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Free Histones and the Cell Cycle

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1. Introduction

DNA replication, the basis of biological inheritance, is a fundamental process occurring during the S-phase of the cell cycle in all eukaryotes. In the nucleus, DNA is associated with histones, basic proteins that help package the lengthy genome to form nucleoprotein filaments called chromatin. Histones are essential for viability as they pack DNA into the nucleus and regulate access to the genetic information contained within the DNA. Due to their strong positive charge, non-chromatin-bound histones can bind non-specifically to negatively charged molecules in the cell, including nucleic acids such as DNA and RNA, as well as negatively charged proteins. Therefore, histone levels are tightly regulated to prevent harmful effects of free histone accumulation: this regulation takes place transcriptionally, posttranscriptionally, translationally and posttranslationally (reviewed in Gunjan et al., 2005). Despite this regulation, different situations can induce an accumulation of non-chromatin-bound histones, called "free" or "excess" histones (Gunjan & Verreault, 2003). In the budding yeast *Saccharomyces cerevisiae*, elevated free histones levels lead to increased DNA damage sensitivity and genomic instability in the form of enhanced mitotic chromosome loss (Gunjan & Verreault, 2003; Singh et al., 2009; Meeks-Wagner & Hartwell, 1986).

A delicate balance between histone and DNA synthesis during the package of the genome into chromatin is essential for cell viability. For this reason, a key regulatory event during the G1/S transition is the induction of histone genes, which allows the coupling of bulk histone synthesis to ongoing DNA replication. In proliferating cells, the synthesis of the vast majority of histones occurs during the S-phase of the cell cycle (Osley, 1991). Moreover in recent years, a novel surveillance mechanism has been described in budding yeast that monitors the accumulation of non-chromatin-bound histones and promotes their rapid degradation by the proteasome in a Rad53 kinase-dependent manner (Gunjan & Verrault, 2003).

In this chapter, we will focus on the model yeast *Sacharomyces cerevisiae* to review how an excess of free histones may be generated in the cell and the different regulatory mechanisms, preventing free histones accumulation in proliferating cells. An overview of the field would be useful to better understand and discuss how essential processes such as chromatin reassembly, transcription elongation, DNA replication and the cell cycle can be coupled through free histone levels. A putative role for Rad53 as a super-integrator of different signals will be discussed.

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2. DNA replication: a crucial event integrated in the cell cycle

DNA replication takes place during the S-phase of the cell cycle. To transmit genetic information over generations, DNA must be precisely replicated before chromosomal segregation takes place. For this purpose, the eukaryotic cell has regulatory mechanisms to limit chromosomal DNA duplication to once per cell cycle, to decide the onset of a new round of DNA replication and to respond to situations in which the genome is at risk.

2.1 Early events in chromosome replication

Accurate and complete DNA replication in each cell cycle and repair of DNA lesions are critical for the maintenance of genetic stability (Aguilera & Gomez-Gonzalez, 2008; Branzei & Foiani, 2008). Failures in this process reduce cell survival and lead to cancer and other diseases in higher metazoans (Hoeijmakers, 2001; Friedberg, 2003). Chromosomal DNA replication in eukaryotes initiates from multiple specific regions of chromosome DNA, known as origins of replication. Therefore, it is crucial to understand how each individual origin is regulated during the cell cycle.

In the budding yeast *S. cerevisiae,* as in higher eukaryotics, activation of multiple replication origins occurs as a two-step reaction (for reviews, see Diffley, 1996, 2004; Bell & Dutta, 2002; Tanaka & Araki, 2010). In the first reaction, called "licensing", a specific protein-DNA complex, known as the pre-replicative complex (pre-RC), is loaded onto origins in the G1 phase. The pre-RC comprises an ORC (origin recognition complex), Cdc6, Cdt1, and the replicative helicase Mcm2-7, which is inactive at this stage. In the second reaction, licensed origins fire at different times during the S-phase to each initiate a pair of replication forks. This origin "firing", or initiation, requires the action of two kinases: cyclin-dependent kinases (CDKs) and Cdc7/Dbf4 (DDK). At the mitotic exit through the G1 phase, CDKs and DDK activities are low, so the replicative helicase Mcm2-7 remains inactive. S-phase-CDKs and DDK activities become more intense during late G1 and promote the assembly of the active replicative helicase. S-CDKs phosphorylate Sld2 and Sld3, enabling them to bind to Dpb11 (Tanaka et al., 2007; Zegerman & Diffley, 2007), whereas DDK acts by phosphorylating subunits of the Mcm2-7 helicase (Sheu & Stillman, 2010). Once the active helicase is assembled, replication origin DNA is unwound and replication forks are formed to synthesise DNA.

In order to coordinate these processes, a regulatory link between DNA replication and cell cycle progression must exist. Firstly, faithful inheritance of the genetic material requires DNA replication to be precisely controlled so that it occurs once per cell cycle. If not, the pre-RC would be reassembled at origins that have already fired, resulting in an over-replication of some parts of the genome. The key to this regulation lies in the initiation of DNA replication and regulatory cell cycle elements control during the M/G1 and G1/S transitions. CDKs play an important role in separating these two reactions (Arias & Walter, 2007). During the mitotic exit and G1, CDK activity is reduced by two different mechanisms: down-regulation in the level of these cyclins and accumulation of the CDK inhibitor Sic1 (Stegmeier & Amon, 2004). Under these conditions, the pre-RCs are assembled at replication origins, but initiation does not occur because CDK activity is low. In the following S phase, S-CDK is activated and DNA replication initiates. At the same time, and very importantly, reassembly of the pre-RC at origins is blocked by CDK to inhibit re-replication (Diffley, 2004; Tanaka et al., 2007): CDK can phosphorylate all the pre-RC components, ORC, Cdc6, Cdt1, and Mcm2-7, to down-regulate their activities for the pre-RC formation (reviewed in

Tanaka & Araki, 2010). Less is known, however, about the dephosphorylation of initiation proteins, whether it is necessary for replication origin resetting and the acting phosphatase(s) that might control this process and, therefore, replication licensing. Recently it has been demonstrated that Cdc14p resets the competency of replication licensing by dephosphorylating multiple initiation proteins during the mitotic exit in budding yeast (Zhai et al., 2010).

2.2 The importance of the G1/S transition

In *S. cerevisiae*, commitment to a new round of cell division takes place towards the end of the G1 phase of the cell cycle, a process called START (Hartwell & Kastan, 1994). This is the main regulatory event of the G1 phase of the cell cycle, and involves an extensive transcriptional programme driven by transcription factors SBF (Swi4-Swi6) and MBF (Mbp1-Swi6) (Costanzo et al., 2004; de Bruin et al., 2004). Activation of these factors depends ultimately on G1 cyclin Cln3.

There are three G1 cyclins in *S. cerevisiae*: *CLN1*, *CLN2* and *CLN3*. MBF and SBF activation in START depends on the cyclin/cyclin-dependent-kinase (CDK) complex Cln3-Cdc28 which phosphorylates the negative regulator of START, Whi5, by promoting its release from SBF (Swi4-Swi6) (Costanzo et al., 2004; de Bruin et al., 2004). Activation of the MBF-dependent transcription by Cln3-Cdc28 is thought to act through a mechanism that is independent of Whi5 which involves the phosphorylation of Mbp1. Accordingly, a recent work has determined the transcriptional targets of Cln3 and their dependence on the SBF or MBF factors (Ferrezuelo et al., 2010). This analysis has produced more than 200 transcription factor-target assignments validated by ChiP assays and by functional enrichment, and supports a model whereby Cln3 differentially activates SBF and MBF. Activation leads to the modification and recruitment of the factors involved in transcription initiation and, therefore, to transcription activation (Costanzo et al., 2004; de Bruin et al., 2004; Takahata et al., 2009). Activation of these factors results in the accumulation of G1 and S-phase cyclins, which promotes S-phase entry (reviewed in Wittenberg, 2005). The kinase activity of Cln1,2-Cdc28 notably triggers the degradation of cyclin-dependent kinase inhibitor Sic1, which no longer inhibits the S phase-promoting complex Clb5,6-Cdc28 (Schneider et al., 1996; Schwob et al., 1994). In addition, a positive feedback mechanism involving Cln1 and Cln2 has been proposed to operate under physiological conditions in SBF/MBF activation (Skotheim et al., 2008). The main events in the *S. cerevisiae* G1-to-S transition are schematised in Figure 1.

Cells tightly regulate the different cell cycle transitions to ensure the correct transmission of genetic information. Checkpoints are surveillance mechanisms that prevent one cell cycle stage from starting if a previous cell cycle stage has not been successfully completed. Checkpoints can be considered as signal transduction cascades with three components: sensors to detect incomplete or aberrant cell cycle events; transducers of the checkpoint signal; and targets that are modified by transducers to cause cell cycle arrest (Elledge, 1996). As the G1-to S phase transition (START) signifies a commitment to complete cell division, eukaryotic cells are capable of undergoing transient arrest during the G1/S transition if conditions which would be unfavourable for cell division, such as nutrient limitation (Gallego et al., 1997), environmental toxins (Philpott et al., 1998) or damaged DNA, are encountered. Impaired ability to either initiate the arrest or to subsequently recover from the arrest and to resume cell division appears to be detrimental (Hartwell & Kastan, 1994; Lydall & Weinert, 1995; Shaulian et al., 2000). In recent years, the way different DNA-

damage situations can trigger cell cycle checkpoint machinery has been studied in great detail. DNA damage or replicative stress, depending on where the cell happens to be in the cell cycle, can cause cell cycle arrest via the "G1/S" checkpoint, the "intra-S" checkpoint or the "G2/M" checkpoint (Jares et al., 2000; Segurado & Tercero, 2009).

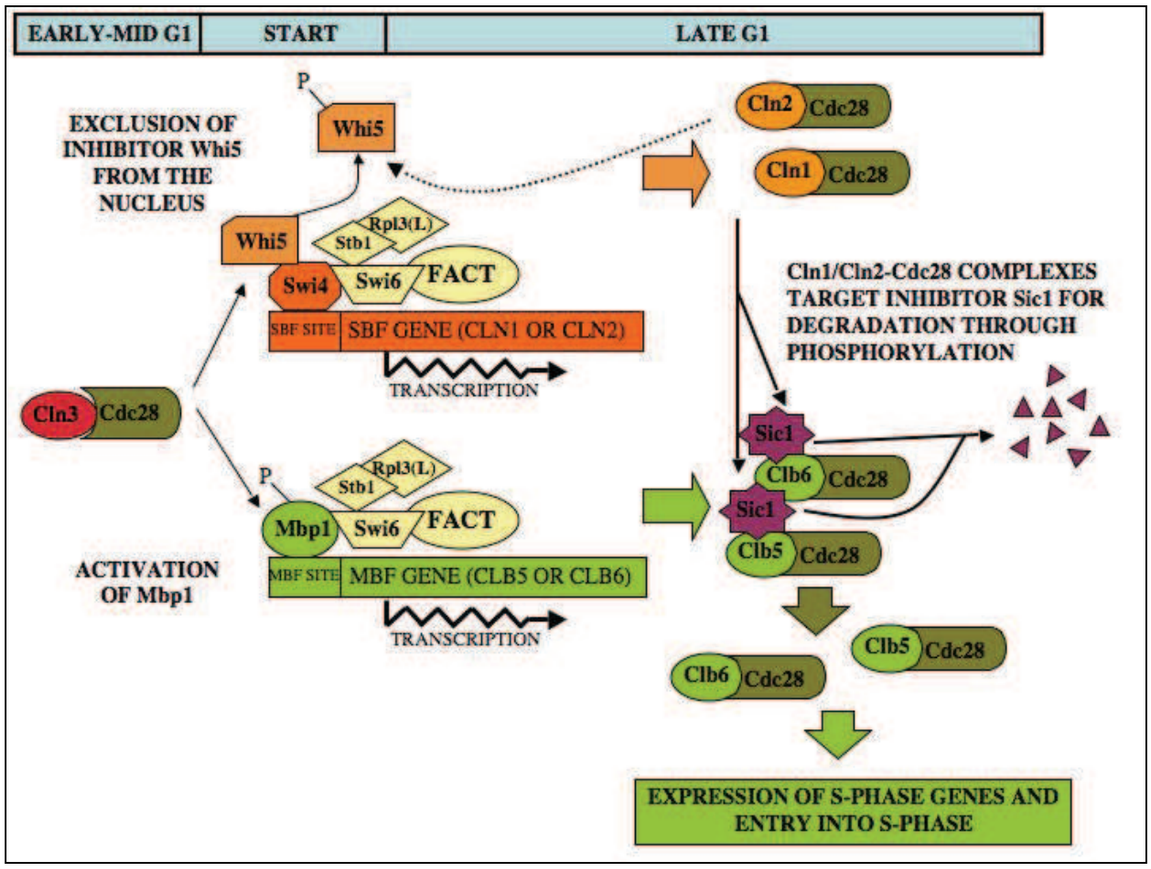

Fig. 1. The G1/S transition in *S. cerevisiae*

2.3 The "intra-S-phase" checkpoint response

The DNA-damage signalling pathway is highly conserved throughout eukaryotes (Lydall & Weinert, 1996). Under DNA damage situations, kinase Mec1 in *S. cerevisiae* is phosphorylated (Morrow et al., 1995; Siede et al., 1996) and causes the phosphorylation of the kinase encoded by *RAD53* (Sanchez et al., 1996; Sun et al., 1996). Then Rad53p phosphorylates the transcription factor Swi6p, causing a delay in the accumulation of mRNA for G1 cyclins and, thus, transient cell cycle arrest (Sidorova & Breeden, 1997). Below we will discuss the function of Rad53 if a DNA-damage situation is detected after START. An essential role for DEAD-Box Helicase DHH1 in G1/S DNA-damage checkpoint recovery has been proposed (Bergkessel & Reese, 2004).

Another relevant response to cope with situations where the genome is at risk, owing to DNA damage or replicative stress, takes place during DNA replication, this being the so-called S-phase or replication checkpoint pathway (also called "intra-S-phase", which refers to cells that have already passed START and begun replication) (Nyberg et al., 2002; Osborn

et al., 2002; Paulovich & Hartwell, 1995; Segurado & Tercero, 2009). Two central players in this checkpoint in budding yeast are the aforementioned kinases Mec1 and Rad53. They are homologues of Rad3 and Cds1, respectively, in the fission yeast *Schizosaccharomyces pombe*, or ATR and Chk2 in mammalian cells, which are deficient in many cancer cells.

In budding yeast, the signalling cascade triggered under replication stress culminates with the phosphorylation of Rad53 (Branzei & Foiani, 2009). This kinase is essential for the activation of the molecular mechanisms required to cope with replication arrest: (1) it promotes the stabilisation of stalled replication forks and allows DNA replication restart after removal of the blocking agent (Santocanale & Diffley, 1998; Tercero & Diffley, 2001); (2) it is also responsible for inducing the transcription factors of ribonucleotide reductase genes or DNA damage response genes (Allen et al., 1994; Huang et al., 1998; Zhao et al., 2001); (3) finally, Rad53 prevents the firing of late replication origins (Duch et al., 2011; Zegerman & Diffley, 2010) and restrains spindle elongation, thus preventing mitosis (Allen et al., 1994; Bachant et al., 2005; Weinert et al., 1994). Kinase Cdc7/Dbf4 is a target of the intra-S-phase checkpoint (Jares et al., 2000).

3. Replicating chromatin: DNA is associated with histones

The DNA of eukaryotic cells fits the confines of the nucleus by a hierarchical scheme of folding and compaction into chromatin. Nucleosomes, the repeating structural units of chromatin, consist in an octameric histone core comprising two copies each of H2A, H2B, H3 and H4, around which 147 bp of DNA are wrapped in 1.65 superhelical turns (Andrews & Luger, 2011; Luger et al., 1997). A linker or H1 histone molecule then associates with the nucleosome core particle (Brown, 2003). Thus nucleosomes are formed into regularly spaced arrays along DNA and can be mobilised by different ATP-dependent remodelling complexes, such as SWI/SNF or RSC, or ATP-independent ones, like the FACT complex. Histones are essential for viability as they pack DNA into the nucleus and regulate access to the genetic information contained within it.

The chromatin structure plays a central role in gene regulation and other nuclear processes, including DNA replication (Groth et al., 2007). During replication, the cell must replicate not only its DNA, but also its chromatin. Accordingly, another regulatory process during the G1/S transition is the induction of histone genes, which allows the coupling of bulk histone synthesis with ongoing DNA replication. In proliferating cells, the synthesis of the vast majority of histones occurs during the S-phase of the cell cycle. Inhibition of DNA synthesis results in a rapid repression of histone genes, indicating that it is tightly coupled with DNA replication.

An interesting question underlying this close coupling between DNA replication and histones expression is: *Why are histone protein levels subject to such a high degree of regulation?* Insufficiency of histones has been seen to be nonviable for the cell (Han et al., 1987). Interestingly, excess histones have also proved deleterious for cell growth, and they provoke genomic instability (Meeks-Wagner & Hartwell, 1986), increased DNA damage sensitivity and cytotoxicity (Gunjan & Verreault, 2003; Singh, Kabbaj, et al., 2009). These effects could be due to the strong positive charge of histones. Non-chromatin-bound histones, named "excess" or "free" histones, could bind non-specifically to negatively charged molecules in the cell, including nucleic acids such as DNA and RNA, as well as to negatively charged proteins. Recently in-depth research has been done into the mechanisms via which excess or free histones exert their deleterious effects *in vivo* (Singh et al., 2010). Using microarray

analysis, the authors of this work found that excess histones mediate their deleterious effects via multiple mechanisms in budding yeast, largely by inappropriate electrostatic interactions with the cellular macromolecules carrying the opposite charge. This analysis also revealed that around 240 genes were either up- or down-regulated by 2-fold, or more, overexpression of the histone gene pair H3/H4. After considering all this information, it is easy to think that cells need to have a very tight regulation of histone protein levels to maintain genomic stability and cell viability.

4. Avoiding free histones in yeast: controlling histones levels

To prevent deleterious effects of free histone accumulation, histone proteins are regulated transcriptionally, posttranscriptionally, translationally and posttranslationally (reviewed in Gunjan et al., 2005).

4.1 Transcriptional regulation

Cells must replicate not only their DNA during the S-phase, but also their chromatin. Accordingly, the transcription of histone genes is activated at the beginning of the S-phase to provide sufficient core histones to assemble replicated DNA. Correspondingly, inhibition of DNA synthesis results in a rapid repression of histone genes, indicating that it is tightly coupled with DNA replication (Osley, 1991; Breeden, 2003; reviewed in Gunjan et al., 2005).

The major core histone genes in *S. cerevisiae* are organised into four loci, each containing two histones genes that are divergently transcribed from a central promoter: two loci encode H2A and H2B (*HTA1-HTB1* and *HTA2-HTB2*) (Hereford et al., 1979), while the other two encode H3 and H4 (*HHT1-HHF1* and *HHT2-HHF2*) (Sutton et al., 2001). The tight cell cycle regulation of the histone genes results from their transcriptional repression in phases G1 and G2, their transcriptional activation just before the S-phase and the posttranscriptional regulation of their mRNAs. During the S-phase, histone genes can also respond to changes; for instance, accumulation of histones in response to the genotoxic agents interfering with DNA replication induces their repression (reviewed by Gunjan et al., 2005).

Three of the four divergent histone gene promoters (the two gene pairs that encode H3/H4, and *HTA1-HTB1*, one of the two gene pairs encoding H2A/H2B) are repressed by Hir proteins: Hir1, Hir2, Hir3 and Hpc2. Thus, the *S. cerevisiae* cells lacking any of the four Hir proteins are incapable of efficiently repressing these histone genes outside the S-phase or by following the replication arrest during this phase (Osley & Lycan, 1987; Xu et al., 1992; Sherwood et al., 1993). This repression is mediated through a negative *cis*-acting sequence (*NEG*) present in the histone promoters, except in *HTA2-HTB2* (Osley et al., 1986). The fourth histone gene pair (*HTA2-HTB2*) shows a similar regulation pattern to the other three, but its repression is independent of the Hir proteins. It is also important to highlight that the SBF transcription factor, a regulator of the aforementioned G1/S-specific genes, also plays a role in *HTA1-HTB1* regulation. Evidence for this role includes, among others, that SBF mutants exhibit modestly reduced HTA1 and HTB1 mRNA levels (Hess & Winston, 2005), and that Swi4 has been detected by chromatin immunoprecipitation at *HTA1-HTB1* (ChiP) (Simon et al., 2001).

4.2 Posttranscriptional regulation

The increase in histone mRNAs during the S-phase is not only due to a cell cycle-regulated promoter in histone genes, but also to a regulated stability of histone messengers: histone

mRNAs accumulate in the S-phase and are rapidly degraded as cells progress to the G2 phase of the cell cycle. This regulation mode is better understood in higher eukaryotes (Marzluff & Duronio, 2002), although the mechanisms to modulate the stability of histone RNAs differ among eukaryotic organisms. In *S. cerevisiae,* histone mRNA abundance oscillates and clearly peaks during the S-phase, even when histones are expressed from a constitutive promoter (Lycan et al., 1987; Xu et al., 1990; Campbell et al., 2002). This stability is regulated through the 3´ elements of the genes. Moreover, loss of Trf4 and Trf5 (polyA polymerases), or of Rrp6 (a component of the nuclear exosome), results in elevated levels of the transcripts encoding DNA replication-dependent histones. *TRF4*, *TRF5* and *RRP6* have been identified as new players in the regulation of histones mRNA levels in yeast (Reis & Campbell, 2007).

4.3 Controlling histone protein levels by proteolysis

In recent years, a novel mechanism to prevent the accumulation of free histones, which is superimposed upon the regulation of histone gene transcription and mRNA stability, has been described in budding yeast (Gunjan & Verreault, 2003; reviewed in Gunjan et al., 2006). The authors demonstrated that Rad53, but not Mec1, is required for the degradation of the excess histones that are not packaged into chromatin. Consequently, *rad53* mutants accumulate abnormally large amounts of soluble histones and are sensitive to histone overexpression. Remarkably, DNA damage sensitivity, slow growth and chromosome loss of *rad53* mutants, can be significantly suppressed by a disruption of one of the two loci encoding histones H3/H4; thus it may be argued that these phenotypes are partially due to the presence of excess histones. This relevant work also demonstrated that Rad53 associates with histones *in vivo* and that this interaction is modulated by its kinase activity. In summary, this new surveillance mechanism not only monitors the accumulation of excess histones, but also induces their degradation. Excess histones associate with Rad53 *in vivo* and undergo modifications such as tyrosine phosphorylation and polyubiquitination before their proteolysis by the proteasome. A tyrosine 99 residue of H3 has been identified as being critical for the proficient ubiquitylation and degradation of this histone. Finally, different proteins have been identified as enzymes involved in the ubiquitylation of free or excess histones like the E2 proteins Ubc4 and Ubc5, as well as E3 ubiquitin ligase Tom1 (Singh, Kabbaj, et al., 2009; reviewed in Singh & Gunjan, 2011).

5. Generating free histones during the cell cycle

So far we have discussed how cells tightly regulate histone levels to prevent harmful effects of free histones from binding non-specifically to nucleic acids and from interfering with processes that require access to genetic information. Firstly, delicate transcriptional and posttranscriptional regulations of histone genes, coupled with DNA replication during the S-phase of the cell cycle, efficiently avoid an accumulation of non-chromatin-bound histones. This kind of mechanisms is evolutionarily conserved (Osley, 1991; Marzluff & Duronio, 2002). Secondly, despite this regulation, situations where free histones appear exist and a posttranslational mechanism mediated by Rad53 induces the proteolysis of excess histones. Finally, therefore, it is interesting to wonder about the processes generating excess histones during the cell cycle (reviewed in Singh et al., 2009).

Firstly, it has been well-established that all eukaryotes have multiple genes encoding each histone protein. Histones are primarily synthesised in the S-phase and deposited by

chromatin assembly factors or histone chaperones on replicating DNA to form chromatin in a process known as chromatin assembly (Gunjan et al., 2005). Different hypotheses have attempted to explain why eukaryotic cells carry such a large number of histone genes. The most simple explanation seems to be that the high demand of histones for chromatin assembly on newly replicated DNA can only be achieved by multiple histone genes. However, it has been shown that *S. cerevisiae* only requires half its complement of histone genes for viability (Osley, 1991). Moreover, the full complement of histones genes in budding yeast synthesises excess histones which are deleterious to cells, making them more sensitive to a variety of DNA damaging agents (Gunjan & Verreault, 2003). Even if it may be a challenge to comprehensively integrate all this information, it is clear that yeast cells synthesise histones in excess in accordance requirements for chromatin assembly during the S-phase. Thus, yeast cells ensure that all the genome is fully packaged into chromatin following the DNA replication that synthesises excess histones during the S-phase by degrading unincorporated histones at the end of DNA replication.

Secondly, rigorously coupling histone synthesis with DNA replication (Stein & Stein, 1984) ensures the rapid incorporation of histones into newly synthesised DNA to form chromatin. However, different situations can generate DNA replication slow down or arrest, resulting in an accumulation of unincorporated newly synthesised histones (Bonner et al., 1988). To better illustrate these situations, replication inhibitors bring about a drastic drop in DNA synthesis and chromatin assembly. Moreover, DNA damage results in DNA replication slowing down or stalling, which is due to either the physical impediment posed by DNA lesions or, more likely, the activation of the intra-S-phase DNA damage checkpoint that prevents new origins from firing (Paulovich & Hartwell, 1995; Tercero & Diffley, 2001).

A third source of excess histones (non-chromatin-bound ones) may be those histones removed during DNA damage, repair and recombination. When DNA damage occurs in the chromatin context, repair factors have to gain access to the damaged site to carry out necessary repairs. In this sense, there is evidence suggesting that histones may be evicted locally from a DNA double strand break (DSB) site to allow access to the repair machinery (Tsukuda et al., 2005). A minor contribution of this last process to free histone accumulation may be expected.

6. A novel source of excess free histones: evicted from transcription

DNA is tightly packed into chromatin. Nucleosomes need to be disassembled and reassembled to allow efficient transcription by RNA polymerases. There are many different factors relating to this process. One very well described essential factor involved in RNA pol II transcription is the FACT complex (reviewed by Reinberg & Sims, 2006; Formosa, 2008). This complex is the only factor known to date that stimulates RNA Pol II-dependent transcription elongation through chromatin in a highly purified system (Orphanides et al., 1998; Pavri et al., 2006) and also *in vivo* (Jimeno-Gonzalez et al., 2006; Biswas et al., 2006; Formosa, 2003; Mason & Struhl, 2003; Saunders et al., 2003). In the budding yeast *S. cerevisiae*, the FACT complex is composed of two essential proteins: Spt16/Cdc68/Ssf1 (hereafter referred to as Spt16) and Pob3 (Stuwe et al., 2008; Orphanides et al., 1999). yFACT and the HMG-box protein Nhp6 form a heterodimer that is capable of binding nucleosomes (Formosa et al., 2001) and of recognising them *in vitro* (Rhoades et al., 2004; Xin et al., 2009). Both Spt16 and Pob3 are able to bind H3/H4 tetramers and H2A/H2B dimers, sometimes in a functionally redundant manner (Stuwe et al., 2008; VanDemark et al., 2008). These

interactions are thought to destabilise nucleosomes during transcription (Belotserkovskaya & Reinberg, 2004; Xin et al., 2009). Thus, yFACT plays a role in maintaining the integrity of the chromatin structure during transcription (Mason & Struhl, 2003; Kaplan et al., 2003; Cheung et al., 2008; Vanti et al., 2009; Jamai et al., 2009). In addition to transcription defects, defects on Spt16 can also lead to cell cycle defects. *SPT16* was originally identified during a screening looking for *cdc* (cell division cycle) mutants (Malone et al., 1991; Rowley et al., 1991). Mutant *cdc68* (also named *spt16-197* and *spt16G132D*) is a thermosensitive mutant with a very clear accumulation of G1 cells.

Our group, in collaboration with the labs of Geli and Gunjan, has recently demonstrated that a dysfunction in chromatin reassembly during active Pol II transcription through defects on the Spt16 protein can generate an accumulation of free histones. We have shown that a strong genetic interaction takes place between the *spt16-197* mutant and those mutants affected in the kinase activity of Rad53. This interaction does not seem to relate to the DNA damage response since other very well-established proteins involved in this response, like Mec1 or Rad9, show no interaction. Since Rad53, but not Mec1 or Rad9, is involved in the detection and subsequent degradation of excess histones, we hypothesised that a dysfunction of Spt16 might lead to an increase in free histones, which would need to be targeted for degradation via Rad53. According to this model, we observed that deleting the *HTA2-HTB2* locus can partially suppress the *ts* phenotype of *spt16-197*. Using co-immunoprecipitation assays to detect free non-chromatin-bound histones, we demonstrated that Pol II-dependent transcription in the absence of active FACT causes an accumulation of evicted histones, which could become toxic for the cell if not targeted for degradation by Rad53 (Morillo-Huesca et al., 2010).

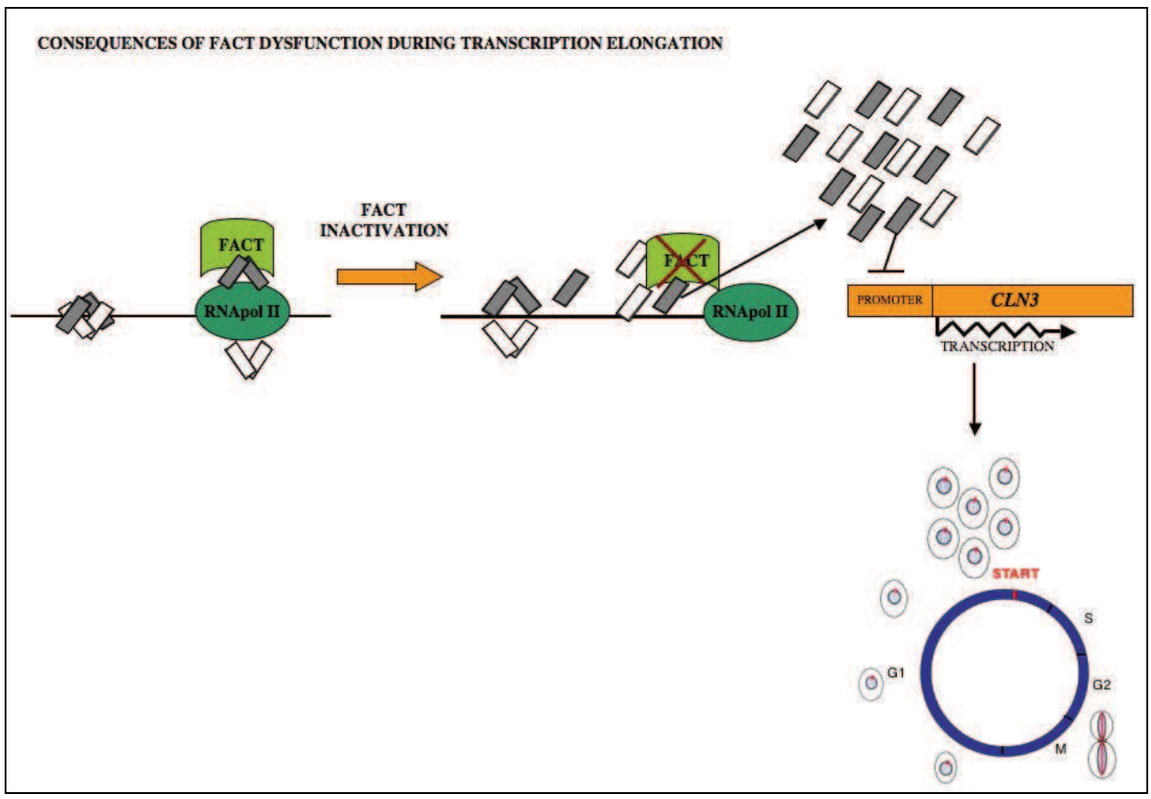

Fig. 2. FACT inactivation causes accumulation of free histones and a subsequent G1 delay.

Beyond the S-phase, transcribed chromatin is probably the main source of free histones in yeast cells, presumably due to the minor imbalances between histone supply and demand during chromatin reassembly. Our results indicate a novel and important role for FACT in yeast, that of a protective factor against the toxic risk represented by evicted histones. This model agrees with a recent publication which reports how Spt16 promotes the redeposition of the original H3 and H4 histones evicted by elongating Pol II (Jamai et al., 2009). The protective role against evicted histones is probably not an exclusive function of FACT, but a function of the other factors that cooperate during chromatin reassembly, like Spt6, for which we have also provided some evidence (Morillo-Huesca et al., 2010).

7. A novel signal regulating the G1/S transition: free histone levels

Our work has allowed us to propose that a dysfunction of chromatin reassembly factors, like FACT and Spt6, generates an accumulation of the excess histones evicted from transcription. In addition to this, we have found an interesting connection between free histone levels and cell cycle defects in the G1/S transition. We postulate that free or non-chromatin-bound histones can trigger the down-regulation of *CLN3*, thereby arresting cells at G1 (START) and contributing to control free histone levels before starting DNA replication (Morillo-Huesca et al., 2010). Our results indicate a so far unknown connection between chromatin dynamics and cell cycle regulation. Firstly, genetic and molecular evidence indicates that, in the absence of FACT or the Spt6 function, the expression of *CLN3* is down-regulated by a mechanism that specifically represses its transcriptional promoter. Secondly, and significantly, the G1 delay studied was not mediated by the DNA-damage checkpoint, although a *rad53* mutant enhances both the thermosensitivity of the *spt16* mutant and its G1 phenotype. This result, in combination with the lack of phosphorylation of Rad53 after FACT inactivation, indicates that excess histones are involved in this phenomenon. This conclusion is strengthened by the results of the experiments that manipulate *in vivo* histone levels: (i) deletion of one of the two loci encoding H2A and H2B partially suppressed the *ts* phenotype and the accumulation of G1 cells of the *spt16* mutant, indicating that a reduction in histone levels can alleviate the *cdc* phenotype due to a FACT dysfunction; (ii) overproduction of histone levels in wild-type cells leads to a clear accumulation of the cells in G1 (asynchronous culture) and a more marked delay in the entry of synchronised cells in the S-phase (Morillo-Huesca et al., 2010). This delay in the G1/S transition also correlates with *CLN3* down-regulation. These results, obtained in wild-type cells, demonstrate that a histone-mediated G1 delay can be obtained in a background with no possible indirect effects mediated by either the role of FACT in the expression of the G1-S regulators or the function of Rad53 in the control of early replication events.

In mammalian cells, histone overexpression slows down entry into and progression through the S-phase (Groth et al., 2007). Interestingly, depletion of human Spt16 leads to the repression of H1, H2A and H2B genes (Li et al., 2007), which could be the result of the accumulation of the free histones in human cells after FACT dysfunction. Given the analogy between the G1-S regulators in yeast (Cln3-SBF-Whi5-Rpd3) and mammals (CyclinD1-E2F-Rb-HDAC1) (Wang et al., 2009; Takahata et al., 2009), the functional link between the accumulation of free histones and the regulation of the G1-S transition may be evolutionarily conserved.

This chapter emphasises that excess free histones may have serious implications for the normal progression of DNA replication when the toxicity of free histones is maximal in the

S-phase (Gunjan & Verreault, 2003). According to this scenario, a G1 delay in response to excess histones favours cell viability. In our model, represented in Figure 2, the G1 delay should allow cells to reduce the free histones levels through the Rad53/Tom1-mediated histone degradation pathway before entering the S-phase. It is interesting to note that Rad53 participates in different linked functions, such as the DNA damage checkpoint, the excess histone degradation pathway, and at the initiation of DNA replication. A model has been recently proposed in which Rad53 acts as a "nucleosome buffer" by interacting with origins of replication to prevent excess histones from binding to origins and to maintain a proper chromatin configuration (Holzen & Sclafani, 2010). For this reason, we propose the term *chromatin repair* to denote a combination of DNA repair, chromatin reassembly and excess histone degradation. An attractive role for Rad53 as a super-integrator of all chromatin repair functions can be presumed.

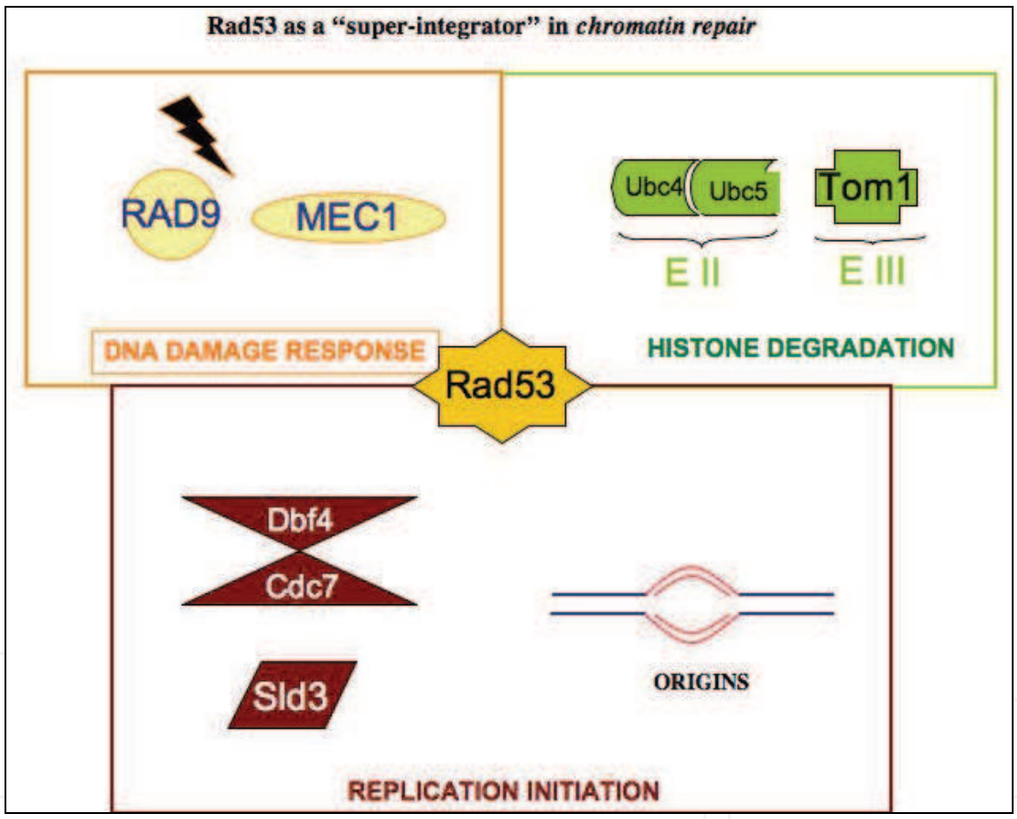

Fig. 3. Rad53 functions

8. Conclusion

In this chapter we have reviewed the contribution of transcription to the levels of free histones and their influence on the cell cycle and DNA replication. Nucleosomes need to be disassembled to allow DNA transcription by RNA polymerases. An essential factor for disassembly/reassembly process during DNA transcription is the FACT complex. We concluded, using loss-of-function FACT mutants, that FACT dysfunction provokes downregulation of *CLN3*, one of the cyclins that plays a key role in the control of the G1/S

transition. FACT dysfunction increases the level of the free histones released from chromatin during transcription, and the G1 delay of the FACT mutant is enhanced by a second mutation affecting Rad53, a protein that regulates DNA repair and excess histone degradation. The overexpression of histones in wild type cells also causes a cell cycle delay before DNA replication. All this experimental evidence points out to a so-far unknown connection between chromatin dynamics and the regulation of the cell cycle, mediated by free histones.

Finally, we propose an attractive overall concept, *chromatin repair*, to signify the combination of DNA repair, chromatin reassembly and excess histone degradation. An attractive role for Rad53 as a super-integrator of all chromatin repair functions can be presumed as this protein plays key roles in the mentioned linked functions.

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10. References

Aguilera, A., and B. Gomez-Gonzalez. (2008). Genome instability: a mechanistic view of its causes and consequences. Nat Rev Genet, Vol. 9, No. 3, pp. (204-17).

Allen, J. B., Z. Zhou, W. Siede, E. C. Friedberg, and S. J. Elledge. (1994). The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev, Vol. 8, No. 20, pp. (2401-15).

Andrews, AJ., and K. Luger. (2011). Nucleosome Structure(s) and Stability: Variations on a Theme. Annu Rev Biophys, Vol. 40, No., pp. (99-117).

Arias, E. E., and J. C. Walter. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. Genes Dev, Vol. 21, No. 5, pp. (497-518).

Bachant, J., S. R. Jessen, S. E. Kavanaugh, and C. S. Fielding. (2005). The yeast S phase checkpoint enables replicating chromosomes to bi-orient and restrain spindle extension during S phase distress. J Cell Biol, Vol. 168, No. 7, pp. (999-1012).

Bell, S. P., and A. Dutta. (2002). DNA replication in eukaryotic cells. Annu Rev Biochem, Vol. 71, No., pp. (333-74).

Belotserkovskaya, R., and D. Reinberg. (2004). Facts about FACT and transcript elongation through chromatin. Curr Opin Genet Dev, Vol. 14, No. 2, pp. (139-46).

Bergkessel, M., and J. C. Reese. (2004). An essential role for the Saccharomyces cerevisiae DEAD-box helicase DHH1 in G1/S DNA-damage checkpoint recovery. Genetics, Vol. 167, No. 1, pp. (21-33).

Biswas, D., R. Dutta-Biswas, D. Mitra, Y. Shibata, B. D. Strahl, T. Formosa, and D. J. Stillman. (2006). Opposing roles for Set2 and yFACT in regulating TBP binding at promoters. Embo J, Vol. 25, No. 19, pp. (4479-89).

Bonner, W. M., R. S. Wu, H. T. Panusz, and C. Muneses. (1988). Kinetics of accumulation and depletion of soluble newly synthesized histone in the reciprocal regulation of histone and DNA synthesis. Biochemistry, Vol. 27, No. 17, pp. (6542-50).

Branzei, D., and M. Foiani. (2008). Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol, Vol. 9, No. 4, pp. (297-308).

Branzei, D., and M. Foiani. (2009). The checkpoint response to replication stress. DNA Repair (Amst), Vol. 8, No. 9, pp. (1038-46).

Breeden, L. L. (2003). Periodic transcription: a cycle within a cycle. Curr Biol, Vol. 13, No. 1, pp. (R31-8).

Brown, D. T. (2003). Histone H1 and the dynamic regulation of chromatin function. Biochem Cell Biol, Vol. 81, No. 3, pp. (221-7).

Campbell, S. G., M. Li Del Olmo, P. Beglan, and U. Bond. (2002). A sequence element downstream of the yeast HTB1 gene contributes to mRNA 3' processing and cell cycle regulation. Mol Cell Biol, Vol. 22, No. 24, pp. (8415-25).

Cheung, V., G. Chua, N. N. Batada, C. R. Landry, S. W. Michnick, T. R. Hughes, and F. Winston. (2008). Chromatin- and transcription-related factors repress transcription from within coding regions throughout the Saccharomyces cerevisiae genome. PLoS Biol, Vol. 6, No. 11, pp. (e277).

Costanzo, M., J. L. Nishikawa, X. Tang, J. S. Millman, O. Schub, K. Breitkreuz, D. Dewar, I. Rupes, B. Andrews, and M. Tyers. (2004). CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. Cell, Vol. 117, No. 7, pp. (899-913).

de Bruin, R. A., W. H. McDonald, T. I. Kalashnikova, J. Yates, 3rd, and C. Wittenberg. (2004). Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. Cell, Vol. 117, No. 7, pp. (887-98).

Diffley, J. F. (1996). Once and only once upon a time: specifying and regulating origins of DNA replication in eukaryotic cells. Genes Dev, Vol. 10, No. 22, pp. (2819-30).

Diffley, J.F. (2004). Regulation of early events in chromosome replication. Curr Biol, Vol. 14, No. 18, pp. (R778-86).

Duch, A., G. Palou, Z. O. Jonsson, R. Palou, E. Calvo, J. Wohlschlegel, and D. G. Quintana. (2011). A Dbf4 mutant contributes to bypassing the Rad53-mediated block of origins of replication in response to genotoxic stress. J Biol Chem, Vol. 286, No. 4, pp. (2486-91).

Elledge, S. J. (1996). Cell cycle checkpoints: preventing an identity crisis. Science, Vol. 274, No. 5293, pp. (1664-72).

Ferrezuelo, F., N. Colomina, B. Futcher, and M. Aldea. (2010). The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle. Genome Biol, Vol. 11, No. 6, pp. (R67).

Formosa, T. (2003). Changing the DNA landscape: putting a SPN on chromatin. Curr Top Microbiol Immunol, Vol. 274, No., pp. (171-201).

Formosa, T. (2008). FACT and the reorganized nucleosome. Mol Biosyst, Vol. 4, No. 11, pp. (1085-93).

Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman. (2001). Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. Embo J, Vol. 20, No. 13, pp. (3506-17).

Friedberg, E. C. (2003). DNA damage and repair. Nature, Vol. 421, No. 6921, pp. (436-40).

Gallego, C., E. Gari, N. Colomina, E. Herrero, and M. Aldea. (1997). The Cln3 cyclin is down-regulated by translational repression and degradation during the G1 arrest caused by nitrogen deprivation in budding yeast. Embo J, Vol. 16, No. 23, pp. (7196-206).

Groth, A., A. Corpet, A. J. Cook, D. Roche, J. Bartek, J. Lukas, and G. Almouzni. (2007). Regulation of replication fork progression through histone supply and demand. Science, Vol. 318, No. 5858, pp. (1928-31).

Gunjan, A., J. Paik, and A. Verreault. (2005). Regulation of histone synthesis and nucleosome assembly. Biochimie, Vol. 87, No. 7, pp. (625-35).

Gunjan, A., J. Paik, and A. Verreault. (2006). The emergence of regulated histone proteolysis. Curr Opin Genet Dev, Vol. 16, No. 2, pp. (112-8).

Gunjan, A., and A. Verreault. (2003). A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in S. cerevisiae. Cell, Vol. 115, No. 5, pp. (537-49).

Han, M., M. Chang, U. J. Kim, and M. Grunstein. (1987). Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication, and transcription. Cell, Vol. 48, No. 4, pp. (589-97).

Hartwell, L. H., and M. B. Kastan. (1994). Cell cycle control and cancer. Science, Vol. 266, No. 5192, pp. (1821-8).

Hereford, L., K. Fahrner, J. Woolford, Jr., M. Rosbash, and D. B. Kaback. (1979). Isolation of yeast histone genes H2A and H2B. Cell, Vol. 18, No. 4, pp. (1261-71).

Hess, D., and F. Winston. (2005). Evidence that Spt10 and Spt21 of Saccharomyces cerevisiae play distinct roles in vivo and functionally interact with MCB-binding factor, SCB-binding factor and Snf1. Genetics, Vol. 170, No. 1, pp. (87-94).

Hoeijmakers, J. H. (2001). Genome maintenance mechanisms for preventing cancer. Nature, Vol. 411, No. 6835, pp. (366-74).

Holzen, T. M., and R. Sclafani. (2010). Genetic interaction of RAD53 protein kinase with histones is important for DNA replication. Cell Cycle, Vol. 9, No. 23, pp. (4735-47).

Huang, M., Z. Zhou, and S. J. Elledge. (1998). The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell, Vol. 94, No. 5, pp. (595-605).

Jamai, A., A. Puglisi, and M. Strubin. (2009). Histone chaperone spt16 promotes redeposition of the original h3-h4 histones evicted by elongating RNA polymerase. Mol Cell, Vol. 35, No. 3, pp. (377-83).

Jares, P., A. Donaldson, and J. J. Blow. (2000). The Cdc7/Dbf4 protein kinase: target of the S phase checkpoint? EMBO Rep, Vol. 1, No. 4, pp. (319-22).

Jimeno-Gonzalez, S., F. Gomez-Herreros, P. M. Alepuz, and S. Chavez. (2006). A gene-specific requirement for FACT during transcription is related to the chromatin organization of the transcribed region. Mol Cell Biol, Vol. 26, No. 23, pp. (8710-21).

Kaplan, C. D., L. Laprade, and F. Winston. (2003). Transcription elongation factors repress transcription initiation from cryptic sites. Science, Vol. 301, No. 5636, pp. (1096-9).

Li, Y., S. X. Zeng, I. Landais, and H. Lu. (2007). Human SSRP1 has Spt16-dependent and -independent roles in gene transcription. J Biol Chem, Vol. 282, No. 10, pp. (6936-45).

Luger, K., T. J. Rechsteiner, A. J. Flaus, M. M. Waye, and T. J. Richmond. (1997). Characterization of nucleosome core particles containing histone proteins made in bacteria. J Mol Biol, Vol. 272, No. 3, pp. (301-11).

Lycan, D. E., M. A. Osley, and L. M. Hereford. (1987). Role of transcriptional and posttranscriptional regulation in expression of histone genes in Saccharomyces cerevisiae. Mol Cell Biol, Vol. 7, No. 2, pp. (614-21).

Lydall, D., and T. Weinert. (1995). Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. Science, Vol. 270, No. 5241, pp. (1488-91).

Lydall, D., and T. Weinert. (1996). From DNA damage to cell cycle arrest and suicide: a budding yeast perspective. Curr Opin Genet Dev, Vol. 6, No. 1, pp. (4-11).

Malone, E. A., C. D. Clark, A. Chiang, and F. Winston. (1991). Mutations in SPT16/CDC68 suppress cis- and trans-acting mutations that affect promoter function in Saccharomyces cerevisiae. Mol Cell Biol, Vol. 11, No. 11, pp. (5710-7).

Marzluff, W. F., and R. J. Duronio. (2002). Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. Curr Opin Cell Biol, Vol. 14, No. 6, pp. (692-9).

Mason, P. B., and K. Struhl. (2003). The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol Cell Biol, Vol. 23, No. 22, pp. (8323-33).

Meeks-Wagner, D., and L. H. Hartwell. (1986). Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell, Vol. 44, No. 1, pp. (43-52).

Morillo-Huesca, M., D. Maya, M. C. Munoz-Centeno, R. K. Singh, V. Oreal, G. U. Reddy, D. Liang, V. Geli, A. Gunjan, and S. Chavez. (2010). FACT prevents the accumulation of free histones evicted from transcribed chromatin and a subsequent cell cycle delay in G1. PLoS Genet, Vol. 6, No. 5, pp. (e1000964).

Morrow, D. M., D. A. Tagle, Y. Shiloh, F. S. Collins, and P. Hieter. (1995). TEL1, an S. cerevisiae homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene MEC1. Cell, Vol. 82, No. 5, pp. (831-40).

Nyberg, K. A., R. J. Michelson, C. W. Putnam, and T. A. Weinert. (2002). Toward maintaining the genome: DNA damage and replication checkpoints. Annu Rev Genet, Vol. 36, No., pp. (617-56).

Orphanides, G., G. LeRoy, C. H. Chang, D. S. Luse, and D. Reinberg. (1998). FACT, a factor that facilitates transcript elongation through nucleosomes. Cell, Vol. 92, No. 1, pp. (105-16).

Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey, and D. Reinberg. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature, Vol. 400, No. 6741, pp. (284-8).

Osborn, A. J., S. J. Elledge, and L. Zou. (2002). Checking on the fork: the DNA-replication stress-response pathway. Trends Cell Biol, Vol. 12, No. 11, pp. (509-16).

Osley, M. A. (1991). The regulation of histone synthesis in the cell cycle. Annu Rev Biochem, Vol. 60, No., pp. (827-61).

Osley, M. A., J. Gould, S. Kim, M. Y. Kane, and L. Hereford. (1986). Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell, Vol. 45, No. 4, pp. (537-44).

Osley, M. A., and D. Lycan. (1987). Trans-acting regulatory mutations that alter transcription of Saccharomyces cerevisiae histone genes. Mol Cell Biol, Vol. 7, No. 12, pp. (4204-10).

Paulovich, A. G., and L. H. Hartwell. (1995). A checkpoint regulates the rate of progression through S phase in S. cerevisiae in response to DNA damage. Cell, Vol. 82, No. 5, pp. (841-7).

Pavri, R., B. Zhu, G. Li, P. Trojer, S. Mandal, A. Shilatifard, and D. Reinberg. (2006). Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell, Vol. 125, No. 4, pp. (703-17).

Philpott, C. C., J. Rashford, Y. Yamaguchi-Iwai, T. A. Rouault, A. Dancis, and R. D. Klausner. (1998). Cell-cycle arrest and inhibition of G1 cyclin translation by iron in AFT1-1(up) yeast. Embo J, Vol. 17, No. 17, pp. (5026-36).

Reinberg, D., and R. J. Sims, 3rd. (2006). de FACTo nucleosome dynamics. J Biol Chem, Vol. 281, No. 33, pp. (23297-301).

Reis, C. C., and J. L. Campbell. (2007). Contribution of Trf4/5 and the nuclear exosome to genome stability through regulation of histone mRNA levels in Saccharomyces cerevisiae. Genetics, Vol. 175, No. 3, pp. (993-1010).

Rhoades, A. R., S. Ruone, and T. Formosa. (2004). Structural features of nucleosomes reorganized by yeast FACT and its HMG box component, Nhp6. Mol Cell Biol, Vol. 24, No. 9, pp. (3907-17).

Rowley, A., R. A. Singer, and G. C. Johnston. (1991). CDC68, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. Mol Cell Biol, Vol. 11, No. 11, pp. (5718-26).

Sanchez, Y., B. A. Desany, W. J. Jones, Q. Liu, B. Wang, and S. J. Elledge. (1996). Regulation of RAD53 by the ATM-like kinases MEC1 and TEL1 in yeast cell cycle checkpoint pathways. Science, Vol. 271, No. 5247, pp. (357-60).

Santocanale, C., and J. F. Diffley. (1998). A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. Nature, Vol. 395, No. 6702, pp. (615-8).

Saunders, A., J. Werner, E. D. Andrulis, T. Nakayama, S. Hirose, D. Reinberg, and J. T. Lis. (2003). Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. Science, Vol. 301, No. 5636, pp. (1094-6).

Schneider, B. L., Q. H. Yang, and A. B. Futcher. (1996). Linkage of replication to start by the Cdk inhibitor Sic1. Science, Vol. 272, No. 5261, pp. (560-2).

Schwob, E., T. Bohm, M. D. Mendenhall, and K. Nasmyth. (1994). The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in S. cerevisiae. Cell, Vol. 79, No. 2, pp. (233-44).

Segurado, M., and JA. Tercero. (2009). The S-phase checkpoint: targeting the replication fork. Biol Cell, Vol. 19, No. 101(11), pp. (617-27).

Shaulian, E., M. Schreiber, F. Piu, M. Beeche, E. F. Wagner, and M. Karin. (2000). The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth arrest. Cell, Vol. 103, No. 6, pp. (897-907).

Sherwood, P. W., S. V. Tsang, and M. A. Osley. (1993). Characterization of HIR1 and HIR2, two genes required for regulation of histone gene transcription in Saccharomyces cerevisiae. Mol Cell Biol, Vol. 13, No. 1, pp. (28-38).

Sheu, Y. J., and B. Stillman. (2010). The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4. Nature, Vol. 463, No. 7277, pp. (113-7).

Sidorova, J. M., and L. L. Breeden. (1997). Rad53-dependent phosphorylation of Swi6 and down-regulation of CLN1 and CLN2 transcription occur in response to DNA damage in Saccharomyces cerevisiae. Genes Dev, Vol. 11, No. 22, pp. (3032-45).

Siede, W., J. B. Allen, S. J. Elledge, and E. C. Friedberg. (1996). The Saccharomyces cerevisiae MEC1 gene, which encodes a homolog of the human ATM gene product, is required for G1 arrest following radiation treatment. J Bacteriol, Vol. 178, No. 19, pp. (5841-3).

Simon, I., J. Barnett, N. Hannett, C. T. Harbison, N. J. Rinaldi, T. L. Volkert, J. J. Wyrick, J. Zeitlinger, D. K. Gifford, T. S. Jaakkola, and R. A. Young. (2001). Serial regulation of transcriptional regulators in the yeast cell cycle. Cell, Vol. 106, No. 6, pp. (697-708).

Singh, R. K., and A. Gunjan. (2011). Histone tyrosine phosphorylation comes of age. Epigenetics, Vol. 6, No. 2, pp. (153-60).

Singh, R. K., M. H. Kabbaj, J. Paik, and A. Gunjan. (2009). Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. Nat Cell Biol, Vol. 11, No. 8, pp. (925-33).

Singh, R. K., D. Liang, U. R. Gajjalaiahvari, M. H. Kabbaj, J. Paik, and A. Gunjan. (2010). Excess histone levels mediate cytotoxicity via multiple mechanisms. Cell Cycle, Vol. 9, No. 20, pp. (4236-44).

Singh, R. K., J. Paik, and A. Gunjan. (2009). Generation and management of excess histones during the cell cycle. Front Biosci, Vol. 14, No., pp. (3145-58).

Skotheim, J. M., S. Di Talia, E. D. Siggia, and F. R. Cross. (2008). Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature, Vol. 454, No. 7202, pp. (291-6).

Stegmeier, F. , and A. Amon. (2004). Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet, Vol. 38, No., pp. (203-232).

Stein, G. S., and J. L. Stein. (1984). Is human histone gene expression autogenously regulated? Mol Cell Biochem, Vol. 64, No. 2, pp. (105-10).

Stuwe, T., M. Hothorn, E. Lejeune, V. Rybin, M. Bortfeld, K. Scheffzek, and A. G. Ladurner. (2008). The FACT Spt16 "peptidase" domain is a histone H3-H4 binding module. Proc Natl Acad Sci U S A, Vol. 105, No. 26, pp. (8884-9).

Sun, Z., D. S. Fay, F. Marini, M. Foiani, and D. F. Stern. (1996). Spk1/Rad53 is regulated by Mec1-dependent protein phosphorylation in DNA replication and damage checkpoint pathways. Genes Dev, Vol. 10, No. 4, pp. (395-406).

Sutton, A., J. Bucaria, M. A. Osley, and R. Sternglanz. (2001). Yeast ASF1 protein is required for cell cycle regulation of histone gene transcription. Genetics, Vol. 158, No. 2, pp. (587-96).

Takahata, S., Y. Yu, and D. J. Stillman. (2009). The E2F functional analogue SBF recruits the Rpd3(L) HDAC, via Whi5 and Stb1, and the FACT chromatin reorganizer, to yeast G1 cyclin promoters. Embo J, Vol. 28, No. 21, pp. (3378-89).

Tanaka, S., and H. Araki. (2010). Regulation of the initiation step of DNA replication by cyclin-dependent kinases. Chromosoma, Vol. 119, No. 6, pp. (565-74).

Tanaka, S., Y.S. Tak, and H. Araki. (2007). The role of CDK in the initiation step of DNA replication in eukaryotes. Cell Div., Vol. 5, No. Jun, pp. (2-16).

Tercero, J. A., and J. F. Diffley. (2001). Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. Nature, Vol. 412, No. 6846, pp. (553-7).

Tsukuda, T., A. B. Fleming, J. A. Nickoloff, and M. A. Osley. (2005). Chromatin remodelling at a DNA double-strand break site in Saccharomyces cerevisiae. Nature, Vol. 438, No. 7066, pp. (379-83).

VanDemark, A. P., H. Xin, L. McCullough, R. Rawlins, S. Bentley, A. Heroux, D. J. Stillman, C. P. Hill, and T. Formosa. (2008). Structural and functional analysis of the Spt16p N-terminal domain reveals overlapping roles of yFACT subunits. J Biol Chem, Vol. 283, No. 8, pp. (5058-68).

Vanti, M., E. Gallastegui, I. Respaldiza, A. Rodriguez-Gil, F. Gomez-Herreros, S. Jimeno-Gonzalez, A. Jordan, and S. Chavez. (2009). Yeast genetic analysis reveals the involvement of chromatin reassembly factors in repressing HIV-1 basal transcription. PLoS Genet, Vol. 5, No. 1, pp. (e1000339).

Wang, H., L. B. Carey, Y. Cai, H. Wijnen, and B. Futcher. (2009). Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets. PLoS Biol, Vol. 7, No. 9, pp. (e1000189).

Weinert, T. A., G. L. Kiser, and L. H. Hartwell. (1994). Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev, Vol. 8, No. 6, pp. (652-65).

Wittenberg, C. (2005). Cell cycle: cyclin guides the way. Nature, Vol. 434, No. 7029, pp. (34-5).

Xin, H., S. Takahata, M. Blanksma, L. McCullough, D. J. Stillman, and T. Formosa. (2009). yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. Mol Cell, Vol. 35, No. 3, pp. (365-76).

Xu, H., U. J. Kim, T. Schuster, and M. Grunstein. (1992). Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in Saccharomyces cerevisiae. Mol Cell Biol, Vol. 12, No. 11, pp. (5249-59).

Xu, H. X., L. Johnson, and M. Grunstein. (1990). Coding and noncoding sequences at the 3' end of yeast histone H2B mRNA confer cell cycle regulation. Mol Cell Biol, Vol. 10, No. 6, pp. (2687-94).

Zegerman, P., and J. F. Diffley. (2007). Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. Nature, Vol. 445, No. 7125, pp. (281-5).

Zegerman, P., and J.F. Diffley. (2010). Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation. Nature, Vol. 467, No. 7314, pp. (474-8).

Zhai, Y., P. Y. Yung, L. Huo, and C. Liang. (2010). Cdc14p resets the competency of replication licensing by dephosphorylating multiple initiation proteins during mitotic exit in budding yeast. J Cell Sci, Vol. 123, No. Pt 22, pp. (3933-43).

Zhao, X., A. Chabes, V. Domkin, L. Thelander, and R. Rothstein. (2001). The ribonucleotide reductase inhibitor Sml1 is a new target of the Mec1/Rad53 kinase cascade during growth and in response to DNA damage. Embo J, Vol. 20, No. 13, pp. (3544-53).

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The study of DNA advanced human knowledge in a way comparable to the major theories in physics, surpassed only by discoveries such as fire or the number zero. However, it also created conceptual shortcuts, beliefs and misunderstandings that obscure the natural phenomena, hindering its better understanding. The deep conviction that no human knowledge is perfect, but only perfectible, should function as a fair safeguard against scientific dogmatism and enable open discussion. With this aim, this book will offer to its readers 30 chapters on current trends in the field of DNA replication. As several contributions in this book show, the study of DNA will continue for a while to be a leading front of scientific activities.

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