DNA REPLICATION GROUP

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OVERVIEW

Despite the biochemical complexity of the DNA replication process, the molecular machinery that duplicates our genome displays a remarkable capacity to adapt to different cell types, each one with its own transcriptional programme and specific patterns of chromatin organisation. In addition, the replisome proteins react to endogenous and exogenous factors that induce replicative stress (RS) and may cause DNA breaks, recombination events, and genomic instability. Our Group studies the mechanisms that confer operational flexibility to the replicative process, combining molecular and cellular approaches in human and mouse cells. In 2022, we completed two studies describing the cellular responses to specific situations of stress, which involve the regulation of origin activity and the control over replication fork progression. We also continued to study the dynamics of DNA replication and the impact of RS in other cellular contexts, including the acquisition of metastatic capacity by tumour cells.

"We have described how PRIMPOL facilitates DNA synthesis during stress-induced proliferation of haematopoietic stem cells, allowing the haematopoietic system to reconstitute itself after a bone marrow transplantation."

RESEARCH HIGHLIGHTS

Three-dimensional chromatin organisation underlies the efficiency of replication origins

In earlier work, we had reported that a fraction of mammalian replication origins remains inactive ("dormant") in S phase but can be activated as a backup mechanism in response to RS. To investigate the regulation of active *vs* dormant origins, we mapped origin activity in mouse embryonic stem cells (mESCs) undergoing mild RS triggered by aphidicolin, a DNA polymerase inhibitor, or by the ectopic expression of CDC6, an origin licensing factor. The main stress-induced response was an increase in the frequency of activation of existing initiation sites that were used with lower efficiency in unchallenged conditions. This phenotype reflects, at the cell population level, the combined effect of the activation of dormant origins in millions of individual cells. By intersecting origin mapping and Hi-C chromosomal conformation data, we found that origin efficiency is directly proportional to the number of three-dimensional (3D) contacts established between origin-containing chromatin fragments. Origins that cluster in 3D tend to fire with similar efficiencies and share their timing of replication, supporting the organisation of origins in higher-level replication factories (Jodkowska *et al.*, 2022; see FIGURE 1).

PRIMPOL-mediated repriming of DNA synthesis during stress-induced proliferation of haematopoietic stem cells

Since its discovery in 2013, our laboratory has been involved in the characterisation of the PRIMPOL enzyme, a DNA primase specialised in damage tolerance. In a recent study, we described how PRIMPOL mediates the replicative tolerance of DNA inter-strand crosslinks (ICLs; González-Acosta *et al.*, *EMBO J* 2021). Inefficient ICL repair causes Fanconi Anaemia (FA), a rare but severe disease characterised by frequent congenital defects, bone marrow failure, aplastic anaemia and cancer predisposition. In 2022, we completed a study in collaboration with M. Lopes (Institute of Molecular Cancer Research, University of Zurich), showing that mouse haematopoietic stem cells (HSCs) that are forced to proliferate by a simulated viral infection display accelerated fork progression and accumulate extensive DNA damage. In this critical situation, HSCs rewire their DNA damage response and engage PRIMPOL primase, favouring re-priming of DNA synthesis over fork reversal. Competitive bone marrow transplantations confirmed that PRIMPOL activity is required for HSC amplification and efficient reconstitution of the haematopoietic system (Jacobs *et al.*, 2022; FIGURE 2). This study opens the possibility that in some cases, PRIMPOL-mediated bypass of damaged DNA could also contribute to the onset of leukaemia. In this regard, we are pursuing the identification of small inhibitors of PRIMPOL, in collaboration with the CNIO Experimental Therapeutics Programme.

DNA replication and RS in other cellular contexts

We have participated in two collaborative studies related to the main research topics described above: (a) the characterisation of a protective function of human p38 SAP kinase to maintain genome integrity in response to osmo-stress, mediated by claspin/Mrc1 phosphorylation (Ulsamer *et al.*, 2022); and (b) the analysis of DNA replication in cells harbouring a truncated variant of RAD51B associated with primary ovarian insufficiency (Franca *et al.*, 2022).

Other ongoing projects in the DNA Replication Group include: (i) a genome-wide analysis of the formation of pre-replicative complexes in human and mouse cells, using CUT&RUN with initiator proteins; (ii) a comparative analysis of replisome composition in *naive* and *primed* mESCs, which could explain the changes in fork speed observed during cell reprogramming; (iii) an investigation of the influence of RS during epithelial-to-mesenchymal transition, a process that underlies the acquisition of resistance to chemotherapy in some tumour cells. ■

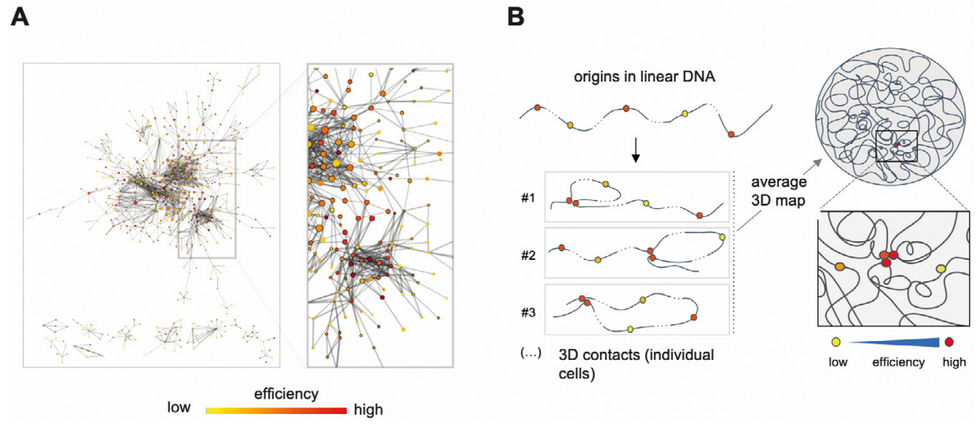

FIGURE 1 Integration of origin maps into 3D chromatin interaction networks. (**A**) Network of chromatin contacts derived from Hi-C data in mESCs (chrom 1). Origins (coloured circles) located at more connected hubs are activated with higher frequency. (**B**) Model of a replication factory formed by clustered origins. Adapted from Jodkowska *et al.* (2022).

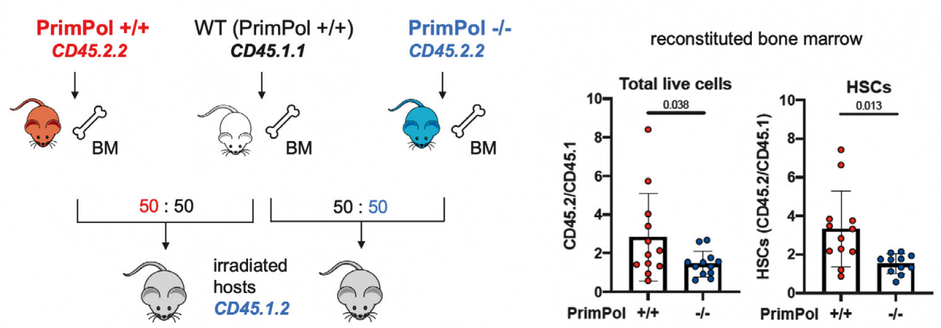

FIGURE 2 Efficient reconstitution of the haematopoietic system requires PRIMPOL activity. (**A**) Experimental design of a competitive bone marrow (BM) transplantation. (**B**) Donor chimerism in the reconstituted BM (total live cells and HSCs). Red, PRIMPOL-proficient; blue, PRIMPOL-deficient cells. Adapted from Jacobs *et al.* (2022).

PUBLICATIONS

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