 is itself activated and how it can influence its substrates in the absence of a dispensable kinase, this paper definitely provides new insights into the mechanisms behind the regulation of DNA replication by Plk1 during the S phase.

There is no question over the fact that Plk1 plays an extremely important role in DNA replication during the S phase despite the longstanding misnomer of it being an exclusively mitotic kinase. While research in this direction has only just begun, work done by Shen *et al*. and the like are shedding light on the mechanisms by which Plk1 can regulate the critical and highly complex process of replicating nearly 3.2 billion base pairs that compose the mammalian genomic DNA.

Moreover, centrosomes duplicate only once per cell cycle to warrant that daughter cells only inherit one centrosome. As this is also true for chromosomal DNA, the cell division cycle can be viewed as two cycles in parallel that might be coordinated. Mal-coordination of both cycles is a cause of oncogenic transformation. We like to propose that Plk1 contributes to the coupling of both cycles as it promotes centrosome functions and DNA replication in S phase. Novel evidence by Shen and colleagues suggests that Plk1 contributes to the regulation of licensing of S phase progression and DNA replication. The question remains whether there is a similar licensing event in centrosome duplication, which ensures that duplication occurs only once per cell cycle, and whether Plk1 in addition to Plk4 and SAS6 is a novel player in this mechanism.

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