Ramón Areces Foundation
International Symposium

Cell Proliferation and Genome Integrity

April 3rd-4th, 2014

Palace of La Magdalena, Santander, Spain

ORGANIZERS:

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Abstract book

1 DEFECTIVE MITOSIS CONTROL, GENOMIC INSTABILITY AND MALIGNANCY OF SKIN CARCINOMA CELLS

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Basal Cell carcinoma (BCC) and Squamous cell carcinoma (SCC) are the main forms of non-melanoma skin cancer and the most frequent human malignancies. SCC but not BCC usually conserves an altered component of squamous differentiation. Paradoxically, SCC has worse prognostic than BCC and is associated with a higher risk of metastasis. Ectopic expression of the DNA replication regulator Cyclin E in normal human epidermal keratinocytes induces DNA damage, mitosis failure and terminal differentiation (1). This response to replication stress constitutes a novel mitosis-differentiation checkpoint. In seek of understanding of their different degree of malignancy we have studied the alterations of this checkpoint in skin carcinoma cells before and after mitosis arrest or upon cell cycle hyperactivation. The results suggest that more and less aggressive carcinoma cells are fundamentally different in their potential to maintain genome stability.

(1) Freije et al, *Oncogene*, 2012.
http://albertogandarillaslab.blogspot.com.es/

2 CHROMOSOMAL STRUCTURAL DEFECTS INHIBIT CYTOKINESIS THROUGH THE NoCut CHECKPOINT BY INTERFERING WITH EXIT FROM MITOSIS

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Coordination of cytokinesis with chromosome segregation is essential to maintain genome stability during cell proliferation. As an instance of this coordination, anaphase chromatin bridges in yeast and animal cells trigger inhibition of abscission through the NoCut checkpoint, which relies on Aurora-B/Ipl1 activity at the spindle midzone. Here we show that Aurora-B delays budding yeast cytokinesis when chromatin bridges are induced by replication stress or by inactivation of condensin or topoisomerase II, but not by introduction of a dicentric chromosome. This indicates that lagging chromatin alone is not sufficient to trigger NoCut. In addition replication stress-dependent and condensin / top2 defective bridges, but not dicentrics, cause a delay in exit from mitosis and in spindle disassembly. Supporting a role of spindle stabilization in NoCut function, deletion of the microtubule depolymerase kip3 leads to a delay in cytokinesis specifically in the presence of dicentric chromatin bridges. We propose that chromosomal structural defects generated under replication stress induce stabilization of the anaphase spindle, allowing midzone bound Aurora-B to detect chromatin bridges and inhibit cytokinesis.

3 WHAT IS THE SIGNIFICANCE OF RIBONUCLEOTIDE REDUCTASE LOCALISATION TO THE CYTOPLASM?

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Ribonucleotide reductase (RNR) catalyses the rate-limiting step in the production of deoxyribonucleoside triphosphate (dNTPs) precursors for DNA synthesis. Proper regulation and maintenance of dNTP levels in the cell is crucial for DNA synthesis and repair following damage. Insufficient or imbalanced dNTP pools can lead to replication errors, genetic abnormalities and cell death. *S. pombe* RNR consists of two subunits, Cdc22 (large) and Suc22 (small), and is well conserved between yeast and humans. RNR functional importance in the cell is reflected by multiple regulatory mechanisms, including transcriptional regulation, proteolysis of subunits and controlling the association of subunits by cellular localisation. The activity of the enzyme and its substrate specificity can be also modulated by allosteric control by nucleotides. In yeasts, there is an additional regulatory mechanism via intrinsically disordered protein (IDP) inhibitors such as Sml1 and Spd1.

Functional RNR is active in the cytoplasm in yeasts and mammalian cells, but the biological significance of this localisation is unclear. We have targeted Cdc22 and Suc22 RNR subunits, expressed from native promoters, to the nucleus individually and in combination and studied the effects of this non-physiological localisation. We have confirmed the nuclear localisation of the GFP-tagged proteins in normal growth conditions and upon treatment with the RNR inhibitor hydroxyurea (HU). All strains are viable and show relatively normal growth under unstressed conditions. However, the strains exhibit high sensitivity to DNA damage and are also sensitive to HU. Furthermore, the strains are resistant to camptothecin (topoisomerase I inhibitor) and sensitive to caffeine. The cells also exhibited poor growth at high temperature. Fluctuation analyses showed an increased mutation rate for all the strains and flow cytometry of the cells showed a defective S phase.

Our results highlight the importance of the cytoplasmic localisation of RNR, since there are deleterious consequences when this norm is perturbed. Further experiments measuring the potential disruption in dNTP levels in the strains are necessary in order to acquire a clearer picture as to why cells have evolved such a tightly regulated system of dNTPs homeostasis with RNR functioning mainly in the cytoplasm rather than in the nucleus.

4 MECHANISMS MEDIATING REPLICATION CHECKPOINT INACTIVATION

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The replication checkpoint plays a key role in the maintenance of genome integrity by sensing problems in replication fork progression and orchestrating a cellular response that delays cell cycle progression and protects replication fork stability. While the mechanisms through which the checkpoint senses and signals replication stress have been extensively characterized, little is known about the pathways that inactivate checkpoint signalling to re-establish cellular physiology.

We identified *BUL2* in a budding yeast genome-wide genetic screen for factors involved in the checkpoint response to replication stress. Bul2 is the adaptor component of the Bul2/Rsp5 ubiquitin ligase complex, involved in the inactivation of different targets through polyubiquitination and subsequent degradation or sub-cellular sorting. Along with *BUL2* we isolated the Pph3/Psy2 and Ptc2 phosphatase coding genes, suggesting a direct involvement of Bul2/Rsp5 in the checkpoint response. We found that *bul2* and *rsp5* mutants show checkpoint inactivation defects and are sensitive to replication stress induced by hydroxyurea treatment, in a fashion synergistic with *pph3* and *ptc2* mutants. This evidence suggests that the Bul2/Rsp5 complex contributes to cell viability mediating timely checkpoint shut-off in a mechanism alternative to Rad53 dephosphorylation. Mass spectrometric analysis of Bul2 physical interactors identified Mec1, the apical replication checkpoint kinase. Mec1 acts as a stalled fork sensor through its recruitment to RPA-coated ssDNA stretches via its partner Ddc2. We found that the interaction between the Bul2/Rsp5 and Mec1/Ddc2 complexes is enhanced in response to replication stress.

We propose that Mec1/Ddc2 directs Bul2/Rsp5 activity to stalled replication forks thus driving the local polyubiquitination and inactivation of key checkpoint signal transducers. The impact of persistent checkpoint signalling on replication fork dynamics and genome integrity will be discussed.

5 UBIQUITIN PROTEASES REGULATING PCNA (UBIQUITYLATION) IN YEAST

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DNA damage is a major source of genome instability. To deal with DNA damage cells have evolved three major pathways that are conserved among eukaryotic organisms: checkpoint response, tolerance and DNA repair. The mechanism of DNA damage tolerance is based on translesion synthesis (TLS) carried out by specialized low fidelity DNA polymerases capable of replicating over DNA lesions during S-phase. Translesion synthesis requires the switch between replicative and TLS DNA polymerases, and this switching is controlled through the ubiquitylation of the proliferating-cell nuclear antigen (PCNA), a processivity factor for DNA synthesis. It is thought that DNA polymerase switching is a reversible process that has positive outcomes for cells as the prevention of irreversible DNA replication forks collapse. However, the low fidelity nature of TLS polymerases has also unfavorable consequences like the increased risk of mutations opposite to DNA lesions. Our group is interested in understanding the role of reversible PCNA ubiquitylation in the process of DNA polymerase switching during S phase. To this end, we have recently identified Ubp10 as an enzyme controlling PCNA deubiquitylation in the model yeast *S. cerevisiae*. The identification of the ubiquitin proteases involved in this process is a first step that will allow us to understand its potential role in a latent safeguard mechanism limiting the residence time of TLS DNA polymerases on replicating chromatin in eukaryotes.

6 CONTROL OF MEIOTIC RECOMBINATION BY CELL CYCLE KINASES

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Meiosis is a type of cellular division in which a single round of DNA replication is followed by two nuclear divisions, originating four haploid cells from a diploid parental one, and therefore reducing the chromosomal number to half. Homologous recombination is a hallmark of meiosis that is essential for the correct chromosome segregation and the generation of genetic variability. It is initiated by the formation of double-strand breaks (DSBs) in the DNA, carried out by a conserved topoisomerase-like protein Spo11 (Rec12 in fission yeast). Rec12 (Spo11) needs the collaboration of accessory proteins, among them the ones forming the pre-recombination SFT complex (Rec7, Rec24, and Rec15). Recombination occurs in the context of the synaptonemal complex (Linear Elements in fission yeast) formed by Rec10, Rec25, Rec27 and Mug20. LinEs determine where DSBs occur and serve as platform where Rec12-accessory proteins are loaded. To understand how DSB formation is controlled is crucial to know how genome integrity is maintained during meiosis.

In particular, we are interested in how meiotic progression is coordinated with recombination, and how DSB formation is prevented until cells have completed DNA replication. This aspect of meiosis is largely unknown and few data have been only reported in budding yeast, where Mer2 (Rec15 in fission yeast) is regulated by CDK and DDK (conserved kinase involved in cell cycle progression) to activate DSB formation.

Currently, we are studying how LinE-components and Rec12-accessory proteins are regulated by CDK and DDK activity, and also by Rad3 (DNA-damage checkpoint kinase). We are taking different approaches. We are studying the influence of these kinases activities in LinE organization and foci formation of Rec12-accessory proteins by cytology. In addition, we are generating mutants in consensus phosphorylation sites in Rec7 by these kinases to explore possible effects on the localization of protein as well as in recombination. Finally, we are studying the impact of different cyclin deletion mutants (regulatory subunits of the CDK activity) in recombination and DSB formation.

7 DISSECTING THE ROLE OF SPINDLY IN THE SPINDLE ASSEMBLY CHECKPOINT AND CHROMOSOME ALIGNMENT

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During the cell cycle the mitotic phase is crucial to ensure generation of two daughter cells with an equal number of chromosomes and identical genetic material. For this purpose, machinery and surveillance mechanisms have evolved to control proper attachment between microtubules (MTs) and kinetochores (KTs) and so prevent errors in chromosome segregation. For this reason a checkpoint exists between metaphase and anaphase, that prolongs mitosis until all chromosomes are correctly aligned on the metaphase plate and correctly bi-orientated. The **spindle assembly checkpoint** (SAC) needs then to be rapidly inactivated through a mechanism that has not been elucidated yet. It appears that the recruitment of the motor protein dynein on kinetochores is one of the keys to SAC silencing. How dynein is targeted to the kinetochore is not fully understood, however we do know that the RZZ complex and Spindly are essential for this event. Spindly was found in a RNAi screen of *Drosophila melanogaster* S2 cells and it has been shown as an important player in the recruitment of the dynein-dynactin motor complex to KTs in mitosis. Its depletion, indeed, leads to metaphase arrest and retention of the SAC components on aligned KTs in flies. Curiously, in human cells, this depletion does not generate a similar retention but it produces strong chromosome alignment defects. This suggests that in human cells there is either a pathway that mediates SAC inactivation independently of dynein stripping, or that Spindly is required for maintaining SAC components on aligned kinetochores in the absence of dynein. Moreover, it was shown that a mutation in the Spindly box (the only highly conserved motif) generates a protein retained on KTs that cannot recruit the dynein-dynactin complex and that persistently activates the checkpoint. These data indicate that the removal of Spindly from aligned kinetochores is essential for SAC silencing.

In this study we test the ability of Spindly to bind the dynein-activator protein dynactin. We analysed different subunits of this big complex, revealing a crucial role for the p150 subunit. Previous studies have shown the importance of the Ser256 in the Spindly box for the recruitment of the dynein/dynactin complex. We therefore wonder whether the phenotype revealed after the mutation, could be due to the impossibility to phosphorylate that Serine. Here we show how Aurora B, Aurora A, Plk1, and Cdk1/cyclin B phosphorylate Spindly, but that this residue does not seem to be a target of known mitotic kinases.

8 THE TPR-LIKE PROTEIN ALM1 IS REQUIRED FOR PROPER CHROMOSOME SEGREGATION IN FISSION YEAST

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TPR proteins are evolutionary conserved large coiled-coil proteins that localize at the nucleoplasmic side of the nuclear pore complex and regulate multiple aspects of mitosis including recruitment and activation of spindle assembly checkpoint components. In yeast, TPR are also essential for spindle pole body integrity. In this work, we characterized the role of the TPR-like protein Alm1 during mitosis in *Schizosaccharomyces pombe*. Alm1 localizes to the nuclear envelope and colocalizes with nuclear pore complexes. Alm1 deletion is viable but leads to asymmetric nuclear divisions and to defects in DNA segregation. Analysis of kinetochore behaviour by time lapse microscopy in *alm1Δ* cells reveals high frequency of lagging chromosomes during Anaphase B. Consistently, *alm1Δ* cells show a delay in the metaphase/anaphase transition which depends on Bub1 and n Mad2, two key activators of the spindle assembly checkpoint. In-depth microscopic and genetic analysis of *alm1Δ* phenotype will be presented. Our data suggest a new role for the TPR Alm1 in chromosome segregation and genome stability.

9 REDUCTION OF ORIGIN LICENSING INDUCES A DNA DAMAGE RESPONSE DURING S PHASE IN BUDDING YEAST

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It has been shown that Cdc6 is required directly for checkpoint activation in *S.pombe* and mammalian cells. To determine whether the same is true in budding yeast we repressed Cdc6 expression and tested for activation of the checkpoint response (hyperphosphorylation of Rad53) in the ensuing S phase. In doing this we noted two things; Firstly, we found that cells without Cdc6 were able to elicit a full checkpoint response in the presence of both HU and MMS indicating that Cdc6 is not required for checkpoint activation. Secondly, the checkpoint was activated in cells released into S phase even without exogenous damaging agent when Cdc6 was absent indicating that reduced licensing might induce DNA damage. Depletion of other preRC components namely MCMs, Cdt1 or ORC had a similar effect showing the effect is not specific for Cdc6. By contrast, partial inactivation or depletion of various 'firing factors' including Cdc7, Sld3 and Dpb11 did not activate Rad53, nor did extending S phase by deleting Clb5. The observed Rad53 phosphorylation after Cdc6 depletion requires Rad9 and Ddc1 but not Mrc1, suggesting DNA damage may be generated and this is supported by the appearance of Rad52 foci and H2AX phosphorylation concomitant with the appearance of hyperphosphorylated Rad53. *RAD9* deletion strains lose viability after Cdc6 depletion but this loss of viability can be rescued with nocodazole treatment suggesting that the DNA damage can be repaired/resolved with time.

Together our results show that reduced licensing can lead to DNA damage during S phase, which may account for the high levels of gross chromosome rearrangements seen in *cdc6*ts (Bruschi C.V., McMillan J.N., Coglievina M., Esposito M.S.,1995) mutants and when G1 cyclins are overexpressed (Tanaka S, Diffley JF. Genes Dev. 2002).

10 REGULATION OF THE RAD51 RECOMBINASE BY CELL CYCLE KINASES

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The essential recombinase Rad51 protects genome stability through the faithful repair of DNA breaks by homologous recombination, and through the protection of stressed DNA replication forks. The importance of timely activation of Rad51 is highlighted by the observations that either down-regulation or up-regulation of Rad51 leads to genome instability phenotypes such as chromosomal rearrangements, loss of heterozygosity, gene amplification and gene deletion. However, it remains largely unknown how Rad51 is regulated spatio-temporally in proliferating human cells.

Here, we report that the breast cancer susceptibility protein BRCA2, a key Rad51 binding partner, coordinates the activity of central cell cycle drivers CDKs and Plk1 to promote Rad51-mediated genome stability control. The soluble nuclear fraction of BRCA2 binds Plk1 directly in a cell cycle- and CDK-dependent manner, and acts as a molecular platform to facilitate Plk1-mediated Rad51 phosphorylation. Using genome-wide ChIP-seq analyses, iPOND (isolation of proteins on nascent DNA) and a single molecule DNA fibre technique, we further demonstrate that Rad51 phosphorylation by Plk1 is important for the enhanced Rad51 association with stressed replication forks and hence protects the genomic integrity of proliferating human cells.

The study reveals the unexpectedly elaborate but highly organized molecular interplay between Rad51 regulators, and has significant implications for understanding tumorigenesis and therapeutic resistance in patients with BRCA2 deficiency and/or elevated activity of Plk1.

11 CYTOKINETIC PROTEINS INN1, CYK3 AND CHS2 FORM A COMPLEX TO COORDINATE PLASMA MEMBRANE INGRESSION AND SEPTUM FORMATION IN BUDDING YEAST

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Yeast cells coordinate contraction of the actomyosin ring with primary septum formation, which is produced by the enzyme chitin synthase Chs2. Formation of the septum is precisely an attractive target for therapeutic intervention, as mammalian cells lack the enzymes that synthesise the septum carbohydrates such as chitin. Using the budding yeast *Saccharomyces cerevisiae* as a model organism, we have described that the actomyosin ring components Inn1 and Cyk3 regulate the catalytic domain of Chs2 during cytokinesis, however the details of such regulation are poorly understood.

To try to understand how yeast cells coordinate actomyosin ring contraction, plasma membrane ingression and septum formation we have isolated key actomyosin rings components from yeast cells undergoing cell division. We have identified cytokinetic proteins Inn1, Cyk3 and Chs2, together with other actomyosin ring components including myosin type II, the IQGAP protein Iqg1 and Hof1. We have shown that Inn1 is essential for Chs2 dynamics at the site of division and for Cyk3 to be localized at the bud neck. In addition we have found that Inn1 directly interacts with the catalytic domain of Chs2, and such interaction is not disrupted by point mutations that affect Chs2 activity. Interestingly, we have found that Cyk3 directly interacts with the catalytic domain of Chs2. Together with other groups, we have previously shown that Cyk3 and Inn1 directly interact, and we have now the data indicating that indeed Inn1, Chs2 and Cyk3 form a stable complex. Finally we have determined that the C2 domain of Inn1 is able to increase the chitin activity associated to Chs2 *in vitro*. Our data clearly demostrate that cytokinetic proteins Inn1, Cyk3 and Chs2 form a complex at the end of mitosis, which plays a key role in coordinating plasma membrane ingression and septum formation.

12 THE BUL2/RSP5 UBIQUITIN LIGASE MEDIATES REPLICATION CHECKPOINT INACTIVATION

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Genome instability, characterized by the dynamic accumulation of mutations and gross chromosomal rearrangements, is a hallmark of cancer cells. In response to replication stress, the replication checkpoint senses replication fork stalling and orchestrates a cellular response aimed at delaying cell cycle progression, stabilizing fragile replication structures and promoting DNA repair. While the mechanisms leading to checkpoint activation in response to genotoxic insults have been thoroughly studied, little is known on how cells shut-off checkpoint signalling to re-establish cellular physiology and cell cycle progression.

A genome-wide genetic screen carried out in *S. cerevisiae* identified factors involved in checkpoint inactivation such as the Pph3/Psy2 and Ptc2 phosphatases, required to dephosphorylate the checkpoint effector kinase Rad53. Mutants in *BUL2*, coding for the adaptor of the Bul2/Rsp5 ubiquitin ligase complex, were also identified in the screen suggesting the involvement of Bul2/Rsp5 ubiquitination in the checkpoint response. *bul2* and *rsp5* mutants are sensitive to replication stress induced by hydroxyurea and, similarly to *pph3*, *psy2* or *ptc2* mutants, show checkpoint inactivation defects. This evidence suggests that the Bul2/Rsp5 complex contributes to cell viability by mediating timely checkpoint inactivation through regulation of key targets. Mass Spectrometric analysis of Bul2 physical interactors identified Mec1, a key apical checkpoint kinase that acts as a replication stress sensor through recruitment to stalled replication forks via its partner Ddc2. We found that Bul2/Rsp5 interaction with the Mec1/Ddc2 complex is enhanced in cells experiencing replication stress.

We hypothesize that Bul2/Rsp5-mediated poly-ubiquitynation can be directed to stalled replication forks through interaction with Mec1/Ddc2 and drive checkpoint inactivation by inhibiting key signal transducers. The influence of defective checkpoint inactivation on replication fork dynamics and genome integrity will be discussed.

13 ROLE OF TUMOUR SUPPRESSOR p53 IN THE EPIDERMAL MITOSIS-DIFFERENTATION CHECKPOINT

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Epidermis is a self-renewal epithelium and the outer barrier against mutagenic agents mainly ultraviolet (UV) light. This makes the skin the most frequent target of human neoplasia. 80% of epithelial skin malignancies bear mutations of tumour suppressor p53. P53 is referred to as *the guardian of the genome* for its key function in maintaining ploidy. Although p53 is able to trigger apoptosis in sunburn epidermis, its role in skin proliferation and differentiation is unclear. How skin homeostasis is preserved in spite of a high rate of genomic insult is also intriguing (1). Patches of cells expressing mutant p53 are frequently detected in otherwise normal skin. We have inactivated this pathway in human epidermal cells and studied the consequences on cell cycle and cell growth. The results provide new clues for the role of p53 in epidermal proliferation and for the cell cycle mechanisms protecting the skin against genetic damage.

(1) Freije et al, *Oncogene*, 2012.
http://albertogandarillaslab.blogspot.com.es/

14 MCM7 UBIQUITYLATION AND DNA REPLICATION TERMINATION

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To ensure duplication of the whole genome DNA replication initiates from thousands of origins of replication. The pre-loaded inactive helicase (Mcm2-7 double hexameric complexes) becomes activated during replication initiation by loading of additional components: Cdc45 and GINS in a carefully regulated process. The progressing replication fork moves through the chromatin until it encounters fork from the neighbouring origin. During the termination of replication forks the replisomes disassemble by an unknown mechanism and topisomerase II resolves the daughter DNA molecules. If not resolved properly, the terminating forks are at high risk of stalling and fork reversal, leading to DNA damage and genomic instability.

Using the *Xenopus laevis* egg extract system, we have shown that blocking polyubiquitylation results in the prolonged association of the active helicase with replicating chromatin. This is not accompanied by defect in nascent DNA synthesis, S-phase checkpoint activation nor excessive origin firing. Instead, we propose that without effective polyubiquitylation it is the disassembly of the active helicase at the termination of replication forks that is defective.

The Mcm7 subunit of the Mcm2-7 complex is the only component of the active helicase that we find polyubiquitylated during S-phase. Mcm7 ubiquitylation coincides with the presence of replication forks on chromatin and is blocked when forks cannot terminate. Both the disassembly phenotype and Mcm7 ubiquitylation are driven by lysine 48-linked ubiquitin chains, but not proteasomal degradation. Instead we propose an alternative model of replisome removal from chromatin.

Altogether, our data provides first insight into the mechanism of replisome disassembly during termination of eukaryotic DNA replication.

15 CELL CYCLE MECHANISMS MAINTAINING GENOME INTEGRITY IN HUMAN SKIN

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The skin is continuously exposed to environmental mutagenic hazard and is the most frequent target for human cancer. It is unclear how epidermis is molecularly protected from UV irradiation of Sunlight causing frequent mutations in cell cycle regulators. For instance, patches of cells bearing mutant forms of the tumour suppressor P53 are frequently detected in sun-exposed normal skin. Loss of P53 often leads to genomic instability. In addition, oncogenic activations such as MYC or Cyclin E in epidermal keratinocytes stimulate differentiation rather than proliferation. We have uncovered a novel human epidermal checkpoint that responds to cell cycle deregulation and triggers differentiation and polyploidy (1). This response involves replication stress, mitosis slippage and DNA damage pathways. We have found a striking role for P53 in this checkpoint that reveals novel mechanisms of the epidermis in protecting the proliferative compartment. Our studies suggest that alterations in this checkpoint might determine the aggressiveness of skin neoplastic phenotypes via genomic instability.

(1) Gandarillas. *Cell Cycle*, 2012.
http://albertogandarillaslab.blogspot.com.es/

16 INDUCTION OF p27 DEGRADATION BY MYC THROUGH CYCLIN A/CDK1 COMPLEXES

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$p27^{KIP1}$ (p27 herein after), member of the KIP/CIP family of CDK inhibitors, accumulates in the nucleus of quiescent cells provoking cell cycle arrest at the G1 phase by inactivating Cyclin E-A/CDK2 and Cyclin D/CDK4-6 complexes. Upon mitogenic stimulation, expression of p27 is downregulated leading to cell cycle progression. The best characterized pathway leading to p27 downregulation involves phosphorylation at threonine 187, an event that targets p27 for SCF^{SKP2}–mediated ubiquitination and degradation in proteasomes. The only kinase known so far to mediate this phosphorylation is the Cyclin E/CDK2 complex. Myc is known to antagonize the inhibitory effect of p27 on the cell cycle. Moreover, there is a correlation between high levels of Myc expression with low levels of p27 in many human tumors. However, the mechanisms by which Myc decreases p27 levels are not well understood. We have previously shown that Myc induces the expression of *SKP2* as well as the phosphorylation of p27 at Thr187 (Bretones et al., J Biol Chem, 2011).

To explore the mechanisms involved we used the Kp27MER cell line, a K562 derivative cell line carrying a $ZnSO_4$-inducible p27 construct and the chimerical MycER protein which can be activated by 4-hydroxy-tamoxifen (4HT). We first showed that induction of p27 in Kp27MER cell line inhibited less efficiently the kinase activity of CDK1 whereas it completely inhibited CDK2. Activation of Myc by 4HT leads to an increase of phospho-T187-p27 and the induction of Cyclin A. Also, Myc activation increases the in vitro kinase activity of immunocomplexes containing CDK1 and CDK2 Interestingly, CDK1 complexes from cells overexpressing p27 were able to phosphorylate p27 at T187 *in vitro* upon Myc activation, but CDK2 complexes were not. The result is consistent with the weak CDK1 inactivation exerted by p27 in these cells. Cyclin B/CDK1 has been reported to phosphorylate p27 *in vitro*, but the involvement of Cyclin A is unknown. As this cyclin is induced by Myc in our model, we asked for the role of Cyclin A/CDK1 in p27 phosphorylation. *In vitro* kinase assays performed with immunoprecipitated Cyclin A complexes showed the same p27 phosphorylation pattern as the observed with CDK1 complexes. Altogether these results suggest that Myc induction of p27-T187 phosphorylation can be carried out not only by Cyclin E/CDK2 as previously reported, but also by Cyclin A/CDK1. This phosphorylation of p27 is so far unreported.

To confirm these results, we used three mouse embryonic fibroblast (MEF) derived cell lines lacking functional CDK genes: CDK2$^{-/-}$, Cyclin E$^{-/-}$ and TKO (CDK2$^{-/-}$; CDK4$^{-/-}$; CDK6$^{-/-}$). Overexpressing Myc-stable cell lines were generated for each cell line to study p27 phosphorylation. CDK1 and Cyclin A complexes from CDK2$^{-/-}$ MEFs subjected to *in vitro* kinase assays showed increased phospho-T187-p27 when Myc was overexpressed. Similarly, CDK1, CDK2 and Cyclin A complexes from Cyclin E$^{-/-}$ MEFs also showed increased phospho-T187-p27 when Myc was overexpressed. CDK1 complexes from TKO MEFs were unable to phosphorylate p27 *in vitro* while overexpression of Myc induced this phosphorylation. Purvalanol A (a CDK1 inhibitor) abolished the p27 phosphorylation *in vitro* but not CDK9 inhibitors. Consistent with the *in vitro* data, extracts from CDK2$^{-/-}$ and TKO MEFs showed higher levels of phospho-T187-p27 levels when Myc was activated and silencing of Myc in Myc-TKO MEFs decreased phospho-T187-p27, dismissing CDK2, CDK4 or CDK6 as responsible for this phosphorylation in this model.

In conclusion, Myc promotes p27 degradation by inducing its phosphorylation at the Thr187, which is mediated not only by Cyclin E/CDK2 complexes, but also by Cyclin A/CDK1 complexes.

17 REPLICATION DEFECTS IN THE ABSENCE OF THE APC/C COFACTOR CDH1

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The anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase that promotes the degradation of different substrates by the proteasome. Its activity is limited to the cell cycle period comprised between anaphase and the end of the G1 phase, and is controlled by the regulated binding of two co-factors: Cdc20 or Cdh1. APC/C-Cdc20 is active during anaphase, while APC/C-Cdh1 is active from anaphase until the end of G1.

APC/C-Cdh1 is known to play an important role in cell cycle progression, controling the levels of mitotic cyclins and other critical cell-cycle regulators. To get further insight into the biological function of Cdh1 in mammals, our group has generated a Cdh1 *knockout* mouse model (García-Higuera et al, 2008) that we are characterising at the cellular as well as the organismal level.

Our results indicate that cells derived from mutant embryos (MEFs) accumulation of DNA damage, and activation of the DNA damage response, suggesting the presence of replication stress in Cdh1 deficient cells. To verify that this was the case, we performed a direct analysis of the DNA replication process and found altered replication dynamics in Cdh1 mutant cells. In particular, Cdh1 deficiency reduces the rate of replication fork progression while increasing the frequency of origing firing. These observations further confirm the importance of the APC/C-Cdh1 complex in preventing replication defects. We are now trying to understand at the molecular level how the absence of Cdh1 triggers these defects.

Reference:

García-Higuera et al (2008) Genomic Instability and Tumour Suppression by the APC/C Cofactor Cdh1. *Nat. Cell Biol.* 10, 802-811.

18 REGULATORY ROLES OF NUCLEOSOMES IN GENOME DUPLICATION AND INTEGRITY

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The sites where DNA synthesis starts from are important regulatory regions to ensure proper genome duplication. Yet, their role in transmitting chromatin structures that influence gene expression patterns and thus, cell fate and genome stability, are not clear. During the last years several efforts to map replication origins genome-wide in mammalian cells revealed that, although DNA synthesis can start at multiple genomic sites, those co-localizing with gene promoters are more efficiently activated and more conserved across cell types. We have recently demonstrated that replication initiation sites at efficient promoter-origins occur at positions of high nucleosome occupancy. These results open new avenues relating initiation of DNA replication and nucleosome positioning that we are currently exploring. Our working hypothesis is that nucleosome positioning dictates the start sites of leading strand synthesis and that the reposition of these specific nucleosomes behind the replication fork could provide the opportunity to change the chromatin structure that could promote a switch during cell differentiation and development. To address this hypothesis we are manipulating the balance between nucleosomes, pre-replication complexes and DNA, and analysing the corresponding output on the replication landscape. The results obtained and their implications on the complex role of chromatin in regulating the genome´s versatility and stability will be discussed.

19 DISCOVERING FUNCTIONS OF THE H2A.Z HISTONE VARIANT IN MEIOSIS

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Chromatin structure is regulated by post-translational modifications of histones as well as incorporation of histone variants. This regulation is important for chromosome dynamics during meiotic prophase, in which synapsis and recombination between homologs are crucial events to promote accurate chromosome segregation. Defects in these processes activate the so-called pachytene checkpoint or meiotic recombination checkpoint, which blocks meiotic cell cycle progression to avoid formation of aneuploid gametes.

We are studying the possible meiotic function(s) of the histone variant H2A.Z, which replaces the canonical histone H2A at some positions in the genome, due to the action of the SWR1 complex. We found that, in *S. cerevisiae*, H2A.Z is incorporated into meiotic chromatin in a SWR1-dependent manner, except in the unsynapsed rDNA region. To explore the role of this histone variant, we have examined various meiotic events in the *htz1, swr1* and *htz1 swr1* mutants. The *htz1* mutant displays delayed and inefficient meiotic progression and shows reduced spore viability. These meiotic defects are at least partially abolished by mutation of *SWR1,* indicating that they could be caused by instability of nucleosomes generated in absence of H2A.Z. Nevertheless, when the pachytene checkpoint is activated (as in a synapsis-defective *zip1* mutant) the absence of H2A.Z generates a total block of meiosis, and this block is not rescued by the absence of *SWR1*. Several lines of evidence indicate that the tight arrest of the *zip1 htz1* and *zip1 swr1* mutants results from sustained activation of the meiotic recombination checkpoint resulting in persistent Swe1-dependent inhibitory phosphorylation of CDK, and point to a role for H2A.Z in the adaptation or recovery from the checkpoint-induced meiotic block. We are currently investigating the possible targets of H2A.Z regulating this process.

On the other hand, we are also studying the function of H2A.Z during unperturbed meiosis. In vegetative cells, an interaction between H2A.Z and the SUN-domain Mps3 protein has been reported. Since Mps3 is required for the rapid prophase movements (RPMs) of meiotic chromosomes driven by the telomeres attached to the nuclear envelope, we have explored the role of H2A.Z in this process. Using a Zip1-GFP fusion to visualize prophase chromosomes, we found that, indeed, the velocity of RPMs is reduced in the *htz1* and *htz1 swr1*, but not in *swr1* mutant. This suggests that this particular H2A.Z function in promoting chromosome movements is independent of its incorporation into chromatin. We are currently analyzing the contribution of H2A.Z to the organization of meiotic nuclear architecture.

In summary, our results reveal a variety of SWR1-dependent and independent H2A.Z functions orchestrating multiple aspects of chromosome dynamics during meiosis.

20 STUDY OF THE COHESION COMPLEX ROLES IN CORNELIA DE LANGE SYNDROME

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Cornelia de Lange syndrome (CdLS) is a rare, genetically heterogeneous characterized by growth and mental retardation. Almost all cases are sporadic and dominant. Mutations in genes that correspond to three subunits of the cohesin complex *SMC1A, SMC3, RAD21* and two regulatory proteins of the cohesin complex *NIPBL* and *HDAC8* have been identified in individuals with clinically diagnosed CdLS. Cohesin complex acts as the chromosomal “glue” and is essential for sister chromatids cohesion and their posterior segregation. Cells derived from CdLS patients show no obvious defects in sister chromatid cohesion, suggesting that other functions of cohesion complex are involved in CdLS. Increasing evidence indicates that cohesin acts as a global organizer of chromatin architecture that influences many processes in interphase cells as gene regulation. In this work, one of the main objectives is to study how the cohesin mutations found in CdLS patients cause the disease phenotypes. Here we show that Cohesin subunits are expressed properly in cycling CdLS cells. Moreover, the integrity of the core cohesion complex is not altered in fibroblasts derived from CdLs patients. Even so, we identify that CdLS fibroblasts have altered transcription of genes involved in developmental process. Our results suggest that the role of cohesin complex in gene expression underlies the CdLS phenotypes.

21 THE DDX19 HELICASE FUNCTIONS IN MAINTENANCE OF GENOME STABILITY

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DNA damage is a challenge to genome integrity, which cells deal with by activating elaborate DNA damage response (DDR) pathways that delay cell division and facilitate DNA repair. Recently it has been proposed in yeast that DDR activation is also important to coordinate transcription and replication, thus avoiding formation of aberrant structures byproducts of transcription/replication interference (R-loops) that can generate double strand breaks. By means of an *in vitro* screen aimed at identifying new DDR genes using Xenopus egg extracts, we isolated Ddx19, a DEAD-Box helicase involved in mRNA export at the nucleopore, as a novel DDR gene candidate. We found that Ddx19 downregulation in mammalian cells activates specifically the ATM-Chk2 pathway, delays cells in S and G2-M phases, and induces accumulation of R-loops. We will present evidence for a novel, DDR-dependent function of Ddx19 in genome maintenance, independent from its role in mRNA export, in the clearance of aberrant R -loops, whose persistence threatens genomic stability.

22 CK2-DEPENDENT PIH1D1 INTERACTIONS DEFINE SUBSTRATE SPECIFICITY OF THE R2TP CO-CHAPERONE COMPLEX

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R2TP complex regulates assembly of large protein complexes and impacts on stability of different proteins, including key regulators of DNA damage response ATM, ATR and DNA-PKcs. We are currently describing a novel phospho-binding domain PIH-N in the PIH1D1 subunit of the R2TP complex, important for mediating the function of the R2TP complex. A co-crystal structure of the PIH-N domain bound to a TEL2 phospho-peptide reveals a very specific recognition mechanism of highly acidic peptides phosphorylated by casein kinase 2 (CK2). As a proof of concept, we performed a proteomic and in silico screen to look for new PIH1D1 phospho-dependent binding partners. Among others we identified several cell cycle and DNA damage response proteins and confirmed direct phospho-dependent interaction with ECD, protein implied in stabilization of the tumour suppressor p53. Our study brings new insights on mechanims of substrate recognition by R2TP compolex and its role in DNA damage signalling and cell cycle.

23 DOWNREGULATION OF PP2A^{Cdc55} AT ANAPHASE ONSET BY ZDS1 AND SEPARASE

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Exit from mitosis and completion of cytokinesis require the inactivation of mitotic cyclin-dependent kinase (Cdk) activity. In budding yeast, Cdc14 phosphatase is a key mitotic regulator that is activated in anaphase to counteract Cdk activity. In metaphase, Cdc14 is kept inactive in the nucleolus sequestered by its inhibitor Net1. At anaphase onset, downregulation of PP2A^{Cdc55} phosphatase by separase and Zds1 protein promotes Net1 phosphorylation and consequently, Cdc14 release from the nucleolus. The mechanism by which Zds1 and separase impinge on PP2A^{Cdc55} activity remains to be elucidated. Previous results show that Zds1 exert its biological function as PP2A^{Cdc55} regulator, by controlling the subcellular localisation of the PP2A regulatory subunit Cdc55. Our previous results suggest that the activity of PP2A^{Cdc55} cannot be modulated solely through regulation of its localization, and that an additional regulatory step may be required to control PP2A^{Cdc55} activity during mitotic exit. Here we show that Cdc55 regulatory subunit is phosphorylated during anaphase upon PP2A^{Cdc55} downregulation. Our results suggest that PP2A^{Cdc55} activity is modulated throughout Cdc55 posttranslational modifications in a separase and Zds1-dependent manner.

24 REGULATION OF REPLICATION BY USP7

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USP7 is a protein deubiquitinase with essential roles in development. Known targets of this deubiquitinase include the p53-MDM2 axis, PTEN and several Polycomb group proteins, highlighting the diversity of its cellular functions. The implication of USP7 in DNA replication is supported by the slow proliferation of USP7 knockout cells and its potential interaction with components of the MCM helicase, as described in a proteomic analysis. Our results confirm that USP7 interacts with the MCM complex and localizes to PCNA foci. Inhibition of its enzymatic activity with P22077, a specific inhibitor of USP7, results in a fast arrest in DNA replication due to a reduction in fork velocity and new origin firing. These effects are independent of the presence of p53, and the inhibitor does not change the levels of MCM components. Upon USP7 inhibition and fork stalling, the replication stress response is activated, with temporal increase in ubiquitination of PCNA, followed by activation of ATR and Chk1 and phosphorylation of RPA2. The cytotoxic effects of the USP7 inhibitors are further potentiated when ATR is also blocked, suggesting that these compounds may be effective against tumours with high levels of replication stress.

25 A CONSERVED ROLE FOR NUCLEOTIDE BIOSYNTHESIS ON ATR BIOLOGY IN MAMMALS

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ATR is an essential kinase that coordinates the response to replication stress (RS). In *S cerevisiae*, deletion of the ATR ortologue Mec1 becomes viable through the concomitant deletion of Sml1, an inhibitor of the ribonucleotide reductase (RNR) complex. However, given that no Sml1 orthologues exist in mammals, to what extent the dNTP biosynthesis pathway contributes to ATR functions in higher organisms remain unknown. Our data show that, *in vitro*, nucleosides can rescue RS and growth on fibroblasts from a mouse model of the ATR-Seckel syndrome, which present reduced levels of ATR. Importantly, BAC transgenic mice carrying extra alleles of the RNR regulatory subunit RRM2 (SuperR2) increase significantly the lifespan of ATR-Seckel mice. Moreover, increased levels of R2 limit the breakage at genomic fragile sites in response to ATR inhibitors, including the recently described Early Replicating Fragile Sites. Altogether, our data reveal an important role for dNTP biosynthesis on ATR-mediated suppression of RS in mammals. The characterization of these mice, as well as our latest findings on the impact of dNTP levels on oncogene-induced transformation and ATR activity will be presented.

26 DOUBLE IMPAIRMENT OF THE STRUCTURE-SELECTIVE ENDONUCLEASES MMS4-MUS81 AND YEN1 LEADS TO PERSISTENT ANAPHASE BRIDGES

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DNA damage and chromosome missegregation are the two main sources of genetic instability. In normal cells it is assumed that concomitant DNA damage and deficiencies in the homologous recombination (HR) pathway causes gross chromosomal rearrangements (GCR) through illegitimate DNA repair (IDR). We will present data showing that yeast mutants for two structure selective endonucleases involved in the HR pathway, the Mms4-Mus81 complex and Yen1, give rise to both gross and fine anaphase bridges both spontaneously and under continuous DNA damage. We will further show that these bridges mainly comprise the nicked form of the Holliday Junction molecule and that either resolvase can complement one another in their prevention and resolution. We will discuss how these findings refine our current models on HR and on the formation of anaphase bridges.

27 UBIQUITYLATION OF CDC45-MCM-GINS DNA HELICASE IS COUPLED TO CDC48-DEPENDENT DISASSEMBLY DURING COMPLETION OF CHROMOSOME REPLICATION

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The Cdc45-MCM-GINS (CMG) DNA helicase is regulated extensively by phosphorylation, but until now it has been unclear whether CMG is also controlled by other post-translational modifications such as the addition of small-protein modifiers. We have found that the Cdc45-MCM-GINS helicase is modified *in vitro* in extracts of S-phase budding yeast cells by K48-linked polyubiquitylation. *In vitro* ubiquitylation is restricted to the Mcm7 subunit and is highly specific, since it only occurs in the context of the Cdc45-MCM-GINS helicase complex. Since K48-linked polyubiquitin chains *in vivo* often target proteins to the proteasome, or to the ubiquitin-selective segregase known as Cdc48 in yeast and p97/VCP in animal cells, we investigated the consequences of inactivating the proteasome or Cdc48 in asynchronous cultures of yeast cells. Strikingly, inactivation of Cdc48 led to the accumulation *in vivo* of Cdc45-MCM-GINS complexes with ubiquitylated Mcm7, suggesting that ubiquitylation of CMG normally precedes Cdc48-dependent disassembly. We performed a screen with all E3 ligases known to regulate genome stability, which showed that *in vivo* ubiquitylation of Mcm7 was dependent on SCF^{Dia2}. To investigate the timing of these events during a single cell cycle, we inactivated Cdc48 in HU-arrested cells, before removal of HU to allow cells to resume DNA replication. Upon release from HU arrest, DNA replication resumed but inactivation of Cdc48 blocked the subsequent disassembly of CMG. These findings identify an essential role for Cdc48 in disassembly of CMG when cells complete chromosome replication. Our data indicate that disassembly of the Cdc45-MCM-GINS helicase is just as carefully regulated as the assembly step during the initiation of chromosome replication.

28 ROLE OF STRUCTURE-SPECIFIC DNA ENDONUCLEASES IN TOPOISOMERASE LESIONS REMOVAL

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Topoisomerases are essential for DNA metabolism, since they release topological stress during processes as replication, transcription and recombination. To do so, they first cleave DNA and form a covalent bond between a tyrosine in the active site and the 3' (for topoisomerase I) or 5' (for topoisomerase II) phosphoryl group of DNA. Relaxation and re-ligation of DNA then follow, together with concomitant liberation of the protein. However, the failure to release the topoisomerase from DNA represents a serious threat to the cell. Two proteins have been identified to be responsible for their removal through the hydrolysis of the 3' or 5'-phosphodiester bond, i.e. tyrosyl-DNA phosphodiesterase 1 and 2 (Tdp1 and Tdp2), respectively. Nevertheless, studies in budding yeast indicate that topoisomerase lesions can be also removed by alternative repair pathways involving structure-specific DNA endonucleases as Mus81-Mms4 and Rad1-Rad10. In order to study this from a biochemical point of view, we prepared several DNA structures containing streptavidin or covalently linked topoisomerase I. These substrates were used to analyse the *in vitro* activity of human Mus81-Eme1 and Mus81-Eme2, as well as yeast Mus81-Mms4 proteins. Aiming to gain insight into the molecular mechanism, the role of Mus81 in the removal of toxic topoisomerase complexes was also assessed *in vivo*.

29 RIFAMPICIN SUPPRESSES THYMINELESS DEATH BY BLOCKING THE TRANSCRIPTION-DEPENDENT STEP OF CHROMOSOME INITIATION

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Thymineless death (TLD), a phenomenon in which thymine auxotrophy becomes lethal when cells are starved of thymine, can be prevented by the presence of rifampicin, an RNA polymerase inhibitor. Several lines of evidence link TLD to chromosome initiation events. This suggests that rifampicin-mediated TLD suppression could be due to the inhibition of RNA synthesis required for DNA chromosomal initiation at oriC, although other mechanisms cannot be discarded. In this work, we show that the addition of different rifampicin concentrations to thyminestarved cells modulates TLD and chromosomal initiation capacity (ChIC).

Time-lapse experiments find increasing levels of ChIC during thymine starvation correlated with the accumulation of simple-Y, double-Y and bubble arc replication intermediates at the oriC region as visualized by two-dimensional DNA agarose gel electrophoresis. None of these structures were observed following rifampicin addition or under genetic-physiological conditions that suppress TLD, indicating that abortive chromosome replication initiations under thymine starvation are crucial for this lethality. Significantly, the introduction of mioC and gid mutations which alter transcription levels around oriC, reduces ChIC and alleviates TLD. These results show that the impairment of transcription-dependent initiation caused by rifampicin addition, is responsible for TLD suppression. Our findings here may provide new avenues for the development of improved antibacterial treatments and chemotherapies based on thymine starvation-induced cell death.

30 E2F7 CONTROLS DNA INTERSTRAND CROSSLINK REPAIR

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The cellular response to DNA damage is essential for maintaining the integrity and stability of the genome. DNA damage activates a checkpoint response that prevents cell cycle progression until the lesion has been repaired. Recent evidence has described E2F7 as a novel target of p53 and suggests a role for E2F7 in DNA damage-dependent transcriptional repression of cell cycle genes. However, the contribution of E2F7 to the cellular response to genotoxic damage is still poorly defined. Our RNA-seq analyses in E2F7-depleted cells show that E2F7 represses the expression of a set of genes involved in the maintenance of genomic stability. We identify E2F7 as a factor that controls cellular recovery during an ongoing DNA damage response in G2 phase of the cell cycle. This role of E2F7 is specific to certain types of DNA damage. Short-term depletion of E2F7 confers an increased recovery competence upon treatment with interstrand crosslink-inducing agents but not upon γ-irradiation. We present evidence that E2F7 controls the repair of cisplatin- and mitomycin C-induced chromosomal aberrations, suggesting that, upon certain types of DNA damage, E2F7 not only contributes to p53-dependent cell cycle arrest as previously described, but also keeps DNA repair pathways under tight control.

31 ROLE OF DORMANT ORIGINS IN THE RESOLUTION OF INTERSTRAND CROSSLINKS

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Replication of the DNA has to be accurately achieved in order to preserve genome stability. This process is highly regulated from initiation through to the last steps for checking and correcting mistakes in the genome, before entering into mitosis. Origins of DNA replication are distributed throughout the genome, and are licensed at the end of mitosis and during G1 by the loading of Mcm2-7 complexes. Only around a 10% of them are used in an unperturbed S-phase, whilst the others remain dormant. These dormant origins are important when cells experience replicative stress during S phase that leads to replication fork stalling. In an event of double fork stalling, the region of DNA in between cannot be replicated. Under these circumstances, a dormant origin become active and complete the replication of this unreplicated segment of DNA to maintain genome integrity. One of the many different sources of replicative stress that replication forks encounter during DNA replication are interstrand crosslinks (ICL), highly toxic DNA lesions that inhibit DNA strand separation and block replication fork progression. Patients suffering from a rare genetic disorder, Fanconi anemia (FA), are particularly sensitive to ICLs. The study of FA pathway has broadens our understanding of the mechanisms involved in the repair of ICLs. In our study, we show an active role for dormant origins in completing DNA replication after encountering ICLs. Nevertheless, very little is known about how dormant origins besides completing replication can play an active role in the repair of the damage. To investigate this, we knocked down the levels of Mcm2-7 and FA proteins to analyse the effects during replication. Our results show that in the presence or absence of ICL agents, both pathways are necessary for maintaining cell proliferation. These results suggest that dormant origins play an essential role in the ICL repair that could suppose a new target for cancer therapy.

32 CONTROL OF ORIGIN ACTIVITY *IN VIVO*: MOUSE MODELS FOR THE OVEREXPRESSION OF INITIATOR PROTEINS CDC6 AND CDT1

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Replication origins are licensed by the coordinated action of ORC, Cdc6 and Cdt1 proteins, which promote the loading of MCM complexes onto DNA in the G1 phase of the cell cycle. Insufficient origin licensing impairs DNA replication and causes genomic instability in mammalian organisms. In turn, overexpression of Cdc6, Cdt1 or individual MCM subunits in cultured cell lines induces DNA damage and cell transformation. However the effects of deregulated expression of mammalian licensing factors *in vivo* are still unknown.

We have generated two mouse models (TetON-Cdc6 and TetON-Cdt1) that allow the inducible overexpression of Cdc6 and Cdt1 proteins in most tissues. First, the effect of Cdc6 and Cdt1 overexpression -alone or in combination- was analyzed in mouse embryonic fibroblasts (MEFs). Both Cdc6 and Cdt1 overexpression altered the dynamics of DNA replication by increasing the frequency of origin firing and decreasing the median rate of fork progression. These effects did not prevent cell proliferation, and MEFs did not show apparent DNA damage or apoptosis. Interestingly, when Cdc6 and Cdt1 overexpression was combined in MEFs derived from a single strain (TetON-Cdc6/Cdt1), cells underwent partial DNA re-replication, showing a partial G2 arrest and activation of the DNA damage response and apoptotic programs.

Regarding overexpression of Cdc6 and Cdt1 in living animals, individual overexpression of either factor was well tolerated by adult tissues, whereas TetON-Cdc6/Cdt1 mice displayed morbidity signs in a very short time (1-2 weeks), with most proliferative tissues showing increased mitotic activity and severe cytoarchitectural abnormalities. The presence of DNA damage and apoptotic figures in several proliferative tissues and the cellular DNA content suggest that partial re-replication is occurring *in vivo*. We have also observed that Cdt1, but not Cdc6 overexpression during development is sufficient to induce embryonic lethality.

Our work provides evidence that simultaneous deregulation of both Cdc6 and Cdt1 induces aberrant events of origin re-firing in adult tissues, showing that pre-replicative proteins co-operate in different ways to promote re-replication depending of the proliferative status of different cell types.

33 THE MCM8-9 HELICASE PROMOTES DNA STRAND SYNTHESIS IN RECOMBINATION REPAIR INDUCED BY DNA INTER-STRAND CROSSLINK

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DNA inter-strand crosslink (ICL) impedes progression of replication forks during S phase. It has been shown that the ICL repair involves a concerted action of multiple repair machineries. When the replicative Mcm2-7 helicase encounters ICL, the Fanconi anemia (FA) proteins are activated for subsequent recruitment of structure-specific nucleases and translesion synthesis DNA polymerases. Unless two opposing forks are encountered at the ICL site, it is likely that the arrested single fork would generate a 'one-ended' DNA double-strand break (DSB). Subsequently, the resected DSB end invades into the sister chromatid, assisted by the Rad51 recombinase, and homologous recombination (HR) regenerates the replication fork to restart DNA synthesis. It is still elusive, however, how the replication fork is regenerated and which machinery(s) promotes this reaction.

Using chicken DT40 cells, we previously revealed that Mcm8 and Mcm9, paralogues of Mcm2-7, form a hexamer-like complex that promotes HR in the downstream of Rad51 during the ICL repair (Nishimura et al., *Mol Cell*, 2012). To further investigate the role of the Mcm8-9 complex, we generated *MCM8* or *MCM9* knockout of human cells by taking advantage of the CRISPR/Cas genome engineering technology. As is the case with DT40 cells, both *MCM8-/-* and *MCM9-/-* cells are hypersensitive to ICL-inducing agents, MMC and cisplatin, whereas these cells are only modestly sensitive to ionizing radiation, suggesting the role of the Mcm8-9 complex is conserved in chicken and human cells. Our genetic and biochemical results are consistent with the idea that the Mcm8-9 complex promotes DNA strand synthesis during HR as a DNA helicase. We would like to propose a model for the role of the Mcm8-9 complex and discuss the relationship between DNA replication and a hidden HR-dependent DNA synthesis.

34 REGULATION OF DNA REPLICATION – INSIGHTS FROM COMPARATIVE GENOMICS

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Accurate and complete replication of the genome is crucial for life. DNA replication is initiated at discrete chromosomal sites called replication origins, with eukaryotic genomes replicated from hundreds or thousands of origins. These origins initiate replication at particular times during S phase, giving rise to a characteristic temporal order for chromosome replication. Aberrant DNA replication timing is associated with increased mutagenesis and genomic instability. We have compared genome replication in a range of yeast species to investigate the mechanisms regulating genome replication timing. Using deep sequencing to measure DNA copy number changes during S phase has allowed us to determine the temporal order of genome replication in eleven different yeast species. Comparisons between closely related species revealed that the location and activity of replication origins are highly conserved. Divergence in the activity of a minority of replication origins has allowed us to identify novel mechanisms regulating origin activity. In comparisons between more divergent species, we find that the location and activity of individual origins are rarely conserved. However, these comparisons identify conserved features of genome replication that reveal the fundamental requirements for regulating the temporal order of genome replication.

35 A NOVEL INHIBITOR OF MRE11-RAD50-NBS1 COMPLEX: FROM STRUCTURE-ACTIVITY RELATIONSHIP TO *IN VIVO*

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Cellular DNA is constantly exposed to various types of the DNA damaging stresses resulting in damaged DNA and need to be efficiently corrected by DNA repair machinery. Defects in DNA repair pathway often lead to cancer predisposition as a consequence of genomic instability and accumulation of chromosomal mutations. MRE11 protein, as component of MRE11-RAD50-NBS1 (MRN) complex, acts as a sensor of DNA double-strand breaks (DSBs) and regulates activity of two alternative repairing pathways: non-homologous end-joining (NHEJ) and homologous recombination (HR). MRN complex also recruits ATM kinase and RPA to the sites of DNA damage, which play an essential role in the maintenance of genome integrity. A chemical inhibitor of 3' to 5' MRE11 exonuclease activity was identified, however it acts by an unknown mechanism with many off target effects in the cells.

To identify more potent and specific inhibitor of MRE11 activities we performed structure-activity relationship screen for the molecules with shape similar to mirin. Three chemicals (M2, M5 and M6) inhibited the activity of Mre11 at significantly lower concentrations then mirin *in vitro*. In addition, these inhibitors did not affect the activity of other nucleases involved in DNA repair, such as Exo1 and Fen1. We also examined the effects of newly identified inhibitors on HR pathway in mammalian cells using I-SceI-inducible DSBs assay. Treating these cells with M2 inhibitor at the dose that do not have an impact on cell proliferation resulted in dramatic inhibition of HR. Similar effects were observed upon Mre11 depletion by siRNA and at 5 times higher concentration of mirin. In contrast, M5 and M6 have no effect on DNA repair. Moreover we observed prevention of ATM autophosphorylation and decrease in amount of chromatin-bound RPA in G2 cells by M2 upon DNA damaging stresses. To achieve similar to M2 affects on RPA 10 times higher concentration of mirin had to be used. These experiments suggest that novel structural analog of mirin, M2, has significantly higher potency to inhibit MRN complex and can serve as a new biological probe to dissect molecular mechanism of action of MRE11 as well as redundancy of DNA repair pathways both *in vivo* and *in vitro*.

36 RAD5 PLAYS A MAJOR ROLE DURING CHROMOSOME REPLICATION IN THE PRESENCE OF DNA DAMAGE

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The evolutionarily conserved *RAD6/RAD18* pathway allows the tolerance to DNA-polymerase blocking lesions, thereby contributing to genomic stability and cell survival. This pathway is subdivided in two different branches: translesion synthesis (TLS), which is error-prone for most types of DNA damage, and the alternative error-free DNA damage avoidance subpathway (DA). In the model organism *Saccharomyces cerevisiae*, TLS is carried out by specialized low-fidelity DNA polymerases that replicate over and past the lesions: Polζ (Rev3/Rev7), Rev1 and Polη (Rad30). The error free branch of DNA damage avoidance uses the newly synthesized, undamaged strand of the sister chromatid as a template for replication across the lesions. In *S. cerevisiae*, this process is triggered by the polyubiquitilation of PCNA by the Mms2-Ubc13 ubiquitin-conjugating complex in conjunction with the E3 ubiquitin-ligase Rad5, which has also ATPase/helicase activity.

We analysed the relative contribution of each branch of the *RAD6/RAD18* pathway to cell survival during chromosome replication in the presence of methyl methanesulfonate (MMS)-induced DNA damage. We found that the DNA damage-avoidance branch, mediated by Rad5, has the major role in this response, with TLS polymerases having just a minor contribution. Thus, whereas cells lacking TLS are slightly affected when treated with MMS during S-phase, Rad5-deficient cells show a high sensitivity under the same conditions. In fact, *rad5*D mutant cells show loss of viability and defects in cell cycle progression even at low MMS doses at which wild type or *tls*D cells are unaffected, suggesting a specific requirement for Rad5 in copying with this kind of damage. Moreover, we determined that both the helicase and the Ubiquitin-ligase activities of Rad5 are required and appear equally important for the response to DNA damage during S-phase. Furthermore, we show that Rad5 is required for the progression of DNA replication forks through MMS-damaged DNA. Consistent with a role during chromosome replication in the presence of DNA damage, we found that Rad5 levels peak during S-phase and that the protein accumulates forming nuclear foci upon cellular exposure to MMS in S-phase. We also show that, despite its specific role during S-phase in coping with DNA damage, Rad5 function can be uncoupled from DNA replication.

37 THE DYRK KINASE POM1 REGULATES CDR2 MEMBRANE ANCHORING AND MID1-DEPENDENT CLUSTERING TO POSITION THE DIVISION PLANE

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Proper division plane positioning is essential to achieve faithful DNA segregation and control daughter cell size, positioning or fate within tissues. In *S. pombe*, division plane positioning is controlled positively by export of the division plane positioning factor Mid1/Anillin from the nucleus and negatively by the Pom1/DYRK kinase gradients emanating from cell tips. Pom1 restricts to the cell middle cortical cytokinetic ring precursors organized by the SAD-like kinase Cdr2 and Mid1/Anillin through an unknown mechanism. Here, we show that Pom1 modulates Cdr2 association with membranes by phosphorylation of a basic region cooperating with the lipid binding KA-1 domain. Pom1 also inhibits Cdr2 interaction with Mid1, reducing its clustering ability, possibly by down-regulation of Cdr2 kinase activity. We propose that the dual regulation exerted by Pom1 on Cdr2 prevents Cdr2 assembly into stable nodes in the cell tip region where Pom1 concentration is high, to position cytokinetic ring precursors to the cell geometrical center and produce daughter cells of equal size.

38 IMPAIRED FORMATION OF DIVERSE TYPES OF Fe-S CLUSTERS CAUSES DIFFERENT DNA DAMAGE RESPONSES IN *Saccharomyces cerevisiae*

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Mitochondria are essential for the synthesis of iron-sulfur clusters (ISCs) independently of the ISC cellular targets. Mitochondrial dysfunctions stimulate nuclear genome instability and DNA damage through inhibition of the production of ISC-containing proteins. In response, cells have developed mechanisms, collectively known as the DNA damage response, to coordinately regulate cellular events (cell cycle arrest and replication/transcription block) and parallel activation of DNA repair pathways. In yeast, the DNA damage checkpoint is the main responsible for enabling cells to confront DNA damage and DNA replication stress. This work is focused on the DNA damage checkpoint and the post-replicative stress responses when ISC synthesis is impaired, by using *S. cerevisiae tet-grx5* and *tet-iba57* conditional mutants affected at separate stages of mitochondrial ISC biogenesis. We demonstrate that cells lacking Grx5 show constitutive activation of the DNA damage checkpoint, and a delayed cell cycle S-phase. In addition, in combination with mutations in the DNA replicative stress pathways, cell viability becomes strongly compromised in the absence of Grx5. In contrast, effects are different when cells lack Iba57, which is specialized in formation of [4Fe-4S] clusters.

39 ATPase-DEPENDENT CONTROL OF A SUMO LIGASE DURING DNA REPAIR

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Modification of proteins by SUMO is essential for the maintenance of genome integrity. The Mms21 SUMO ligase docks to the arm region of the Smc5 protein in the Smc5/6 complex; together, they cooperate during recombinational DNA repair. However, very little is known about how this collaboration is brought about. Here we show that the Smc5/6-Mms21 complex operates as a giant SUMO ligase, requiring all of its subunits for its activity in vivo. Sumoylation requires the ATPase function of the SMC subunits, a step that is part of the ligase mechanism that assists Ubc9 function. Our results demonstrate that the ATPase activity of the Smc5/6-Mms21 complex is coordinated with its SUMO ligase, through the coiled coil domain of Smc5 and the physical remodeling of the molecule, to promote sumoylation during DNA repair.

40 A BRIEF DE-REGULATION OF THE CELL CYCLE PHOSPHATASE CDC14 LEADS TO DRAMATIC GENOMIC REARRANGMENTS IN DIPLOID YEAST CELLS

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One of the hallmarks of cancer cells is genomic instability. Genes that prevent the appearance of cancer-related phenotypes are usually known as tumour suppressor genes. Among them, those involved in the maintenance of the genome integrity during cell division are commonly referred as caretakers. Decreased activity of caretakers leads to the appearance of genomic instability events, a reduced lifespan and an increase in the risk of cancer. Human Cdc14 phosphatases are candidates to be considered caretakers, as it has been reported to play a role in DNA repair, mitotic spindle assembly, regulation of the centrosome cycle, and cytokinesis, among others. In budding yeast we and others have previously reported that the transient inactivation of Cdc14 in haploid cells leads to a de-coordination of anaphase events that leads in turn to the appearance of a DNA anaphase bridge comprised mostly of the rDNA-bearing chromosome XII. In a previous work, we have shown that this anaphase bridge breaks upon Cdc14 re-activation, compromising cell viability and the genome integrity, especially for the chromosome XII. In this work, we show that, in diploid yeast cells, Cdc14 plays a role in the maintenance of the genomic integrity that is not only restricted to the chromosome XII. In addition, a brief inactivation of this key cell cycle phosphatase, and not necessarily its permanent depletion, has dramatic consequences on the progeny, related to a long-term decreased viability, reduced lifespan and genomic integrity.

40 PROTEIN KINASE C CONTROLS ACTIVATION OF THE DNA INTEGRITY CHECKPOINT

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The protein kinase C (PKC) superfamily plays key regulatory roles in numerous cellular processes. *Saccharomyces cerevisiae* contains a single PKC, Pkc1, whose main function is cell wall integrity maintenance. In this work, we connect the Pkc1 protein to the maintenance of genome integrity in response to genotoxic stresses. Pkc1 and its kinase activity are necessary for the phosphorylation of checkpoint kinase Rad53, histone H2A and Xrs2 protein after DNA damage, indicating that Pkc1 is required for activation of checkpoint kinases Mec1 and Tel1. Furthermore, Pkc1 electrophoretic mobility is delayed after inducing DNA damage, which reflects that Pkc1 is posttranslationally modified. This modification is a phosphorylation event mediated by Tel1. The expression of different mammalian PKC isoforms at the endogenous level in yeast *pkc1* mutant cells revealed that PKCδ is able to activate the DNA integrity checkpoint. Finally, downregulation of PKCδ activity in HeLa cells caused a defective activation of checkpoint kinase Chk2 when DNA damage was induced. Our results indicate that the control of the DNA integrity checkpoint by PKC is a mechanism conserved from yeast to humans.

41 BEYOND THE FIRST CELL DIVISION IN *top2*-DEFECTIVE MUTANTS IN *Saccharomyces cerevisiae*

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The main function of topoisomerase proteins is to disentangle DNA in all its biological processes. Topoisomerase II (Topo II) is an essential protein because of its unique ability to cut double stranded DNA and resolve catenations. When the Top2 gene is deactivated the cell division suffers from a massive entanglement of the chromosomes which results in anaphase bridges that cannot be resolved and that leads, in most cases, to the death of both daughter-to-be cells. Importantly, Top2 is one of the main molecular targets of antitumour chemotherapy.

We have studied the cell cycle in several yeast mutant strains where Top2 can be deactivated. Some mutants maintained DNA bridges for several hours when trying cell division, suggesting an arrest in anaphase, and they had a high rate of survival provided that Top2 gene is reactivated within the first 3 hours. Other mutants resolved these bridges shortly after their appearance, presumably by cutting the DNA and thus creating massive damage. The survival rate of these mutants decreased faster than in the first ones. Flow cytometry analysis showed in both cases DNA degradation, although it is more prominent in the second class of mutants.

Rad9 is a checkpoint protein that mediates the arrest of the cell cycle in the presence DNA damage. Deleting this gene in the Top2 mutants led to a decrease in the survival rate, suggesting that Rad9 may sense DNA topological problems arising from the Top2 deficiency without causing a G2/M cell cycle arrest. Accordingly, Rad52 foci often formed upon Top2 deactivation and these foci appear in cell after the DNA bridge disappears. Strikingly though, rad52 deletion not modify the survival rate of both mutants.

Our results suggest that downregulation of Top2 activity, a common outcome during acquisition of secondary resistance to anti-Top2 chemotherapy, could be accomplished through two classes of mutation in terms of how cells can continue dividing. The characterization of such mutants will provide new personalized targets in anti-Top2 resistant tumours.

42 ISOLATION OF A NEW SPLICEOSOME MUTANT WITH CELL CYCLE DEFECTS BY A NOVEL CONDITIONAL SCREENING IN *S. pombe*

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Conditional screenings have so far identified hundreds of mutants in many biological processes that allow the study of loss-of-function under the restrictive condition, but also propagation and genetic crosses of these strains under the permissive condition. Although drug sensitivity mutants have been isolated, the most spread condition used is temperature.

We have searched for formamide sensitive mutants, a novel conditionality in fission yeast, that display cell cycle defects. Among others, we have identified one mutation in a NTC (Nineteen core) spliceosome component *cwf15* (Complexed with cdc5 protein 15) that is an essential and evolutionary conserved RNA processing factor from yeast to humans. It has been previously shown to physically interact to other spliceosome components but no further characterization has been done. Here we further characterize *cwf15.32*^{Fs} cell cycle defects and test for genetic interactions with other splicing mutants. Our conditional mutation allows growth in normal media but it blocks cell cycle progression in the presence of formamide. At the restrictive condition, mutant cells transit the whole interphase normally and initiate mitosis but they are unable to elongate the mitotic spindle, leading to dramatic genome segregation defects. This phenotype could suggest that one or more elements that regulate spindle elongation are particularly sensitive to splicing defects or unveil an interesting role for RNA processing in the control of spindle dynamics essential for proper chromatin segregation.

43 SIN-DEPENDENT DISSOCIATION OF THE SAD-LIKE KINASE CDR2 FROM THE DIVISION SITE IN MITOSIS AND IMPLICATIONS ON LATE CYTOKINESIS EVENTS

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In most eukaryotes, cytokinesis depends on an acto-myosin based contractile ring which constriction promotes cleavage furrow ingression between the two daughter cells. In fission yeast, assembly of the acto-myosin ring is intiated from precursor nodes organised on the medial cell cortex by the Sad-like kinase Cdr2. These nodes contain the anillin-like division plane definition factor Mid1 as well as other future components of the contractile ring such as Blt1, Gef2 or Nod1. At mitotic entry, node precursors recruit additional key ring components such as myosin II, the actin-nucleator Cdc12 and the F-BAR protein Cdc15, before compacting into the nascent acto-myosin ring. Surprisingly, Cdr2 dissociates from the ring precursors and becomes cytoplasmic during contractile ring assembly. Here we show that Cdr2 detachment from the cortex depends on the SIN, a signalling pathway that coordinates mitotic exit and cytokinesis. Our proteomic analysis and mutagenesis studies indicate that the NDR kinase Sid2, the last component of the SIN cascade, may directly phosphorylate two RXXS motifs on Cdr2, creating binding sites for the 14-3-3 protein Rad24. Accordingly, Rad24 co-immunoprecipitates with Cdr2 from cell extracts and participates in Cdr2 mitotic cortical detachment. Finally, our preliminary analysis of a Cdr2 mutant resistant to SIN activity revealed delays in ring constriction. We conclude that the SIN-dependent detachment of the Cdr2 scaffold for precursor nodes during mitosis contributes to reliable late cytokinesis events.

44 REB1 PROTEIN, FROM REPLICATION BLOCKAGE TO TRANSCRIPTION REGULATION

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The fission yeast Reb1 protein binds to two specific sites near the 3´-end of the rRNA genes contributing to the termination of rDNA transcription by RNA polymerase I [1], and in the blockage of replication forks approaching from the direction opposite to transcription [2].

We have recently found that Reb1 also binds *in vivo* and *in vitro* to a sequence upstream the promoter of *ste9*$^{+}$ participating in the up-regulation of the expression of *ste9*$^{+}$ under nitrogen starvation [3]. Ste9 cooperates in the response to nutritional stress, which arrests the cells in the G1 phase, by activating the anaphase-promoting complex/cyclosome which targets B-type cyclin for proteasome [4]. This enables cells to proceed through sexual differentiation, mating and spore formation to resist the harsh environment. Reb1 deleted cells show a reduced arrest in G1 leading to a diminish fertility [3].

When cells need to lengthen the G1 phase to reach the minimal size to begin replication, as in the termosensitive *wee1-50* mutant, Reb1 becomes essential to maintain cell viability. The combined *reb1Δ wee1-50* mutants die of mitotic disaster after a couple of rounds of premature entrance into replication and mitosis. Reb1 is also required for the viability of cells with defects on proteins required for the initiation of replication, as in *cdc10-129* mutants. These data indicates that Reb1 seems to be required to avoid a premature passage through *start*.

Our current work is centered on a high-throughput screening for other Reb1 binding sites on the fission yeast´s genome. A preliminary study has revealed that Reb1 binds upstream many genes, preferentially at their transcription start sites. These results have shown that Reb1 binds to the promoter of the cell cycle regulators *cig2+* and *nim1+*, and takes part in their transcriptional regulation upon nitrogen depletion.

These results indicate that *S.pombe´s* Reb1 may be acting as transcriptional regulator protein for multiple genes.

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45 EXPLORING SYNTHETIC LETHAL INTERACTIONS BETWEEN CHECKPOINT KINASE 1 (CHK1) AND DNA REPLICATION

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Complex networks of redundant surveillance mechanisms, namely evolutionarily conserved DNA damage response (DDR) and checkpoints, maintain genomic integrity following various genomic insults. Exploring synthetic lethal interactions between DNA repair pathways have wide appliance to the treatment of many types of malignancies. One of the key players in genome surveillance pathways is a protein kinase, Chk1. DNA damage and replication stress supposedly induces activation of Chk1, which then transduces the checkpoint signal and aids cell cycle arrest allowing time for DNA repair. Hence, Chk1 represents druggable molecular target for inhibition following replication stress induced by chemotherapeutic agents. Such synthetic lethal interaction leads to enhanced sensitivity of cancer cells with additional burden of damaged DNA, without cytotoxic effects to the normal cells.

Our results suggest that in human tumor cell lines, ablation of CHK1 function by either knock down or inhibition during replication stress created by depletion of replicative DNA Polymerase α (DNA Pol α) or antimetabolite exposure leads to cancer cell death. SCH 900776 was identified as a highly potent and functionally optimal CHK1 inhibitor with minimal intrinsic antagonistic properties via high content screening (Guzi et al., 2011). We have been able to develop a metabolically stable analog of SCH 900776, OH209EN1 which phenocopies short interfering RNA-mediated CHK1 ablation and genetically interacts with DNA antimetabolite agents and sensitizes cancer cells approximately 4 times more efficiently. OH209EN1 rapidly suppresses accumulation of CHK1-p296 auto-phosphorylation following HU treatment and also induces fatal amount of Double Strand Breaks (DSBs) accumulation as assessed by γH2AX. These results suggest that the new metabolically stable analog OH209EN1 may be used as a tool to elucidate the role of CHK1 at the replication checkpoint and provide an enhanced therapeutic window for cancer treatment in near future.

46 MITOTIC INDUCTION OF DNA DAMAGE AND RECOMBINATION IN CELLS CONTAINING UNDER-REPLICATED DNA

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It has long been assumed that cells would not enter mitosis until chromosome replication is completed. This concept derives mainly from studies in which the progression of replication forks was halted by chemical (MMS, HU) or enzymatic impediments (e.g. ligase mutants). This causes exposure of long stretches of single-stranded DNA followed by activation of the DNA damage or replication checkpoint pathways that delay mitotic entry until the problem is fixed.

In this work we tested in yeast whether cells that have not yet finished DNA replication, but in which fork progression is normal, can enter mitosis and become genetically unstable. To extend S phase without causing replication stress we used *cdc6-1* and *cdt1-21* ts mutants, which form fewer pre-replication complexes (pre-RCs) at semi-permissive temperatures. We showed using DNA combing that the density of active origins dropped proportionally to the rising temperature, along with a longer S phase and increased DNA damage response (Rad52 recombination foci, phospho-Rad53). Interestingly, DDR activation always took place 60 min after G1 release, the time at which cells normally enter mitosis. Crucially, we showed that DNA damage in cells containing under-replicated DNA was dependent on mitotic CDK and Cdc5/Plk1 kinase activities, as well as on phosphorylation of a replication fork protein. Our results demonstrate that i) cells can enter mitosis with under-replicated DNA, undetected by checkpoints, ii) mitotic entry is responsible for DNA damage or fork collapse in cells that replicate late, iii) completion of DNA synthesis occurs at least in part through illegitimate recombination-dependent mechanisms. Indeed Rad52 foci persist and anaphase is delayed for a long time in these cells, as expected for one end homology-driven repair. Accordingly, gross chromosome rearrangement (GCR) rates are very high in *cdc6-1* and *cdt1-21* mutants transiently grown at semi-permissive temperature. Our observations are reminiscent of the premature chromosome condensation phenotype caused by fusion of mitotic cells to S-phase nuclei. This work challenges the dogma that cells cannot enter mitosis before S phase is finished. It also provided a mechanism for the breakage of late-replicating common fragile sites (CFS) as well as a conceptual framework to explain the high chromosomal instability of cancer cells with altered replication programmes.

47 DIFFERENT NUCLEOSOMAL LANDSCAPES AT EARLY AND LATE REPLICATING ORIGINS IN *Saccharomyces cerevisiae*

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Eukaryotic genomes are replicated during S phase according to a temporal program. Several determinants seem to control the timing of origin firing, including the chromatin environment and epigenetic modifications. By performing high-resolution analysis of genome-wide nucleosome positioning we have identified different chromatin architectures at early and late replication origins. The different nucleosome patterns from early and late origins are already established in G1 and are tightly correlated with the genomic context. Moreover, specific early and late nucleosomal patterns seem to be fixed robustly, even in *rpd3* mutants in which histone acetylation and origin timing have been significantly altered. Nevertheless, higher histone acetylation levels correlate with the local modulation of chromatin-associated features, leading to increased origin accessibility. In addition, we conducted parallel analyses of replication and nucleosome dynamics that revealed that origin conformation is modulated during origin activation.

48 SEARCHING FOR REGULATORS OF THE MAMMALIAN dNTP POOL

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The ribonucleotide reductase (RNR) is an evolutionary conserved enzyme responsible for the reduction of all four types of nucleosides to deoxynucleosides. A shortage of dNTPs leads to replication stress and has recently been suggested to be the cause of oncogene-induced DNA damage. Moreover, misregulation of RNR is associated with various types of cancer. In yeast, the lethality associated with Mec1 (ATR) deletion can be rescued by the concomitant deletion of Sml1, an inhibitor of the RNR. All of the above indicate that the regulation of RNR activity is essential on the context of replication stress. We are currently looking for novel regulators of the RNR activity in mammalian cells, and their putative connections to ATR. To this end we are combining three different approaches that involve the generation of mouse models with altered RNR function, proteomics of RNR regulators and genetic screenings for modulators of RNR activity. Our progress in these projects will be presented.

49 FISSION YEAST MHF1 (CENP-S) AND MHF2 (CENP-X) PLAY A ROLE IN GENOME STABILITY

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Our laboratory is interested in understanding the complex molecular mechanisms that regulate cell division and its coordination with cell growth and DNA replication in eukaryotes. We have undertaken a functional genomic approach to identify novel cell cycle regulators and gain greater insight into the mechanisms that control cell cycle progression in fission yeast. For this purpose we have used a synthetic lethal approach (SpSGA) to investigate the genetic interactions between the collection of non-essential fission yeast mutants (Bioneer version 3.0) and the query mutant strains *wee1-50* and *cdc2-3w* that have *wee* phenotypes characterized by a short G2 phase and small cell size.

We identified *mhf1Δ* and *mhf2Δ* among the 70 mutants that displayed negative genetic interactions with *wee1-50* and *cdc2-3w*. Mhf1 (CENP-S) and Mhf2 (CENP-X) are two histone-fold proteins, highly conserved from yeast to mammalian cells, that play an only partially understood role in the kinetochore architecture and kinetochore-microtubule dynamics. In mammalian cells MHF1 and MHF2 are also involved in DNA repair, specifically in the Fanconi anaemia pathway. For these reasons, we are functionally characterising these proteins in fission yeast. Our preliminary results show that both Mhf1 and Mhf2 are required for proper chromosome segregation during mitosis and meiosis. We have observed that the mutants lacking *mhf1*+ or *mhf2*+ show genomic instability and a significant increase in the rate of chromosome loss. We are now beginning to understand the role of these proteins in the maintenance of genome stability during chromosome segregation at cell division and during DNA repair/remodelling processes upon replicative stress.

50 A SUGARY TASTE TO DNA DAMAGE TOLERANCE VIA TORC2 SIGNALING

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The Target Of Rapamycin (TOR) kinase belongs to the conserved eukaryotic family of phosphatidylinositol 3-kinase related kinases (PIKKs). While all members of this family play a pivotal role in response to genotoxic stress, TOR is mainly known for its role in nutrient-dependent signaling. TOR proteins are found at the core of at least two evolutionary conserved complexes, TORC1 and TORC2. Recently, we reported a novel role for TORC2 in tolerance to DNA damage and maintenance of genome stability in fission yeast. A recent study in budding yeast also suggested a role for TORC2 in guarding genome integrity in the face of double strand breaks (DSB). The conservation of the role of TORC2 in these two highly divergent yeasts suggests that this role may be conserved in higher eukaryotes.

Despite the wide variety of functions in which TORC2 has been implicated, the signals that regulate TORC2 activity have so far remained obscure. TORC2 has one known direct substrate, the kinase Gad8, which is phosphorylated by TORC2 at Ser546. We have found that glucose is required and sufficient to induce Gad8 Ser546 phosphorylation *in vivo* and Gad8 kinase activity *in vitro*. The glucose sufficiency signal that activates TORC2-Gad8 is mediated via the cAMP/PKA pathway, a major glucose-sensing pathway. By contrast, Pmk1, the mitogen activated protein kinase (MAPK) in the cell integrity pathway, inhibits Gad8 phosphorylation and activation in response to glucose starvation. Interestingly, mutations in the cAMP-Pka1 pathway render cells sensitive to DNA damage. This sensitivity is repressed by over-expression of Gad8, suggesting a novel link between glucose sensing, TORC2 and tolerance to DNA damaging conditions.

51 REPLICATION FORK STALLING AT SITE-SPECIFIC BARRIERS IN *S. pombe* IS MEDIATED BY THE DNA-BINDING DOMAIN OF MRC1 IN A CHECKPOINT-INDEPENDENT MANNER

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The S-Phase checkpoint mediator protein Mrc1 (*S. cerevisiae* Mrc1, metazoan Claspin) forms a trimeric sub-complex within the eukaryotic replisome together with Swi1 (*S. cerevisiae* Tof1, metazoan TIMELESS) and Swi3 (*S. cerevisiae* Csm3, metazoan TIPIN) and full-fills several important functions during replisome progression. Firstly, Mrc1 is necessary to activate the S-Phase effector kinase Cds1 (*S. cerevisiae* Rad53) after the replisomes stalls due to DNA damage or replication stress. Secondly, studies in *S. cerevisiae* have shown that Mrc1 is required for the restart of replication forks after HU arrest. Thirdly, *S. cerevisiae* Mrc1 is necessary for the replication of difficult templates such as inverted or tri-nucleotide repeats. In the absence of Mrc1 these sequences cause an increase in fork stalling. However, replisome pausing at site specific, protein mediated replication barriers does not depend on Mrc1 in *S. cerevisiae*, but on Tof1/Swi1 in both *S. cerevisiae* and *S. pombe*.

Here we use a new genetic screening tool to surprisingly identify Mrc1 as a novel factor involved in stalling at site specific, protein mediated replication barriers in *S. pombe*. This screening tool make use of the effect that a reduction of pausing at the *MPS1* pause site reduces the rate of mating type switching and sporulation, resulting in an easily detectable phenotype. We go on to show that pausing at several other *S. pombe* replication barriers also depends on Mrc1 and that this function is independent of Mrc1's role as S-phase checkpoint mediator but is dependent on its DNA binding activity. An analysis of the protein sequences of Mrc1/Claspin from several species suggests that this DNA-binding domain is well conserved within the eukaryotic lineage, but absent in *S. cerevisiae*. Furthermore, our data indicate that this effect is due to a decrease in duration of the fork stalling, rather than due to a decrease in the percentage of forks stalled.

In summary, we show that *S. pombe* Mrc1 unlike *S. cerevisiae* Mrc1 mediates replisome pausing at site-specific protein mediated barriers. Therefore, our study offers important new insights into the complexity of the mechanism of replication fork stalling in eukaryotes.

52 A NOVEL ROLE FOR MICROTUBULE CYTOSKELETON IN FISSION YEAST DNA REPAIR

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DNA damage repair is key to maintaining genomic integrity and preventing cancer. While biochemical mechanisms involved in DNA damage signaling and repair have been well characterized, the role of nuclear architecture and in vivo DNA dynamics in damage response is poorly understood. In the fission yeast Schizosaccharomyces pombe, cytoplasmic microtubule bundles that are attached to the nuclear envelope and spindle pole body move the nucleus in an oscillatory manner during interphase. Here, we show that these cytoplasmic microtubules are required for efficient DNA repair. Time lapse imaging shows that chromosomal movement and the dynamics of DNA repair centers during interphase are microtubule-dependent. We propose that cytoplasmic microtubules exert forces on nuclear envelope proteins that move the chromosomes, and that these movements affect steps in DNA repair such as homology search during recombination. Our results reveal an unexpected role of cytoplasmic microtubules in genomic integrity.