DNA REPLICATION GROUP

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OVERVIEW

Our laboratory studies the molecular mechanisms that underlie genomic duplication in mammalian cells. The 'replisome' complex in charge of DNA replication encounters natural obstacles (e.g. unusual DNA structures, collisions with transcription proteins), as well as exogenous challenges such as ionising radiation, UV light and chemicals that modify the DNA structure and block DNA polymerases. The situations in which replication forks are forced to slow down, stall or collapse are generically referred to as replicative stress (RS). Our Group investigates the 'DNA damage tolerance' pathways that facilitate DNA replication in the presence of RS or damaged DNA. In recent years, we have identified 2 mechanisms that counteract RS: (1) the conditional activation of dormant replication origins; (2) the participation of PrimPol, a primase-polymerase enzyme, in the restart of stalled forks. We continue to characterise the different cellular responses to RS (FIGURE 1).

"We have developed genetic tools to investigate the physiological impact of defective DNA replication, including mouse strains that suffer from a high incidence of haematological cancers due to their inefficient response to DNA damage during replication."

RESEARCH HIGHLIGHTS

Cellular functions of PrimPol protein

We have continued to characterise PrimPol, a DNA primase-polymerase that participates in DNA damage tolerance during chromosomal replication. With this aim in mind, we have generated human and mouse cells in which PrimPol expression is either downregulated or completely ablated. PrimPol-deficient cells display a marked sensitivity to UV irradiation, including the accumulation of unrepaired Thy dimers (CPDs and 6,4pp) in the DNA. The skin of PrimPol KO mice also presents an inefficient healing response to UV irradiation and a higher frequency of benign papillomas. The importance of PrimPol as a tumour suppressor gene is currently being investigated.

Effects of DNA re-replication *in vivo*

CDC6 and CDT1 proteins are responsible for the loading of MCM2-7, the DNA helicase, at replication origins. After CDC6 and CDT1 execute their 'origin licensing' functions in the G1 phase, their activities are inhibited until mitosis is complete in order to prevent origin reactivation and DNA over-replication within the same cell cycle. However, these strict control mechanisms may be partially overridden in some cancer types, notably non-small cell lung carcinomas, by the overexpression of *Cdc6* and/or *Cdt1* genes.

We have recapitulated the deregulated expression of *Cdc6* and *Cdt1* using mouse strains that allow the inducible expression of both proteins, alone or in combination. While individual deregulation of CDC6 or CDT1 has only mild effects, their combination is lethal for developing embryos and also for adult individuals. Using single-molecule analysis of DNA replication, high-throughput confocal microscopy and histopathology, we have identified origin re-firing events that are sufficient to cause DNA over-replication and DNA damage in different tissues. These mouse models will allow a complete study of the physiological impact of DNA re-replication *in vivo*.

Evidence for replicative stress in early embryonic cell cycles

Replicative stress is normally studied in the context of cells undergoing external challenges. However, it also occurs in the unperturbed S phase when the replication machinery reaches special DNA structures (e.g. G-quadruplexes) that are difficult to replicate, or when it collides with a transcriptional fork. In 2016, we participated in a collaborative study, led by Dr M. Lopes (University of Zurich, Switzerland), that identified unexpected levels of physiological RS. Mouse embryonic stem cells and early embryos at the blastocyst stage display a constitutive accumulation of RPA-covered ssDNA, fork slowing and fork remodelling events, all hallmarks of RS. These characteristics are related to the short duration of the G1 phase in embryonic stem cells and are lost upon the onset of cell differentiation. This result underscores the importance of the G1 phase to fully repair DNA that had been damaged in the previous cell cycle, before entering a new round of replication (Ahuja *et al.*, 2016).

Single-molecule analyses of DNA replication

As replicative stress impinges on many cellular processes, the possibility of analysing DNA replication at the single-molecule level continues to attract the interest of many research groups at the CNIO and other institutions. In 2016, we collaborated in two projects led by Oscar Fernández-Capetillo (CNIO Genomic Instability Group) to demonstrate that USP7 ubiquitin protease targets SUMO and is essential for DNA replication (Lecona *et al.*, 2016), and that PolD3 is haploinsufficient for DNA replication in mice (Murga *et al.*, 2016). In the latter project, the analyses of replication in stretched DNA fibres revealed a striking accumulation of asymmetric forks in the absence of POLD3, a regulatory subunit of DNA polymerase δ (FIGURE 2). Finally, a collaboration with R. Freire (*Hospital Universitario de Canarias*, Tenerife) revealed a novel function for USP37 ubiquitin protease in the control of DNA replication (Hernández-Pérez *et al.*, 2016). ■

PUBLICATIONS

- Murga M, Lecona E, Kamileri I, Díaz M, Lugli N, Sotiriou SK, Anton ME, Méndez J, Halazonetis TD, Fernández-Capetillo O (2016). POLD3 is haploinsufficient for DNA replication in mice. *Mol Cell* 63, 877-883.
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- Ahuja AK, Jodkowska K, Teloni F, Bizard AH, Zellweger R, Herrador R, Ortega S, Hickson I, Altmeyer M, Méndez J, Lopes M (2016). A short G1 phase imposes constitutive replication stress and extensive fork remodeling in mouse embryonic stem cells. *Nat Commun* 7, 10660.
- Hernández-Pérez S, Cabrera E, Amoedo H, Rodríguez-Acebes S, Koundrioukoff S, Debatisse M, Méndez J, Freire R (2016). USP37 deubiquitinates Cdt1 and contributes to regulate DNA replication. *Mol Oncol* 10, 1196-1206.
- Muñoz S, Méndez J (2016). DNA replication stress: from molecular mechanisms to human disease. *Chromosoma*. PMID: 26797216.
- Boskovic J, Bragado-Nilsson E, Prabhakar BS, Yefimenko I, Martínez-Gago J, Muñoz S, Méndez J, Montoya G (2016). Molecular architecture of the recombinant human MCM2-7 helicase in complex with nucleotides and DNA. *Cell Cycle* 15, 2431-2440.

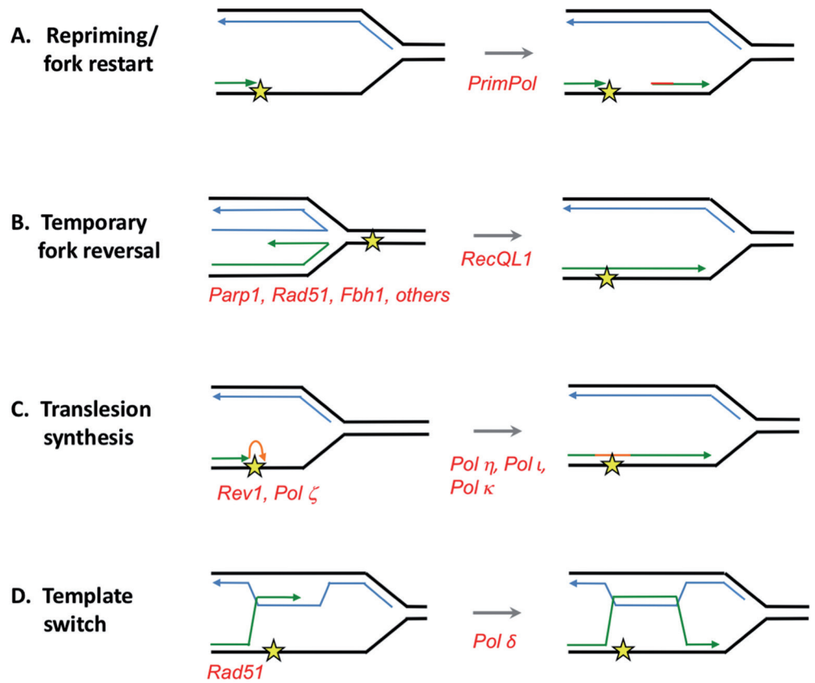

Figure 1 Pathways that facilitate fork progression through damaged DNA. The yellow star represents a polymerase-blocking lesion. The main proteins involved in each pathway are mentioned. (**A**) Repriming downstream of the lesion. (**B**) Fork reversal and restart. (**C**) Translesion synthesis DNA polymerases. (**D**) Lesion skipping by template-switch. Adapted from Muñoz and Méndez (2016).

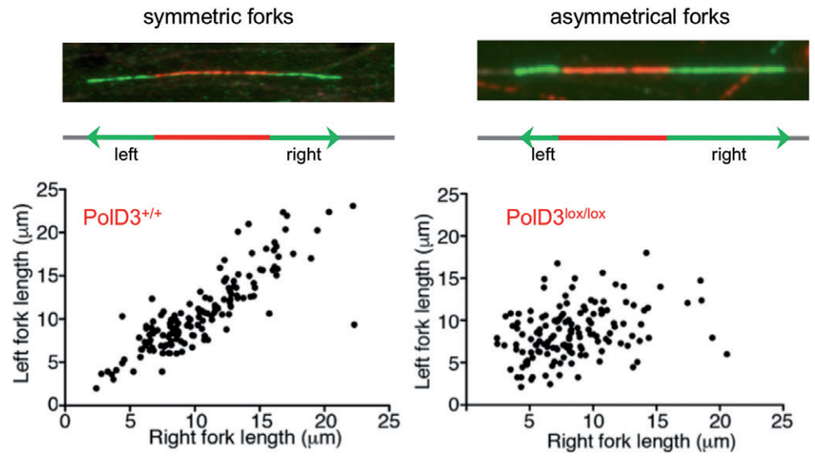

Figure 2 Detection of fork asymmetry in stretched DNA fibres. Top: representative images of replicating DNA molecules labelled with CldU (red) and IdU (green). Each image shows 2 forks moving away from a central origin. Bottom: quantification of fork asymmetry in DNA fibres prepared from B-cells from PolD3-competent (left) or PolD3-deficient (right) mice. Adapted from Murga *et al* (2016).