Inestabilidad genética asociada a R loops

Francisco García Benítez

Tesis Doctoral

Universidad de Sevilla

2018

Inestabilidad genética asociada a R loops

Trabajo realizado en el Departamento de Genética, Facultad de Biología (Universidad de Sevilla) y en el departamento de Biología del Genoma, CABIMER (Universidad de Sevilla-CSIC-UPO), para optar al grado de Doctor en Biología Molecular, Biomedicina e Investigación Clínica por el licenciado Francisco García Benítez.

Sevilla, 2018

El doctorando

Francisco García Benítez

Los directores de tesis

Andrés Aguilera López

Hélène Gaillard

ABBREVIATIONS

5-FOA	5-fluorotic acid
53BP1	p53 binding protein 1
A.U.	Arbitrary units
AID	Activation-induced cytidine deaminase
ARS	Autonomously replicating sequence
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3 related
BER	Base-excision repair
bp	Base pairs
BrdU	Bromedeoxyuridine
cDNA	complementary DNA
ChIP	Chromatin immunoprecipitation
CSR	Class switch recombination
DDR	DNA damage response
DNA	Deoxyribonucleic acid
DRIP	DNA:RNA immunoprecipitation
DSB	Double-strand break
dsDNA	double strand DNA
FACS	Fluorescence-activated cell sorting
FACT	Facilitates chromatin transcription
FG	Phe-Gly rich
FLC	Full-linear chromosome
Gal	Galactose
GCR	Gross chromosomal rearrangements
GFP	Green fluorescence protein
Gly	Glycine
GO	Gen ontology
H3ser10-P	Histone H3 phosphorylation at serine 10
HA	Hemagglutinin
HBD	Hybrid binding domain

HDAC	Histone de-acetylase
His	Histidine
HR	Homologueous recombination
HU	Hydroxyurea
kb	Kilobase
Leu	Leucine
min	Minutes
MMR	DNA mismatch repair
MMS	Methyl methanesulfonate
MRN	Mre11-Rad50-Nbs1
mRNA	messenger RNA
mRNP	mRNA ribonucleoprotein particle
NER	Nucleotide-excision repair
NHEJ	Non-homologueous end joining
NPC	Nuclear pore complex
NLC	Non-linear chromosome
NTS	Non-transcribed strand
Nup	Nucleoporin
ORF	Open reading frame
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
Phe	Phenylalanine
qPCR	quantitative PCR
RBP	RNA binding protein
rDNA	Ribosomal DNA
RF	Replication fork
RNA	Ribonucleic acid
RNAi	interference RNA
RNAP	RNA polymerase
RNase	Ribonuclease
RPA	Replication protein A
SC	Synthetic complete medium
SEM	Standard error of the mean
siC	Non-targeting siRNA control

siRNA	Small interfering RNA
SPO	Sporulation medium
ssDNA	single stranded DNA
TAGIN	Transcription-associated genome instability
TAM	Transcription-associated mutation
TAR	Transcription-associated recombination
TS	Template strand
UV	Ultraviolet light
WT	Wild type
YFP	Yellow fluorescent protein
YNB	Yeast nitrogen base

INDEX OF CONTENT

INDEX OF FIGURES

INTRODUCTION

RESULTS

DISCUSSION

MATERIALS AND METHODS

RESUMEN

La inestabilidad genómica es una patología celular, la cual se produce cuando una célula acumula variaciones genéticas que alteran su genoma. La acción de agentes genotóxicos externos, así como el propio metabolismo celular dan lugar a daños en el ADN. Esto daños pueden producir alteraciones genéticas que pueden abarcar desde mutaciones puntuales a reordenaciones cromosómicas. La inestabilidad genómica se asocia con el envejecimiento, la tumorigénesis y múltiples enfermedades genéticas. Numerosos procesos celulares, como la transcripción o la replicación, actúan sobre el ADN, siendo necesaria la coordinación de los mismos para evitar y/o solucionar aquellos problemas que pueden comprometer la estabilidad del genoma.

Durante la transcripción, la acumulación de superenrollamientos negativos detrás de la polimerasa de ARN, facilitan el desenrollamiento de la hélice del ADN. Esta apertura transitoria del ADN favorece que el ARN naciente rehibride con la cadena molde de ADN para formar un híbrido de ADN:ARN, dando lugar a una estructura llamada bucle R (*R loop*). La formación de *R loops* conlleva que la cadena de ADN no transcrita sea desplazada quedando como en forma de cadena sencilla. Los *R loops* se producen de forma natural como un intermediario en procesos específicos que incluyen la transcripción y la replicación del ADN mitocondrial o el cambio de isotipo de las inmunoglobulinas en los linfocitos B. Sin embargo, la acumulación de estas estructuras es una fuente de inestabilidad genómica asociada a la transcripción, como se ha observado en bacterias, levaduras y células de mamífero. De hecho los *R loops* constituyen un obstáculo para el avance de la horquilla de replicación del ADN. Igualmente, la transcripción puede constituir un obstáculo para la progresión de la horquilla de replicación. Estos conflictos entre la transcripción y la replicación pueden desencadenar un incremento de las roturas de doble cadena como fuente de inestabilidad genómica, que pueden ser agravados por los *R loops*.

INTRODUCTION

In living cells, DNA is continuously exposed to endogenous agents such as cellular metabolism products or failures during cellular processes including replication, transcription, DNA damage signalling and DNA repair. In addition, DNA is exposed to exogenous agents such as ultraviolet light (UV), ionizing radiation or chemical agents. The action of these different agents may lead to DNA damage such as modified bases, DNA-protein adducts, abasic sites or DNA breaks. Unless repaired, these DNA lesions may interfere with replication and finally lead to mutations. Although mutations and changes in the DNA sequence are necessary for evolution and genetic variation, they may be harmful for the cells and organisms. Genome instability is a general term that refers to the accumulation of a high variety of genetic alterations that may occur on the DNA, from changes in a unique nucleotide to changes at chromosomal scale. Depending on the nature of the alteration, we can differentiate between base substitutions, insertions or deletions of short sequences, micro and minisatellite contractions or expansions, genome reorganization, loss of heterozygosity, copy number variants or changes in the chromosome number. Pathological mutations and elevated genome instability are associated with human diseases and a major predisposition to cancer. Therefore, coordination between the cellular processes that use DNA as a template, such as transcription, replication and DNA repair, is required to maintain genome stability.

1. – DNA Damage Response

All kind of DNA lesions need to be detected and signalled to preserve the integrity of the genome. The cellular response to DNA damage, which is called DNA damage response (DDR), allows the signalling and repair of DNA lesions and facilitates DNA replication restart (Harper and Elledge 2007). The DDR activates DNA repair and promotes a phosphorylation signalling cascade that causes a cell cycle delay or block to enable the repair of DNA damage. Moreover, if the damage is persistent, apoptotic pathways can be engaged (Sertic, Pizzi et al. 2012).

Different repair mechanisms are required depending on the kind of DNA damage. Base lesions are repaired by base excision repair (BER); lesions that distort the DNA helix such as bulky adducts are repaired by nucleotide excision repair (NER) or

by transcription-coupled NER (TC-NER) when the DNA lesion is recognized by the RNA polymerase (RNAP) in the transcribed strand during transcription. DNA mismatches occurring during replication are repaired by post replicative mismatch repair (MMR). Finally, two principal pathways to repair double-stranded DNA breaks (DSBs) have been reported, non homologous end joining (NHEJ) and homologous recombination (HR).

Although all DNA lesions are injurious and need to be repaired, DSBs are the most harmful kind of damage that may take place in the DNA because they affect both strands of a given DNA molecule and are therefore difficult to repair. DSBs have the potential to cause mutations, chromosomal rearrangements and finally genomic instability. In *Saccharomyces cerevisiae*, DSBs are detected by a complex formed by Mre11, Rad50 and Xrs2 (MRX, MRN in human). The MRX complex plays a role as a bridge maintaining both DSB ends close to each other. This complex promotes the recruitment of Tel1 (ATM in human cells). On the other hand, Mec1 (ATR in human cells) is activated by replication impairments in which the generated single-stranded DNA (ssDNA) is quickly coated by replication protein A (RPA). Mec1/ATR activation may also be promoted by DNA breakage and the ssDNA resulting from 5´-end resection. Both proteins, Tel1/ATM and Mec1/ATR trigger the activation of DDR. This activation induces a signalling cascade mediated by the activation of the downstream kinases Rad53 and Chk1 that allows the amplification of the DNA damage signal and the repair of the later. As a consequence of DDR activation, C-terminal tails of histone H2A are phosphorylated promoting chromatin remodelling. In addition, many proteins implied in the repair of DSBs are phosphorylated (Pardo, Gómez-González et al. 2009; Sulli, Di Micco et al. 2012; Gobbini, Cesena et al. 2013).

1.1.- DSB repair

NHEJ and HR are the principal pathways to repair DSBs. During NHEJ, direct re-ligation of the two ends of the DSBs takes place. HR needs an intact homologous donor sequence to repair the DSB. The sister chromatid is the preferred donor molecule since it provides a sequence exactly identical to the damaged DNA. Therefore, HR is

restricted to the S and G2 phases of the cell cycle, being the only ones in which a sister chromatid is present.

The Ku complex, composed of yKu70 and yKu80, binds at DSB ends and may play a role in protecting the DNA end. During NHEJ, the Ku complex prevents 5'-end resection and promotes the ligation of the two ends by ligase Lig4. Additionally the Xrs2 component of the MRX complex mediates the interaction with the Lig4 complex, promoting NHEJ (Matsuzaki, Shinohara et al. 2008). However, during S/G2, if the Ku complex is removed from the DSB, 5'-end resection takes places and repair becomes committed to HR. The 5´-end resection generates a 3'-end ssDNA that is readily coated by RPA. Rad51 is then incorporated and displaces RPA from the 3'-end ssDNA, a step catalysed by the HR factor Rad52. The formation of this Rad51 filament promotes the invasion of an homologous double stranded DNA (dsDNA), forming a DNA-DNA hybrid called D-loop (Pardo, Gómez-González et al. 2009; Heyer, Ehmsen et al. 2010; Shibata 2017). To repair the DSB, two principal HR pathways are distinguished, synthesis-dependent strand annealing (SDSA) and double strand break repair (DSBR). During SDSA, DNA synthesis of one resected DSB-end occurs at the D loop. The DNA synthesis restores the damaged DNA and provides a complementary strand for the 3´-end ssDNA of the other resected DSB-end, allowing D loop reversal and the re-annealing of both strands (Figure I1). However, during DSBR the D loop is extended and the second resected DSB-end hybridizes with the D loop promoting the DNA synthesis of both resected DSB-ends using both strands of the template DNA molecule. DNA synthesis from the D loop generates intermediate structures called Hollyday junctions (HJ), which are then resolved by specific structure-dependent nucleases, leading to crossover or non-crossover products depending on the specific cleavage mechanism used. Additionally, other pathways have been described to repair DSB. Break-induced replication (BIR), which can take place in the absence of the second end of the DSB, is a mechanism in which the D-loop functions as a replication fork (RF) initiating the DNA synthesis in one direction. Finally, if the DSB occurs between repeated sequences, both DSB ends resected up to the repeats can anneal with each other, in a process termed single strand annealing (SSA) (Pardo, Gómez-González et al. 2009; Heyer, Ehmsen et al. 2010).

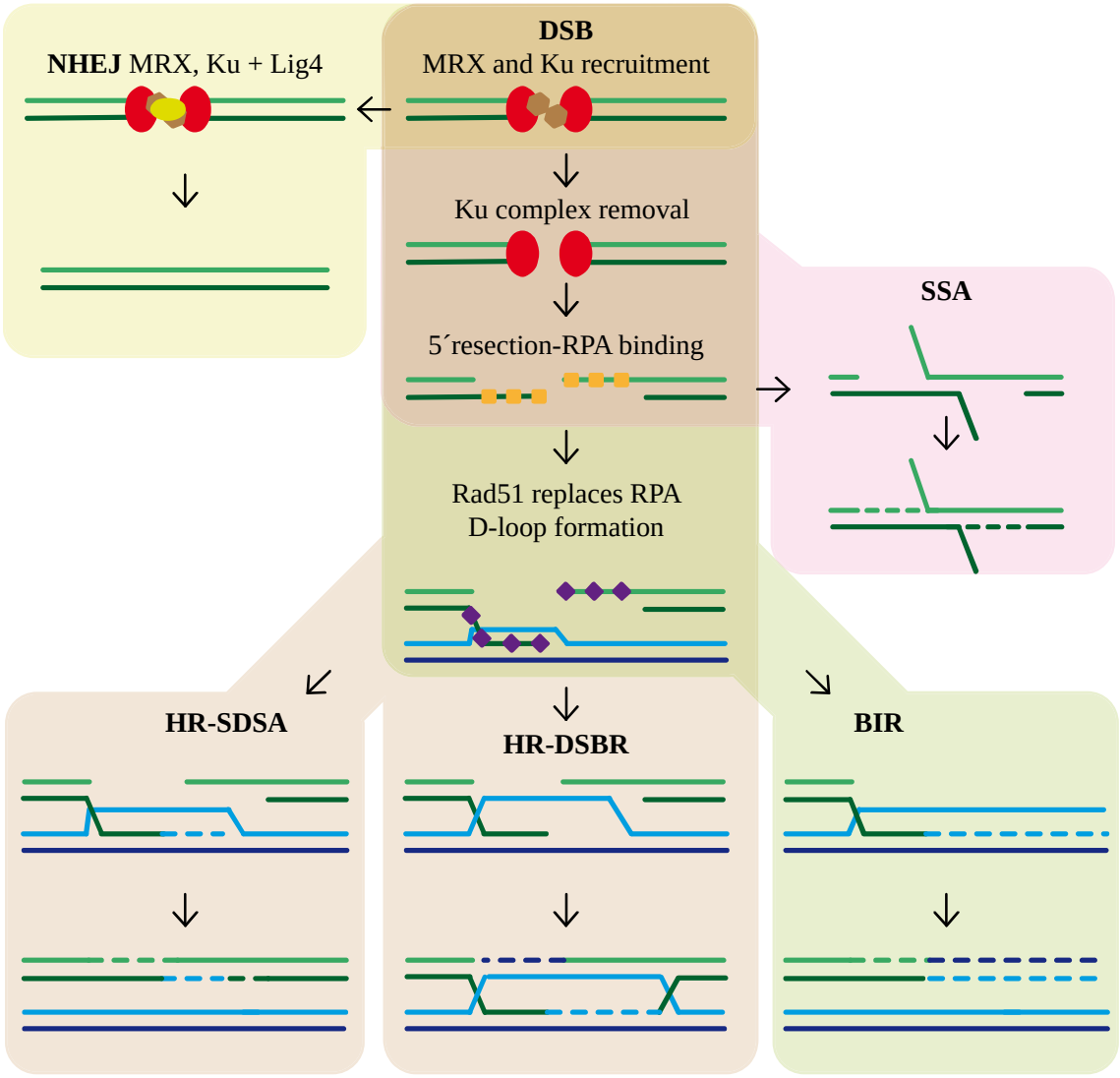

Figure I1 Double strand break repair

Double strand break (DSB) can be repaired by non-homologous end joining (NHEJ), homologous recombination (HR), single-strand annealing (SSA) or break-induced replication (BIR). In NHEJ, processed ends are joined by ligation. HR repair is initiated by 5′ to 3′ resection at the DSB. If the DSB occurs between direct repeats, annealing of the direct repeats may follow resection resulting in SSA repair. Alternatively, the resected 3′ overhang (green) may invade an homologous template (blue) to initiate repair synthesis. In synthesis-dependent strand annealing (SDSA) the newly synthesized strand (dashed blue) anneals back to the other end of the broken DNA (green). In double strand break repair (DSBR), the second strand of the DSB (green) anneals with the displaced strand of the donor DNA (blue), repair synthesis takes place, and then the newly synthesized strands are ligated to form a double Holliday junction (dHJ). Depending on how the dHJ is cleaved, resolution can result in a crossover or a non-crossover.

2. - Transcription associated genome instability

Transcription is the initial step in gene expression, in which genomic information is converted into RNA by RNAP. The transcription process can be divided in initiation, elongation and termination. During transcription, the DNA strands transiently separate from each other to allow RNA synthesis, generating a transcription bubble that contains the transcribed DNA strand (TS), approximately 9 ribonucleotides from the nascent messenger RNA (mRNA), and the non transcribed strand (NTS), which remains single-stranded (Aguilera and Gómez-González 2008; Gaillard, Herrera-Moyano et al. 2013). Synthesis of the RNA molecule during eukaryotic gene expression by RNAPII is coupled with maturation processes of pre-mRNAs that include 5´-capping, splicing, 3´-cleavage and polyadenylation, as well as the binding of different RNA-binding proteins (RBP), which together form the messenger ribonucleoprotein particle (mRNP). Different protein complexes that act all along the path from the transcription site to the nuclear pore complex (NPC) enable the coordination of mRNP biogenesis and export (Perales and Bentley 2009).

Transcription of a DNA sequence enhances its propensity to suffer mutations, in particular on the NTS, a process referred to as transcription-associated mutation (TAM). First evidence of TAR was obtained in *Escherichia coli* in which transcription activity was shown to modify the mutation rate within the lactose operon (Herman and Dworkin 1971). Later work in *S cerevisiae* using an inducible promoter to control transcription demonstrated that the rate of mutations is increased (35- fold) when transcription is induced (Datta and Jinks-Robertson 1995). Similarly, increased transcription levels of a gene encoding the green fluorescence protein (GFP) from the repressible *tet* promoter caused an increased mutation rate in human cells (Bachl, Carlson et al. 2001). Altogether, this data shows that transcription of DNA leads to an increase in the rate of mutations, compromising genomic stability. On the other hand, transcription also leads to increased recombination frequencies, a process referred to as transcription-associated recombination (TAR). First evidence of TAR were provided by λ phage recombination studies in *E. coli* (Ikeda and Matsumoto 1979). TAR is also observed in *S. cerevisiae*, in which transcription by RNAPI, RNAPII and RNAPIII were shown to lead to hyper-recombination (Thomas and Rothstein 1989; Huang and Keil 1995; Pratt-Hyatt, Kapadia et al. 2006). In addition, TAR has been described in higher

eukaryotes (Nickoloff and Reynolds 1990). Altogether, TAM and TAR constitute two types of genome instability that depend on active transcription and are referred to as transcription-associated genome instability (TAGIN). Several conditions associated with transcription such as topological changes associated with the advance of the transcriptional machinery, the formation of R loops, the correct formation of the export-competent mRNP or conflicts with the replication machinery may lead to TAGIN.

2.1.- Transcription-associated topological changes

During transcription, positive and negative supercoils accumulate ahead and behind the advancing RNAP, respectively. Topoisomerases 1 (Top1) and 2 (Top2) are needed to resolve the negative and positive supercoils. The action of topoisomerases is particularly important at highly transcribed loci, such as the rDNA cluster. Indeed topoisomerase mutants show higher recombination frequencies at the rDNA (Christman, Dietrich et al. 1988) and the accumulation of DNA supercoils leads to transcription blockage at the 18S rDNA (El Hage, French et al. 2010). A similar situation has been observed at convergent transcription units, which accumulate positive supercoils and show transcription defects as well as recombination increases in the absence of Top1 and Top2 (García-Rubio and Aguilera 2012). On the other hand, during transcription elongation negative supercoils lead to DNA double helix opening, generating ssDNA. ssDNA is more susceptible to be damage than dsDNA, and spontaneous deamination, which converts dCTPs into dUTPs, occurs more frequently in ssDNA, thus implying that DNA unwinding sensitizes to deamination (Frederico, Kunkel et al. 1990). Transcription also increases recombination induced by treatment with 4-nitroquinoline 1-oxide (4-NQO) and methyl methanesulfonate (MMS) DNA damaging agents supporting the idea that transcribed DNA is more susceptible to be damaged as a consequence of the occurrence of negative DNA supercoiling and ssDNA stretches (García-Rubio, Huertas et al. 2003). Another consequence of DNA supercoils is that it favours non-canonical DNA conformation such as parallel four-stranded G quartets (G-quadruplex) (Duquette, Handa et al. 2004). These structures stabilize the ssDNA hindering the restoration of the dsDNA. The resolution of negative supercoils by Top1 avoid the folding of the G-quadruplex in the NTS (Yadav, Owiti et al. 2016).

Altogether, the action of topoisomerases thus plays a role in genome stability maintenance.

2.2. R loops

The accumulation of negative supercoils in transcribed genes favours the hybridization of the nascent mRNA with the DNA template behind the RNAP, forming a DNA-RNA hybrid and a displaced ssDNA, a structure called R loop (Figure I2). Beside negative supercoiling, another condition favouring R loop formation is the DNA sequence itself, as a high guanine density in the displaced ssDNA strand promotes R-loop formation (Roy and Lieber 2009). The presence of DNA nicks may also promote the mRNA re-hybridization into the DNA (Roy, Zhang et al. 2010). DNA:RNA hybrids are more stable than dsDNA (Roberts and Crothers 1992) and are naturally formed during transcription and replication, short hybrid sequences (8-11 nucleotides) being necessary intermediates inside the transcription bubbles and during replication. R loops can occur naturally as a physiologically relevant intermediate in specific processes such as *E. coli* plasmid replication, transcription regulation, mitochondrial DNA replication, class switch recombination of immunoglobulin genes or telomere homeostasis. However, in some circumstances, R loops can be generated as transcriptional by-products that endanger the genome stability, generating replication problems and chromatin alterations (Aguilera and García-Muse 2012; Bhatia, Herrera-Moyano et al. 2017).

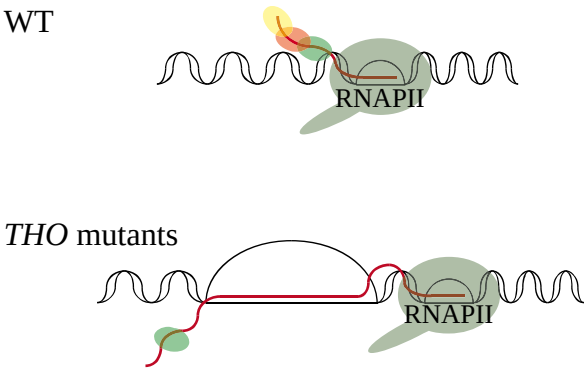

Figure I2 R loop
An R loop is a three-strand nucleic acid structure formed by an DNA:RNA hybrid plus a displaced DNA strand (ssDNA). In wild type cells (WT) the mRNA is co-transcriptionally package into an mRNP. In the absence of the THO complex, which is involved in the mRNP formation, the mRNA not properly packaged may lead to R loop formation.

2.2.1. - mRNP biogenesis and R loop-dependent TAR

During transcription by RNAPII, the nascent mRNA is coated by different RBP. The binding of the RBPs and the processing of the nascent mRNA lead to the formation of the mRNP and its co-transcriptional export to the cytoplasm (Köhler and Hurt 2007). Several factors play an important role in this process, such as the THO complex, an evolutionary conserved complex composed of the Tho2, Hpr1, Mft1, Thp2 and Tex1 proteins in yeast (Chávez, Beilharz et al. 2000; Peña, Gewartowski et al. 2012). Mutations in THO components lead to a transcription elongation impairment and mRNA export defects. Moreover, deletions of these proteins confer transcription-dependent hyper-recombination in *S. cerevisiae* (Prado, Piruat et al. 1997; Chávez, Beilharz et al. 2000; Jimeno, Rondon et al. 2002; Rondon, Jimeno et al. 2003). The first evidence that TAR may be dependent on the accumulation of co-transcriptional R loops was obtained from studies of yeast THO mutants. TAGIN observed in these mutants is suppressed by the over-expression of RNase H1, an enzyme which removes the RNA moiety from the DNA:RNA hybrid, supporting the idea that co-transcriptional R loops accumulate when mRNP biogenesis is deficient (Huertas and Aguilera 2003). R loop-dependent TAR has also been shown in *Caenorhabditis elegans* THO mutants and THO-depleted human cells (Domínguez-Sánchez, Barroso et al. 2011; Castellano-Pozo, Santos-Pereira et al. 2013).

On the other hand, the THO complex can interact with the RNA helicases Sub2 and the RBP Yra1. Both proteins are conserved in human as UAP56 and ALY, respectively. These proteins bound to the THO complex constitute the TREX complex (transcription-export). The TREX complex is specifically recruited to transcribed genes, travelling with the polymerase during transcription and binds to the nascent mRNA during elongation (Sträßer, Masuda et al. 2002). Deletion of TREX components confer similar phenotypes of transcription impairment (Jimeno, Rondon et al. 2002; Rondon, Jimeno et al. 2003), mRNA export defects (Zenklusen, Vinciguerra et al. 2002) and TAR than THO mutants.

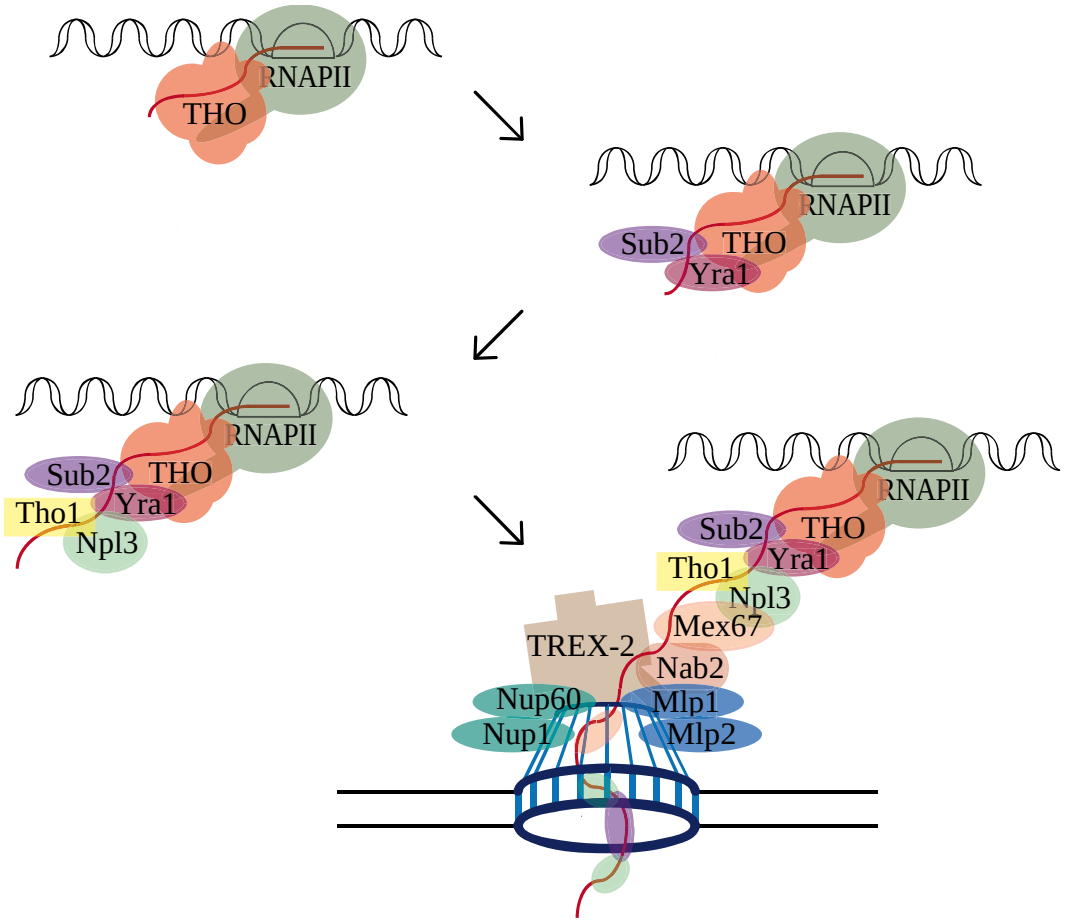

Figure I3 mRNP biogenesis
During transcription elongation, the THO complex is recruited at transcribed genes, facilitating the binding of Sub2 and Yra1. Npl3 interacts with the RNAPII and it is loaded on the mRNA. Mex67 is also recruited by interaction with THO and/or Npl3. The TREX2 complex is associated with the nuclear pore complex (NPC) by interaction with Nup1. Nab2 is loaded on the mRNP and interacts with Mlp1 at the NPC basket.

In addition to the TREX complex, different RBPs such as Tho1, Nab2 or Npl3 are recruited to mRNAs at the early stages of the transcription-export pathway (Luna, Gaillard et al. 2008). Similarly to other mRNP biogenesis factors, mutations of Npl3 and Nab2, but not Tho1, show TAR phenotypes (Piruat and Aguilera 1998; Gallardo, Luna et al. 2003; Santos-Pereira, Herrero et al. 2013). Although over-expression of Sub2 or Tho1 rescues the genome instability phenotype observed in *hpr1Δ* mutants (Jimeno, Luna et al. 2006), over-expression of Npl3 and Yra1 leads to hyper-recombination in wild type cells (Santos-Pereira, Herrero et al. 2013; Gavaldá, Santos-Pereira et al. 2016). Altogether, this data suggest that a proper stoichiometry of these proteins is important to maintain genome stability. In addition to THO, mutations in several RNA metabolism proteins have been involved in R loop formation. Thus,

RNase H1-mediated rescue of hyper-recombination has been shown for mutations of yeast Sub2, (González-Aguilera, Tous et al. 2008) or the RBP Npl3 (Santos-Pereira, Herrero et al. 2013). Deletion of the human 5´-3´ exoribonuclease XRN2 leads to R loop-dependent DSBs and genome instability (Morales, Richard et al. 2016). The correct splicing of the nascent mRNA is also important to avoid R loop-dependent genome instability, as seen by depletion of the ASF/SF2 splicing factor (Li and Manley 2005). Top1-mediated recruitment of the ASF/SF2 splicing factor in the mRNP avoids the formation of R-loop (Tuduri, Crabbe et al. 2009). Additionally, the spliceosome U2 snRNP factor has also been related to the prevention of R loop-dependent genome instability phenotypes (Tanikawa, Sanjiv et al. 2016). In agreement with the co-transcriptional R loop accumulation observed in THO mutants and several other RNA metabolism factors, different genome-wide screenings in yeast and human cells have revealed that a growing number of factors working at different stages of mRNP biogenesis and export function in preventing R loop formation and maintaining genome integrity (Paulsen, Soni et al. 2009; Wahba, Amon et al. 2011; Stirling, Chan et al. 2012). Altogether, these observations support that an accurate formation of the mRNP is necessary to prevent the R loop-dependent genome instability.

2.2.2.- R loops and chromatin

Histones are subjected to many different post-translational modifications such as methylation, acetylation and phosphorylation, which participate in the regulation of all DNA template processes and chromatin compaction. Genome wide analysis in human cells has shown that histone H3 methylation (H3K14me1, H3K14me2 and H3K36me3) and histone H3 (H3K9 and H3K27) acetylation are significantly enriched over R loop containing regions (Sanz, Hartono et al. 2016). High levels of histone H3 acetylation facilitate R-loop accumulation as observed in human cells after treatment with trichostatin A (TSA) or suberoylanilide hydroxamic acid, two different histone de-acetylase inhibitors. Moreover, depletion of the THO complex or the Sin3A histone de-acetylase complex leads to hyper-acetylated opened DNA that facilitates the formation of R loops. THO and Sin3A physically interacts, suggesting that THO prevents the formation of R loops by ensuring optimal mRNP biogenesis and by interacting with

Sin3A, therewith promoting histone de-acetylation and chromatin closing after transcription (Salas-Armenteros, Pérez-Calero et al. 2017).

Heterochromatin at centromeric region in *Schizosaccharomyces pombe* is mediated by DNA:RNA hybrids formed by the association of ncRNAs with chromatin. This association induces the transcriptional silencing complex, which involves the accumulation of the H3K9me2, heterochromatin mark (Nakama, Kawakami et al. 2012). This H3 di-methylation is also observed in *C. elegans* THO mutants. In addition to H3K9me2, the absence of *C. elegans* THO complex leads to pathological R loop accumulation, which triggers the phosphorylation of histones H3 at serine10 (H3-ser10P), a mark of chromatin condensation. A similar correlation between H3-ser10P and R loops has also been observed in yeast and human cells (Castellano-Pozo, Santos-Pereira et al. 2013). This data led to the proposal that R loops trigger local chromatin condensation, which in turn represent an obstacle to the RF progression and leads to genome instability. Importantly, not all mutations that lead to an increase in R loops formation trigger the accumulation of H3-ser10P. Mutations in several yeast histone residues lead to the accumulation of non-pathological R loops. H3K9-14-28-23A, H3Δ1-28 or H4K31Q mutants show no transcription or replication defects, moreover no H3-ser10P accumulation are observed, in contrast to other mutants such as *hpr1Δ*, in which R loop-dependent genome instability and H3-ser10P accumulation are observed. These results suggest that R loops by themselves are not always a threat to genome integrity, and that H3-ser10P enrichment is necessary for R loop-dependent DNA breaks (García-Pichardo, Cañas et al. 2017).

2.2.3. - Enzymatic activities that target R loops structures

A number of enzymatic activities can act on R loops, targeting either the displaced ssDNA strand or the DNA:RNA hybrid. The activities acting on the displaced ssDNA such as nucleases, deaminase or other base modifying enzymes have the potential to increase the impact of R loops on TAGIN, as their action generates DNA damage at such R loop structures. Activities acting on the DNA:RNA hybrid, however, comprise ribonucleases and helicase activities that contribute to R loop removal and therewith prevent the accumulation of pathological R loops.

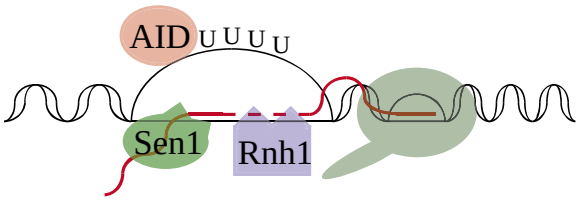

Figure I4 Enzymatic activities that target R loops
The ssDNA within an R loop is a substrate for enzymatic activities that target ssDNA, such as the human cytidine induced deaminase (AID) that convert cytidine into uracil. Helicases such as Sen1 play a role in unwinding DNA:RNA hybrids. RNase H1 (Rnh1) specifically degrades the RNA moiety from the DNA:RNA hybrid.

AID

The human activation-induced cytidine deaminase (AID) is a protein that is specifically expressed in B lymphocytes**.** AID converts dCTPs into dUTPs on the displaced ssDNA but not on dsDNA substrates (Bransteitter, Pham et al. 2003; Chaudhuri, Tian et al. 2003). Transcription through the B cells variable S regions promotes R loop formation thereby exposing stretches of ssDNA on the NTS, providing a substrate for AID (Yu, Chedin et al. 2003). AID is necessary for somatic hypermutation and class switching recombination and thus playing a central role in antibody diversification in B cells (Muramatsu, Kinoshita et al. 2000). Physical interaction with RPA, which binds at ssDNA, enables the recruitment of AID to co-transcriptional R-loops (Chaudhuri, Khuong et al. 2004). Ectopic expressed AID also interacts directly with the elongation complex in *E. coli*, suggesting the transcriptional machinery might recruit AID (Besmer, Market et al. 2006).

dUTPs within the DNA is excised by uracil DNA glycosylase leaving an abasic site, which can be recognized by an apurinic/apyrimidic endonuclease that nicks the DNA (Petersen-Mahrt, Harris et al. 2002; Guikema, Linehan et al. 2007). DSBs are formed when these nicks occur in close proximity on opposite DNA strands. Additionally DNA resection by Exo1 during MMR might generate a DSB from two separated dUTPs (Ehrenstein and Neuberger 1999; Stavnezer, Guikema et al. 2009; Fear 2013). Erroneous DSBs repair can lead to be resolved as chromosomal translocations, providing a rationale for the predisposition of B cells to undergo

tumorigenesis processes. DSBs induced by AID have been shown to be responsible for translocations between *c-myc* and the Ig variable region (Ramiro, Jankovic et al. 2004; Dorsett, Robbiani et al. 2007). AID expression in *S. cerevisiae* bearing heterologous S region and *c-myc* transcribed sequences also leads translocations between both regions (Ruiz, Gómez-González et al. 2011). Moreover, such AID-mediated translocations are enhanced in THO mutants. Using a recombination system as a reporter, AID over-expression was shown to increase recombination in R loop-accumulating yeast strains such as the THO mutants (Gómez-González and Aguilera 2007), indicating that AID expression can be used as a tool to detect R loops.

Ribonucleases

The main enzymatic activities preventing R loop accumulation is the RNase H1 that breaks down the RNA moiety of DNA:RNA hybrids. This protein has two different domains, which are conserved from yeast to human. The N-terminal domain recognizes the DNA:RNA hybrids and is called hybrid binding domain (HBD). The C-terminal domain contains the catalytic subunit. Although this protein is conserved from yeast to human, some important differences exist across species. For example, both domains are linked by a variable region. Notably, human RNase H1 has one N-terminal domain, however, two copies of the domain are present in *S. cerevisiae* and *C. elegans* RNase H1, both copies being necessary to bind the DNA:RNA hybrid. Double stranded RNA (dsRNA) is also recognized by RNase H1, although with a lower efficiency (Cerritelli and Crouch 1995; Evans and Bycroft 1999; Nowotny, Cerritelli et al. 2008).

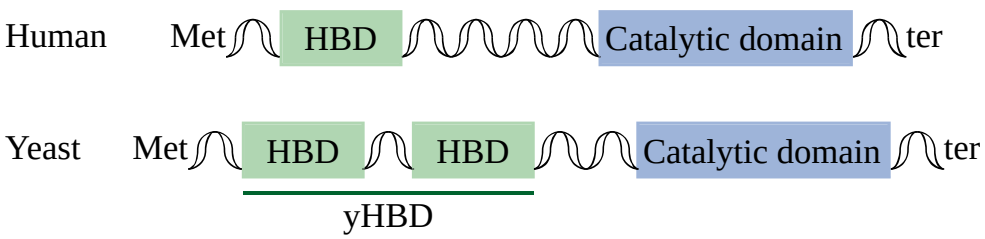

Figure I5 Structure of human and yeast RNase H1
Human RNase H1 contains two domains, the hybrid-binding domain (HBD) and the catalytic domain. Yeast RNase H1 presents a similar structure but it possesses two HBD domains that share homologies to human HBD. The HBD and the catalytic domains are separated by a variable region in both species.

Helicases

R loops can also be removed by the action of some DNA:RNA helicases, such as yeast Sen1 and its human homologue SETX. This helicase is involved both in the unwinding of co-transcriptional DNA:RNA hybrids formed during pause-dependent transcription termination and in favouring RF progression through RNAPII-transcribed regions (Mischo, Gomez-Gonzalez et al. 2011; Skourti-Stathaki, Proudfoot et al. 2011; Alzu, Bermejo et al. 2012). Other helicases involved in R loop unwinding are the human DDX21 and DDX23 helicases, which are recruited to DNA:RNA hybrids throughout the gene body during transcription elongation (Song, Hotz-Wagenblatt et al. 2017; Sridhara, Carvalho et al. 2017). Human DDX19, which is relocated to the nucleus under replication stress via ATR, was also shown to unwind DNA:RNA hybrids (Hodroj, Recolin et al. 2017). Other helicases such as human RNA helicase Aquarius (AQR) (Sollier, Stork et al. 2014), yeast DNA helicase Sgs1 and its human homolog BML (Chang, Novoa et al. 2017) or human DHX9 as some examples of helicases involved in DNA:RNA unwinding. DHX9 was also shown to be important for the unwinding of DNA-bases at G-quadruplex (Chakraborty and Grosse 2011). The yeast helicase Pif1 is another example related with G-quadruplex of an helicases able to unwind DNA:RNA hybrids and to favour RF progression (Tran, Pohl et al. 2017).

2.3.- Transcription-replication conflicts

The replication and transcription machineries share the same template, the DNA; therefore interference between both processes may occur. Transcription-replication conflicts are an important source of genome instability as they may cause an increase in RF stalling and DNA breaks (Mirkin and Mirkin 2007). Depending on the orientation of transcription with respect to the direction of replication, transcription-replication conflicts can be co-directional or in head-on orientation. Collisions between replication and transcription were first studied in *E. coli*, with the conclusion that RF movement rates are not affected by co-directional transcription but are reduced in the case of head-on encounters (French 1992).

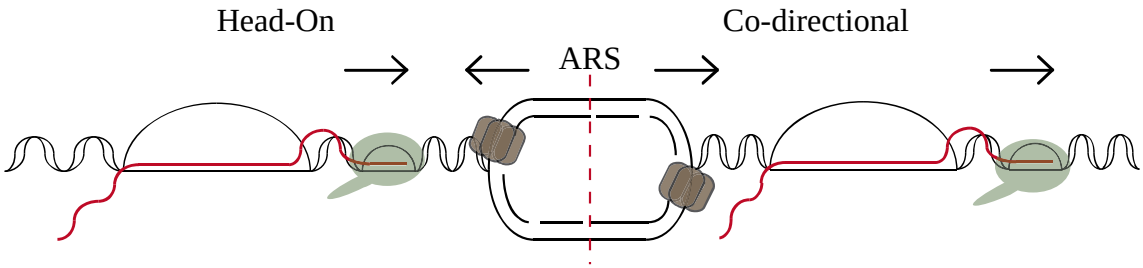

Figure I6 Transcription-Replication Conflicts
Replication machineries advancing from a bi-directional replication origin lead to conflicts with the transcription machinery at active genes. Depending on the orientation of transcription with respect to the direction of replication, transcription-replication conflicts can be co-directional or in head-on orientation.

Transcription of highly expressed genes such as those encoding rRNAs, which are organized in repeats at the rDNA locus in yeast, may constitute a serious obstacle to RF progression. To prevent these conflicts and their possible consequences, a specific region called replication fork barrier (RFB) is present in the rDNA repeat and blocks RF progression upstream of transcribed regions (Brewer, Lockshon et al. 1992). This RF blockage is achieved through binding of the Fob1 protein to the RFB and prevents head-on collisions between RNA and DNA polymerases at the rDNA locus (Kobayashi 2003). However, transcription-replication conflicts are not restricted to the rDNA. Using different plasmid systems, in which transcription is orientated either in co-directional or in head-on orientation from the replication origin, it has been observed that transcription convergent to replication leads to a replication pause site (Prado and Aguilera 2005). Furthermore, an increase in recombination was observed in these conditions, indicating that transcription-mediated replication impairment may be responsible for TAR. Subsequently, transcription of the *lacZ* gene was also shown to lead to replication impairment in wild type cells. Moreover this replication impairment at the transcribed *lacZ* sequence was exacerbated in the absence of the THO component Hpr1 and was partially rescued by cleavage of the nascent mRNA, suggesting that the presence of R loops is the cause of the observed replication impairments (Wellinger, Prado et al. 2006). Consistently R loop-formation at an immunoglobulin S region, cloned in a plasmid in both orientations, caused RF stalling and chromosomal rearrangements (Gan, Guan et al. 2011), indicating that co-transcriptional R-loop formation is a conserved threat for replication.

Several mechanisms have been described that help the advance of RF through transcribed sequences. For example, the Rrm3 helicase is involved in the progression of RF through non-nucleosomal protein-DNA complexes (Ivessa, Lenzmeier et al. 2003). Moreover, an accumulation of Rrm3 has been observed at highly transcribed genes during S phase, supporting the idea that transcription is a obstacle to the RF progression (Azvolinsky, Giresi et al. 2009). In the same line Rrm3 over-recruitment has been observed in transcribed genes in THO mutants, which was suppressed by RNase H1 over-expression (Gómez-González, García-Rubio et al. 2011). On the other hand, replication stress caused by treatment with hydroxyurea (HU), which inhibits the ribonucleotides reductase causing dNTP depletion and slows down replication, trigger RNAPII release from transcribed genes near firing origins of replication. This removal depends on Mec1, the chromatin remodelling complex Ino80 and the RNAPII-associated complex PAF1 (Poli, Gerhold et al. 2016). Furthermore, RNAPII can reduce the effects of these conflicts on genome integrity facilitating its release from chromatin after a putative collision with the RF and preventing RF collapse (Felipe-Abrio, Lafuente-Barquero et al. 2015), supporting the idea that the transcription machinery is an obstacle to RF progression which has to be overcomed to complete correctly the replication of DNA.

In addition, different chromatin remodelling factors such as the FACT complex whose deletion leads to R loop accumulation, may prevent conflicts assisting RF progression at transcribed regions and is necessary for replication through R loop-containing region (Herrera-Moyano, Mergui et al. 2014). Similarly, Fanconi anemia factors, BRCA1, BRCA2 and FANCD2 are critical for RF progression on R loop-containing DNA template. BRCA2 and FANCD2 have been proposed to bind to R loop-containing regions where there is a replication problem and to promote replication restart thus preventing R loop-dependent DNA damage (Bhatia, Barroso et al. 2014; Hill, Rolland et al. 2014; García-Rubio, Pérez-Calero et al. 2015; Schwab, Nieminuszczy et al. 2015). In addition, FANCM, may lead to the resolution of DNA:RNA hybrids and replication blocks, and its yeast homologue Mph1 also plays a crucial role in DNA:RNA hybrid resolution (Schwab, Nieminuszczy et al. 2015; Lafuente-Barquero, Luke-Glaser et al. 2017). The replicative helicase MCM has been also been related with R loop formation and RF progression. Mutation of Mcm2 shows R loop accumulation only in S phase, suggesting that replication defects in this mutants

lead to R loop accumulation (Vijayraghavan, Tsai et al. 2016). In addition, a number of interactions between replisome-associated proteins and different factors that remove R loops have been described. For example, the recruitment of BRCA1 to R loops mediates the recruitment of Senataxin (Hatchi, Skourti-Stathaki et al. 2015) while RPA contribute to the recruitment of RNase H1 (Nguyen, Yadav et al. 2017). Thus different pathways involving different replisome-associated proteins participate in R loop removal to facilitate RF progression and avoid TAR.

In conclusion, the capacity of R loops to stall RF progression may be a major cause of DNA breaks, therefore the coordination between the replication and transcription machineries and the ability of RF to replicate correctly the DNA through transcribed genes and R loops is a guarantee to preserve the stability of the genome.

3.- The Nuclear Pore Complex

The nuclear pore complex (NPC) is a macromolecular structure embedded in the nuclear membrane that is composed of a set of approximately 30 different proteins called nucleoporins, most of which are conserved from yeast to human. The NPC is composed of two octameric symmetric outer rings, one on the cytoplasmatic side and the other one on the nuclear side of the nuclear envelope. Both rings are connected by linker nucleoproteins and a third ring that allows anchoring to the nuclear membrane. In addition, phenylalanine-glycine repeats-rich FG nucleoporins are located in the NPC central channel. These FG nucleoporins form a selective barrier and regulate nucleocytoplasmatic transport. Different filament nucleoporins, such as Nup82 and Nup159 that binds different mRNA binding factors, are anchored to the symmetric core of the NPC on the cytoplasmic side. A nuclear basket structure that is composed of Nup1, Nup2, Nup60, Mlp1 and Mlp2 (NUP50, NUP153 and TPR in human cells) is located on the nuclear side of the NPC. These asymmetrically located nucleoporins are key components in establishing the directionality of the nucleocytoplasmic transport (Alber, Dokudovskaya et al. 2007; Hoelz, Debler et al. 2011; Beck and Hurt 2017; Sakiyama, Panatala et al. 2017). In human cells, a nuclear scaffold called lamina is located on the nucleoplasmic side of the nuclear envelope. This lamina is formed of a net of proteins covering the nuclear envelope and acts as a bridge between different pores, whose primary function is to assist the re-assembly of the nucleus after mitosis. No lamina is found in *S. cerevisiae*, in which the nuclear envelope is not disrupted during mitosis. However, several lamina-associated proteins are conserved from yeast to human, and locate at the nuclear envelope in yeast, such as the nuclear envelope protein Mps3 (Taddei and Gasser 2012).

NPC has functions beyond the control of nucleo-cytoplasmic transport, such as chromosome organization, transcription and DNA repair. NPCs coincide with heterochromatin exclusion areas suggesting that the NPCs and nuclear envelope-associated proteins play a determinant role in chromatin organization. In agreement with this idea, yeast centromeres are clustered together at the nuclear periphery-associated spindle pole body, while chromosome arms are located in the nucleoplasm and telomeres anchored at three to six nuclear envelope-associated locations. In addition, the yeast nucleolus, which contains the single rDNA locus located on chromosome XII, is

located close to the nuclear envelope and opposite to the spindle pole body. The location of the human nucleolus with respect to the nuclear envelope is not as well defined as in yeast. In human cells, nucleoporins are not spatially restricted to the NPC and rDNA is located at several chromosomes (Vaquerizas, Suyama et al. 2010; Gay and Foiani 2015; Ibarra and Hetzer 2015; Beck and Hurt 2017).

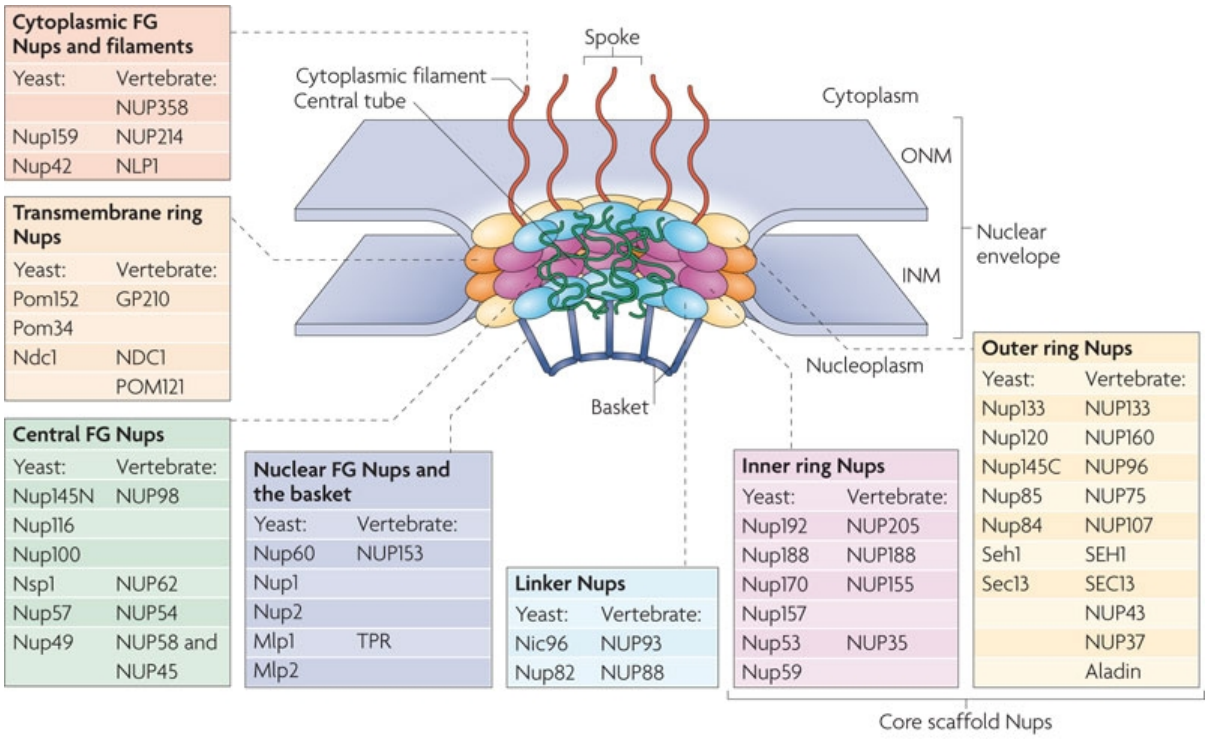

Figure I7 Nuclear pore complex
Each NPC is a cylindrical structure comprised of eight spokes surrounding a central tube that connects the nucleoplasm and cytoplasm. The NPC is anchored to the nuclear envelope by a trans-membrane ring structure. Linker nucleoporins (Nups) help to anchor the Phe-Gly (FG) Nups which fill the central tube. NPC-associated peripheral structures consist of cytoplasmic filaments, the basket and a distal ring. The Nups that are known to constitute each NPC sub-structure are listed. Both inner and outer ring Nups are known to form biochemically stable NPC sub-complexes, which are thought to have a role in NPC biogenesis and nuclear envelope assembly. Figure taken from (Strambio-De-Castillia, Niepel et al. 2010).

The NPC complex also plays a role in mRNA biogenesis. Concomitant with transcription, the mRNA is exported to the cytoplasm. To facilitate the export, the RBP Yra1, Nab2 and Npl3 mediate the interaction between the export receptor Mex67-Mrs2 and the mRNP. The mRNA is transferred to the Mex67-Mtr2, which interacts with FG repeat-containing nucleoporins, essential for mRNA translocation through the NPC

(Köhler and Hurt 2007; Luna, Gaillard et al. 2008; Iglesias, Tutucci et al. 2010). Additionally to Mex67-Mtr2, the TREX2 complex that is composed of Sac3, Thp1, Sus1, Cdc31 and Sem1 is also involved in the export of the mRNA (Luna, Gaillard et al. 2008; Faza, Kemmler et al. 2009). This complex integrates earlier steps in the gene expression pathway with the last one, the export of mature transcripts. Sus1 is also a component of the SAGA histone acetylase complex, which has a function in transcription initiation (Rodríguez-Navarro, Fischer et al. 2004), although the role of the TREX2 complex in transcription elongation was shown to be independent of SAGA (González-Aguilera, Tous et al. 2008). TREX2 localizes on the inner side of the nuclear envelope, binding to the NPC through the interaction between Sac3 and Nup1. In addition, Sac3 interacts with Mex67 to promote mRNP translocation through the NPC (Lei, Stern et al. 2003; Ellisdon, Dimitrova et al. 2012; Jani, Valkov et al. 2014). Others RNA binding proteins are important for RNA processing and export through the NPC. Nab2, which is functionally related with TREX2 complex, can bind to Mex67 and Mlp1 in the basket of the NPC playing a determinant role in the RNA export. Nab2 is necessary to protect the nascent mRNA against decay by the nuclear exosome (Gallardo, Luna et al. 2003; Fasken, Stewart et al. 2008; Iglesias, Tutucci et al. 2010; Schmid, Olszewski et al. 2015). Worthy of note that mutations of Mex67, Nab2 or the TREX2 components Thp1, Sac3, Sus1 or Sem1 proteins induce RNase H1-sensitive TAR (Gallardo and Aguilera 2001; Jimeno, Rondon et al. 2002; Gallardo, Luna et al. 2003; González-Aguilera, Tous et al. 2008; Faza, Kemmler et al. 2009), similarly to THO complex, suggesting that the correct formation and export of the mRNP is a key aspect in the genomic stability maintenance.

Transient localization of transcribed DNA in the proximity of the NPC has been proposed to serves as a scaffold to build and assemble the mRNP, coordinating transcription, processing, and export of mRNAs, this transient localization is referred as the gene gating hypothesis (Blobel 1985). This process can be important for inducible genes that require a rapid and high level of expression and efficient mRNA export. Indeed a handful of loci (*INO1*, *GAL* genes, *HSP104*, *HXK1*, *MFA2*, *TSA2* and *SUC2*) were shown to be recruited from the nucleoplasmic interior to the NPC upon transcriptional RNAPII activation in yeast (Brickner and Walter 2004; Casolari, Brown et al. 2004; Cabal, Genovesio et al. 2006; Dieppois, Iglesias et al. 2006; Schmid, Arib et al. 2006; Taddei, Van Houwe et al. 2006; Sarma, Haley et al. 2007). In addition to

RNAPII inducible genes, *tRNA* genes transcribed by RNA polymerase III have been shown to re-localize to the NPC as a consequence of their transcriptional increase in M phase (Chen and Gartenberg 2014). Several factors with functions in mRNP biogenesis and export, such as Mex67 (Dieppois, Iglesias et al. 2006), TREX-2 (Drubin, Garakani et al. 2006), SAGA (Cabal, Genovesio et al. 2006; Luthra, Kerr et al. 2007), some nucleoporins and the nuclear basket proteins Mlp1/2 have been shown to be required for efficient gene gating, helping the preferential processing and export of mRNA (Dieppois, Iglesias et al. 2006). In some cases gating at the NPC seems to rely on specific DNA sequences. Upstream of the *GAL1* genes there is an upstream activating sequences (UAS) involved in *GAL1* transcription activation. When cells are grown in the absence of glucose and presence of galactose, SAGA is recruited at the UAS. This interaction leads to SAGA-dependent recruitment of Mlp1 to the UAS, showing that UAS is necessary for the physical association with Mlp1 at the NPC (Luthra, Kerr et al. 2007). Interestingly, two sequences that target the *INO1* and *TSA2* genes to the nuclear periphery have been identified, one at the promoter and the other are upstream of the gene. Mutations of both sequences abolish the nuclear periphery location of these genes and impair their transcription. However, introduction of these sequences at the *GAL1* gene have no effect on its activation (Ahmed, Brickner et al. 2010). In addition, another sequence has been described at the *HSP104* which is necessary to target this gene to the nuclear periphery. Moreover introduction of the recruitment sequences of *INO1* or *HSP104* at the *URA3* gene, leads to recruitment of *URA3* at the same location of this respective introduced sequences gene (Brickner, Ahmed et al. 2012) supporting an additional level of genetic information that controls the spatial gene organization and expression.

3.1.- Mlp1/Mlp2/TPR

The yeast myosin-like protein 1 and 2 (Mlp1 and Mlp2) and their human ortholog ‘translocated promoter region’ (TPR) are major structural components of the nuclear pore basket that form fibers anchored at the NPC that protrude toward the nucleoplasm (Cordes, Reidenbach et al. 1997; Strambio-de-Castillia, Blobel et al. 1999). Mlp1/2 might form an extended interacting network radiating from the basket and interlinking neighbouring NPCs suggesting that these basket projections might play

the role of the eukaryotic lamina in yeast and being the docking site to spindle pole body organizer, silencing factors, the proteasome, and components of mRNP (Niepel, Molloy et al. 2013). The N-terminal domain of Nab2 mediates physical interactions with the C-terminal domain of Mlp1 (Green, Johnson et al. 2003; Grant, Marshall et al. 2008). Mlp1 physically interacts with Esc1 and is necessary for the correct Esc1 localization at the NPC (Niepel, Molloy et al. 2013). Mlp1 is also found to interact with Sub2 (Sträßer, Masuda et al. 2002) and Yra1, the TREX-2 components Sac3, Thp1 and Cdc31, the Mex67 export receptor (Niepel, Molloy et al. 2013) and the Npl3 RBP (Green, Johnson et al. 2003). These physical interactions between mRNP and Mlp1/2 proteins allows the preferential NPC association with highly transcribed genes in an RNA-dependent manner contributing to gene NPC anchoring by interacting with nascent transcripts (Casolari, Brown et al. 2004; Casolari, Brown et al. 2005; Vinciguerra, Iglesias et al. 2005; Niepel, Molloy et al. 2013). Interestingly, Mlp2 binds directly to spindle pole body components Spc110, Spc42 and Spc29 (Niepel, Strambio-de-Castillia et al. 2005).

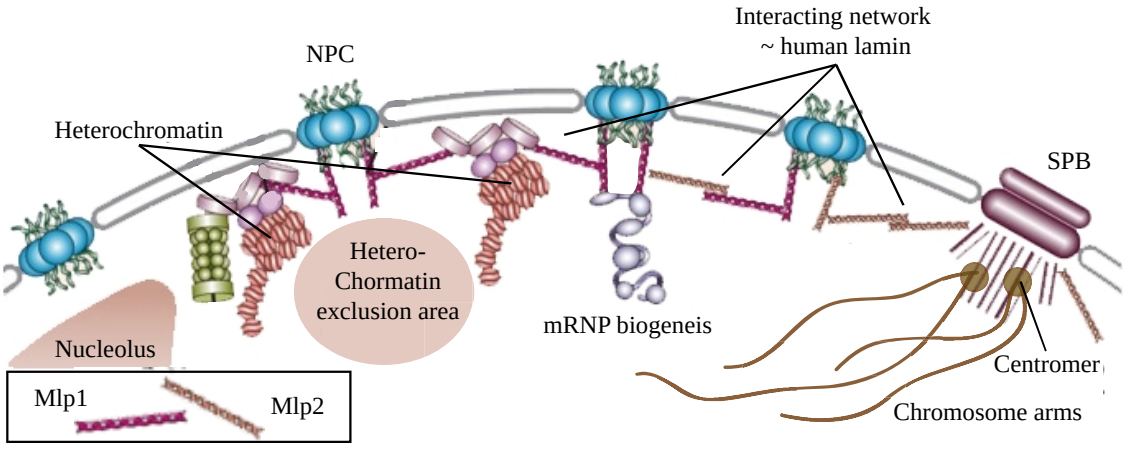

Figure I8 Mlp proteins form the nuclear basket and an interaction network underlying the nuclear envelope.
Mlp1 and Mlp2 assemble into coiled-coil dimers that form the nuclear pore complex (NPC) basket and extend horizontally to link adjoining NPCs. The basket serves as a site for mRNP binding (presumably facilitating mRNA proofreading), keeps the area beneath the NPC central tube free from dense chromatin, and might aid in the organization of these structures around the NPC. Mlps underlying the nuclear envelope (NE) connect NPCs and the spindle pole body (SPB) into a network and physically support the structure of the nucleus. Esc1p is integrated into the network and anchors silenced telomeric DNA and the proteasome to the NE. Taken from (Niepel, Molloy et al. 2013).

Over-expression of Mlp1 or TPR block the export of polyadenylated mRNA from the nucleus in yeast and human cells, respectively (Bangs, Burke et al. 1998; Kosova, Pante et al. 2000; Galy, Gadal et al. 2004). On the other hand, yeast strains lacking Mlp1/2 proteins show mild or no export defects (Kosova, Pante et al. 2000; Powrie, Zenklusen et al. 2011). However, deletions of Mlp/TPR present a failure in retaining unprocessed or faulty mRNA in the nucleus (Galy, Gadal et al. 2004; Rajanala and Nandicoori 2012). In the retention of unprocessed mRNA, ubiquitination of Yra1 by Tom1 promotes the dissociation of Yra1 from the Nab2-bound mRNPs. Yra1 has to be removed from the mRNPs prior to its export to the cytoplasm. This release facilitates the binding of mRNPs to the NPC and their export, where Nab2 helps to target the mRNP complex to the nuclear pore via its interaction with Mlp1 (Iglesias, Tutucci et al. 2010). Additionally, des-phosphorylation of Npl3 in the nucleus promotes the interaction with the export receptor Mex67 (Gilbert and Guthrie 2004). Therefore, different modifications of RBPs such as Yra1, Nab2 or Npl3, modify the affinity of theses protein to the export receptor Mex67 and to Mlp1, enabling to recognize and retain aberrant mRNAs in the nucleus (Vinciguerra, Iglesias et al. 2005; Fasken, Stewart et al. 2008; Soheilypour and Mofrad 2016). Similar behaviours were observed in human cells (Saroufim, Bensidoun et al. 2015), indicating the participation of Mlp1/2 and TPR proteins in mRNPs quality control.

Mlp1 has been shown to be required for gene gating of the *GAL* locus and *HSP104* gene upon transcriptional activation (Dieppois, Iglesias et al. 2006). *GAL1* gene exhibits a transcriptional memory process of the previous transcriptional state. This memory confers the ability to re-induce faster *GAL1* transcription initiation and thus faster gene expression when re-induced following a short intervening period of repression (Kundu, Horn et al. 2007). Mlp1 is also necessary for the maintenance of transcriptional memory after transcription induction (Tan-Wong, Wijayatilake et al. 2009), supporting a role for Mlp1 in gene gating. A predicted consequence of the association of transcribed genes at the NPC is the possible increase of torsional stress at the concerned chromosomal loci. Indeed, the association to a fixed structure might impede the free rotation of the DNA, leading to the accumulation of positive supercoiling and therefore constituting a topological barrier to RF progression. During replication stress, the checkpoint activities of Mec1 and Rad53 are necessary to stabilize RF and promote RF restart. Deletion of Mlp1, which abolishes gene gating, was shown

to suppress the HU-sensitivity of Rad53-deficient cells (Bermejo, Capra et al. 2011). This data suggest that S-phase checkpoint activation is necessary to resolve topological barriers that can arise at sites where transcribed genes are associated to fixed nuclear envelope structures. Furthermore, Bermejo and colleagues have shown that Rad53-dependent Mlp1 phosphorylation takes place in such situations, leading to the release of the DNA from the NPC and replication resumption.

OBJECTIVES

The main goal of this thesis was to improve our understanding of the mechanisms underlying R loop-mediated genome instability. To this end, we have pursued the following objectives:

1.- Identification of new proteins involved in the control of R loop formation using AID expression as a tool to potentiate R loop-dependent recombination.

2.- Analyse the consequences of Mlp1 and/or Mlp2 mutations on R loop accumulation and genome stability.

3.- Gain new insights into the role of physical proximity of chromatin to the nuclear pore in preventing R loop-mediated genome instability.

4.- Explore the function of TPR in the maintenance of genome stability in human cell lines.

5.- Develop a new tool for the detection and quantification of R loops in *S. cerevisiae*.

RESULTS

DISCUSSION

CONCLUSIONS

MATERIALS AND METHODS

1.- Growth media and conditions

1.1- Bacteria culture media

Rich medium LB: 0.5% yeast extract, 1% bacto-tryptone, 1% NaCl. LB was supplemented with 100 µg/ml ampicillin when it was necessary for plasmid selection.

1.2.- Yeast culture media

Rich medium YPAD: 1% yeast extract, 2% bacto-peptone, 2% glucose, 20 mg/L adenine.

Minimum medium SD: 0.17% yeast nitrogen base (YNB) without amino acids nor ammonium sulphate, 0.5% ammonium sulphate, 2% glucose.

Complete medium SC: SD medium supplemented with amino acids leucine, tryptophan, histidine, lysine, methionine, aspartate and threonine and the nitrogen bases adenine and uracil at concentrations described in (Sherman, Fink et al. 1986). The absence of one or more of the requirements is specified when required.

Complete medium SGal: identical to SC but containing 2% galactose instead of glucose as carbon source.

Complete medium SRaff: identical to SC but containing 2% raffinose instead of glucose as carbon source.

Complete medium SG/L: identical to SC but containing 3% glycerol and 2% sodium lactate instead of glucose as carbon source.

Sporulation medium (SPO): 1% potassium acid, 0.1% yeast extract, 0.005% glucose, supplemented with a quarter of the concentration of requirements described for SC medium.

Solid mediums were prepared adding 2% agar before autoclaving.

1.3.- Cell culture media

Human cells were cultured in DMEM (Gibco, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/ml streptomycin, 60 µg/ml penicillin and 0.25 µg/ml amphotericin B.

1.4.- Growth conditions

Yeast strains were incubated at 30°C. Bacteria strains were incubated at 37°C. Human cells were incubated at 37ºC and 5% CO_2. Bacteria and yeast liquid cultures were incubated on horizontal orbital shakers at 200rpm. Diploid yeast strains were sporulated at 26°C in SPO medium for 3-4 days.

2.- Antibiotics, drugs, inhibitors, enzymes and antibodies

2.1.-Antibiotics

Ampicillin, Amp (Sigma): β-lactam antibiotic that inhibits cell division in *E. coli*, preventing the cell wall synthesis. It was used for plasmid selection in *E. coli*. Working concentration: 100 µg/ml.

Geneticin, G418 (USB): aminoglycoside antibiotic that inhibits protein synthesis by binding to the ribosome. It was used in yeast strains to select, follow and maintain the kanamycin resistance marker *KanMX4*. Working concentration: 100 µg/ml.

Hygromycin B, Hyg (Roche): aminoglycoside antibiotic from *Streptomyces hygroscopicus* that inhibits protein synthesis. It was used in yeast strains to select, follow and maintain the hygromycin resistance cassette *HhpMX4.* Working concentration: 250 µg/ml.

Nourseothricin, Nat (Werner BioAgents): aminoglycoside antibiotic from *Streptomyces noursei*. It was used in yeast strains to select, follow and maintain the *nourseothricin* resistance *NatMX4* cassette. Working concentration: 100 µg/ml.

Penicillin, streptomycin, and amphotericin B (Biowest): antibiotics used to prevent growth of bacteria, yeast and fungi in human cell culture. Penicillin inhibits bacterial cell wall synthesis (Concentration: 60 µg/ml). Streptomycin inhibits prokaryote protein synthesis by preventing the transition from initiation complex to chain-elongating ribosome and causes miscoding (Concentration: 100 µg/ml). Amphotericin B interferes with fungal membrane permeability (Concentration: 0.25 µg/ml).

2.2.- Drugs and inhibitors

Phenylmethanosulfonyl fluoride PMSF (Sigma): inhibitor of serine (trypsin and chymotrypsin) and cysteine proteases. Concentration: 1 mM.

Complete Protease Inhibitor cocktail (Roche): mix of several protease inhibitors including serine, cysteine and metalloproteases. Used according to manufacturer's recommendations.

Diethyl pyrocarbonate DEPC (Sigma): RNase inhibitor. Concentration: 1/1000 v/v.

Hydroxyurea HU (USB): compound that inactivates ribonucleotides reductase by forming a free radical nitroxide that binds a tyrosyl free radical in the active site of the enzyme. This blocks the synthesis of deoxynucleotides, which inhibits DNA synthesis.

5-fluorotic acid, FOA (USB): toxic analogue of uracil that poisons *URA3* yeast cells but not *ura3* mutants (Boeke, La Croute et al. 1984). Concentration: 500mg/L.

Bromodeoxiuridine BrdU (Sigma): synthetic nucleoside analogue of thymidine (Lengronne, Pasero et al. 2001). Concentration: 200 µg/ml.

Cordycepin (SIGMA): adenosine antagonist 3' deoxyadenosine, inhibitor of RNA chain elongation. Concentration: 50 µM.

2.3.- Enzymes

Spermidine (Sigma): polyamine involved in cell metabolism. It binds and precipitates DNA and protein-bound DNA. Concentration: 0.5 mM.

Spermine (Sigma): polyamine involved in cell metabolism present in all eukaryotic cells. It binds nucleic acids and contributes to stabilize the helix structure. Concentration: 0.15 mM.

Klenow (Roche): major fragment of the *E. coli* DNA polymerase I, with 5'-3' polymerase and 3'-5' exonuclease activities. Used for labelling radioactive probes.

Alkaline phosphatase (Roche): hydrolyzes 5'-monophosphate groups from DNA ends generated after an enzymatic cut. Dephosphorylation hampers religation of cut vector, favouring insertion of the fragment of interest.

T4 phage DNA ligase (Roche): enzyme that catalyzes the covalent union of dsDNA ends.

Expand®High-Fidelity DNA polymerase (Roche): mix of Taq (from *Thermus aquaticus*) and Pwo (from *Pyrococcus woesei*) polymerases. It was used for high fidelity PCRs with 5'-A overhang ends.

Phusion®High-Fidelity DNA polymerase (Finnzymes): a *Pyrococcus*-like polymerase fused with a processivity-enhancing domain. It was used for high fidelity PCRs with blunt ends.

Go-Taq®Flexi DNA polymerase (Promega): it was used for DNA probes and checking PCRs.

MyTaq™ *DNA polymerase* (Bioline): it was used for DNA probes and checking PCRs.

iTaq™ Universal SYBR® Green Supermix (Biorad): mix for quantitative PCR amplification that contains the ampliTaq Gold®DNA polymerase and the LD DNA polymerase, dNTPs with a dUTP/dTTP mixture and the ROX fluorochrome, used in an optimized buffer for the qPCR reaction.

Pronase (Sigma): *Streptomyces griseus* proteases.

Proteinase K (Roche): serine protease from *Pichia pastoris*.

Restriction enzymes (New England Biolabs and Takara): sequence-specific DNA endonucleases.

RNase A (Sigma): endonuclease that degrades single-stranded RNA.

RNase H (New England Biolabs): endonuclease that specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. This enzyme does not digest single or double-stranded DNA.

Zymolyase 20T (USB): mix of enzymes from *Arthrobacter luteus* used to digest *S. cerevisiae* cell wall. Concentration: 2 mg/ml.

Lysozyme (Sigma): enzyme from chicken egg white that hydrolyzes bacterial peptidoglycans.

Protein A/G Dynabeads (Life Technologies): magnetic beads with recombinant Protein A or G coupled to its surface. Protein A/G binds to the Fc region of IgG, IgA and IgM immunoglobulins. It was used for immunoprecipitation assays.

pGEM®-T Easy Vector Systems (Promega): kit used to clone PCR products that contains pGEM®-T vector, T4 DNA ligase and the appropriate buffer for rapid ligation.

2.4.- Antibodies

Antibodies used in this thesis are listed in Table M1 and Table M2

Table M1 Primary antibodies

Antibody	Source	Epitope	Reference	Use
Anti-FLAG	Mouse	N-Asp-Tyr-Lys-Asp-Asp-Asp Asp-Lys-C	F3165 (SIGMA)	ChIP (3µl)
RNA Pol II (8WG16)	Mouse	C-terminal heptapeptide repeat present on the largest subunit of PolII	MMS-126R (Covance)	ChIP (20µl)

H3ser10-P	Rabbit	Linear peptide corresponding to human Histone H3 at Ser10. It recognizes Histone H3 when phosphorylated at Ser10	06-570 (Millipore)	ChIP (20µl) in 10µl of Protein A
S9.6	Mouse	DNA-RNA hybrids	Hybridoma cell line HB-8730	DRIP (10µg)
BrdU	Mouse	BrdU	MBL	ChIP (3µl)
Anti-GFP	Mouse	A mixture of two monoclonal antibody (7.1 and 13.1 clones) that recognizes both wild type and mutant forms of GFP	11814460001 (Roche)	IF (3µg) ChIP (3µg)
Anti Nsr1	Mouse	C-terminal yeast Nsr1	ab4642 (Abcam)	IF (1/500)
antiHA			(Abcam)	
β-Actin	Rabbit	Synthetic peptide derived from within residues 1-100 of Human beta Actin	ab8227 (Abcam)	WB (1:1000)
Anti TPR	Mouse	N-terminal amino acids 1-99 of Human TPR	ab58344 (Abcam)	IF (1/500) WB (1/500)
Anti TPR	Rabbit	C-terminal amino acids 2300-2349 of Human TPR	ab84516 (Abcam)	IF (1/500) WB (1/500)
Phospho-H2A.X (Ser139), JBW301	Mouse	Synthetic peptide corresponding to amino acids 134-142 of human histone H2A.X	05-636 (Millipore)	IF (1:500)
53BP1	Rabbit	Amino acids 350 and 400 of Human 53BP1	NB100-304 Novus Biologicals	IF (1:500)
Nucleolin	Rabbit	Synthetic peptide conjugated to KLH, corresponding to N terminal amino acids 2-17 of Human Nucleolin with a C-terminal added cysteine	ab50279 (Abcam)	IF (1:1000)

WB: Western Blot; IF: Immunofluorescence; ChIP: Chromatin Immunoprecipitation

Table M2 Secondary antibodies

Specificity	Conjugation	Reference	Use
Rabbit	Peroxidase	A6154 (Sigma)	WB (1/2000)
Mouse	Peroxidase	A4416 (Sigma)	WB (1/2000)
Rabbit	Alexa fluor 488	Molecular Probes	IF (1/1000)
Rabbit	Alexa fluor 568	Molecular Probes	IF (1/1000)
Rabbit	Alexa fluor 647	Molecular Probes	IF (1/1000)
Mouse	Alexa fluor 488	Molecular Probes	IF (1/1000)
Mouse	Alexa fluor 546	Molecular Probes	IF (1/1000)
Mouse	Alexa fluor 594	Molecular Probes	IF (1/1000)
Mouse	Alexa fluor 647	Molecular Probes	IF (1/1000)

WB: Western Blot; IF: Immunofluorescence

3.- Strains and plasmids

3.1.- Bacterial strains

All experiments with *E. Coli* were carried out using the DH5α strain: *F¯ endA1 gyr96 hsdR17 ΔlacU169(f80lacZΔM15) recA1 relA1 supE44 thi-1* (Hanahan 1983).

3.2.- Yeast strains

Yeast strains used for the screening are listed in Table M3.

Table M3 Mini KO collection

ORF	Name	ORF	Name	ORF	Name
YAL040C	CLN3	YOR038C	HIR2	YKL032C	IXR1
YAL021C	CCR4	YPL167C	REV3	YKL054C	DEF1
YAL015C	NTG1	YPL164C	MLH3	YKL057C	NUP120
YAL011W	SWC3	YPL139C	UME1	YKL068W	NUP100
YAR002W	NUP60	YPL138C	SPP1	YKL110C	KTI12
YAR003W	SWD1	YPL121C	MEI5	YKL114C	APN1
YLL019C	KNS1	YPL116W	HOS3	YKL149C	DBR1
YLR013W	GAT3	YPL101W	ELP4	YKL160W	ELF1
YLR014C	PPR1	YBR188C	NTC20	YGR044C	RME1
YLR016C	PML1	YBR195C	MSI1	YGR057C	LST7
YLR085C	ARP6	YBR223C	TDP1	YGR066C	---
YLR095C	IOC2	YBR228W	SLX1	YGR067C	---
YLR113W	HOG1	YBR233W	PBP2	YGR102C	GTF1
YML081W	TDA9	YBR245C	ISW1	YGR104C	SRB5
YML062C	MFT1	YDR108W	TRS85	YOR111W	---
YMR153W	NUP53	YDR117C	TMA64	YOR123C	LEO1
YML060W	OGG1	YDR121W	DPB4	YOR144C	ELG1
YML011C	RAD33	YDR146C	SWI5	YOR166C	SWT1
YMR044W	IOC4	YDR359C	EAF1	YOR197W	MCA1
YMR019W	STB4	YDR399W	HPT1	YOR213C	SAS5
YML061C	PIF1	YDR419W	RAD30	YOR228C	MCP1
YMR167W	MLH1	YDR423C	CAD1	YOR246C	ENV9
YMR179W	SPT21	YDR432W	NPL3	YOR258W	HNT3
YMR190C	SGS1	YEL037C	RAD23	YOR288C	MPD1
YMR201C	RAD14	YEL056W	HAT2	YJL206C	---
YMR219W	ESC1	YER032W	FIR1	YJL176C	SWI3
YMR224C	MRE11	YER041W	YEN1	YLR381W	CTF3
YMR284W	YKU70	YER045C	ACA1	YLR385C	SWC7
YNL330C	RPD3	YER051W	JHD1	YLR392C	ART10
YNL309W	STB1	YER068W	MOT2	YLR398C	SKI2

YNL253W	TEX1	YER085C	---	YLR401C	DUS3
YOR023C	AHC1	YGR123C	PPT1	YLR407W	---
YOR032C	HMS1	YGR129W	SYF2	YLR418C	CDC73
YPL178W	CBC2	YGR200C	ELP2	YLR247C	IRC20
YOR051C	ETT1	YGR212W	SLI1	YLR318W	EST2
YOR080W	DIA2	YHL009C	YAP3	YDR163W	CWC15
YOR083W	WHI5	YHR087W	RTC3	YDR169C	STB3
YOR339C	UBC11	YHR124W	NDT80	YDR192C	NUP42
YOR344C	TYE7	YHR134W	WSS1	YDR217C	RAD9
YOR346W	REV1	YHR206W	SKN7	YGL222C	EDC1
YOR386W	PHR1	YCL061C	MRC1	YGL241W	KAP114
YOL001W	PHO80	YCR014C	POL4	YGL244W	RTF1
YOL004W	SIN3	YLR451W	LEU3	YGL251C	HFM1
YOL051W	GAL11	YLR135W	SLX4	YGR001C	EFM5
YPL230W	USV1	YLR154C	RNH203	YGR006W	PRP18
YPL216W	---	YKL009W	MRT4	YPL086C	ELP3
YPL213W	LEA1	YKL020C	SPT23	YPL064C	CWC27
YPL055C	LGE1	YIL079C	AIR1	YJL127C	SPT10
YPL048W	CAM1	YIL084C	SDS3	YJL115W	ASF1
YPL042C	CDK8	YIL008W	URM1	YJL092W	SRS2
YPL037C	EGD1	YFL049W	SWP82	YJL065C	DLS1
YPL015C	HST2	YFL052W	ZNF1	YJL056C	ZAP1
YPL008W	CHL1	YGR270W	YTA7	YJL049W	CHM7
YPL001W	HAT1	YGR275W	RTT102	YJL047C	RTT101
YPR135W	CTF4	YGR288W	MAL13	YER063W	THO1
YPR164W	MMS1	YIR018W	YAP5	YHR041C	SRB2
YPR179C	HDA3	YIR033W	MGA2	YJR078W	BNA2
YPR196W	---	YKL033W-A	---	YJR082C	EAF6
YDR363W-A	SEM1	YKR077W	MSA2	YJR147W	HMS2
YFR034C	PHO4	YKR080W	MTD1	YKR092C	SRP40
YGR249W	MGA1	YMR075W	RCO1	YKR099W	BAS1
YBL006C	LDB7	YMR078C	CTF18	YKR101W	SIR1
YBL008W	HIR1	YMR080C	NAM7	YLR435W	TSR2
YBL019W	APN2	YMR091C	NPL6	YML021C	UNG1
YBL032W	HEK2	YOL090W	MSH2	YNR063W	---
YBL066C	SEF1	YOL100W	PKH2	YJR094C	IME1
YBL088C	TEL1	YOL104C	NDJ1	YJR124C	---
YGL025C	PGD1	YER064C	VHR2	YJR140C	HIR3
YGL013C	PDR1	YER088C	DOT6	YKL005C	BYE1
YPL022W	RAD1	YER092W	IES5	YDL200C	MGT1
YGL043W	DST1	YER095W	RAD51	YDL214C	PRR2
YGL082W	---	YGR134W	CAF130	YDR004W	RAD57
YGL087C	MMS2	YHR191C	CTF8	YBR271W	EFM2
YGL096W	TOS8	YHR193C	EGD2	YBR274W	CHK1
YNL236W	SIN4	YLR032W	RAD5	YCR065W	HCM1
YNL230C	ELA1	YLR035C	MLH2	YCR066W	RAD18

YNL218W	MGS1	YLR052W	IES3	YCR077C	PAT1
YNL156C	NSG2	YMR312W	ELP6	YJR035W	RAD26
YKL213C	DOA1	YNL250W	RAD50	YJR043C	POL32
YKL214C	YRA2	YML095C	RAD10	YJR050W	ISY1
YDR253C	MET32	YML102W	CAC2	YJR060W	CBF1
YDR255C	RMD5	YML103C	NUP188	YNL004W	HRB1
YDR273W	DON1	YML109W	ZDS2	YNL021W	HDA1
YDR279W	RNH202	YML113W	DAT1	YNL025C	SSN8
YDR295C	HDA2	YML121W	GTR1	YNL046W	---
YDR307W	PMT7	YMR106C	YKU80	YNR010W	CSE2
YDR314C	RAD34	YMR125W	STO1	YNR024W	MPP6
YDR317W	HIM1	YPR018W	RLF2	YBL103C	RTG3
YDR334W	SWR1	YPR051W	MAK3	YBR065C	ECM2
YIL017C	VID28	YPR052C	NHP6A	YNL136W	EAF7
YIL024C	---	YPR068C	HOS1	YNL133C	FYV6
YIL040W	APQ12	YPR070W	MED1	YIL128W	MET18
YIL072W	HOP1	YPR101W	SNT309	YIL130W	ASG1
YIR005W	IST3	YJL184W	GON7	YIL149C	MLP2
YIR009W	MSL1	YPR072W	NOT5	YIR002C	MPH1
YIR013C	GAT4	YLR226W	BUR2	YGR056W	RSC1
YNL072W	RNH201	YNL059C	ARP5	YOR141C	ARP8
YNL076W	MKS1	YMR263W	SAP30	YOR162C	YRR1
YNL082W	PMS1	YCR081W	SRB8	YOR161C	PNS1
YNL085W	MKT1	YBR278W	DPB3	YOR172W	YRM1
YNL090W	RHO2	YBR285W	---	YOR191W	ULS1
YNL107W	YAF9	YNL146W	---	YOR208W	PTP2
YNL121C	TOM70	YBR112C	CYC8	YBR033W	EDS1
YDR174W	HMO1	YLL054C	---	YCR084C	TUP1
YGR040W	KSS1	YLR011W	LOT6	YLR399C	BDF1
YGR063C	SPT4	YLR098C	CHA4	YGL240W	DOC1
YKR023W	---	YML080W	DUS1	YGR229C	SMI1
YKR029C	SET3	YMR280C	CAT8	YGR252W	GCN5
YBR131W	CCZ1	YOR001W	RRP6	YAL027W	SAW1
YBR150C	TBS1	YOR006C	TSR3	YNL278W	CAF120
YBR275C	RIF1	YOR295W	UAF30	YOR290C	SNF2
YBR289W	SNF5	YOR297C	TIM18	YOR363C	PIP2
YLR394W	CST9	YOL028C	YAP7	YOR368W	RAD17
YML027W	YOX1	YOL043C	NTG2	YDR066C	RTR2
YML036W	CGI121	YOL068C	HST1	YDR069C	DOA4
YML041C	VPS71	YPL194W	DDC1	YDR075W	PPH3
YMR048W	CSM3	YPL129W	TAF14	YDR092W	UBC13
YMR137C	PSO2	YPL096W	PNG1	YDR099W	BMH2
YIL030C	SSM4	YBR175W	SWD3	YDR369C	XRS2
YOR270C	VPH1	YBR182C	SMP1	YKL113C	RAD27
YOR274W	MOD5	YBR184W	---	YHR034C	PIH1
YOR276W	CAF20	YDR050C	TPI1	YHR079C	IRE1

YOR179C	SYC1	YDR076W	RAD55	YGL058W	RAD6
YKR095W	MLP1	YDR078C	SHU2	YJL103C	GSM1
YLR442C	SIR3	YDR097C	MSH6	YHR204W	MNL1
YNR052C	POP2	YDR123C	INO2	YCL011C	GBP2
YPR023C	EAF3	YDR364C	CDC40	YGR036C	CAX4
YDR443C	SSN2	YDR392W	SPT3	YOL148C	SPT20
YGL151W	NUT1	YDR408C	ADE8	YGR258C	RAD2
YGL173C	XRN1	YDR414C	ERD1	YGL070C	RPB9
YER161C	SPT2	YER035W	EDC2	YDR296W	MHR1
YDL074C	BRE1	YGR159C	NSR1	YGR262C	BUD32
YFL013C	IES1	YGR180C	RNR4	YJL013C	MAD3
YJR047C	ANB1				

Other strains used in this work are listed in Table M4. *mlp* mutants were generated by replacement of the *MLP1* or *MLP2* genes with the *KanMX* cassette in strain SYRB1-4C. The URA3/GPD-Tk (7x) sequence integrated at the *ura3* locus was popped out by growing the strains in FOA-containing SC-URA plates to generate yFGB04, yFGB05 and yFGB06 strains. Strains baring the chromosomal *his3*-based recombination system were obtained by genetic crosses with strain 344-15B-Leu+ (García-Pichardo, Cañas et al. 2017). Strains baring the artificial anchoring construction were obtained by genetic crosses with strains FSY5216 and FSY5217 (Texari, Dieppois et al. 2013).

Strains yFGB13, were obtained by genetic crosses with FSY5216 and BY4741 YOR128C (*Ade2ΔKan).* The strain obtained was back-crossed twice with FSY5216 to get an isogenic W303 background. Then, the *LlacZ-Ade2* recombination system was amplified by PCR and the *TRP1* marker replaced by homologueous recombination with the recombination system in FSY5216. The LlacZ-ADE2 was generated by amplification of the *ADE2* gene with primers containing *Aor*51HI restriction sites. This construction was cloned into the Aor51HI site of pSCH204 (Chávez and Aguilera 1997). The *mlp1* mutant strain was obtained by direct disruption of the *MLP1* gene with the *NatMX* cassette. The *hpr1* mutant strain was obtained by genetic crosses.

Table M4 Yeast strains used in this thesis.

Strain	Relevant genotype	Reference
SYRB1-4C	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX ura3::URA3/GPD-TK (7x)*	J.M.Santos-Pereira
yFGB01	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX ura3::URA3/GPD-TK (7x) mlp1Δ::KanMX*	This study
yFGB02	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX ura3::URA3/GPD-TK (7x) mlp2Δ::KanMX*	This study
yFGB03-1A	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX ura3::URA3/GPD-TK (7x) mlp1Δ::KanMX mlp2Δ::KanMX*	This study
yFGB04	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX*	This study
yFGB05	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX mlp1Δ::KanMX*	This study
yFGB06	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX mlp2Δ::KanMX*	This study
yFGB07	*MAT**a** ura3-1 ade2-1 his3-11,15 leu2-3,112 trp1-1 can1-100 RAD5 bar1Δ::HygMX mlp1Δ::KanMX mlp2Δ::KanMX*	This study
HPBAR-1R	*MAT**a** ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2-3,112 hpr1Δ3::HIS3, bar1Δ, RAD5*	M. San Martín
CY11555	*MAT**a** ura3-1 his3-11,15 leu2-3,112 trp1-1 ADE2 CAN1 RAD5 mlp1S1710A*	(Bermejo, Capra et al. 2011)
CY11531	*MAT**a** ura3-1 his3-11,15 leu2-3,112 trp1-1 ADE2 CAN1 RAD5 mlp1S1710D*	(Bermejo, Capra et al. 2011)
FSY5216	*MAT**a** ura3 ade2 his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 TRP*	(Texari, Dieppois et al. 2013)
yFGB07-1A	*MAT**α** ura3 ade2 his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 TRP*	This study
yFGB07-1B	*MAT**α** ura3 ade2 his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 TRP mlp1Δ::KanMX*	This study
yFGB08	*MAT**α** ura3 ade2 his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 TRP hpr1Δ::HIS3*	This study
344115B leu+	*Mat**α** his3-513::TRP1::his3-537 ura3-52 trp1*	(García-Pichardo, Cañas et al. 2017)
yFGB09	*Mat**a** his3-513::TRP1::his3-537 ura3 trp1 bar1Δ::HygMX*	This study
yFGB10	*Mat**a** his3-513::TRP1::his3-537 ura3 trp1 bar1Δ::HygMX mlp1Δ::KanMX*	This study
yFGB11	*Mat**a** his3-513::TRP1::his3-537 ura3 trp1 bar1Δ::HygMX mlp2Δ::KanMX*	This study
yFGB12	*Mat**a** his3-513::TRP1::his3-537 ura3 trp1 mlp1Δ::KanMX mlp2Δ::KanMX*	This study

yFGB13	*MATα ura3 ade2Δ::KanMX his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 L-lacZ-ADE2*	This study
yFGB14	*MATα ura3 ade2Δ::KanMX his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 L-lacZ-ADE2 mlp1Δ::NatMX*	This study
yFGB15	*MATα ura3 ade2Δ::KanMX his3 leu2 trp1 Nup49-GFP LacI-GFP-HIS3 LexA BS LacO at GAL10 L-lacZ-ADE2 hpr1Δ::HIS3*	This study

3.3.- Human cell lines

Human cells used in this study are listed in the Table M5

Table M5 Human cell lines used in this thesis.

Cell Line	Description	Medium	Source
HeLa	Human cervical adenocarcinoma epithelial cells	DMEM	ECACC
U2OS	Human bone osteosarcoma epithelial cells	DMEN	ATCC

ECACC: European Collection of Authenticated Cell Cultures.

3.4.- Plasmids

Plasmids used in this work are listed in Table M6.Plasmid p313LZGAID was generated by cloning the *HIS3*-containing *Apa*I-*Dra*III fragment from pRS313 into *Apa*I-*Dra*III digested pLZGAID (García-Pichardo, Cañas et al. 2017).

HBD from yeast (yHBD) was amplified by PCR using specific primers with two different restriction sites (*Bgl*II and *Sal*I) to replace the human HBD for the yHBD in frame with GFP and the tetracycline repressible promoter in plasmid pCM198. On the other hand, the yeast HBD was amplified by PCR using specific primers containing a *Pac*I restriction site to allow its cloning into the *Pac*I site of pFA6a-*TRP-pGAL1-3xHA*. After that, the construction was amplified in *E. coli* and digested with *Bgl*II and *Sal*I and cloned into the pRS314 expression vector digested with *BamH*I and *Sal*I .

Table M6 Plasmids used in this thesis

Plasmids	Description	Source
pLZGAID	YCp containing the p*L-lacZ* system and the human *AID* gene under the *GAL1* promoter	(García-Pichardo, Cañas et al. 2017)
pRS313	YCp vector based on the *HIS3* marker	(Sikorski and Hieter 1989)
p313LZGAID	YCp containing the p*L-lacZ* system and the human *AID* gene under the *GAL1* promoter	This study
pRS313-GALRNH1	YCp containing the *RNH1* gene under the *GAL1* promoter	B. Gómez-González
pRS416	YCp vector based on the *URA3* marker	(Sikorski and Hieter 1989)
pRS416 GALRNH1	YCp containing the *RNH1* gene under the *GAL1* promoter	(Epshtein, Potenski et al. 2016)
pRS414GALAID-FLAG	YCp p414GALAID with FLAG epitope adding in AID C-terminus protein	M. García Rubio
pRS314GLlacZ	YCp containing the *L-lacZ* system under the *GAL1* promoter	(Piruat and Aguilera 1998)
pCM184	YCp plasmid containing the *tetO* promoter and *TRP1* marker	(Gari, Piedrafita et al. 1997)
pCM189	YCp plasmid containing the *tetO* promoter and *URA3* marker	(Gari, Piedrafita et al. 1997)
pCM184AID	YCp containing the *AID* ORF cloned from pRS316GALAID (Gómez-González and Aguilera 2007) into pCM184 *Not*I site	D. García-Pichardo
pCM189AID	YCp containing *AID* ORF under the *tetO* promoter	(Santos-Pereira, Herrero et al. 2013)
pCM184RNH1	YCp containing *RNH1* ORF under the *tetO* promoter	(Santos-Pereira, Herrero et al. 2013)
pWJ1213	YCp containing the RAD52-YFP fusion	(Feng, During et al. 2007)
pWJ1344	YCp containing the RAD52-YFP fusion	(Lisby, Rothstein et al. 2001)
p414GAL	YCp containing the *GAL1* promoter	(Mumberg, Muller et al. 1994)
pRS414-GALAID	YCp containing the human *AID* ORF under the *GAL1* promoter	(Gómez-González and Aguilera 2007)
pRS315	YCp vector based on the *LEU2* marker	(Sikorski and Hieter 1989)
pRS315-GALRNH1	YCp carrying the *RNH1* ORF under the *GAL1* promoter	(Gómez-González, García-Rubio et al. 2011)
pARSGLB-OUT	YCp containing the OUT recombination system under the *GAL1* promoter	(Prado and Aguilera 2005)
pARSGLB-IN	YCp containing the IN recombination system under the *GAL1* promoter	(Prado and Aguilera 2005)
pCM189 GFP-HBD	YCp containing *GFP-HBD* construction under the *tetO* promoter	This study

pRS314 GHBDHA	YCp containing *HBD-HA* construction under the *GAL1* promoter	This study
pBTM116-URAr-LexA	Yeast vector containing LexA DNA-binding ORF	(Texari, Dieppois et al. 2013)
pBTM116-URAr-LexA-Nup60	Yeast vector containing *NUP60* ORF fused to LexA DNA-binding ORF	(Texari, Dieppois et al. 2013)
pCDNA3	Mammalian expression vector containing the cytomegalovirus promoter (P_{CMV})	(ten Asbroek, van Groenigen et al. 2002)
pCDNA-RNaseH1	Mammalian expression vector containing human RNase H1 gene under the control of P_{CMV}	(ten Asbroek, van Groenigen et al. 2002)
pEGFP-C2	Mammalian expression vector to express N-terminal EGFP fusion protein under the control of the P_{CMV}	Clontech
pEGFP-M27-H1	RNase H1 coding sequence starting at M27 (w/o the putative mitochondrial localization signal)	(Cerritelli, Frolova et al. 2003)

4.- Yeast methodology

4.1.- Transformation

Yeast transformation was performed as previously described using the lithium acetate/single-stranded DNA/polyethylene glycol method (Gietz, Schiestl et al. 1995).

Large-scale transformation was performed as previously described (García-Pichardo, Cañas et al. 2017). Briefly, cells were grown in 200 µl 2x YPAD for 2 days at 30ºC. Cells were diluted into fresh 2X YPAD media and incubated 3-4 h at 30ºC with shaking. Then cells were centrifuged 5 min at 2000 rpm and washed in 150 µl 0.1 M LiAc 10 mM TE before transformation. Cells were resuspended and incubated 30 min at 30ºC and 20 min at 42ºC in the transformation mix (500 ng plasmid DNA, 30% PEG, 100 mM LiAc, 1x TE, 80 µg/ml salmon sperm DNA). Transformed cells were washed and grown in appropriate selective medium.

4.2.- Recombination assays

Recombination frequencies were determined as the average value of the median frequencies obtained from at least three independent fluctuation tests with the indicated recombination systems. Recombinants were obtained by platting appropriate

dilutions in applicable selective medium. To calculate total number of cells, they were plated in the same media as the original transformation used. Each fluctuation test was performed from six independent colonies according to standard procedures (Gomez-Gonzalez, Ruiz et al. 2011).

4.2.1.- Recombination systems

L-lacZ. This system is based on *leu2*Δ*3'*and *leu2*Δ*5'* truncations of the *LEU2* gene that share 600 bp of homology. The sequence of the 3 kb long *lacZ* gene from *E. coli* was cloned between the direct repeats. Recombinants are selected in plates without leucine. Recombinants are selected in plates without leucine (Chávez and Aguilera 1997).

pLZGAID. Plasmid with the direct-repeat recombination system *L-lacZ* combined the AID gene under the *GAL1* inducible promoter. Recombinants are selected in plates without leucine.

his3-513::TRP1::his3-537. This system is based on the duplication of a 6.1-kb *EcoRI-SalI* DNA fragment at the *HIS3* locus on the right arm of chromosome *XV*. One copy of this sequence carries the allele *his3-513,* generated by mutation of the *KpnI* at the 3' site of *HIS3* coding region, and the other copy carries the allele *his3-537* generated by mutation of a *HindIII* site *his3-51* distal to the *KpnI* site. Between the duplications, the sequence of the pBR322 plasmid containing yeast *ARS1-TRP1* sequence has been inserted at the unique *EcoRI* site. This system allows to measure gene conversion and recombination between the repeats. Recombinants are selected in plates without histidine (Aguilera and Klein 1988).

GLB IN/OUT. This system is based on *leu2*Δ*3'*and *leu2*Δ*5'* truncations of the *LEU2* gene under the *GAL1* inducible promoter. The *leu2* repeats are oriented according to their transcription either inward (IN) or outward (OUT) with respect to the unique *ARSH4* replication origin contained in the plasmid. Recombinants are selected in plates without leucine (Prado and Aguilera 2005).

LlacZ-ADE2. This chromosomal system inserted at the *GAL* locus is based on the *LlacZ* recombination system and contains the *ADE2* gene cloned into the *Aor57HI* restriction site. Recombinants are selected in plates without leucine.

4.3.- Detection of Rad52-YFP foci

Rad52-YFP foci were visualized in cells transformed with plasmid pWJ1213 or pWJ1344 with a DM600B microscope (Leica) as previously described (Lisby, Rothstein et al. 2001) with minor modifications. Individual transformants were grown to early-log-phase, fixed for 10 minutes in 0.1 M K_iPO_4 pH 6.4 containing 2.5% formaldehyde, washed twice in 0.1 M K_iPO_4 pH 6.6, and resuspended in 0.1 M K_iPO_4 pH7.4. At least 200 S/G2 cells were analyzed for each transformant. Average values obtained from at least 3 independent transformants are plotted for each genotype.

4.4.- Plasmid loss assay

Percentage of plasmid loss was determined as the average value of the median percentages of cells that lost the centromeric plasmid pRS315 upon growth in non-selective media obtained from at least three independent fluctuation tests. Each test was performed with six independent colonies.

4.5.- Cell cycle synchronization and FACS analysis

For cell cycle synchronization, overnight cultures were diluted to an OD_{600nm} of 0.2 and grown until mid-log at 30ºC in synthetic medium. Cells were synchronized in G1 adding 0.125 µg/ml of α-factor (Biomedal) for *bar1*Δ mutants. After 2.5 h, cells were released from G1 in rich medium with 1 µg/ml pronase. Samples were taken at the indicated times.

For FACS analysis, cells were processed as previously described (Moriel-Carretero, Tous et al. 2011). Briefly, 1mL of the culture was centrifuged and washed with 1 ml 1x PBS, resuspended in 1 ml 70% ethanol and stored at 4ºC. Cells were washed with 1 ml 1x PBS, resuspended in 100 µl 1x PBS-RNase A 1 mg/ml and

incubated overnight at 37ºC. Then, cells were washed again with 1x PBS and resuspended in 1 ml of 5 µg/mL propidium iodide in 1x PBS, incubated in the dark for 30 min, sonicated 5 seg at 10% of amplitude and scored in a FACScalibur (Becton Dickinson, CA).

4.6.- Pulse-Field gel electrophoresis (PFGE)

For DNA extraction, mid-log cultures grown in YPAD were centrifuged and washed with 5 ml EDTA 50 mM pH 8. Each sample was resuspended in 180 µl CPES (40 mM citric acid pH 6, 10 mM sodium phosphate pH 6, 20 mM EDTA pH 8, 1.2 M sorbitol, 1 mg/ml zymoliase 20T), and mixed with 300 µl 2% low-melting-point agarose dissolved in CPE (40 mM citric acid pH 6, 120 mM sodium phosphate pH6, 20 mM EDTA pH 8). This mix was poured into moulds and allowed to solidify for 10 min at 4ºC. Plugs were subsequently incubated as follows: overnight in 5 ml CPE with 1% v/v β-mercaptoethanol at 30ºC, then overnight in 3 ml buffer L (0.1 M EDTA pH 8, 0.01 M Tris-Cl pH 7.6, 0.02 M NaCl, 0.5 mg/ml proteinase K, 1% w/v sarkosyl) at 50ºC, and finally washed twice 1 h at 50ºC in 10 ml TE pH 7.6 containing 40 µg/ml PMSF, and 1h at RT in 10 ml TE pH 7.6. Electrophoresis was performed at 12ºC in a Bio-Rad CHEF Mapper, using a voltage gradient of 5.5 V/cm, switch times from 5 to 30 s, switch angle of 115º, in 1% agarose gel in 0.5X TBE for 30 h, as previously described (Cha and Kleckner 2002). Gels were treated and transferred to a Hybond XL membrane (GE Healthcare) by standard procedures.

4.7.- Chromatin Immunoprecipitation (ChIP)

ChIP was performed as previously described (Gaillard, Wellinger et al. 2015). Briefly asynchronous or G1-synchronized mid-log cultures grown in YPAD or synthetic medium at 30ºC were used. 50 ml of the cultures were cross-linked in 1% formaldehyde after 15 min, glycine was added to a final concentration of 125 mM, washed twice with cold PBS. Pellets were resuspended in 500 µl of lysis buffer (50 mM HEPES/KOH, 140 mM NaCl, 1 mM EDTA pH 8. 0, 1% Triton X-100, 0.1% sodium deoxicholate) supplemented with protease inhibitors (1 mM PMSF and 1X Complete protease inhibitor cocktail). Cells were broken in an orbital shaker (Vibrax VXR basic, IKA)

using glass beads. Samples were separated from the beads. The precipitate was resuspended in lysis buffer supplemented with protease inhibitors and sonicated using a Bioruptor (Diagenode). Samples were centrifuged to eliminate cell debris. 20 µl were used as a control of total DNA (Input) and 300 µl were processed for immunoprecipitation.

The immunoprecipitation was performed overnight at 4ºC using Dynabeads Protein A/G (Life Technologies) previously incubated with the antibody. Samples were washed twice with every following buffer: lysis buffer, lysis buffer with 500 mM NaCl, buffer III (10 mM Tris-HCl pH8, 1 mM EDTA pH8.0, 250 mM LiCl, 0.5% NP40 (IGEPAL), 0.5% sodium deoxicolate) and TE (10 mM Tris-HCl pH7.6, 1 mM EDTA pH8.0). Proteins were eluted in 250 µl elution buffer (10 mM Tris-HCl pH7.6, 1 mM EDTA pH8.0, 1% SDS) at 65ºC for 10 min. Samples were treated with 6 µl of 50 mg/ml pronase for 1 h at 42ºC to remove proteins and decrosslinked for 5 h at 65ºC. MACHEREY-NAGEL DNA kit was used to clean DNA that was eluted in 100 µl of MQ water.

4.7.1.- Replication analysis

Incorporation of BrdU was performed by ChIP with some modifications. Cells were grown in SC medium and synchronized with α-factor, washed twice in pre-warmed SC medium and released from G1 arrest in the presence of 200 µg/ml BrdU (Sigma) by addition of 1 µg/ml pronase. Where indicated, release was performed in the presence of 20 mM hydroxyurea. Addition of formaldehyde and glycine was replaced, 0.1% Sodium Azide was added to each sample after samples were collected. Immunoprecipitation was performed using Protein A-Dynabeads coated with monoclonal anti-BrdU antibody (MBL). Input and immunoprecipitated DNA were analyzed by real-time qPCR. Relative BrdU incorporation at a given region was calculated relative to the signal at a late replicating region (Chr. V, position 242210-242280, (Gómez-González, García-Rubio et al. 2011)) in the same sample.

4.8.- DRIP assays

DNA-RNA Immunoprecipitation was performed as previously described (Garcia-Rubio, Barroso et al. 2018). Cells were resuspended in spheroplasting buffer (1 M sorbitol, 2 mM Tris–HCl pH 8.0, 100 mM EDTA pH 8.0, 0.1% v/v beta-mercapto-ethanol. 0.2% w/v zymoliase 20T). DNA was treated with RNase A (30 min at 37ºC) and Proteinase K (1h at 56ºC) in G2 buffer (0.8 mM Guanidine HCl, 30 mM Tris–HCl pH 8.0, 30 mM EDTA pH 8.0, 5% Tween20, 0.5% Triton X-100) and carefully extracted with chloroform:isoamylalcohol (24:1) followed by isopropanol precipitation. Precipitated DNA was spooled on a glass rod, washed twice with 70% EtOH, gently resuspended in TE and enzymatically digested with *Hind*III, *Eco*RI, *BsrGI*, *XbaI* and *SspI* in an appropriate commercial buffer. Samples were split and treated with *E. coli* RNase H (NEB) or mock treated. DNA:RNA hybrids immunoprecipitation was performed by overnight incubation with Protein A-Dynabeads (Invitrogen) coated with the S9.6 antibody at 4ºC using 1x binding buffer (100 mM NaPO4 pH 7.0, 1.4 M NaCl, 0.5% Triton X-100). DNA was eluted with elution buffer (50 mM Tris pH 8.0, 10 mM EDTA, 0.5%SDS), treated with proteinase K and purified with the MACHEREY-NAGEL DNA kit. Real-time qPCR was performed at the indicated regions. S9.6 signal was determined by dividing the immunoprecipitated signal to the input for each sample.

4.9.- Northern Blot

Yeast cell were grown in SRaff medium and were transferred to galactose-containing medium for *GAL1* gene activation. RNA was extracted from mid-log cultures using acidic phenol (Köhrer and Domdey 1991). Total RNAs were separated by agarose gel electrophoresis and transferred to Hybond-N nitrocellulose membranes (GE Healthcare), which were subsequentially hybridized with ^{32}P-labelled DNA probes. The DNA primers used to amplify the probes are listed in Table M7. Radioactive signals were acquired using a FLA-5100 Imager Fluorescence Analyzer (Fujifilm) and were quantified using the MultiGauge 2.0 analysis software (Science Lab). In Northern blot analyses, signals were normalized to the *SCR1* gene, transcribed by RNAPIII and whose transcripts are very stable. Signal was plotted as arbitrarily units (A.U.).

5.- Human cells methodology

5.1.- siRNA and plasmid transfection

All assays were performed 48 or 72 hours after small interfering RNA (siRNA) transfection and 48 hours after plasmid transfection. The siRNA pool used to deplete TPR was purchased from Dharmacon (010548) and contains the four individual siRNA listed in Table M7. Cells were transfected with siRNA using DharmaFECT 1 (Dharmacon) at 30-50% confluence. 24 hours before transfection, cells were cultured in antibiotic-free medium. Transfection in one well of a 6-well plate was performed using a combination of two mixtures. The first one contains 50 µl culture serum-free medium (medium without antibiotics or FBS), 36 µl H2O, 9 µl 5X siRNA buffer (Dharmacon) and 5 µl siRNA 20 µM (100 nM). The second one contains 95 µl serum-free medium and 5 µl DharmaFECT 1. Each mixture was incubated at room temperature (RT) for 5 min, mixed and incubated for 20 min. Meanwhile, medium was replaced by 800 µl serum-free medium. Transfection solution was added carefully drop by drop to the cell culture that was then incubated for 4-5 hours. Afterwards, 2 ml of a high-concentrate complete medium was added to have a final normal concentration of all the medium components.

For plasmid transfection, cells were transfected at 80% confluence. 24 h before transfection cells were cultured in antibiotic-free medium (3 ml for 6-well plates). Transfection in one well of a 6-well plate was performed using a mixture (final volume 150 µl) with 9 µl FuGENE 6 (Roche) in Opti-MEM, incubated for 5 min at RT. Then, 3 µg of DNA was added and the mixture was incubated for 20 min at RT. Transfection solution was finally added drop by drop to the cell culture.

5.2.- Human cells protein extraction

Cells were collected using accutase, washed in cold PBS and resuspended in ice-cold lysis buffer (10 mM Tris-HCl pH7.5, 150 mM NaCl, 0.5 mM EDTA pH8, 0.5% v/v NP-40, 1 mM PMSF, and 1x protease inhibitor cocktail) (100 µl/1x10^6 cells) during 30 min on ice. The lysate was centrifuged 10 min at 16000g and the supernatant

was transfered to a new tube. Prior to gel loading, 4X laemmi buffer (200 Mm Tris-HCl, 40% glycerol, 8% SDS, 0.4% Bromophenol Blue, 400 mM β-mercaptoethanol) was added to 1X final concentration and samples were boiled for 5 min.

Proteins were separated in 29:1 acrylamide:bis-acrylamide gels with concentrations appropriate to the molecular size of the proteins of interest and SDS-PAGE was performed according to previously described method (Laemmli 1970). Electrophoreses were performed in a Mini-PROTEAN 3 Cell with Running Buffer (25 mM Tris base pH 8.3, 194 mM glycine, 0.1% SDS buffer at 100 V). Page RulerTM (Fermentas) was used as a protein marker.

For Western blot, proteins were wet-transferred using Trans-Blot system (Biorad) in 1X Transfer Buffer (6 g/L Tris base, 28.8 g/L glycine and 0.5% SDS) with 20% methanol o/n at 30V 4°C. Proteins were transferred to a nitrocellulose membrane (Hybond-ECL, GE Healthcare). Membranes were blocked (1X TBS, 0.1% Tween 20, 5% milk) for 1 h. Primary antibodies were incubated overnight at 4 °C (1X TBS, 0.1% Tween 20, 5% milk). After 3 wash of 10 min each one, membranes were incubated with the corresponding secondary antibodies conjugated with the horseradish peroxidase for 1h hour and washed again. Finally, Immobilon Western Chemiluminescent HRP Substrate (Millipore) was used for chemiluminescence detection depending on the expected strength of the signal.

5.3.- Immunofluorescence

Cells were cultured on glass coverslips, fixed in formaldehyde or methanol and specific target molecules were visualized in a fluorescence microscope DM600B (Leica) or confocal CS SP5 (Leica) after incubation with specific primary antibodies and with fluorophore-conjugated secondary antibodies.

For TPR, γH2AX and 53BP1 antibodies, cells were fixed in 2% formaldehyde in PBS for 20 min at RT and permeabilized with 70% ethanol for 5 min at -20 °C, 5 min at 4 °C, and washed twice in PBS. Only for TPR antibody cells were pre-permeabilized with Triton-X 100 before blocking. After blocking with 3% bovine serum albumin (BSA) in PBS, the coverslips were incubated with primary antibodies diluted in 3%

BSA in PBS for 2 h at RT. For antibody dilutions see Table M1. Then cells were washed three times in PBS for 5 min. Secondary antibodies conjugated with Alexa Fluor diluted (1:1000) in 3% BSA in PBS were incubated for 1 h at RT. Coverslips were washed twice in PBS before and after the staining of the DNA with 1 µg/ml DAPI (2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride) for 5 min. Finally, coverslips were washed in water and a drop of Immu-mount mounting medium (Thermo) was used for mounting.

For S9.6 and nucleolin immunofluorescence, cells were fixed in cold absolute methanol for 7 min at -20 °C and washed twice in PBS. Coverslips were blocked in 2% BSA in PBS overnight at 4 °C, incubated with anti-S9.6 (1:100) and anti-nucleolin (1:1000) primary antibodies diluted in 2% BSA in PBS o/n at 4 °C, washed in PBS and incubated with secondary antibodies conjugated with Alexa Fluor diluted in 2% BSA in PBS (1:1000) for 1 h at RT. Washes, DAPI staining and mounting were performed as described above.

5.3.- Human DRIP

DRIP was performed as previously described (Garcia-Rubio, Barroso et al. 2018). Briefly, cells were treated with SDS and proteinase K, then DNA was carefully extracted with phenol:chloroform:isoamylalcohol (24:24:1) followed by isopropanol precipitation. From this step on, the protocol is the same as for the yeast DRIP assay.

5.4.- Single cell gel electrophoresis (Comet assay)

Comet assay was performed using a commercial kit (Trevigen, Gaithersburg, MD, USA) following the manufacturer´s protocol, 48 h after siRNA transfection. When indicated, RNase H1 was overexpressed or 50 µM cordycepin was added to the culture 4 hours before the experiment. Comet assay with RNaseH1 or cordycepin were performed 72h after transfection.

5.4.1.- Alkaline comet assay

Cells were collected using accutase, washed and resuspended in ice cold PBS, combined with low melting agarose, immobilized on CometSlides (30 min at 4 °C) and lysed for 30 min at 4 °C. Then, DNA was unwound and denatured in freshly prepared alkaline unwinding solution (200 mM NaOH, 1 mM EDTA pH>13) for 30 min at RT and electrophoresis was performed in prechilled alkaline electrophoresis solution (200 mM NaOH, 1 mM EDTA pH>13) at 21 V for 30 min. Next, slides were immersed twice in $_{d}$H2O for 5 min each, then in 70% ethanol for 5 min and dried at RT. DNA was stained with SYBR Green at 4 °C for 5 min.

5.4.2.- Neutral comet assay

Cells were collected and immobilized on CometSlides as in alkaline comet assay. Cells were lysed for 1 h at 4 °C and immersed in prechilled 1X neutral electrophoresis buffer (for 500ml, 60.57 g Tris Base and 204.12 g of sodium acetate were dissolved in H_2O and the pH adjusted to 9.0 with glacial acetic acid) for 30 min at 4 °C. Electrophoresis was performed in prechilled 1X neutral electrophoresis buffer at 35 V for 15 min and then immersed in DNA precipitation solution (1M NH_4Ac in 70% ethanol) for 30 min at RT. Finally, slides were immersed in 70% ethanol for 30 min at RT and dried. DNA was stained with SYBR Green at 4 °C for 30 min.

6.- Polymerase chain reaction (PCR)

6.1.- Non-quantitative PCR

DNA amplification with temperature-stable polymerases for probe generation, strain verification or cloning were performed following standard protocols with the DNA polymerases listed in chapter 2.3. (Materials and Methods).

6.2.- Real-time quantitative PCR (qPCR)

For this thesis, real-time qPCRs were performed using the iTaq™ Universal SYBR® Green Supermix (Biorad). Reactions were set with 6 µl H2O, 2 µl primer mix (0.1 mM each), 2 µl template and 10 µl SYBR® Green Supermix (Biorad). Runs were always performed with the following program: 1 cycle (10 min 95ºC), 40 cycles (15 s 95ºC and 1 min 65ºC) with a final dissociation stage (15 s 95ºC, 1 min 65ºC, 15 s 95ºC and 15 s 60ºC). Samples were run in 7500 Fast Real-Time PCR system (Applied Biosystem). Results were analyzed with 7500 System Software V2.0.6. A calibration curve with five 10-fold serial dilutions for yeast experiments and four 5-fold serial dilutions for human experiments of a standard DNA sample was calculated for absolute quantification.

ChIP samples were diluted in H_2O. 2 µl of 1:25 and 1:5 dilutions of the Input and the IP respectively were typically used. IP/INPUT ratios were calculated for each analysed region. In the case of yeast DRIP experiments, 2 µl of a 1:25 dilution for Input and 2 µl of undiluted sample for IP were used for qPCR. For human DRIP experiments 4 µl of a 1:5 dilution for Input and 4 µl of undiluted sample for IP were used. The relative abundance of DNA:RNA hybrid immunoprecipitated in each region was normalized to the Input signal obtained. Real-time qPCR primers were designed using Primer Express 3.0 Software (Applied Biosystem). Primers used in this thesis, for non-quantitative and quantitative PCR, are described in Table M7.

Table M7 Primers used in this thesis

Primers	Sequence	Use
MLP1 A	CTGATAGATATATTGCTGCC	Primers to check Mlp1 strain
MLP1 B	AACATTCAAAACACAAACCG	
MLP2 A	AAGAAGAAAACAATATCGGCG	Primers to check Mlp2 strain
MLP2 B	ATACTTAACTACTAGTACGG	
SCR1 A	GTTCAGGACACACTCCATCC	*SCR1* probe
SCR1 B	AGGCTGTAATGGCTTTCTGG	
GAL1 A	ACGAGTCTCAAGCTTCTTGC	*GAL1* probe
GAL1 B	TATAGACAGCTGCCCAATGC	
GCN4 3´ A	TTGTGCCCGAATCCAGTGA	DRIP and ChIP *GCN4*
GCN4 3´ B	TGGCGGCTTCAGTGTTTCTA	
PDC1 A	CCTTGATACGAGCGTAACCATCA	DRIP and ChIP *PDC1*
PDC1 B	GAAGGTATGAGATGGGCTGGTAA	
PDR5 A	TACGTCTTGTTTCGGCCTTAATC	DRIP and ChIP *PDR5*
PDR5 B	GTCAGAGGCTATATTTCACTGGAGAA	

ARS508 A	AGATTCTTTGAACACGGTCTGTCA	ChIP BrdU
ARS508 B	TGTGCTAAACCACTCAGTTGGAA	
ARS508 +3508 A	CCCGTGGTAAACCTTTAGAAA	ChIP BrdU
ARS508 +3508 B	ATATGAACGGCAAATTGAGAC	
ARS508 +510 A	AGTCATTAATAGCAAAGCCGT	DRIP and ChIP *SPF1*
ARS508 +510 B	GGTCCTTTGATGTAACGATCA	
ARS1211 A	CGGCTTACCGGTCTTGAAAAT	ChIP BrdU
ARS1211 B	GGAATACTTTTGCTTGAGTTGTTTAGTTT	
ARS1211 +125 A	GTTTCCTCCACCTCCTTTGTGT	ChIP BrdU
ARS1211 +125 B	TGACCGATATATTGTGTTTCTATACTGTGT	
ARS1211 +2505 A	CGTTCAATTCGTTGGCGTTAC	ChIP BrdU
ARS1211 +2505 B	TTAACACCGTTTTCGGTTTGC	
ARS1021 A	CCCATTTCGGCGGCTAAT	ChIP BrdU
ARS1021 B	TAGAAGCCATTGATGGTATTGTACATT	
ARS1021 +4597 A	GGTTGCCCTAACGGTTGTTC	ChIP BrdU
ARS1021 +4597 B	TGGAGCTTTACCAACAAGAGCTAA	
ARS1021 +625 A	TGCTCCCCAAAATAAAGTGTTCTAC	ChIP BrdU
ARS1021 +625 B	AGCCCTTTGAAGGATGAATGAC	
Chr. V A	TGCCTGCACGCCATTGT	ChIP BrdU
Chr. V B	TTCCCCACGGAAAGTTGTATCT	
HXT1 A	AGCTGGCAGAATCGACGAA	ChIP *HXT1*
HXT1 B	GGTCAGGTGGGCATTTGTTAA	
GAL1 3´ A	AAAGAAGCCCTTGCCAATGA	DRIP and ChIP *GAL1*
GAL1 3´ B	CATTTTCTAGCTCAGCATCAGTGATC	
GAL1 5´ A	TGAGTTCAATTCTAGCGCAAAGG	ChIP *GAL1*
GAL1 5´ B	TTCTTAATTATGCTCGGGCACTT	
INT A	TGTTCCTTTAAGAGGTGATGGTGAT	ChIP intergenic region
INT B	GTGCGCAGTACTTGTGAAAACC	
TRP1 A	CGTCCAACTGCATGGAGATG	DRIP recombination
TRP1 B	TGGCAAACCGAGGAACTCTT	
rDNA 18S A	TCAACTTTCGATGGTAGGAT	DRIP rDNA
rDNA 18S B	GGAATCGAACCCTTATTCCC	
ADE2 A +Aor51HI	TTTGCCGACCGCACGCCGCATCCAGCGCTGACGC AAGAAAAACAAGAAAATCGG	Cloning
ADE2 B +Aor51HI	CCGTACAGCGCTTACTTGTTTTCTAGATAAGCTTC GTAACCGACA	
INS REC SYS A	ATGTCTGTTATTAATTTCACAGGTAGTTCTGGTCC ATTGGTGAAAGTTTGCGGCTTGCAGAGCACAGAG GAAGTAATTGTTGTTTGGCC	Cloning
INS REC SYS B	AGCATTTTTGACGAAATTTGCTATTTTGTTAGAGT CTTTTACACCATTTGTCTCCACACCTCCGCTTACA CGACTACGTCGTTAAGGCCG	
yHBD-GFP-BglII	TCCGGACTCAGATCTGGGATGTTCCGAAGGAACT ATCGATTCCTAATT	Cloning
yHBD-GFP-SalI	GATGCGGCCCTCCTGCAGGGCCCTGAGCTTGTAA TCATGCGCACTCAT	
yHBD-HA A	ATGTCTTTAATTAACATGGCAAGGCAAGGGAACT TCTAC	Cloning
yHBD-HA B	GTTAATTAAAGACATCGTGCCGTTTCCAAAACTT GAACC	

7.- Statistical analyses

Statistical tests (Student's *t*-test, Mann-Whitney *U*-test or *Wilcoxon* signed-rank *test*) were calculated using GraphPad Prism software. In general, a p-value<0.05 was considered as statistically significant.

REFERENCES

Aguilera A. and H. Gaillard (2014). "Transcription and Recombination: When RNA Meets DNA." Cold Spring Harbor Perspectives in Biology 6(8): a016543.

Aguilera A. and T. García-Muse (2012). "R loops: from transcription byproducts to threats to genome stability." Molecular Cell 46(2): 115-124.

Aguilera A. and B. Gómez-González (2008). "Genome instability: a mechanistic view of its causes and consequences." Nature Reviews. Genetics 204 9(3): 204-217.

Aguilera A. and H. L. Klein (1988). "Genetic control of intrachromosomal recombination in Saccharomyces cerevisiae. I. Isolation and genetic characterization of hyper-recombination mutations." Genetics 119(4): 779-790.

Ahmed S., D. G. Brickner, et al. (2010). "DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery." Nature Cell Biology 12(2): 111-118.

Alber F., S. Dokudovskaya, et al. (2007). "The molecular architecture of the nuclear pore complex." Nature 450: 695.

Azvolinsky A., P. G. Giresi, et al. (2009). "Highly transcribed RNA polymerase II genes are impediments to replication fork progression in Saccharomyces cerevisiae." Molecular cell 34(6): 722-734.

Bachl J., C. Carlson, et al. (2001). "Increased Transcription Levels Induce Higher Mutation Rates in a Hypermutating Cell Line." The Journal of Immunology 166(8): 5051-5057.

Bangs P., B. Burke, et al. (1998). "Functional Analysis of Tpr: Identification of Nuclear Pore Complex Association and Nuclear Localization Domains and a Role in mRNA Export." The Journal of Cell Biology 143(7): 1801-1812.

Beck M. and E. Hurt (2017). "The nuclear pore complex: understanding its function through structural insight." Nature Reviews Molecular Cell Biology 18: 73.

Bermejo R., T. Capra, et al. (2011). "The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores." Cell 146(2): 233-246.

Besmer E., E. Market, et al. (2006). "The Transcription Elongation Complex Directs Activation-Induced Cytidine Deaminase-Mediated DNA Deamination." Molecular and Cellular Biology 26(11): 4378-4385.

Bhatia V., S. I. Barroso, et al. (2014). "BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2." Nature 511(7509): 362-365.

Bhatia V., E. Herrera-Moyano, et al. (2017). "The Role of Replication-Associated Repair Factors on R-Loops." Genes 8(7): 171.

Blobel G. (1985). "Gene gating: a hypothesis." Proceedings of the National Academic of Science of the U.S.A. 82(24): 8527-8529.

Boeke J. D., F. La Croute, et al. (1984). "A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast : 5-fluoro-orotic acid resistance. ." Molecular & General Genetics 197(2): 345-346.

Boguslawski S., D. Smith, et al. (1986). "Characterization of monoclonal antibody to DNA.RNA and its application to immunodetection of hybrids." Journal of Immunological Methods 89(1): 123-130.

Bransteitter R., P. Pham, et al. (2003). "Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase." Proceedings of the National Academy of Sciences of the U.S.A. 100(7): 4102-4107.

Brewer B. J., D. Lockshon, et al. (1992). "The arrest of replication forks in the rDNA of yeast occurs independently of transcription." Cell 71(2): 267-276.

Brickner D. G., S. Ahmed, et al. (2012). "Transcription Factor Binding to a DNA Zip Code Controls Interchromosomal Clustering at the Nuclear Periphery." Developmental Cell 22(6): 1234-1246.

Brickner J. H. and P. Walter (2004). "Gene recruitment of the activated INO1 locus to the nuclear membrane." PLoS Biol 2(11): e342.

Brown C. R., C. J. Kennedy, et al. (2008). "Global histone acetylation induces functional genomic reorganization at mammalian nuclear pore complexes." Genes & Development 22(5): 627-639.

Burns L. T. and S. R. Wente (2014). "From Hypothesis to Mechanism: Uncovering Nuclear Pore Complex Links to Gene Expression." Molecular and Cellular Biology 34(12): 2114-2120.

Cabal G. G., A. Genovesio, et al. (2006). "SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope." Nature 441(7094): 770-773.

Casolari J. M., C. R. Brown, et al. (2005). "Developmentally induced changes in transcriptional program alter spatial organization across chromosomes." Genes and Development 19(10): 1188-1198.

Casolari J. M., C. R. Brown, et al. (2004). "Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization." Cell 117(4): 427-439.

Castellano-Pozo M., J. M. Santos-Pereira, et al. (2013). "R loops are linked to histone H3 S10 phosphorylation and chromatin condensation." Molecular Cell 52(4): 583-590.

Cerritelli S. M. and R. J. Crouch (1995). "The non-RNase H domain of Saccharomyces cerevisiae RNase H1 binds double-stranded RNA: magnesium modulates the switch between double-stranded RNA binding and RNase H activity." RNA 1(3): 246-259.

Cerritelli S. M. and R. J. Crouch (2009). "Ribonuclease H: the enzymes in eukaryotes." The FEBS Journal 276(6): 1494-1505.

Cerritelli S. M., E. G. Frolova, et al. (2003). "Failure to Produce Mitochondrial DNA Results in Embryonic Lethality in Rnaseh1 Null Mice." Molecular Cell 11(3): 807-815.

Cordes V. C., S. Reidenbach, et al. (1997). "Identification of protein p270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments." The Journal Cell Biology 136(3): 515-529.

Cha R. S. and N. Kleckner (2002). "ATR Homolog Mec1 Promotes Fork Progression, Thus Averting Breaks in Replication Slow Zones." Science 297(5581): 602.

Chan Y. A., M. J. Aristizabal, et al. (2014). "Genome-wide profiling of yeast DNA:RNA hybrid prone sites with DRIP-chip." PLoS Genetic 10(4): e1004288.

Chang E. Y. and P. C. Stirling (2017). "Replication Fork Protection Factors Controlling R-Loop Bypass and Suppression." Genes (Basel) 8(1).

Chaudhuri J., C. Khuong, et al. (2004). "Replication protein A interacts with AID to promote deamination of somatic hypermutation targets." Nature 430: 992.

Chaudhuri J., M. Tian, et al. (2003). "Transcription-targeted DNA deamination by the AID antibody diversification enzyme." Nature 422: 726.

Chávez S. and A. Aguilera (1997). "The yeast HPR1 gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability." Genes and Development 11(24): 3459-3470.

Chávez S., T. Beilharz, et al. (2000). "A protein complex containing Tho2, Hpr1, Mft1 and a novel protein, Thp2, connects transcription elongation with mitotic

recombination in Saccharomyces cerevisiae." The EMBO Journal 19(21): 5824-5834.

Chen L., J.-Y. Chen, et al. (2017). "R-ChIP Using Inactive RNase H Reveals Dynamic Coupling of R-loops with Transcriptional Pausing at Gene Promoters." Molecular Cell 68(4): 745-757.e745.

Chen M. and M. R. Gartenberg (2014). "Coordination of tRNA transcription with export at nuclear pore complexes in budding yeast." Genes and Development 28(9): 959-970.

Christman M. F., F. S. Dietrich, et al. (1988). "Mitotic recombination in the rDNA of S. cerevisiae is suppressed by the combined action of DNA topoisomerases I and II." Cell 55(3): 413-425.

Datta A. and S. Jinks-Robertson (1995). "Association of increased spontaneous mutation rates with high levels of transcription in yeast." Science 268(5217): 1616-1619.

den Dulk B., S. M. Sun, et al. (2006). "Rad33, a new factor involved in nucleotide excision repair in Saccharomyces cerevisae." DNA Repair 5(6): 683-692.

den Dulk B., P. van Eijk, et al. (2008). "The NER protein Rad33 shows functional homology to human Centrin2 and is involved in modification of Rad4." DNA Repair 7(6): 858-868.

Dieppois G., N. Iglesias, et al. (2006). "Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes." Molecular and Cellular Biology 26(21): 7858-7870.

Domínguez-Sánchez M. S., S. Barroso, et al. (2011). "Genome instability and transcription elongation impairment in human cells depleted of THO/TREX." PLoS Genetic 7(12): e1002386.

Dorsett Y., D. F. Robbiani, et al. (2007). "A role for AID in chromosome translocations between c-myc and the IgH variable region." The Journal of Experimental Medicine 204(9): 2225-2232.

Drubin D. A., A. M. Garakani, et al. (2006). "Motion as a phenotype: the use of live-cell imaging and machine visual screening to characterize transcription-dependent chromosome dynamics." BMC Cell Biology 7: 19.

Duquette M. L., P. Handa, et al. (2004). "Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA." Genes and Development 18(13): 1618-1629.

Ehrenstein M. R. and M. S. Neuberger (1999). "Deficiency in Msh2 affects the efficiency and local sequence specificity of immunoglobulin class-switch recombination: parallels with somatic hypermutation." The EMBO Journal 18(12): 3484-3490.

El Hage A., S. L. French, et al. (2010). "Loss of Topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis." Genes and Development 24(14): 1546-1558.

Ellisdon A. M., L. Dimitrova, et al. (2012). "Structural basis for the assembly and nucleic acid binding of the TREX-2 transcription-export complex." Nature Structural and Molecular Biology 19: 328.

Epshtein A., C. J. Potenski, et al. (2016). "Increased spontaneous recombination in RNase H2-deficient cells arises from multiple contiguous rNMPs and not from single rNMP residues incorporated by DNA polymerase epsilon." Microbial Cell 3(6): 248-254.

Evans S. P. and M. Bycroft (1999). "NMR structure of the N-terminal domain of Saccharomyces cerevisiae RNase HI reveals a fold with a strong resemblance to

the N-terminal domain of ribosomal protein L91." Journal of Molecular Biology 291(3): 661-669.

Fan H.-Y., R. J. Merker, et al. (2001). "High-Copy-Number Expression of Sub2p, a Member of the RNA Helicase Superfamily, Suppresses hpr1-Mediated Genomic Instability." Molecular and Cellular Biology 21(16): 5459-5470.

Fasken M. B., M. Stewart, et al. (2008). "Functional Significance of the Interaction between the mRNA-binding Protein, Nab2, and the Nuclear Pore-associated Protein, Mlp1, in mRNA Export." The Journal of Biological Chemistry 283(40): 27130-27143.

Faza M. B., S. Kemmler, et al. (2009). "Sem1 is a functional component of the nuclear pore complex–associated messenger RNA export machinery." The Journal of Cell Biology 184(6): 833-846.

Fear D. J. (2013). "Mechanisms regulating the targeting and activity of activation induced cytidine deaminase." Current Opinion in Immunology 25(5): 619-628.

Felipe-Abrio I., J. Lafuente-Barquero, et al. (2015). "RNA polymerase II contributes to preventing transcription-mediated replication fork stalls." The EMBO Journal 34(2): 236-250.

Feng Q., L. During, et al. (2007). "Rad52 and Rad59 exhibit both overlapping and distinct functions." DNA Repair 6(1): 27-37.

Feuerbach F., V. Galy, et al. (2002). "Nuclear architecture and spatial positioning help establish transcriptional states of telomeres in yeast." Nature Cell Biology 4: 214.

Frederico L. A., T. A. Kunkel, et al. (1990). "A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy." Biochemistry 29(10): 2532-2537.

French S. (1992). "Consequences of replication fork movement through transcription units in vivo." Science 258(5086): 1362.

Gaillard H., E. Herrera-Moyano, et al. (2013). "Transcription-Associated Genome Instability." Chemical Reviews 113(11): pp 8638–8661.

Gaillard H., R. E. Wellinger, et al. (2015). "Methods to Study Transcription-Coupled Repair in Chromatin." Chromatin Protocols. Methods in Molecular Biology 1288.

Galy V., O. Gadal, et al. (2004). "Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1." Cell 116(1): 63-73.

Gallardo M. and A. Aguilera (2001). "A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination." Genetics 157(1): 79-89.

Gallardo M., R. Luna, et al. (2003). "The Nab2p and Thp1p-Sac3p complex functionally interact at the interface transcription-mRNA metabolism." Journal of Biological Chemistry.

Gan W., Z. Guan, et al. (2011). "R-loop-mediated genomic instability is caused by impairment of replication fork progression." Genes and Development 25: 2041-2056.

García-Benítez F., H. Gaillard, et al. (2017). "Physical proximity of chromatin to nuclear pores prevents harmful R loop accumulation contributing to maintain genome stability." Proceedings of the National Academic of Science of the U.S.A. 114(41):10942-10947

García-Muse T. and A. Aguilera (2016). "Transcription–replication conflicts: how they occur and how they are resolved." Nature Reviews Molecular Cell Biology 17: 553.

García-Pichardo D., J. C. Cañas, et al. (2017). "Histone Mutants Separate R Loop Formation from Genome Instability Induction." Molecular Cell 66(5): 597-609 e595.

García-Rubio M., S. I. Barroso, et al. (2018). "Detection of DNA-RNA Hybrids In Vivo." Methods in Molecular Biology 1672: 347-361.

García-Rubio M., P. Huertas, et al. (2003). "Recombinogenic effects of DNA-damaging agents are synergistically increased by transcription in Saccharomyces cerevisiae. New insights into transcription-associated recombination." Genetics 165(2): 457-466.

García-Rubio M. L. and A. Aguilera (2012). "Topological constraints impair RNA polymerase II transcription and causes instability of plasmid-borne convergent genes." Nucleic Acids Research 40(3): 1050-1064.

García-Rubio M. L., C. Pérez-Calero, et al. (2015). "The Fanconi Anemia Pathway Protects Genome Integrity from R-loops." PLoS Genetics 11(11): e1005674.

Gari E., L. Piedrafita, et al. (1997). "A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in Saccharomyces cerevisiae." Yeast 13(9): 837-848.

Gavaldá S., J. M. Santos-Pereira, et al. (2016). "Excess of Yra1 RNA-Binding Factor Causes Transcription-Dependent Genome Instability, Replication Impairment and Telomere Shortening." PLoS Genetics 12(4): e1005966.

Gay S. and M. Foiani (2015). Chapter Six - Nuclear Envelope and Chromatin, Lock and Key of Genome Integrity. International Review of Cell and Molecular Biology. K. W. Jeon, Academic Press. 317: 267-330.

Gietz R., R. Schiestl, et al. (1995). "Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure." Yeast Apr 15(11(4)): 355-360.

Gilbert W. and C. Guthrie (2004). "The Glc7p Nuclear Phosphatase Promotes mRNA Export by Facilitating Association of Mex67p with mRNA." Molecular cell 13(2): 201-212.

Gobbini E., D. Cesena, et al. (2013). "Interplays between ATM/Tel1 and ATR/Mec1 in sensing and signaling DNA double-strand breaks." DNA Repair 12(10): 791-799.

Gómez-González B. and A. Aguilera (2007). "Activation-induced cytidine deaminase action is strongly stimulated by mutations of the THO complex." Proceedings of the National Academic of Science of the U.S.A. 104(20): 8409-8414.

Gómez-González B., M. García-Rubio, et al. (2011). "Genome-wide function of THO/TREX in active genes prevents R-loop-dependent replication obstacles." The EMBO Journal 30(15): 3106-3119.

Gomez-Gonzalez B., J. F. Ruiz, et al. (2011). "Genetic and molecular analysis of mitotic recombination in Saccharomyces cerevisiae." Methods in Molecular Biology 745: 151-172.

González-Aguilera C., C. Tous, et al. (2008). "The THP1-SAC3-SUS1-CDC31 complex works in transcription elongation-mRNA export preventing RNA-mediated genome instability." Molecular Biology of Cell 19(10): 4310-4318.

Grant R. P., N. J. Marshall, et al. (2008). "Structure of the N-Terminal Mlp1-Binding Domain of the Saccharomyces cerevisiae mRNA-Binding Protein, Nab2." Journal of Molecular Biology 376(4): 1048-1059.

Green D. M., C. P. Johnson, et al. (2003). "The C-terminal domain of myosin-like protein 1 (Mlp1p) is a docking site for heterogeneous nuclear ribonucleoproteins that are required for mRNA export." Proceedings of the National Academic of Science of the U.S.A. 100(3): 1010-1015.

Guikema J. E. J., E. K. Linehan, et al. (2007). "APE1- and APE2-dependent DNA breaks in immunoglobulin class switch recombination." The Journal of Experimental Medicine 204(12): 3017-3026.

Hamperl S., M. J. Bocek, et al. (2017). "Transcription-Replication Conflict Orientation Modulates R-Loop Levels and Activates Distinct DNA Damage Responses." Cell 170(4): 774-786.e719.

Hamperl S. and K. A. Cimprich (2014). "The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and genome instability." DNA Repair (Amst) 19: 84-94.

Hanahan D. (1983). "Studies on transformation of Escherichia coli with plasmids." Journal of Molecular Biology 166(4): 557-580.

Harper J. W. and S. J. Elledge (2007). "The DNA Damage Response: Ten Years After." Molecular Cell 28(5): 739-745.

Hatchi E., K. Skourti-Stathaki, et al. (2015). "BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair." Molecular Cell 57(4): 636-647.

Herman R. K. and N. B. Dworkin (1971). "Effect of Gene Induction on the Rate of Mutagenesis by ICR-191 in Escherichia coli." Journal of Bacteriology 106(2): 543-550.

Herrera-Moyano E., X. Mergui, et al. (2014). "The yeast and human FACT chromatin-reorganizing complexes solve R-loop-mediated transcription-replication conflicts." Genes and Development 28(7): 735-748.

Heyer W.-D., K. T. Ehmsen, et al. (2010). "Regulation of homologous recombination in eukaryotes." Annual review of genetics 44: 113-139.

Hill S. J., T. Rolland, et al. (2014). "Systematic screening reveals a role for BRCA1 in the response to transcription-associated DNA damage." Genes and Development 28(17): 1957-1975.

Hodroj D., B. Recolin, et al. (2017). "An ATR-dependent function for the Ddx19 RNA helicase in nuclear R-loop metabolism." The EMBO Journal 36(9): 1182-1198.

Hoelz A., E. W. Debler, et al. (2011). "The Structure of the Nuclear Pore Complex." Annual Review of Biochemistry 80(1): 613-643.

Horigome C., D. E. Bustard, et al. (2016). "PolySUMOylation by Siz2 and Mms21 triggers relocation of DNA breaks to nuclear pores through the Slx5/Slx8 STUbL." Genes and Development 30(8): 931-945.

Huang G. S. and R. L. Keil (1995). "Requirements for Activity of the Yeast Mitotic Recombination Hotspot Hot1: RNA Polymerase I and Multiple Cis-Acting Sequences." Genetics 141(3): 845-855.

Huertas P. and A. Aguilera (2003). "Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination." Molecular Cell 12(3): 711-721.

Hutchison N. and W. H. (1985). "Localization of DNAase I-sensitive sequence to specific regions of interphase nuclei." Cell Dec 43((2 Pt 1)): 471-482.

Ibarra A., C. Benner, et al. (2016). "Nucleoporin-mediated regulation of cell identity genes." Genes and Development 30(20): 2253-2258.

Ibarra A. and M. W. Hetzer (2015). "Nuclear pore proteins and the control of genome functions." Genes and Development 29(4): 337-349.

Iglesias N., E. Tutucci, et al. (2010). "Ubiquitin-mediated mRNP dynamics and surveillance prior to budding yeast mRNA export." Genes and Development 24(17): 1927-1938.

Ikeda H. and T. Matsumoto (1979). "Transcription promotes recA-independent recombination mediated by DNA-dependent RNA polymerase in Escherichia coli." Proceedings of the National Academic of Science of the U.S.A. 76(9): 4571-4575.

Ivessa A. S., B. A. Lenzmeier, et al. (2003). "The Saccharomyces cerevisiae Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes." Molecular Cell 12(6): 1525-1536.

Jani D., E. Valkov, et al. (2014). "Structural basis for binding the TREX2 complex to nuclear pores, GAL1 localisation and mRNA export." Nucleic Acids Research 42(10): 6686-6697.

Jimeno S., R. Luna, et al. (2006). "Tho1, a Novel hnRNP, and Sub2 Provide Alternative Pathways for mRNP Biogenesis in Yeast THO Mutants." Molecular and Cellular Biology 26(12): 4387-4398.

Jimeno S., A. G. Rondon, et al. (2002). "The yeast THO complex and mRNA export factors link RNA metabolism with transcription and genome instability." The EMBO Journal 21(13): 3526-3535.

Jones R. M., O. Mortusewicz, et al. (2013). "Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress." Oncogene 32: 3744.

Jong A. Y., B. Wang, et al. (1995). "Pulsed Field Gel Electrophoresis Labeling Method to Study the Pattern of Saccharomyces cerevisiae Chromosomal DNA Synthesis During the G1/S Phase of the Cell Cycle." Analytical Biochemistry 227(1): 32-39.

Kabeche L., H. D. Nguyen, et al. (2018). "A mitosis-specific and R loop-driven ATR pathway promotes faithful chromosome segregation." Science Jan 5(359(5631)): 108-114.

Kim N. and S. Jinks-Robertson (2009). "dUTP incorporation into genomic DNA is linked to transcription in yeast." Nature 459(7250): 1150-1153.

Kobayashi T. (2003). "The Replication Fork Barrier Site Forms a Unique Structure with Fob1p and Inhibits the Replication Fork." Molecular and Cellular Biology 23(24): 9178-9188.

Köhler A. and E. Hurt (2007). "Exporting RNA from the nucleus to the cytoplasm." Nature Reviews Molecular Cell Biology 8: 761.

Köhrer K. and H. Domdey (1991). "Preparation of high molecular weight RNA." Methods in enzymology 194: 386-405.

König F., T. Schubert, et al. (2017). "The monoclonal S9.6 antibody exhibits highly variable binding affinities towards different R-loop sequences." PLoS One 12(6): e0178875.

Kosova B., N. Pante, et al. (2000). "Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p." The Journal of Biological Chemistry 275(1): 343-350.

Krull S., J. Dörries, et al. (2010). "Protein Tpr is required for establishing nuclear pore-associated zones of heterochromatin exclusion." The EMBO Journal 29(10): 1659-1673.

Kundu S., P. J. Horn, et al. (2007). "SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster." Genes and Development 21(8): 997-1004.

Laemmli U. K. (1970). "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4." Nature 227: 680.

Lafuente-Barquero J., S. Luke-Glaser, et al. (2017). "The Smc5/6 complex regulates the yeast Mph1 helicase at RNA-DNA hybrid-mediated DNA damage." PLoS Genetics 13(12): e1007136.

Lang K. S., A. N. Hall, et al. (2017). "Replication-Transcription Conflicts Generate R-Loops that Orchestrate Bacterial Stress Survival and Pathogenesis." Cell 170(4): 787-799.e718.

Lei E. P., C. A. Stern, et al. (2003). "Sac3 Is an mRNA Export Factor That Localizes to Cytoplasmic Fibrils of Nuclear Pore Complex." Molecular Biology of the Cell 14(3): 836-847.

Lengronne A., P. Pasero, et al. (2001). "Monitoring S phase progression globally and locally using BrdU incorporation in TK(+) yeast strains." Nucleic Acids Research 29(7): 1433-1442.

Li X. and J. L. Manley (2005). "Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability." Cell 122(3): 365-378.

Liang Y., T. M. Franks, et al. (2013). "Dynamic Association of NUP98 with the Human Genome." PLoS Genetics 9(2): e1003308.

Lisby M., R. Rothstein, et al. (2001). "Rad52 forms DNA repair and recombination centers during S phase." Proceedings of the National Academic of Science of the U.S.A. 98(15): 8276-8282.

Loeillet S., B. Palancade, et al. (2005). "Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast." DNA Repair 4(4): 459-468.

Luna R., H. Gaillard, et al. (2008). "Biogenesis of mRNPs: integrating different processes in the eukaryotic nucleus." Chromosoma 117(4): 319-331.

Luthra R., S. C. Kerr, et al. (2007). "Actively transcribed GAL genes can be physically linked to the nuclear pore by the SAGA chromatin modifying complex." Journal of Biological Chemistry 282(5): 3042-3049.

Marini M., A. Falqui, et al. (2015). "The structure of DNA by direct imaging." Science Advances 1(7): e1500734.

Mason P. B. and K. Struhl (2005). "Distinction and Relationship between Elongation Rate and Processivity of RNA Polymerase II In Vivo." Molecular Cell 17(6): 831-840.

Matsuzaki K., A. Shinohara, et al. (2008). "Forkhead-Associated Domain of Yeast Xrs2, a Homolog of Human Nbs1, Promotes Nonhomologous End Joining Through Interaction With a Ligase IV Partner Protein, Lif1." Genetics 179(1): 213-225.

Maul R. W., H. Saribasak, et al. (2011). "Uracil residues dependent on the deaminase AID in immunoglobulin gene variable and switch regions." Nature Immunology 12(1): 70-76.

Mirkin E. V. and S. M. Mirkin (2007). "Replication Fork Stalling at Natural Impediments." Microbiology and Molecular Biology Reviews : MMBR 71(1): 13-35.

Mischo H. E., B. Gomez-Gonzalez, et al. (2011). "Yeast Sen1 helicase protects the genome from transcription-associated instability." Molecular Cell 41(1): 21-32.

Morales J. C., P. Richard, et al. (2016). "XRN2 Links Transcription Termination to DNA Damage and Replication Stress." PLoS Genetics 12(7): e1006107.

Moriel-Carretero M., C. Tous, et al. (2011). "Control of the function of the transcription and repair factor TFIIH by the action of the cochaperone Ydj1." Proceedings of the National Academic of Science of the U.S.A. 108(37): 15300-15305.

Mumberg D., R. Muller, et al. (1994). "Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression." Nucleic Acids Reserchs 22(25): 5767-5768.

Muramatsu M., K. Kinoshita, et al. (2000). "Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme." Cell 102(5): 553-563.

Nagai S., K. Dubrana, et al. (2008). "Functional Targeting of DNA Damage to a Nuclear Pore–Associated SUMO-Dependent Ubiquitin Ligase." Science 322(5901): 597-602.

Nakama M., K. Kawakami, et al. (2012). "DNA–RNA hybrid formation mediates RNAi-directed heterochromatin formation." Genes to Cells 17(3): 218-233.

Nguyen H. D., T. Yadav, et al. (2017). "Functions of Replication Protein A as a Sensor of R Loops and a Regulator of RNaseH1." Molecular Cell 65(5): 832-847.

Nickoloff J. A. and R. J. Reynolds (1990). "Transcription stimulates homologous recombination in mammalian cells." Molecular and Cellular Biology 10(9): 4837-4845.

Niepel M., K. R. Molloy, et al. (2013). "The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome." Molecular Biology of the Cell 24(24): 3920-3938.

Niepel M., C. Strambio-de-Castillia, et al. (2005). "The nuclear pore complex–associated protein, Mlp2p, binds to the yeast spindle pole body and promotes its efficient assembly." The Journal of Cell Biology 170(2): 225-235.

Nowotny M., S. M. Cerritelli, et al. (2008). "Specific recognition of RNA/DNA hybrid and enhancement of human RNase H1 activity by HBD." The EMBO Journal 27(7): 1172-1181.

Ohle C., R. Tesorero, et al. (2016). "Transient RNA-DNA Hybrids Are Required for Efficient Double-Strand Break Repair." Cell 167(4): 1001-1013.e1007.

Oza P., S. L. Jaspersen, et al. (2009). "Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery." Genes and Development 23(8): 912-927.

Palancade B., X. Liu, et al. (2007). "Nucleoporins Prevent DNA Damage Accumulation by Modulating Ulp1-dependent Sumoylation Processes." Molecular Biology of the Cell 18(8): 2912-2923.

Pardo B., B. Gómez-González, et al. (2009). "DNA double-strand break repair: how to fix a broken relationship." Celullar and Molecular Life Sciencies 66(6): 1039-1056.

Paulsen R. D., D. V. Soni, et al. (2009). "A genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability." Molecular Cell 35(2): 228-239.

Peña Á., K. Gewartowski, et al. (2012). "Architecture and nucleic acids recognition mechanism of the THO complex, an mRNP assembly factor." The EMBO Journal 31(6): 1605-1616.

Perales R. and D. Bentley (2009). ""Co-transcriptionality" - the transcription elongation complex as a nexus for nuclear transactions." Molecular Cell 36(2): 178-191.

Petersen-Mahrt S. K., R. S. Harris, et al. (2002). "AID mutates E. coli suggesting a DNA deamination mechanism for antibody diversification." Nature 418: 99.

Petersen S., R. Casellas, et al. (2001). "AID is required to initiate Nbs1/γ-H2AX focus formation and mutations at sites of class switching." Nature 414(6864): 660-665.

Piruat J. I. and A. Aguilera (1998). "A novel yeast gene, THO2, is involved in RNA pol II transcription and provides new evidence for transcriptional elongation-associated recombination." The EMBO Journal 17(16): 4859-4872.

Poli J., C.-B. Gerhold, et al. (2016). "Mec1, INO80, and the PAF1 complex cooperate to limit transcription replication conflicts through RNAPII removal during replication stress." Genes and Development 30(3): 337-354.

Powrie E. A., D. Zenklusen, et al. (2011). "A nucleoporin, Nup60p, affects the nuclear and cytoplasmic localization of ASH1 mRNA in S. cerevisiae." RNA 17(1): 134-144.

Prado F. and A. Aguilera (2005). "Impairment of replication fork progression mediates RNA polII transcription-associated recombination." The EMBO Journal 24(6): 1267-1276.

Prado F., J. I. Piruat, et al. (1997). "Recombination between DNA repeats in yeast hpr1delta cells is linked to transcription elongation." The EMBO Journal 16(10): 2826-2835.

Pratt-Hyatt M. J., K. M. Kapadia, et al. (2006). "Increased Recombination Between Active tRNA Genes." DNA and cell biology 25(6): 359-364.

Rajanala K. and V. K. Nandicoori (2012). "Localization of Nucleoporin Tpr to the Nuclear Pore Complex Is Essential for Tpr Mediated Regulation of the Export of Unspliced RNA." PLoS One 7(1): e29921.

Ramiro A. R., M. Jankovic, et al. (2004). "AID Is Required for c-myc/IgH Chromosome Translocations In Vivo." Cell 118(4): 431-438.

Richard P. and J. L. Manley (2017). "R Loops and Links to Human Disease." Journal of Molecular Biology 429(21): 3168-3180.

Roberts R. W. and D. M. Crothers (1992). "Stability and properties of double and triple helices: dramatic effects of RNA or DNA backbone composition." Science 258(5087): 1463.

Rodríguez-Navarro S., T. Fischer, et al. (2004). "Sus1, a Functional Component of the SAGA Histone Acetylase Complex and the Nuclear Pore-Associated mRNA Export Machinery." Cell 116(1): 75-86.

Rohner S., V. Kalck, et al. (2013). "Promoter- and RNA polymerase II–dependent hsp-16 gene association with nuclear pores in Caenorhabditis elegans." The Journal of Cell Biology 200(5): 589-604.

Rondón A. G., M. García-Rubio, et al. (2003). "Molecular evidence for a positive role of Spt4 in transcription elongation." The EMBO Journal 22(3): 612-620.

Rondon A. G., S. Jimeno, et al. (2003). "Molecular Evidence that the Eukaryotic THO/TREX complex is required for efficient transcription elongation." Journal of Biological Chemistry Oct 3(278 (40)): 390637-390643.

Roy D. and M. R. Lieber (2009). "G Clustering Is Important for the Initiation of Transcription-Induced R-Loops In Vitro, whereas High G Density without Clustering Is Sufficient Thereafter." Molecular and Cellular Biology 29(11): 3124-3133.

Roy D., Z. Zhang, et al. (2010). "Competition between the RNA Transcript and the Nontemplate DNA Strand during R-Loop Formation In Vitro: a Nick Can Serve as a Strong R-Loop Initiation Site." Molecular and Cellular Biology 30(1): 146-159.

Ruiz J. F., B. Gómez-González, et al. (2011). "AID Induces Double-Strand Breaks at Immunoglobulin Switch Regions and c-MYC Causing Chromosomal Translocations in Yeast THO Mutants." PLoS Genetics 7(2): e1002009.

Sakiyama Y., R. Panatala, et al. (2017). "Structural dynamics of the nuclear pore complex." Seminars in Cell & Developmental Biology 68: 27-33.

Salas-Armenteros I., C. Pérez-Calero, et al. (2017). "Human THO–Sin3A interaction reveals new mechanisms to prevent R-loops that cause genome instability." The EMBO Journal 36(23): 3532-3547.

Samadashwily G. M., G. Raca, et al. (1997). "Trinucleotide repeats affect DNA replication in vivo." Nature Genetics 17: 298.

Santos-Pereira J. M. and A. Aguilera (2015). "R loops: new modulators of genome dynamics and function." Nature Reviews. Genetic 16(10): 583-597.

Santos-Pereira J. M., A. B. Herrero, et al. (2013). "The Npl3 hnRNP prevents R-loop-mediated transcription-replication conflicts and genome instability." Genes and Development 27(22): 2445-2458.

Santos-Rosa H. and A. Aguilera (1994). "Increase in incidence of chromosome instability and non-conservative recombination between repeats in Saccharomyces cerevisiae hpr1 delta strains." Molecular and General Genetics 245(2): 224-236.

Sanz L. A., S. R. Hartono, et al. (2016). "Prevalent, dynamic, and conserved R-loop structures associate with specific epigenomic signatures in mammals." Molecular Cell 63(1): 167-178.

Sarma N. J., T. M. Haley, et al. (2007). "Glucose-Responsive Regulators of Gene Expression in Saccharomyces cerevisiae Function at the Nuclear Periphery via a Reverse Recruitment Mechanism." Genetics 175(3): 1127-1135.

Saroufim M. A., P. Bensidoun, et al. (2015). "The nuclear basket mediates perinuclear mRNA scanning in budding yeast." The Journal of Cell Biology 211(6): 1131.

Schmid M., G. Arib, et al. (2006). "Nup-PI: the nucleopore-promoter interaction of genes in yeast." Molecular Cell 21(3): 379-391.

Schmid M., P. Olszewski, et al. (2015). "The Nuclear PolyA-Binding Protein Nab2p Is Essential for mRNA Production." Cell Reports 12(1): 128-139.

Schwab Rebekka A., J. Nieminuszczy, et al. (2015). "The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription." Molecular cell 60(3): 351-361.

Seeber A. and S. M. Gasser (2017). "Chromatin organization and dynamics in double-strand break repair." Current Opinion in Genetics & Development 43: 9-16.

Sertic S., S. Pizzi, et al. (2012). "NER and DDR: Classical music with new instruments." Cell Cycle 11(4): 668-674.

Sherman S., G. Fink, et al. (1986). "Methods in yeast genetics." Cold Spring Harbor NY.

Shibata A. (2017). "Regulation of repair pathway choice at two-ended DNA double-strand breaks." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 803-805: 51-55.

Sikorski R. S. and P. Hieter (1989). "A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae." Genetics 122(1): 19-27.

Smolka M. B., C. P. Albuquerque, et al. (2007). "Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases." Proceeding of the National Academic of Science of the U.S.A. 104(25): 10364-10369.

Soheilypour M. and M. R. K. Mofrad (2016). "Regulation of RNA-binding proteins affinity to export receptors enables the nuclear basket proteins to distinguish and retain aberrant mRNAs." Scientific Reports 6: 35380.

Sollier J. and K. A. Cimprich (2015). "R-Loops Breaking Bad." Trends in Cell Biology 25(9): 514-522.

Sollier J., C. T. Stork, et al. (2014). "Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability." Molecular Cell 56(6): 777-785.

Stavnezer J., J. E. J. Guikema, et al. (2009). "Mechanism and Regulation of Class Switch Recombination." Annual review of Immunology Jul 8(26): 261-292.

Stirling P. C., Y. A. Chan, et al. (2012). "R-loop-mediated genome instability in mRNA cleavage and polyadenylation mutants." Genes and Development 26(2): 163-175.

Strambio-de-Castillia C., G. Blobel, et al. (1999). "Proteins connecting the nuclear pore complex with the nuclear interior." Journal in Cell Biology 144(5): 839-855.

Strambio-De-Castillia C., M. Niepel, et al. (2010). "The nuclear pore complex: bridging nuclear transport and gene regulation." Nature Reviews Molecular Cell Biology 11: 490.

Sträßer K., S. Masuda, et al. (2002). "TREX is a conserved complex coupling transcription with messenger RNA export." Nature 417: 304.

Su X. A., V. Dion, et al. (2015). "Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability." Genes and Development 29(10): 1006-1017.

Sulli G., R. Di Micco, et al. (2012). "Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer." Nature Reviews Cancer 12: 709.

Taddei A. and S. M. Gasser (2012). "Structure and Function in the Budding Yeast Nucleus." Genetics 192(1): 107-129.

Taddei A., G. Van Houwe, et al. (2006). "Nuclear pore association confers optimal expression levels for an inducible yeast gene." Nature 441(7094): 774-778.

Tan-Wong S. M., H. D. Wijayatilake, et al. (2009). "Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex." Genes and Development 23(22): 2610-2624.

Tanikawa M., K. Sanjiv, et al. (2016). "The spliceosome U2 snRNP factors promote genome stability through distinct mechanisms; transcription of repair factors and R-loop processing." Oncogenesis 5(12): e280.

ten Asbroek A. L. M. A., M. van Groenigen, et al. (2002). "Ribonuclease H1 Maps to Chromosome 2 and Has at Least Three Pseudogene Loci in the Human Genome." Genomics 79(6): 818-823.

Texari L., G. Dieppois, et al. (2013). "The nuclear pore regulates GAL1 gene transcription by controlling the localization of the SUMO protease Ulp1." Molecular Cell 51(6): 807-818.

Thomas B. J. and R. Rothstein (1989). "Elevated recombination rates in transcriptionally active DNA." Cell 56(4): 619-630.

Tresini M., D. O. Warmerdam, et al. (2015). "The core spliceosome as target and effector of non-canonical ATM signaling." Nature 523(7558): 53-58.

Tuduri S., L. Crabbe, et al. (2009). "Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription." Nature Cell Biology 11(11): 1315-1324.

Vaquerizas J. M., R. Suyama, et al. (2010). "Nuclear Pore Proteins Nup153 and Megator Define Transcriptionally Active Regions in the Drosophila Genome." PLoS Genetics 6(2): e1000846.

Vijayraghavan S., F.-L. Tsai, et al. (2016). "A Checkpoint-Related Function of the MCM Replicative Helicase Is Required to Avert Accumulation of RNA:DNA Hybrids during S-phase and Ensuing DSBs during G2/M." PLoS Genetics 12(8): e1006277.

Vinciguerra P., N. Iglesias, et al. (2005). "Perinuclear Mlp proteins downregulate gene expression in response to a defect in mRNA export." The EMBO Journal 24(4): 813-823.

Wahba L., J. D. Amon, et al. (2011). "RNase H and multiple RNA biogenesis factors cooperate to prevent RNA-DNA hybrids from generating genome instability." Molecular Cell 44(6): 978-988.

Wellinger R. E., F. Prado, et al. (2006). "Replication fork progression is impaired by transcription in hyperrecombinant yeast cells lacking a functional THO complex." Molecular and Cellular Biology 26(8): 3327-3334.

Winzeler E. A., D. D. Shoemaker, et al. (1999). "Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis." Science 285(5429): 901-906.

Wong R. W. (2015). "Nuclear Pore Complex: From Structural View to Chemical Tools." Chemistry & Biology 22(10): 1285-1287.

Yadav P., N. Owiti, et al. (2016). "The role of topoisomerase I in suppressing genome instability associated with a highly transcribed guanine-rich sequence is not restricted to preventing RNA:DNA hybrid accumulation." Nucleic Acids Research 44(2): 718-729.

Yu K., F. Chedin, et al. (2003). "R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells." Nature Immunology 4: 442.

Zenklusen D., P. Vinciguerra, et al. (2002). "Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p." Molecular and Cellular Biology 22(23): 8241-8253.

PUBLISHED RESULTS