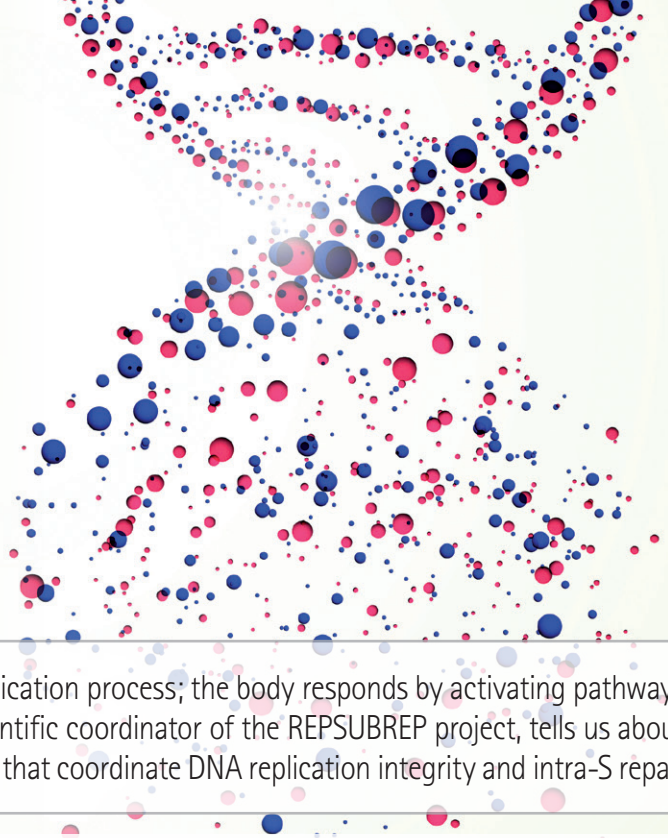
Unravelling the secrets of DNA repair



Damage to DNA often occurs during the replication process; the body responds by activating pathways to repair the damage. **Dr Dana Branzei**, scientific coordinator of the REPSUBREP project, tells us about her research into the regulatory mechanisms that coordinate DNA replication integrity and intra-S repair

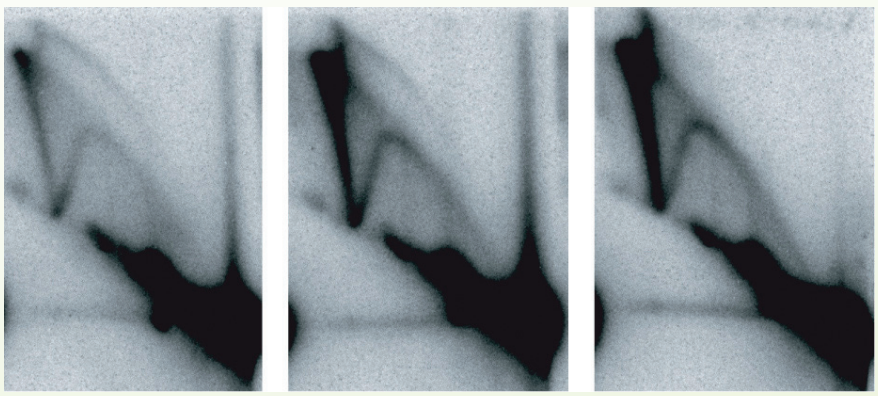
As we grow older our DNA begins to degenerate, a process which is often associated with genetic instability, while it is also regularly exposed to various endogenous and environmentally damaging agents. In order to maintain genome stability, cells are endowed with different DNA repair systems and DNA damage surveillance mechanisms, an area of great interest to Dr Dana Branzei and her colleagues at IFOM, the FIRC Institute of Molecular Oncology. "My lab is interested in how cells are able to repair and tolerate DNA damage, particularly when it arises during DNA replication, and how these different DNA repair mechanisms are coupled with one another and with other cellular process, such as cell-cycle transitions and the establishment of chromatin structure," she outlines. "In the REPSUBREP project we're investigating the mechanisms of DNA damage tolerance in eukaryotic cells, and their impact on genome integrity and regulation."

DNA damage

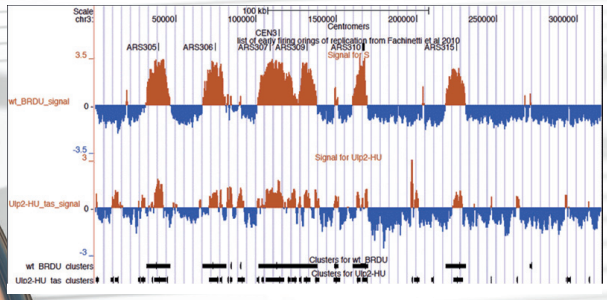
DNA damage tends to occur spontaneously during the replication process. One of the main problems is that replication forks often get blocked; difficulties in fork recovery can lead to chromosome breakage and ultimately genome rearrangements. "The project is studying the repair pathways that get activated during replication in response to these kinds of endogenous stresses, or when cells are exposed to different types of genotoxic stress," explains Dr Branzei. There are several ways to bypass damage or fill in DNA gaps that arise during replication; these mechanisms are generally referred to as DNA damage tolerance pathways. "Most of the time it is possible to prevent chromosome breaks during replication, but occasionally they do happen," continues Dr Branzei. "If a replication fork is not properly restored it is likely to lead to collapse and chromosome breaks, that can then lead to aberrant recombination and genome rearrangements."

There are two main pathways of DNA damage tolerance, one of which is error-free, while the other is error-prone. The error-free pathway uses recombination to bypass DNA damage, while the error-prone pathway uses specialised translesion DNA polymerases. "My lab is interested in how cells are able to bypass damage in an error-free manner involving recombination. This usually happens by switching templates, from the damaged parental DNA to the undamaged, newly synthesized chromatid strand. We have identified that error-free damage tolerance is mediated by the formation of sister chromatid junctions, a type of chromosomal structure," explains Dr Branzei. Researchers at Dr Branzei's lab have been able to visualise these structures and show that they not only promote DNA damage bypass, but also that they need to be resolved effectively to prevent problems later on during chromosome segregation. "One of the major goals of the REPSUBREP project is to characterise this process of template switching and to identify how it influences chromosome structure and integrity," she says.

The aim in this work is to identify the factors and regulatory pathways involved in the formation and metabolism of the chromosomal intermediates that arise



2D gel analysis of replication intermediates.

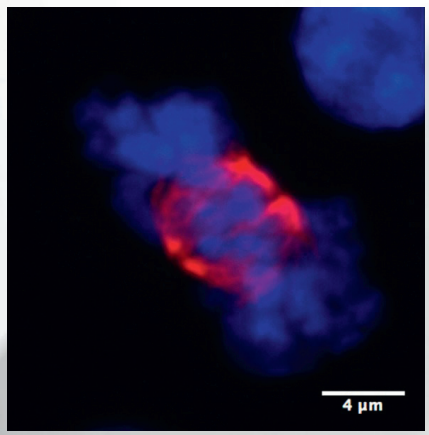


ChIP-on chip analysis (genome-wide analysis of protein cluster or replication profiles).

during template switching. A number of pathways can be activated in response to DNA damage during replication, one of which is mediated by a process called SUMOylation; the Sgs1/BLM protein, mutated in cancer-prone Bloom syndrome patients, is an important focus of research in this regard. "Sgs1 is a RecQ helicase. We know that this helicase plays a very important role in resolving the sister chromatid junctions that arise during replication and damage tolerance," says Dr Branzei. SUMOylation affects a number of the targets that participate in this resolution process, while it also has an impact on the functionality of other chromosomal structural proteins, such as the Structural Maintenance of Chromosome complex, Smc5-6. "There's an important link between Sgs1 activity and SUMOylation, which acts in very intricate ways to promote error-free replication. The exact mechanism of how Sgs1 is regulated via SUMOylation remains unknown, but we do know that several other targets participating in error-free DNA damage tolerance are activated by SUMOylation," continues Dr Branzei.

SUMOylation

The SUMOylation process plays a central role in determining whether damage bypass occurs via an error-free or an error-prone pathway. Alongside its involvement in the resolution of intermediates, Dr Branzei says SUMOylation is also important in terms of how the DNA lesions that occur during replication, primarily DNA gaps, are repaired or filled in. "SUMOylation seems to be the driving force in channeling these lesions into the error-free pathway of template switching," she explains. Dr Branzei and her team have also found that SUMOylation that occurs during replication



Immunostaining of spindle pole bodies (chicken DT40 cells).

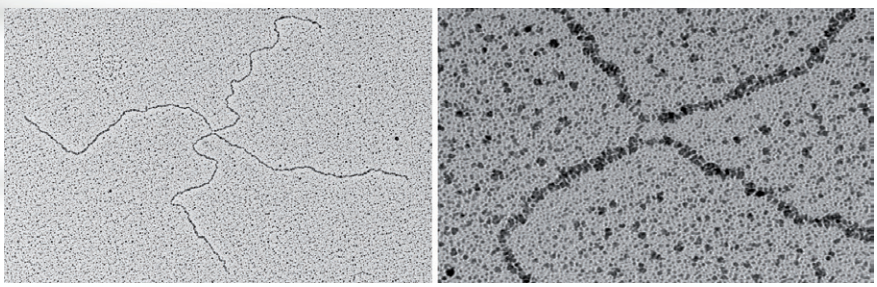
can also affect the chromatin architecture, although this work has not yet been published. "SUMOylation promotes DNA looping, and this looping plays an important role in channelling lesions towards the error-free pathway, and away from other mutagenic pathways," she says. "So it is not the case that just one target gets SUMOylated and activates a whole cascade of events."

The SUMOylation of certain targets plays a positive role in promoting replication or repair, but hyper-SUMOylation can also be toxic to the cell, so the modification process is quite dynamic. The RecQ helicases have a central role in resolving the sister chromatid junctions that arise during DNA damage tolerance, but several other helicases are involved. "We are working on several other families of helicases, such as the iron-sulphur cluster helicases, the Fanconi-anaemia associated family of helicases, and the UvrD family of helicases. We are also looking at several other enzymes, such as structure-specific nucleases," outlines Dr Branzei. This research holds real importance to our understanding of certain types of disease. "For instance, there is a cluster of diseases called cohesinopathies that are caused by mutations in factors that mediate sister chromatid proximity, or cohesion between sister chromatids," says Dr Branzei. "We've established different cell lines that basically mimic the mutations found in human patients and we are trying to characterise their DNA repair defect. We have evidence that those different cohesinopathies might have a recombination defect in common."

The project's main interest in these terms is Fanconi-anaemia and certain cohesinopathies, but they are also trying to understand the DNA repair defects associated with various tumours. It is becoming clear that problems in replication, and in resolving the problems that arise during replication, are an important feature of the oncogenic process. "Our work is important for enabling us to establish the molecular mechanisms behind specific or general tumourigenic processes. Not all tumours or cancers have the same type of DNA repair defect. It is important to study what happens in specific cancers – what is the repair defect of that specific cancer cell and how can we target it to achieve efficient therapy?" says Dr Branzei.

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Over the coming years Dr Branzei expects that her research will focus increasingly on how chromatin structure and architectural changes influence DNA damage tolerance, as well as on the mechanisms behind the activation or adaptation of DNA damage surveillance systems. "I think that there is a connection between chromatin structure and the formation of various recombination structures, and the ability of cells to shut down various safeguard mechanisms that get activated in response to DNA damage. I would be interested in looking at that in future," she says. "We have also developed a new approach for visualising chromosomal structures that we can use to identify the roles of various factors to genome integrity. There is still a lot to do in that regard – we have only just started to get the techniques working and to understand what happens in different genetic backgrounds."



Electron microscopy (EM) analysis of repair intermediates.

At a glance

Full Project Title

Regulatory mechanisms coordinating replication integrity and intra-S repair (REPSUBREP)

Project Objectives

The REPSUBREP project aims at identifying the mechanisms underlying error-free replication and DNA damage tolerance (DDT) when cells are exposed to genotoxic stress. Specific objectives are identifying the genetic pathways and the DNA structures that mediate error-free DDT as well as the regulatory pathways and molecular switches that influence the error-free versus error-prone DDT switch.

Project Funding

€1,841,600 over 5 years

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Project Coordinator

Romanian molecular biologist Dana Branzei did her bachelor and doctoral studies at Tohoku University in Japan, continuing her scientific career in DNA repair and genome stability at the Riken Institute in Japan and then at IFOM in Milan, Italy. From 2008, she became head of the DNA repair laboratory at IFOM in Milan. Her laboratory studies mechanisms of DNA damage response and tolerance pathways in eukaryotes.

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