Exceeding the limits – Cdc45 overexpression turns bad.

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DNA replication needs to exactly duplicate the genomic content of the cell – not more and not less – otherwise severe genomic instability can occur. To keep DNA replication in check a multi-step program controls the assembly of the replication fork and the timing of replication origin firing, while multiple surveillance pathways deal with any replication problems. A key-step during DNA replication is the assembly of the replicative helicase Cdc45-MCM2-7-GINS (CMG), which forms in early S-phase [1]. The CMG performs the bulk of DNA unwinding in the context of the replication fork, generating single-stranded-DNA, the template for DNA polymerases. In human cells 10.000s of potential replication origins form every cell cycle and a subset is used in a temporal defined program to initiate DNA synthesis throughout S-phase. Notably, the human Cdc45 protein is a low abundance protein, with a copy number well below the number of replication origins. Therefore it has been proposed that Cdc45 is a rate limiting factor for the fine-tuned CMG helicase assembly reaction. Interestingly, during tumorigenesis the sophisticated regulatory loops that control complex assembly become compromised, in part due to overexpression of replication factors. The Grosse lab has now investigated how Cdc45 overexpression affects human cells[2]. While in budding yeast multiple factors control the timing of origin firing[1], in this study it was found that elevated Cdc45 protein levels are sufficient to induce enhanced origin firing, which is consistent with a previous research report[3]. Yet, this enhanced origin firing was associated with a severe genome instability phenotype. How is this possible? The data show that cells overexpressing Cdc45 display γH2AX-phosphorylation and induce apoptosis. However, signalling did not follow the ATR/Chk1 route, which is the typical pathway activated during replication stress (e.g. during dNTP depletion), but instead a mild ATM/Chk2 response was observed. Remarkably, upon Cdc45 overexpression, asymmetric fork stalling and single-stranded DNA (ssDNA) accumulation were discovered, hallmarks of a replication catastrophe. These data strongly suggest that the single stranded DNA binding protein RPA had become limiting during DNA synthesis. This situation is extremely dangerous to the cell, as unprotected ssDNA (DNA not covered by RPA) is very fragile and prone to nucleolytic damage. Indeed, exhaustion of the single stranded DNA binding protein RPA[4] or its severe downregulation[5] is known to cause a replication catastrophe associated with a mild ATM/Chk2 response and apoptosis. Thus, the existing data are in support of the novel concept that Cdc45 overexpression limits RPA availability for DNA synthesis (Figure 1). Future work will need to explore the underlying mechanisms in order to validate this appealing concept. Curiously, previous studies suggested that RPA-Cdc45 interact directly[6]. Thus, it will be important to address if a Cdc45-RPA complex could affect RPA's role in DNA synthesis and DNA repair. Alternatively, a different mechanism could be at play e.g. enhanced origin firing could cause an exhaustion of the RPA protein pools, which in turn would promote the generation of unprotected ssDNA. This model appears particular attractive, as a recent report found that in *Xenopus* Myc-induced loading of Cdc45 is associated with similar phenotype[7]. It is still not clear if Cdc45 overexpression affects cancer and normal cells in the same way. Nevertheless, the current study strongly suggests that increased Cdc45 protein levels or activity could be dangerous to the cell, in particular when RPA becomes limiting. It will therefore be exciting to investigate how the RPA and Cdc45 protein levels are regulated during tumor development. Is there a correlation between the Cdc45 - RPA protein balance and genomic stability or even the grading of cancer cells? Clearly Cdc45 expression must be tightly regulated; it will be fascinating to learn more about the regulatory loops and their biological relevance, eventually leading to new therapeutically opportunities.

Figure legend:

Figure 1. Model explaining how Cdc45 overexpression may affect DNA replication and genomic stability

During normal DNA replication origins fire in a predefined pattern throughout S-Phase. Limiting Cdc45 amounts restrict CMG helicase complex formation at each origin, thus a balanced DNA synthesis guarantees that RPA never becomes restricted in its ability to protect single-stranded DNA (ssDNA). Cdc45 overexpression affects the normal regulation by promoting enhanced CMG formation, which results in unbalanced DNA synthesis and the appearance of ssDNA that becomes vulnerable to breaks producing extensive DNA damage.

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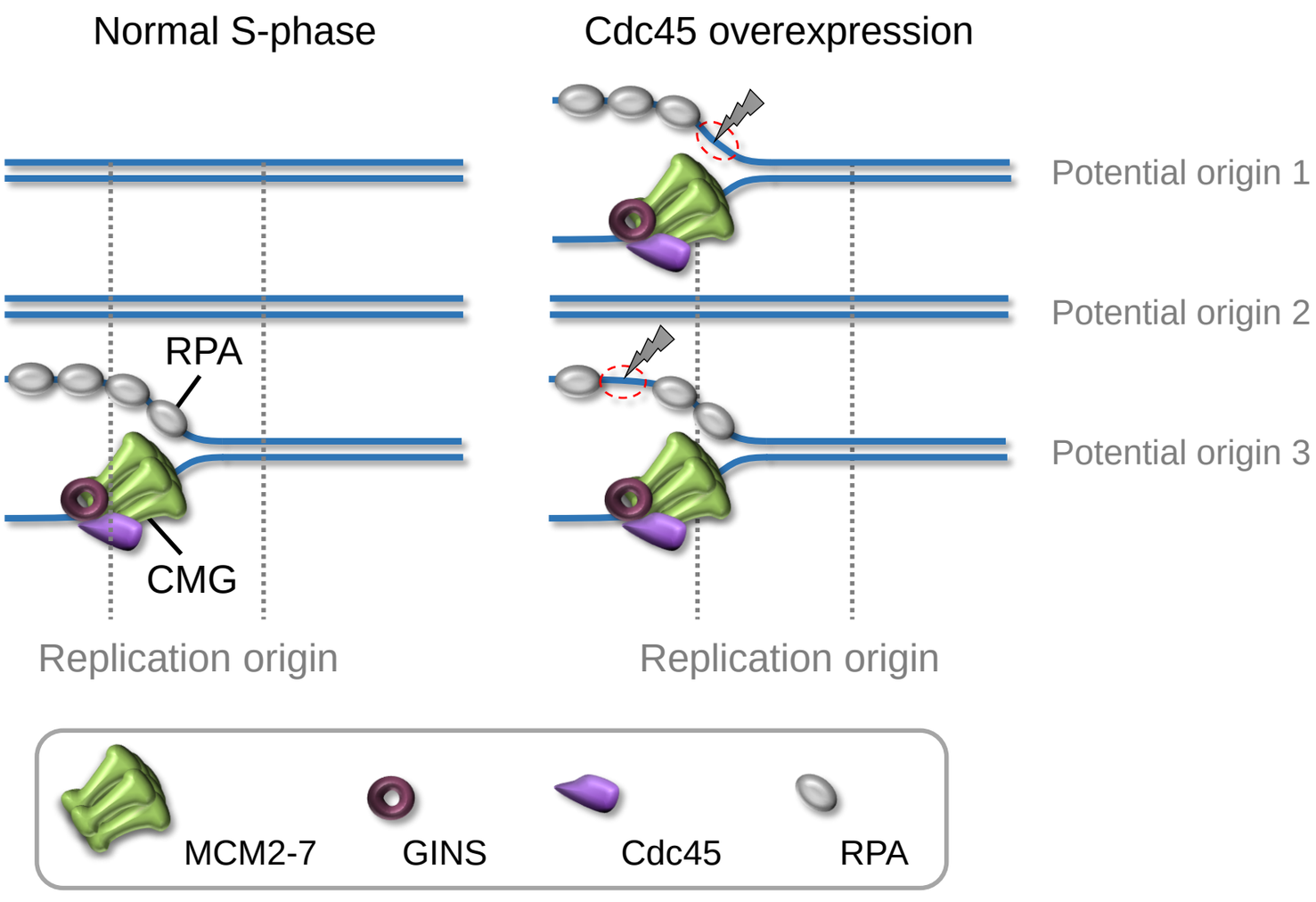
Normal S-phase
Cdc45 overexpression
RPA
CMG
Potential origin 1
Potential origin 2
Potential origin 3
Replication origin
Replication origin
MCM2-7
GINS
Cdc45
RPA