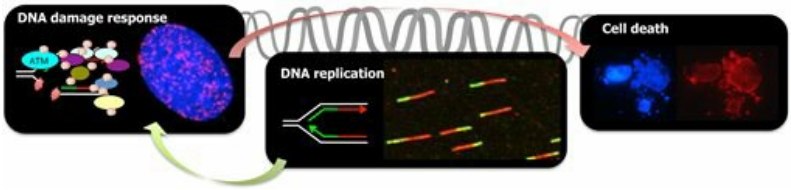
DNA replication and genome stability



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Group overview

DNA replication is the process by which dividing cells copy their genetic information. Replication is very important but also dangerous for cells, because if obstacles inhibit the movement of the replication apparatus, this can lead to DNA damage, mutations or cell death. This is called replication stress. Our group investigates molecular mechanisms of replication stress in cancer development and treatment.

Our research group

The replication apparatus is also called “replication fork” because of its structure (see Figure, right). Replication forks that encounter obstacles can stall and collapse into DNA double-strand breaks (DSBs), a highly mutagenic and toxic form of DNA damage (**Jones and Petermann, 2012 (http://www.ncbi.nlm.nih.gov/pubmed/22417748)** ; **Petermann and Helleday, 2010 (http://www.ncbi.nlm.nih.gov/pubmed/20842177)**). This cascade of replication fork stalling followed by conversion into DNA damage makes up replication stress.

Endogenous replication stress may be an important driving factor of tumour development. Consequently, cellular factors preventing replication stress are often tumour suppressors, while factors promoting replication stress may be oncogenes. Additionally, many DNA-damaging anti-cancer drugs act by slowing or stalling replication forks to kill cancer cells. Our group is interested in understanding how oncogenes, tumour suppressors and anti-cancer treatments influence replication fork progression and how stalled replication forks are converted into toxic DSBs.

We use mammalian cell models and the DNA fibre method, which employs labelling of live cells with nucleoside analogues, which can then be detected by immunofluorescence after DNA fibres have been isolated. This allows us to measure the speed of replication fork progression and whether replication forks stall or restart. Using this approach, we have shown how the oncogene Cyclin E causes replication stress and how the tumour suppressor BRCA2 modulates the therapeutic action of cancer drug gemcitabine (**Jones et al, 2013 (http://www.ncbi.nlm.nih.gov/pubmed/22945645)** ; **Jones et al, 2014 (http://www.ncbi.nlm.nih.gov/pubmed/25053826)**).

Left: Co-localisation of DNA damage (gamma-H2AX, green) and stalled replication forks (RPA, red) in the nucleus of a cell treated with replication inhibitor. Middle: DNA double-strand breaks (arrow) induced by replication fork stalling and visualised by pulse-field gel electrophoresis. Right: Pulse-labelling of live cells with thymidine analogues CldU and IdU allows visualising replication fork movement on isolated DNA fibres.

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Current projects

DNA damage caused by replication inhibitors

Many cytotoxic chemotherapy drugs specifically target cancer cells by interfering with DNA replication, which is essential for cancer proliferation (examples: gemcitabine, 5-fluorouracil, cisplatin). These replication inhibitors stall replication fork progression, which causes toxic double-strand breaks (DSBs). Their therapeutic action can be potentiated by cancer-specific defects in DNA repair, e.g. mutation in the homologous recombination (HR) genes BRCA1 and BRCA2.

BRCA2-mutated cells are generally more sensitive to replication inhibitors than normal cells. Strangely however, they are less sensitive to gemcitabine. We have found that gemcitabine efficiently causes DSBs in normal cells, but leads to fewer DSBs in BRCA2-mutant cells. This suggests that sometimes, DNA repair can promote rather than prevent DSB formation during replication stalling (**Jones et al, 2014 (http://www.ncbi.nlm.nih.gov/pubmed/25053826)**).

To better understand this, we are now investigating the cellular pathways that promote DSB formation during replication inhibitor treatments in more detail. DNA replication is an important target for cancer therapy. Uncovering the molecular mechanisms by which replication inhibition kills cells may help to exploit this target much more effectively in the future.

Endogenous replication stress in cancer

Faithful and complete replication of the genome is essential to maintain genomic stability and prevent cancer-promoting mutations. It has been shown that cancer cells can exhibit elevated DNA damage as a result of faulty chromosome replication, which is also known as replication stress. Replication stress may be a major cause of cancer-driving genomic instability.

De-regulation of proliferation by oncogenes is thought to be the cause of replication stress in cancer. However, very little is known about the mechanisms by which hyper-proliferation in cancer may cause replication stress. My lab is interested in finding such mechanisms. Firstly, we have shown that overexpression of the oncogene Cyclin E causes increased levels of replication initiation, which slows down of replication fork progression, thus promoting replication-associated DNA damage. Secondly, we also observed that a considerable portion of Cyclin E-induced replication stress results from interference between replication forks and the transcription machinery (**Jones et al, 2013 (http://www.ncbi.nlm.nih.gov/pubmed/22945645)**).

We are now further investigating transcription-replication interference as a potential mechanism of replication stress in cancer cells. Knowing which cellular pathways can cause replication stress will help to properly detect and exploit replication stress for cancer therapy.

Recent publications

Jones RM, Kotsantis P, Stewart GS, Groth P, Petermann E (2014) BRCA2 and RAD51 promote double-strand break formation and cell death in response to Gemcitabine. Mol Cancer Ther 13: 2412-21

Jones RM, Mortusewicz O, Afzal I, Lorvellec M, Garcia P, Helleday T, Petermann E (2013) Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress. *Oncogene* 32(32):3744-3753.

Zimmerman KM, Jones RM, Petermann E, Jeggo PA (2013) Diminished origin-licensing capacity specifically sensitizes tumor cells to replication stress. Mol Cancer Res 11: 370-380

Petermann E, Orta ML, Issaeva N, Schultz N, Helleday T (2010)Hydroxyurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair. *Mol Cell* 37(4): 492-502

Petermann E, Woodcock M, Helleday T (2010)Chk1 promotes replication fork progression by controlling replication initiation. *Proc Natl Acad Sci U S A* 107(37): 16090-16095

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