

Science of the cell cycle

Dr Rob de Bruin describes his research on cell cycle-regulated transcription and explains how it may ultimately contribute to a better understanding of cancer and the development of novel therapeutic



Can you elaborate on how your research interests have evolved throughout your early postdoctoral work?

Throughout my career, my work has concentrated on the importance of transcriptional control during development, the cell division cycle, and its response to internal and environmental signals. During my postdoctoral work at the Scripps Research Institute in California, USA, I started to focus on the mechanisms involved in transcriptional regulation during the gap phase-1 and synthesis phase (G1 and S phases).

In 2009, I joined the Laboratory for Molecular Cell Biology at University College London (UCL), UK, as an independent group leader. Since then, cell cycle-regulated transcription has remained my main focus. Studies from my lab have led to the discovery of a simple yet elegant mechanism by which the DNA replication checkpoint can override the G1/S transcriptional programme by directly regulating the transcriptional repressor in both yeast and human cells. Overall, our discoveries have revealed that regulatory mechanisms are conserved in evolutionarily diverged systems.

Why is your research focused on the G1- and S-phases of the cell cycle? Can you briefly outline what these involve and how they fit into the cycle as a whole?

Cell division is a process essential to the propagation of life. At the heart of controlling

cell proliferation lies the transition from the G1-phase of the cell cycle to the S-phase, when the cell 'decides' whether or not to enter a new division cycle and undergo DNA replication. This decision coincides with the activation of G1/S transcription, which comprises a large number of dosage-sensitive co-regulated genes that encode proteins involved in many essential cellular functions such as cell division control, DNA replication, cell growth and maintenance of genome stability.

Importantly, the most commonly deregulated transcriptional networks in cancers are active during the G1- and S-phases of the cell cycle. These include G1/S transcription and the transcriptional response to DNA replication stress during S-phase, which are both the focus of our current work. Our investigations aim to provide unique insight into the role of these transcriptional networks in both healthy cells and pathological conditions.

For what reason have you chosen yeast as a model species?

Parallel work in fission yeast, *Schizosaccharomyces pombe*, and the distantly related budding yeast, *Saccharomyces cerevisiae*, has allowed us to make important discoveries in human cells, which serve as a model for human disease. Our experimental setup allows us to translate the insights obtained in yeast directly into the more complex human system, based on either sequence or functional conservation of the proteins involved.

What hopes do you have for the translational potential of your work?

I have high hopes that our current work will lead to new insights into human cancer and provide novel therapeutic approaches that will translate into benefits for patients and their families. Cancer-associated mutations that drive dysfunctional S-phase entry cause high levels of replication stress, and the cellular response to this is therefore thought to be particularly important for certain cancer cells. Consequently, targeting proteins with an essential role in

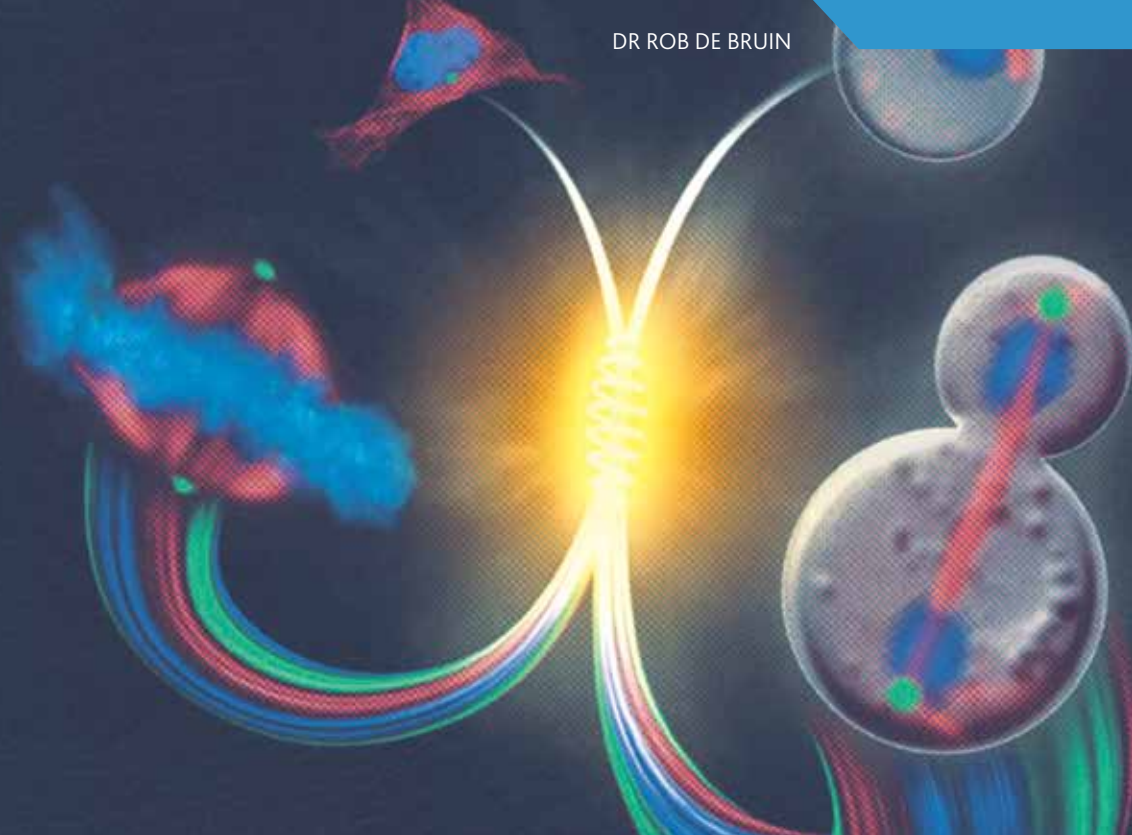


the response to replication stress creates a potentially large therapeutic window for damaging cancer cells without affecting normal cells.

Furthermore, our future work will provide a detailed understanding of how individual G1/S targets shape the outcome of replication stress-induced events, such as cell survival and genome stability. It is therefore expected to help exploit vulnerabilities of cancer cells with high levels of replication stress and guide us towards more effective strategies for cancer therapy.

Much of your work involves fundamental science. How does the importance of this compare to more translational research?

While we aim to translate the insights obtained in yeast directly into humans, we are continuing to build on our findings in yeast. This work is more fundamental and it is therefore less obvious how it will translate into significant benefits for patients. However, if the past has shown us anything it is that this type of fundamental work is essential for making new discoveries that form the basis of translational research in subsequent years. Therefore, it is of the utmost importance to keep funding and carrying out basic research to drive research discoveries that will benefit patients in the future.



Not just a phase

A group of researchers from the Laboratory for Molecular Cell Biology at [University College London](#) are investigating the fundamental biological mechanisms that impact cell cycle progression and genomic stability

DEFECTS IN THE regulation of the gap phase-1 and synthesis phase (G1 and S phases) genes can be detected in every cancer type. The regulation of these genes, collectively known as G1/S transcripts, plays a central role in the cell cycle – the process whereby healthy cells divide and multiply in a regulated manner. Cancer is, by definition, a disease in which cells multiply uncontrollably; therefore the presence of deregulated G1/S transcription in cancerous cells implies that it is an abnormality of the cell cycle regulation pathway that paves the way for tumorigenesis.

This aspect of cell biology is the focus of Dr Rob de Bruin's research group. Based in the Laboratory for Molecular Cell Biology at University College London (UCL), his team conducts both molecular and biochemical experiments, using two species of yeast – *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* – and human cell lines as models of cancer, to elucidate the processes by which changes in gene expression impact cell cycle control and genome stability in both healthy and pathogenic cells.

OUT OF CONTROL

De Bruin's interest in G1/S transcription activation began early in his career while

conducting postdoctoral research at the Scripps Research Institute in California, USA. Using yeast models, de Bruin successfully identified Whi5 as a transcriptional repressor involved in G1/S transcriptional regulation, and revealed that it was analogous to the pRb tumour suppressor pocket protein in mammals. He also identified a negative feedback loop in yeast cells that turns off G1/S transcription during S-phase cycle activity.

Upon joining UCL in 2009, de Bruin was able to establish his own research group using funds from a Medical Research Council Career Development Award. Here, he has continued to investigate G1/S transcription, with the ultimate aim of furthering understanding of cancer pathogenesis. Already, the group has demonstrated that the previously identified negative loop turns off G1/S transcription during S-phase in human cells as well as yeast; established a mechanism by which the DNA-replication checkpoint can override the G1/S transcription programme by directly regulating the transcriptional repressor; and identified different subgroups of G1/S targets that are regulated by distinct molecular mechanisms in budding yeast.

At present, the team is focusing on identifying the cellular functions that cancer cells

specifically depend on as a result of deregulated G1/S transcription. By exploring the various mechanisms at play in yeast models and human cell lines, they hope to develop a deeper understanding of the factors that impact tumour development.

REPLICATION STRESS

In addition, de Bruin's team is investigating transcriptional responses to DNA replication stress during the S-phase of the cell cycle. Replication stress (where insufficient DNA replication occurs, causing DNA replication forks to progress slowly or stop) can occur as a result of a number of factors, including deregulation of components required for DNA synthesis or a problem with the frequency of replication initiation. "The ability of cells to cope with such genotoxic stress is largely dependent on the action of the DNA replication checkpoint – an evolutionarily conserved signalling pathway that constantly monitors for the loss of integrity at the DNA replication fork," explains de Bruin. Again, the researchers are exploring these cellular functions through the use of yeast and human cell lines.

Particular attention is being paid to the Nrm1 transcriptional repressor. De Bruin and

INTELLIGENCE

CAUSES FOR CELL CYCLE TRANSCRIPTION IN CANCER CELLS: USING YEAST TO IDENTIFY DIFFERENCES IN PROTEINS BETWEEN HEALTHY AND CANCEROUS CELLS

OBJECTIVES

This research addresses three fundamental questions related to G1/S transcription:

- What is the role of G1/S cell-cycle transcription in the cellular response to replication stress?
- How does deregulated G1/S transcription contribute to genomic instability and the dependency on particular pathways for cell survival?
- How do chromatin-modifying enzymes control gene expression during the G1 and S-phases of the cell-cycle?

KEY COLLABORATORS

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FUNDING

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his colleagues were the first investigators to successfully delineate the essential role played by Nrm1 as a regulator of the cell cycle – it turns off G1/S transcription when exiting the G1-phase – and to establish its position as a target within the DNA replication checkpoint pathway. Building on these findings, the researchers are now anxious to obtain a more complete understanding of the underlying mechanisms. To achieve this, they are pursuing several avenues of investigation: exploring the biological relevance of restricting gene expression during the G1-phase and the influence of gene expression on checkpoint response; and studying how gene expression during the G1- and S-phases is regulated at the chromatin level.

Additionally, the UCL group is paying particular attention to the family of proteins known as the E2F transcription factors, which regulated G1/S transcription in human cells. "Our work has shown that maintaining E2F-dependent transcription is an essential part of the checkpoint response to prevent replication stress-induced DNA damage," states de Bruin. Gene expression, regulated by E2F transcription factors, plays a

crucial role in the human cell stress response; replication stress as a result of a failure to maintain E2F-dependent transcription can lead to irreparable DNA damage and cell death. For this reason, the team is studying the ways in which E2F target genes can shape the outcome of replication stress in terms of cell survival and genome stability.

LITTLE MECHANISMS, LARGE IMPACT

By bringing to light the mechanisms through which cell cycles become deregulated and genomes unstable, this research is contributing to a wealth of basic and translational knowledge. Such information is not only vital for the identification of novel anticancer drug targets and the development of new therapeutic approaches, it may also hold as yet unknown benefits for future generations. Given that genome instability and loss of transcriptional control are associated with ageing and over 60 inheritable diseases – ranging from neurodegenerative to autoimmune disorders and cancer – there is no telling what may one day be gained from the knowledge being gathered today.

INTERNATIONAL INNOVATION ASKS DR ROB DE BRUIN TO DISCUSS HIS CAREER HIGHS AND LOWS

CAREER HIGHLIGHT: "My proudest academic achievement was as an undergraduate student, when I was involved in the pioneering and fundamental research done on transgene silencing in plants carried out in the Mol laboratory in Amsterdam, The Netherlands. The experiment in which I was involved used some unconventional methods to demonstrate that effective post-transcriptional gene silencing required an inverted T-DNA repeat loci organisation – what is now known as a 'hairpin structure'. This, and other work, eventually led to the development of RNA interference (RNAi) technology, which has been one of the most important new findings in biology over the last decade."

CAREER CHALLENGES: "The biggest difficulty I've encountered in my career has been entering and establishing myself in new fields of research, transiting both from yeast to human cells and from G1/S transcription to the genome integrity checkpoint. While it is very exciting to take your research in new directions, you will always face resistance from people already established in those fields. The only way to overcome this difficulty is to see it as a challenge and continue to do good science, which will eventually lead to becoming a member of a new club."

