Our laboratory studies the process of genome duplication, which is responsible for many of the mutations and genomic alterations found in human cancer. While the protein machinery responsible for DNA replication is normally very accurate, it becomes error-prone when the DNA displays chemical alterations caused by endogenous reactive species, external drugs or ionising radiation. We are interested in the phenomenon of replicative tolerance, i.e. the mechanisms that allow the progression of the ‘replisome’ proteins through damaged DNA, a step that normally precedes the activation of specific DNA repair pathways. In 2019, we focused on two major areas: (1) studying the efficiency of replication origins in response to replication stress in pluripotent cells, in the context of their three-dimensional positions in the nuclear chromatin; and (2) evaluating the function of DNA primase PrimPol in the tolerance of cytotoxic DNA lesions in normal and cancer cells with different genetic backgrounds.

“We have identified that PrimPol primase counteracts the effect of cisplatin and other DNA crosslinking agents and mediates an adaptive response in cancer cells that could be exploited in chemotherapy.”
In previous years, we identified 2 cellular responses to replicative stress (RS), a phenomenon defined by the slowdown of DNA synthesis caused by damaged DNA templates or special conditions that mimic chemotherapy. These pathways are important in BRCA-deficient cells, which have already lost an alternative mechanism to counteract RS (Quinet et al., 2019).

As one of the early proponents of the role of ‘dormant origins’ in counteracting RS, we are interested in their characteristics as well as their localisation and mode of activation. We led a study to map active origins in embryonic stem cells under normal growth conditions or in the presence of RS (FIGURE 1), in collaboration with M. Gómez (Centro de Biología Molecular “Severo Ochoa” in Madrid), Alfonso Valencia (Barcelona Supercomputing Center) and V. Pancaldi (Cancer Research Centre of Toulouse). Because origins are not ‘fired’ in every cell in the population, they can be classified according to their efficiency, i.e. the proportion of cells in which they are activated.

The most efficient origins tend to be located at regions displaying ‘open chromatin’ epigenetic marks, frequently overlapping with CpG islands and transcriptional start sites. In contrast, RS-responsive origins correspond to less efficient initiation sites and are not enriched in these genomic elements. RS triggers a global increase in origin efficiency, maximising the activation of low-usage origins as a backup for replication. To identify structural determinants of origin efficiency, we integrated origin maps into 3D networks of chromatin contacts, finding that origin efficiency is proportional to their connectivity with other origins (FIGURE 2). Interconnected origins tend to display similar efficiency and replicate at the same time in S phase, providing a logical framework for the concept of chromosomal ‘replication factories’ (Jodkowska et al., 2019).

Mapping of ‘dormant’ origins that react to replicative stress

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Replicative tolerance mediated by PrimPol protein

PrimPol is the only primase in mammalian cells besides the canonical Pol/I primase involved in the initiation of DNA replication. In previous work, we reported that PrimPol facilitates replication through UV-induced DNA adducts by synthesising DNA primers downstream of the damaged nucleotides. In 2019, we collaborated with the laboratory of A. Vindigni (Washington University, St Louis, MO, USA) to contribute to the characterisation of TIAR, an RNA-binding protein required for genomic stability that restricts CDK1 activity under RS (Lafarga et al., 2019). We also started a collaboration with Scott Lowe (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) to perform CRISPR-based genetic screenings related to the effect of uncontrolled DNA replication in the process of gene amplification.

In parallel, together with L. Blanco (Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, Madrid), we characterised a variant of PrimPol found in some lung cancers, in which a single point mutation (Y100H) changes the enzyme specificity of nucleotide sugar selection from dNTPs to rNTPs (Díaz-Talavera et al., 2019).

These and other recent results from our Group imply that PrimPol counteracts the effect of DNA-damaging agents. Therefore, PrimPol inhibition may increase the efficacy of chemotherapy treatments. We have started a screening for potential PrimPol small molecule inhibitors, in collaboration with the CNIO Experimental Therapeutics Programme.

DNA replication in other biological contexts

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