Dynamics of Genetic Information: 
Fundamental Basis and Cancer

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Teams in this unit aim at elucidating the regulation of mechanisms that are crucial to the maintenance of genome integrity, like DNA replication, repair and recombination, as well as the role of cell cycle checkpoints and non-coding RNAs in genome and epigenome maintenance. Experimental models include yeast, mammalian and human cell lines, mutant mouse models and tumour samples. A large variety of methods is used including genetic, molecular and cytogenetic techniques, fluorescence in situ hybridisation and DNA ‘combing’, as well as high throughput sequencing approaches such as DNA-seq for mutation landscape analyses, RNA-seq for transcriptome analyses and ChIP-seq mapping of chromatin-associated proteins and their genome-wide modulation in response to DNA damages.
Tumorigenesis is a multistep process in which chromosomal instability plays an important role. We have shown that telomere instability due to excessive shortening is the main driving force of chromosome aberrations during the early stages of cell transformation. Our work has shown that this chromosomal instability is associated with genome-wide changes in the chromatin landscape, which are directly correlated with transcription changes mainly affecting the non-coding genome, including microRNAs. We have discovered that the deregulation of microRNAs following chromosome instability is responsible for the acquisition of tumor related phenotypes and the induction of a metastable state of cell differentiation in which epithelial cells respond to a senescence microenvironment by engaging in transitions deemed to be important for different steps in the metastatic progression of carcinomas. During this process, transformed epithelial cells acquire stem cell characteristics with renewal capacity, become cell-autonomous tumour initiating cells and are endowed of mesenchymal differentiation potential. Our laboratory is interested in exploring the molecular mechanisms that drive the production of cancer stem cells and specifically in dissecting the microRNA/transcription factor circuitry that connects such differentiation metastability to tumour aggressiveness. Identifying key actors in this circuitry will open the possibilities for drug development.

Our laboratory is also interested in understanding the molecular bases of telomerase-independent telomere maintenance mechanisms (ALT). Indeed, a non-negligible proportion of cancer cells use recombination instead of telomerase to maintain telomere length. On the other hand, there is increasing evidence that anti-telomerase therapies may favor the emergence of ALT mechanisms, thus allowing the tumor cell to escape. We have shown that recombination between telomeres occur in specialized PML bodies specifically found in ALT cells. We are currently studying the mechanisms by which telomeres are recruited to these structures and how recombination reactions, which are normally suppressed at telomeres, are allowed in the ALT context. Understanding how ALT mechanisms work will open a new path in anti-tumour strategies.

Finally, we are interested in understanding the role of RTEL1, a helicase whose gene is found associated with severe forms of Hoyeraal-Hreidarsson syndrome. Although the protein has been involved in telomere and genome instability in the mouse, we have found that it plays an important role in non-coding RNA trafficking and RNP biogenesis in human cells. One major aim of our work is to define the contribution of RNA-related defects to the disease manifestations.
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Our research interest is focused on non coding (nc)RNAs, representing the “dark matter” of the genome. In eukaryotes, ncRNAs have been shown to regulate gene expression, chromatin domains and genome stability. A growing number of evidence suggests that they play central roles on cancer development and cellular differentiation. Regulatory ncRNA can be classified in two categories depending on their size. Short interfering (si) RNAs, also known to be part of the RNA interference pathway, have been extensively studied and control gene expression and chromosome segregation. Large (l)ncRNAs participate also in gene silencing and are key players in cell differentiation and development but, in contrast to siRNAs, their mode(s) of action remain poorly characterized. Our lab was one of the first to describe lncRNA-mediated epigenetic regulation controlling transposon proliferation and gene expression in the budding yeast providing powerful genetic and large scale tools to uncover their regulatory mechanisms in this classic model organism.

Figure 1: Model depicting how the trans-acting Ty1 ncRNA inhibits the RNA polymerase II (RNAPII) of the TY1 transposon through Set1 activity.

Figure 2: RNA-seq data of the yeast chromosome 3. Results were obtained on a strain deleted for xrn1, encoding the exonuclease activity. Dark bars represent each transcripts in sense or antisense orientation for coding (blue arrows) and non coding genes (XUTs in red arrows). Gene position is in abscise according to the chromosme coordinates and intensity of RNA level is ordinate. Grey zones represent repeat elements. © Antonin Morillon

Since the beginning of our research project in 2005, we obtained two main results showing the existence of a trans-acting ncRNA controlling the Ty1 transposon in yeast (Figure 1). In addition, we provided evidence that cryptic transcription mediates the deposition of histone marks controlling inducible genes. Finally using RNA-seq technology, we recently defined an entire family of antisense regulatory ncRNA in yeast, that we called XUTs (Figure 2).

Our work shows that yeast is indeed an excellent organism to study regulatory ncRNA that are involved in chromatin regulation. Interestingly, the processing and the mode of action of the yeast large ncRNA implicate pathways important for genome integrity and cell development in mammalian cells, suggesting that their mechanisms might be conserved among the eukaryotic kingdom.

Our future aims are to extensively identify all the regulatory ncRNA in yeast and to further characterize their associated proteins to understand the mechanisms controlling histone modifications both at the euchromatin and heterochromatin domains. Our ongoing work will set up the fundamental basis for future studies in higher eukaryotes, especially during differentiation and cancer.

RECENT/KEY PUBLICATIONS:

KEYWORDS:
Epigenetic, ncRNA, Chromatin, Yeast, Genome Fluidity
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Functional Organization and Plasticity of Mammalian Genomes

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KEYWORDS:
DNA Replication, Genome Instability, Replication Dynamics, DNA Molecular Combing, Replication Checkpoint, Common Fragile Sites

Our team is interested in the process of cellular DNA replication and how it relates to genome instability. The stability of genetic information relies on proper coordination of replication initiation events, ensuring that all regions of the chromosomes are replicated once and only once per cell cycle. However, some regions of the genome, notably common fragile sites (CFSs), raise specific problems. CFSs are loci that recurrently exhibit breaks on mitotic chromosomes following perturbation of DNA replication, and are now increasingly regarded as a driving force of oncogenesis. CFSs are indeed involved in the formation of chromosome rearrangements found in tumor cells (deletions, gene amplifications). In addition, some CFSs overlap tumor suppressor genes, whose inactivation further fuels tumor progression. CFSs now appear as preferential targets for oncogene-induced replication stress in precancerous lesions, which suggests that their instability promotes oncogenesis from early stages of the process. Understanding the molecular mechanisms responsible for the instability of these major actors of cancer-genome remodeling is therefore of prime importance.

Nowadays it is admitted that replication stress delays completion of CFS replication more than the rest of the genome, and that breaks occur at under-replicated sequences upon chromosome condensation at mitosis. My team has recently demonstrated that this delay is due to a specific replication program combining late replication with failure to activate origins along the fragile region. Of note, a major consequence of our findings is that commitment to fragility depends on the tissue-specificity of replication programs.

The epigenetic setting of CFSs lead us to map them, in collaboration with B. Dutrillaux, in a broad variety of human cell types. We found that the map of fragile regions differs markedly between cell types although a limited reservoir of 49 loci accounts for all CFSs we observed. Only a subset of these loci becomes fragile in a given cell type, and their combination varies from one cell type to the other. It has long been observed that some CFSs co-localize with large genes. We have found that 43 out of the 49 CFSs constituting the reservoir localize to chromosome bands containing genes at least 300 kb-long. Interestingly, two recent reports that have catalogued recurrent focal deletions in large cohorts of human tumors and cancer cell lines. Indeed, re-analysis of the results show that large genes host 51% of recurrent cancer deletions, and that many of these genes are associated with CFSs in at least one of the cell types in which we mapped them. Therefore, a majority of recurrent focal deletions found in human cancers originate from CFSs stable in the cell types from which the cancers derive. Deciphering how large genes impact CFS instability is therefore crucial to our understanding of genome remodeling in tumor cells.

Our future work aims to uncover the molecular mechanisms linking transcription status of large genes to the organization of chromatin loops by their regulatory sequences, and how these features impact the setting of replication origins and CFS instability.

Recent/Key Publications:
- Naim V. et al. (2013) ERCC1 and MUS81-EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis. Nat Cell Biol. 15:1008-U270.
Our group aims at identifying and characterizing the biological processes that maintain genomic integrity and ensure the faithful transmission of genetic information during reproduction, as well as endogenous and exogenous events that enhance genome instability. We are focusing our work on two biological situations in which DNA double-strand breaks (DSBs) form in the budding yeast *Saccharomyces cerevisiae.*

First, we study the exchange of genetic material by recombination which occurs between the parental chromosomes during meiosis. Using genome-wide molecular methods, we were able to show that each yeast chromosome has a unique map of meiotic DSBs with alternating ‘hot’ and ‘cold’ domains where recombination occurs more or less frequently, and correlate with enriched region of histone H3-K4 trimethylation, (Figure 1). We recently identified the Spp1 protein, a member of the COMPASS complex, as linking histone methylation to the Mer2 protein, a key protein of the differentiated chromosomal axis required for DSB formation.

Second, we study the mechanisms of genome instability and tested in plants and mice. We established the mutational landscape of yeast mutator strains using next generation sequencing and bioinformatics methods to identify the variants. We also study the instability of human tandem repeated DNA sequences. It gives rise to a large variety of length variants due to expansion and contraction of the repeat units. We have also found that the mitotic stability of CEB1 depends on the activity of the Pif1 helicase. In vitro and in vivo analyses showed that CEB1 repeats formed stable G-quadruplex (G4) secondary structures and that the Pif1 protein unwinds these structures efficiently. This was further confirmed by using the PhenDCs G4-quadruplexes ligands developed by the group of M-P. Teulade-Fichou (Institut Curie, Orsay); These molecules specifically destabilized CEB1 in wild-type treated cells and yielded CEB1 rearrangements similar to that in pif1Δ cells. Mechanistically, the instability of CEB1 occurs during leading-strand replication. The steps leading to CEB1 rearrangements are illustrated in Figure 2. The formation of CEB1 G-quadruplexes also stimulates gross chromosomal rearrangements. We currently pursue a structure-function analysis of the CEB1 repeats and CEB25 G-quadruplexes upon mutagenesis of the guanine tracts and loop regions. We found that short loops leads to more thermodynamically stable G-quadruplex, in correlation with their higher instability in vivo.

Also, we have developed a Gal4-Spo11 fusion protein, which allows us to modify the usual DNA cleavage sites along the recombinands and diversified this method to target different regions of the yeast genome. This method to modify meiotic recombination profiles has been licensed to MEIOGENIX to be tested in plants and mice.

Second, we study the mechanisms of genome instability and mutagenesis. We established the mutational landscape of several yeast mutator strains using next generation sequencing and bioinformatics methods to identify the variants. We also study the instability of human tandem repeated DNA sequences (minisatellites) inserted in the yeast genome. In the *S. cerevisiae* genome, as in the human genome, tandem repeated minisatellite DNA sequences are unstable during meiosis when they may undergo expansion and/or contraction of the number of tandem repeats. To investigate the mechanism(s) underlying tandem-repeat instability, we introduced two human minisatellite CEB1 and CEB25 alleles into the *S. cerevisiae* genome. We found that deletion of the RAD27/FEN1 gene, which is involved in DNA replication and repair, causes a high level of instability of the CEB1-1.8 allele in cells growing mitotically, indicating that replication defects stabilise these repeated sequences. It gives rise to a large variety of length variants due to expansion and contraction of the repeat units. We currently pursue a structure-function analysis of the CEB1 and CEB25 G-quadruplexes upon mutagenesis of the guanine tracts and loop regions. We found that short loops leads to more thermodynamically stable G-quadruplex, in correlation with their higher instability in vivo.

**KEYWORDS:**
Genetic Instability, G-Quadruplex, Recombination, Meiosis, Cancers

**RECENT/KEY PUBLICATIONS:**

**PATENTS AVAILABLE FOR LICENSING:**
- Lopes J. et al. (2014) G-Quadruplex, Recombination, Meiosis, Cancers
The p53 pathway is altered in most, if not all, tumours. In more than half of human cancers, the p53 gene is mutated and, in the other half, the p53 protein is inactivated, often by overexpression of its specific inhibitors MDM2 and MDM4. A better understanding of the pathways that regulate p53 could lead to development of new and broadly applicable anti-cancer strategies. Our group is using mouse models to gain a better understanding of the regulation of p53.

Much of what we know about the regulation of p53 results from biochemical studies and analyses relying on transfection of expression plasmids into cells in culture. In recent years, studies of several mouse models carrying targeted p53 mutations revealed significant differences between the in vivo data and those obtained by earlier in vitro approaches. For example, we found that mutation of threonine and proline residues in p53’s proline rich domain (PRD), which were thought to be essential for regulation of the protein, did not significantly affect the transcription or tumor suppressor function of p53 in the mouse - a finding that may explain the sequence variability of the PRD in evolution.

We also generated the mutant mouse p53ΔP, which expresses a p53 that lacks the proline-rich domain, and has provided tremendous insight into p53 regulation. Studies of this mutant showed that MDM2 and MDM4 have distinct and complementary roles in p53 regulation: MDM2 mainly regulates p53 stability, whereas MDM4 regulates its activity (Fig. 1).

In addition, we have shown that MDM4 is a promising target for anti-cancer strategies, and that the combined use of MDM2 and MDM4 antagonists may reactivate p53 in some cancers. We also recently showed that the capacity of p53 to mediate transcriptional repression is important for strategies against MDM4 to work efficiently in some, but not all tumors. These studies demonstrate just how much information can be gained from studying p53 regulation in vivo, as well as the potential of such approaches for developing effective therapies.

Our group is now generating new mutant mice to pursue the analysis of p53 regulation. This approach recently helped us to demonstrate that the Mdm4-S transcript, often overexpressed in human tumors, is a marker, rather than a driver, of cancer progression. Furthermore, we showed that a nonsense mutation leading to the loss of the p53 C-terminal domain leads to increased p53 activity, and this causes bone marrow failure and pulmonary fibrosis. Importantly, the combined observation of aplastic anemia and lung fibrosis is a hallmark of syndromes caused by abnormally short telomeres. This led us to show that p53 is a major regulator of telomere metabolism, via its capacity to downregulate the expression of several key genes, including Dkc1 (Dyskerin) and Rtel1 (Fig. 2). Indeed, our team has also developed all the necessary tools to identify genes that are directly or indirectly regulated by p53.

**KEYWORDS:** p53 Regulation, MDM4, MDM2, Telomeres, Anti-Cancer Strategies, p53 Target Genes, Mouse Models

**RECENT KEY PUBLICATIONS:**