DNA REPLICATION GROUP

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OVERVIEW

Recent epidemiology studies indicate that up to two thirds of the mutations found in tumours are the consequence of inaccurate DNA replication; the rest are inherited or caused by environmental factors. We study the process of DNA replication and its regulatory pathways, with a particular interest in the phenomenon of replicative stress (RS) caused by the temporal stalling or inhibition of the protein machinery responsible for DNA synthesis. In 2018, we focused on the following areas: (1) the activation of 'dormant' replication origins in response to RS; (2) the molecular connection between the speed of replication forks and the frequency of origin activation, the two main parameters affected by RS; and (3) the function of PrimPol primase in 'replicative tolerance', i.e. the duplication of chemically damaged DNA molecules in order to facilitate their subsequent repair. We have also applied single-molecule methods to analyse the impact of RS in several biological processes.

"We have developed a method to determine the primary cause of replicative stress as a necessary step towards the design of methods to restrict it in primary cells and/or enhance it in tumour cells."

RESEARCH HIGHLIGHTS

Differential activation of replication origins upon replicative stress

Ten years ago, our laboratory reported that stalled replication forks induce the activation of extra origins as a backup mechanism to complete DNA replication. The genomic characteristics of these 'dormant' origins and their mode of activation remained largely unknown. We have now identified, in collaboration with Dr M. Gómez (*Centro de Biología Molecular* "Severo Ochoa", *CSIC-UAM*, Madrid) and Dr V. Pancaldi (formerly at CNIO; currently at the Cancer Research Centre of Toulouse, CRCT), the genomic positions and efficiency of activation of thousands of replication origins in mouse embryonic stem cells, in normal growth conditions or under stress to trigger extra origin activation. This comparative analysis has revealed that the vast majority of 'stress-responsive' origins are active in a fraction of the control cell population, but their efficiency is significantly increased when stalled forks accumulate. The efficiency of activation of each individual origin correlates with its physical proximity to active or bivalent promoters, CpG islands, and the presence of 'open chromatin' epigenetic marks. The integration of linear origin maps into 3D chromatin interaction networks reveals a hierarchical arrangement in which local clusters of origins are brought together by long-range chromatin interactions.

Cause and effect in replicative stress phenotypes

Replicative stress (RS) phenotypes are normally identified by specific nuclear patterns of markers γH2AX and RPA, but their detailed characterisation requires single-molecule analyses of fork speed and frequency of origin activation using DNA fibres. The interpretation of these assays is complicated because primary alterations in fork speed trigger the secondary activation of extra origins, and conversely, primary changes in the number of active origins also affect fork speed. We have designed interventions in which primary effects of RS on fork speed can be distinguished from primary effects on origin firing, and have applied them to our current research on PrimPol protein (FIGURE). Identifying the primary cause of RS may inform us about new methods to enhance it in cancer cells, increasing their susceptibility to chemotherapeutic agents that target DNA repair.

Primpol protein and its potential applications in cancer therapy

Besides Polα/primase, PrimPol is the only other primase in mammalian cells and it facilitates replication through damaged DNA templates. In 2018, we used Crispr/Cas9 technology to eliminate PrimPol expression in cancer cells, making them hypersensitive to DNA crosslinking agents. These results open the possibility of inhibiting PrimPol as a coadjuvant in chemotherapy. In collaboration with Dr L. Blanco (*Centro de Biología Molecular* "Severo Ochoa", *CSIC-UAM*, Madrid), we have characterised a variant of PrimPol in which amino acid Tyr100 is changed to His, a mutation identified in certain types of lung cancer. Tyr100 mediates the enzyme selection of dNTPs over rNTPs, and Y100H is unusually proficient at using the latter, which may provide a cellular advantage during oncogenic transformation when the dNTP/rNTPs balance is disrupted.

Single-molecule analysis of DNA replication: shedding light on relevant biological processes

RS potentially impinges on all biological processes that involve cell proliferation. Over the past year, we participated in two collaborative projects to analyse RS in specific contexts. First, a study led by Dr I. Moreno de Alborán (*Centro Nacional de Biotecnología*, *CSIC-UAM*, Madrid), has uncovered the replicative defects linked to the loss of transcription factors c-Myc and Max during the differentiation of B lymphocytes. The second study, in collaboration with Dr G. Stoecklin (Heidelberg University) and Dr O. Fernández-Capetillo (CNIO), has led to the functional characterisation of TIAR, an RNA-binding protein that controls mitotic entry and is required for genomic stability. ■

PUBLICATIONS

- Rodríguez-Acebes S, Mourón S, Méndez J (2018). Uncoupling fork speed and origin activity to identify the primary cause of replicative stress phenotypes. *J Biol Chem* 293, 12855-12861.
- Pérez-Olivares M, Trento A, Rodríguez-Acebes S, González-Acosta D, Fernández-Antorán D, Román-García S, Martínez D, López-Briones T, Torroja C, Carrasco YR, Méndez J, Moreno de Alborán I (2018). Functional interplay between c-Myc and Max in B lymphocyte differentiation. *EMBO Rep* 19, e45770.

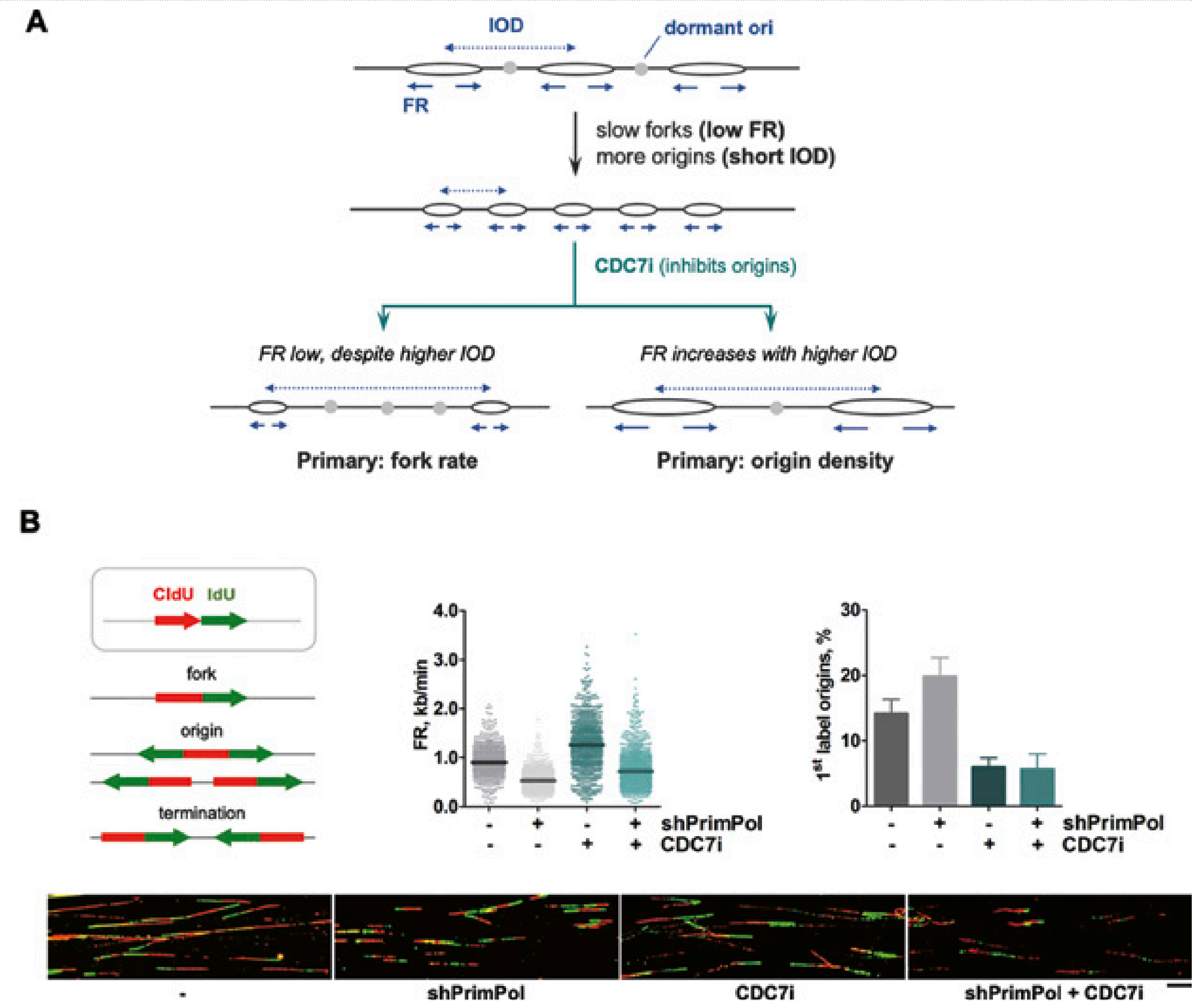

Figure New methods to determine the primary cause of replicative stress. (**A**) Test based on a CDC7 kinase inhibitor (CDC7i) to separate cause and effect when fork speed is reduced and origin density is increased. A complementary test can be applied in the opposite situation (fork rate increased, origin density reduced; not shown). (**B**) CDC7i test applied to U2OS cells undergoing RS after PrimPol downregulation. In this case, RS is due to a primary defect in fork speed. Representative images of DNA fibres used to measure fork speed and origin usage are shown. Bar, 10 µm. Adapted from Rodríguez-Acebes *et al.* (2018).