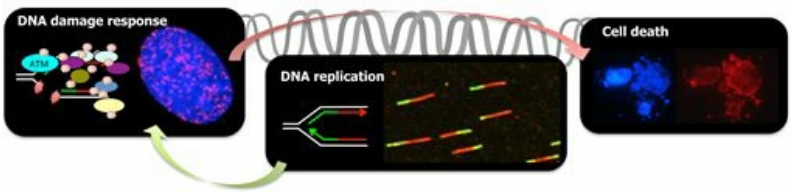
DNA replication and genome stability



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Group overview

DNA replication is the process by which dividing cells copy their genetic information. This process, while very important, is potentially dangerous as obstacles to the movement of the copying apparatus (called “replication fork”) can lead to DNA damage, mutations or cell death. Such events are important for cancer development and - treatment.

Our research group

If replication fork progression is impaired, forks can stall or collapse into DNA double-strand breaks (DSBs), a situation known as replication stress **(Jones and Petermann, 2012 (http://www.ncbi.nlm.nih.gov/pubmed/22417748)** ; **Petermann and Helleday, 2010 (http://www.ncbi.nlm.nih.gov/pubmed/20842177)**). Endogenous replication stress may be an important driving factor of tumour development.

Consequently, cellular pathways responding to replication stress can be expected to act as tumour suppressors, while pathways promoting replication stress may be tumour-promoting. On the other hand, many DNA-damaging anti-cancer drugs act by slowing or stalling replication forks. Our group is interested in understanding how oncogenes, tumour suppressors and anti-cancer treatments influence replication fork progression and what consequences this has for the cell.

Our lab uses 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 (**Jones et al, 2013 (http://www.ncbi.nlm.nih.gov/pubmed/22945645)** ; **Jones et al, 2014 (http://www.ncbi.nlm.nih.gov/pubmed/25053826)** ; **Petermann et al, 2010 (http://www.ncbi.nlm.nih.gov/pubmed/20188668)**).

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

Oncogene-induced replication stress

Faithful and complete replication of the genome is essential to maintain genomic stability and prevent cancer-promoting mutations. It is increasingly becoming clear that activated oncogenes of the growth factor signalling pathways, including Myc, Ras and Cyclin E, cause replication stress, which may be a major cause of cancer-driving genomic instability.

De-regulated G1/S transition, characterised by a shortened G1 phase and accelerated S phase entry, is thought to be a major cause of oncogene-induced replication stress. However, the underlying molecular mechanisms are not yet understood. Our recent work has shown that increased levels of replication initiation during overexpression of the oncogene Cyclin E interfere with replication fork progression, leading to overall slowed replication fork speeds and DNA damage. We found that a considerable portion of Cyclin E-induced replication stress results from interference between transcription and replication. Treatment of Cyclin E-overexpressing cells with transcription inhibitors or RNase H1 to degrade RNA/DNA hybrids increased speeds of replication fork progression and decreased levels of spontaneous homologous recombination (HR). Our data suggest that increased replication initiation and transcription act in the same pathway, possibly because changes to initiation patterns increase spatial conflicts between replication and transcription.

DNA damage response to replication inhibitors

Several anti-cancer drugs specifically target replicating cells by interfering with DNA replication, thus generating lethal DNA damage. Such treatments exploit the high proliferation rates of cancer cells, and can be further potentiated by cancer-specific defects in DNA repair. Much research in recent years has focused on understanding the mammalian DNA damage response to replication inhibitors. We and others have shown that proteins involved in homologous recombination repair (HR) such as Rad51 and XRCC3, and the HR-promoting proteins PARP1, Mre11 and Chk1 promote the efficient restart of stalled replication forks. Prolonged replication inhibition leads to widespread fork collapse and DSB formation, and HR is required for the repair of these DSBs and promotes survival of replication inhibitor treatments.

Such findings could be of clinical importance as several types of cancer can display altered levels of HR activity. Based on this, we are currently investigating the role of BRCA2 in the response to clinical replication inhibitors.

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.
- **Jones RM, Petermann E** (2012) Replication fork dynamics and the DNA damage response. *Biochem J* 443(1): 13-26
- **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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