CELL BIOLOGY

The need to regulate replication fork speed

Fork speed modulation counteracts redox imbalance to safeguard genome integrity

By Belén Gómez-González and Andrés Aguilera

The integrity of DNA is constantly threatened by the molecules that are endogenously generated by cell metabolism, the most common byproduct being reactive oxygen species (ROS) (*1*). This is particularly relevant during DNA replication as highlighted by the overrepresentation of replication-associated spontaneous mutations in cancer (*2*). In a constantly changing environment, cell survival requires a fine-tuned control of replication. Since DNA replication in eukaryotic cells initiates from multiple replication origins, it can be regulated at both the frequency of origin initiation and the rate of replication fork progression. The *in vitro* reconstitution of the eukaryotic replisome with purified proteins has recently boosted our knowledge of both processes in eukaryotes (*3, 4*). However, we still lack a full understanding of the mechanisms and implications of fork rate modulation *in vivo*. Notably, on page XXX of this issue, Somyajit *et al*. (*5*) show that the reduction of replication fork speed by low levels of ROS is a major mechanism to mitigate their negative impact on DNA replication and genome integrity, in a process that may be critical for tumor cell survival.

Somyajit *et al*. report that forks slowed down in human cells treated with low dose hydroxyurea (HU), an inhibitor of the ribonucleotide reductase (RNR). RNR is an essential enzyme in all living organisms that catalyses the production of deoxyribonucleotide triphosphates (dNTPs), the components that make up DNA. Low levels and imbalanced dNTPs challenge genome integrity by inducing replication fork stalling and DNA breaks as well as mutagenesis due to the unequal competition between the dNTPs. Instead, high dNTP levels can impair the polymerase proofreading activity or promote polymerase slippage, also leading to mutagenesis. Hence, dNTP pool changes can promote tumorigenesis, framing RNR as an important target for anti-cancer drugs (*6*). At high doses, HU induces replication fork stalling and activates the DNA damage checkpoint with dramatic consequences on origin firing. These include the global inhibition of new origin firing and the local activation of the so-called 'dormant' origins to enable full chromosome duplication while preventing further replication fork stalling (*7*). Given that dNTPs are limiting for replication fork progression, treatment with high HU also indirectly affects replication fork speed (*8, 9*). However, Somyajit *et al*. show that a low dose of HU, insufficient to stall replication forks or activate the DNA damage checkpoint, was able to modulate replication fork speed directly, rapidly, and independently of dNTP levels. This occurs by the reaction catalysed by RNR itself. RNR converts ribonucleoside 5'-diphosphates (NDPs) into 2'-deoxyribonucleoside 5'-diphosphates (dNDPs) via an electron transfer that when inhibited by HU causes a redox imbalance. The observed replication fork slow down correlated with a rapid dissociation of different components of the replication protection complex, such as TIMELESS and TIPIN, from replication forks. Consistently, several studies with the yeast orthologs of the replication protection complex have shown that fork rates are reduced in their absence in vivo (*10*) and accelerated by their presence in vitro (*3*). Both the slow replication forks and dissociation of TIMELESS observed with low doses of HU were rescued by quenching ROS but not by adding exogenous dNTP precursors, a result that could be recapitulated with H_2O_2 (*5*) undoubtedly arguing that it is redox imbalance that triggers TIMELESS dissociation from the replisome and the resulting replication fork slow down.

ROS are produced during aerobic metabolism and include hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radicals (OH^-). In addition to the pathological consequences of ROS inherent to their high reactivity, subtle changes in ROS elicit signaling responses that are key events in physiological processes such as cellular differentiation, tissue regeneration, and prevention of aging (*11*). Importantly, the coupling between fork speed and redox signaling is mediated by the interaction between TIMELESS and one component of this redox signaling response, peroxiredoxin 2 (PRDX2). This is an antioxidant enzyme from the peroxiredoxin family, which are highly sensitive to subtle fluctuations in ROS levels. Somyajit *et al*. propose that oxidation of PRDX2 oligomers, which occur on exposure to ROS, induces their disruption and dissociation from chromatin, dragging out TIMELESS-TIPIN from ongoing replisomes and thus slowing down replication fork progression in an oxidizing environment (see the figure).

Failures in this coupling mechanism (as in PRDX2-deficient cells) lead to replication-dependent and TIMELESS-mediated genetic instability, as observed by the increased occurrence of DNA lesions typically derived from replicative stress, such as ultrafine anaphase DNA bridges and p53 binding protein 1 (53BP1) nuclear bodies (*5*). The concept of replicative stress may thus need to be revisited beyond replication fork slowdown and/or stalling to include also replication acceleration. This indicates that the PRDX2-TIMELESS interaction prevents the damaging consequences to DNA of metabolite oscillations. Notably, cancer cells show high levels of ROS, slow replication forks and are highly sensitive to PRDX2 inactivation, implying that they exploit this coupling mechanism to allow tumor cell survival (*5*). The necessity of this pathway for tumor cell survival argues that it is not a frequent oncogenic event and supports that ROS-induced replicative stress is not a main driver for tumorigenesis (*12*).

Thus, the coupling of redox signaling with fork speed emerges as an effective strategy to prevent the negative consequences of oxidative stress on replication in strictly aerobic eukaryotic cells, whereas anaerobic facultative cells like yeast can temporarily separate DNA synthesis from ROS-generating respiration processes (*13*). Since cancer cells use this coupling mechanism to bypass their abnormally high oxidative stress derived from its altered metabolism, it seems obvious to see it as a potential target in chemotherapy. It will be interesting to investigate how this mechanism impacts on DNA repair insufficiency-associated manifestations such as premature ageing and genetic diseases (*14*) and at vulnerable DNA regions such as telomeres or sites of transcription-replication collisions, such as ribosomal DNA (rDNA) or fragile sites (*15*). The need for a mechanism of fork speed regulation to counteract redox imbalance supposes a new twist in our understanding of the cellular mechanisms devoted to safeguard genome integrity.

CABIMER, Universidad de Sevilla, Av. Américo Vespucio 24, 41092 Seville, Spain. E-mail: aguilo@us.es

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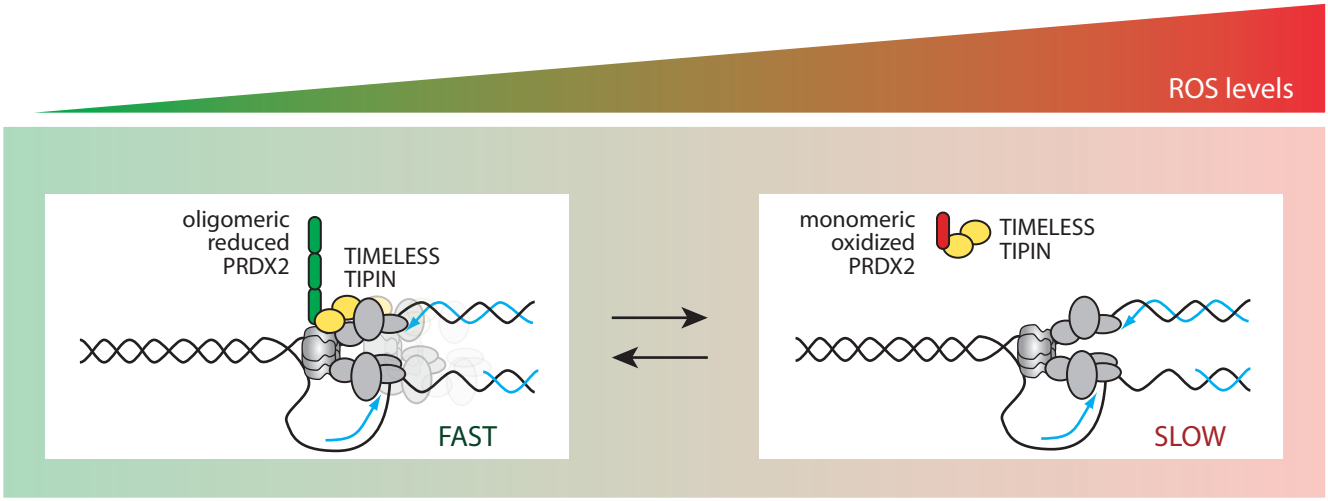
FIGURE LEGEND

Cells adjust the replication fork speed to their redox state. To enable timely duplication of the full genome, the presence of TIMELESS-TIPIN at the replisome speed up replication. However, in the presence of subtle changes in ROS, peroxiredoxin PRDX2 oligomers are oxidized and disrupted, thus being dissociated from chromatin. The interaction between PRDX2 and TIMELESS causes TIMELESS-TIPIN dissociation from ongoing replisomes, so forks slow down as a mechanism to safeguard genome integrity. Cancer cells, with an abnormally high oxidative stress derived from their altered metabolism, use this mechanism of fork slow down for survival.


ROS levels
oligomeric
reduced
PRDX2
TIMELESS
TIPIN
FAST
monomeric
oxidized
PRDX2
TIMELESS
TIPIN
SLOW