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Assembly and Regulation of the Pre-Replication Complex: Increasing Complexity in Sight of Diversified Function and Regulation

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1. Introduction

Perhaps the two most fundamental rules in DNA replication say firstly to replicate the entire genome without leaving even small parts unreplicated and secondly, not to replicate any part, even a small one, more than just once during each cell cycle. In light of the size of an average eukaryotic genome and the time of S-phase, this is an enormous challenge. If in a gedankenexperiment a mammalian cell were scaled up to the size of a football and its DNA proportionally expanded in length, the DNA would span more than the distance from the earth to the sun - more than 1700 millions of kilometers in a football that has to be replicated in approximately eight hours.

In contrast to a (small) bacterial genome on a single chromosome, the replication of an eukaryotic genome (portioned in several chromosomes) has to start from multiple starting points, called origins of replication, in order to be accomplished in a reasonable time. Around 30.000 such origins are activated in an average mamalian cell and give rise to replication forks in each S-phase. In order to hold the fundamental rules of once per cell cycle replication, this complexity demands an extremely sophisticated regulation of replication initiation events. The formation of the pre-replication complex (pre-RC) on potential orgins of replication at a time different from active replication is a key step in this regulation.

Through the formation of the pre-RC, the replicative helicase, the MCM2-7 complex, is loaded onto chromatin. The activity of this helicase separates the DNA double strand at the beginning and during S-phase and permits access of the polymerase machinery to the template strands. In addition, only the presence of the pre-RC proteins on chromatin permits the recruitment of other proteins that are necessary to load the polymerases. Therefore, in order to avoid that replication can start more than once per cell cycle at the same site, which would lead to re-replication, the formation of the pre-RC is restricted to a period with low S-phase kinase activity, that is, at the exit from mitosis and in G1. This system normally assures the once-per cell cycle replication: The license to start replication can only be given before replication itself has started. The cell only has to safeguard in addition that a pre-RC that once gave rise to a replication fork is by this action deactivated for the remaining cell cycle.

However, this very efficient system to avoid rereplication comprises on the other hand the danger that not sufficient replication forks will be created to replicate the entire genome if the cell encounters stress situations that block replication forks or lead to DNA breaks.

Therefore, far more pre-RCs are assembled as will normally be used later in S-phase to start a replication fork. This poses the questions how sites of pre-RC assembly are chosen and how the subpopulation of pre-RCs that will be activated and thus finally lead to the establishment of a replication fork are picked.

In this chapter, I will first review the basic protein machinery of the pre-RC and will then discuss additional factors that were recently characterized, which might influence or even decide the localization and fate of a given pre-RC. I will then give a short insight into situations, where re-replication is physiologically normal and give a summary of how origin licensing is regulated in these special cell cycles. At the end of this chapter, I will present examples that relate the increasing complexity of pre-RC factors in higher eukaryotes with their more and more complex organization of S-phase with respect to cell fate and differentiation.

2. Assembly and function of the pre-RC

The yeast Sacharomyces cerevisiae is special as it assembles its pre-RCs on sites on the chromatin that are defined by sequence motifs, so called *ARS* (**A**utonomous **R**eplication **S**equence) sites. Nevertheless, the yeast pre-RC serves as a paradigm, since its basic machinery is conserved throughout eukaryotes.

Pre-RC assembly begins in G1 with the binding of the hexameric ORC-complex (**O**rigin **R**ecognition **C**omplex, ORC1 - ORC6) to the ARS consensus sequences. The ORC-subunits 1,4 and 5 are ATPases. Once the ORC-complex is chromatin-bound, CDC6, another ATPase, joins and recruits the Cdt1 protein. Cdt1 has no enzymatic activity, but is nevertheless also an essential factor for pre-RC assembly. Importantly, Cdt1 seems to be the most regulated factor of the pre-RC, since it is tightly regulated by chromatin binding, several degradation pathways and finally by binding to geminin (see below).

Pre-RC assembly is then finally accomplished by the loading of the replicative helicase, the MCM2-7 complex, onto chromatin. In S. cerevisiae, Cdt1 is already in its soluble, non-chromatin bound form associated with the MCM2-7 complex, whereas in other organisms, Cdt1 and the MCM2-7 complex bind one after the other to the forming pre-RC. The concerted ATP consuming action of ORC, CDC6 and Cdt1 loads multiple hexameric MCM2-7 complexes, 20 to 40, for each pre-RC, on DNA. A huge amount of work and effort was invested to understand this ATP-driven MCM2-7 loading machinery functionally and (so far partially) also structurally (Mizushima et al, 2000), (Harvey & Newport, 2003), (Bowers et al, 2004), (Speck et al, 2005), (Randell et al, 2006).

ATP hydrolysis by ORC and CDC6 is necessary for iterative MCM2-7 loading and thus for viability. However, ORC bound to ATP, but defective of ATP hydrolysis, can support one single round of MCM2-7 loading, whereas the ATPase activity of CDC6 is strictly required for any MCM2-7 loading (Bowers et al, 2004). The "loaded" chromatin binding state of the MCM2-7 complex is defined as a salt-resistant binding that is, once loading is accomplished, even independent of the presence of the other pre-RC proteins.

Speck et al. added a milestone by presenting the first structural information of the "MCM2-7 loading machine" (Speck et al, 2005). The authors presented an image reconstruction from electron micrographs of the yeast ORC complex alone and in complex with CDC6. These structures with a resolution of about 25 Å show the ORC complex as an elongated entity composed of three domains. Association with CDC6 creates a ring-like structure that has quite similar dimensions to an archeal MCM helicase ring that could be visualized by protein crystallography.

Another fundamental step forward in understanding pre-RC formation functionally and structurally was achieved in 2009 when Evrien et al. and Remus et al. managed independently to reconstitute yeast pre-RC assembly *in vitro* from purified proteins onto immobilized DNA and to follow and dissect this assembly functionally and structurally (Remus et al, 2009), (Evrin et al, 2009). Importantly, they could present for the first time structural data showing how the MCM2-7 complex is topologically "loaded" onto double stranded DNA. The ORC-CDC6 assembly on chromatin recruits Cdt1-MCM2-7 heteroheptamers and loads MCM2-7 complexes stably onto DNA in a way that each time two of them form stable head-to head double hexamers that can freely slide along the DNA. They also presented data arguing that these double hexamers are loaded in a way that the DNA double strand passes through the central channel of the helicase, at least during its inactive state.

3. Regulation of the pre-RC in different organisms: Assembly and activation

As mentioned, the yeast pre-RC is a simple, but without doubt the best understood "MCM2-7 loading machine" known, but as organisms became more and more complex in respect of their development and cellular differentiation, additional factors seemed to be necessary to provide either back-up mechanisms of regulation or the possibility of fine-tuning pre-RC assembly and activation in different cellular contexts.

In this section, I will present these additional factors. Some of them have been studied for more than ten years, others were only very recently discovered or described and their functions still await confirmation and a coherent embedding in the known context of origin licensing and cell identity.

Geminin:

Geminin was discovered 1998 in a screen for proteins being degraded by the anaphase promoting complex (APC) as a protein that blocks DNA replication in Xenopus laevis egg extracts (McGarry & Kirschner, 1998). Soon after the discovery of Cdt1 (Maiorano et al, 2000), it became clear that geminin binds to Cdt1 and inhibits Cdt1 in recruiting the MCM2-7 complex to the assembling pre-RC (Tada et al, 2001).

Whereas in yeast, Cdt1's activity is regulated solely by its degradation at S-phase entry, in higher eukaryotes geminin provides thus another layer of MCM2-7 recruitment control. Geminin is highly expressed at S-phase onset and blocks a second round of pre-RC assembly by binding and inhibiting Cdt1. Not before the end of mitosis, when the APC targets geminin for destruction, a new round of pre-RC assembly is thus permitted. Geminin is absent from the genomes of yeast and fungi and C. elegans might have a diverged form that however still awaits comprehensive functional analysis (Yanagi et al, 2005).

Drosophila has a geminin that shares the functions of controlling and blocking DNA replication by direct interaction with Cdt1 (Quinn et al, 2001). Plants also encode a protein that shares partial homology with geminin that functions in cell differentiation and that also interacts with plant Cdt1, however, a clear role in replication control by blocking Cdt1 needs to be shown (Caro & Gutierrez, 2007).

Importantly, geminin is also implicated in development and cell differentiation, as it interacts with key transcription factors, chromatin remodelers and seems further to be able to act as a transcription factor for developmental genes on its own (see down). An interesting model arises with these findings stating that geminin is placed at the intersection

between the "replication road" and the "differentiation road" and its presence and binding to different partners influences which way a cell goes: Less geminin (or no geminin at a particular localization at a particular moment in the cell cycle) leads the cell to assemble pre-RCs and to license origins and so to engage in another cell cycle. More geminin blocks replication licensing and thus proliferation, but at the same time activates the transcription of genes that lead to cell differentiation like neuronal transcription factors (see below). However, these regulating functions of geminin are surely more complex than known so far and we await in the future more data about how precisely in a given situation geminin influences cell fates.

In the earliest and extremely fast embryonic cell cycles, before transcription is activated, geminin is not (completely) degraded at mitosis, and is present also in G1 during pre-RC formation. Several studies investigated how pre-RC formation in the presence of geminin in these early cell cycles could be possible. Hodgson et al. (Hodgson et al, 2002) reported that the geminin that survived mitosis does not bind to Cdt1 and seems to be modified (as Li et al. suggested later, possibly by ubiquitination (Li & Blow, 2004)) in a way that its interaction with Cdt1 is blocked, thus Cdt1 is kept active in recruiting MCM2-7 complexes to chromatin. After licensing is achieved, the nuclear envelope reforms around chromatin, and only after this step is completed, S-phase promoting Cdks can be concentrated inside the nucleus in order to trigger S-phase. Then, once the nuclear envelope is resealed, also geminin becomes imported into the nucleus and seems to loose its modification by the import process itself so that it is again competent of binding and inhibiting Cdt1.

Another study reports the existence of two different stoichiometries of the Cdt1-geminin complex, one containing less geminin and a second one containing more geminin bound to each Cdt1 molecule (Lutzmann et al, 2006). The two different complexes can be assembled *in vitro,* and by using the Xenopus egg extract system it was shown that both complexes indeed behave differently: Cdt1 with less geminin bound is active in recruiting the MCM2-7 complex, but licenses origins in a strictly controlled "once per cell cycle" manner (under these conditions, Cdt1 alone leads to re-replication) whereas the complex of Cdt1 with more geminin associated is licensing inactive. This change in the stoichiometry seems to take place exactly at the onset of S-phase. These models of how licensing is permitted in the presence of geminin are not mutually exclusive. Several structural studies of Cdt1- and geminin-domains, alone and in complex, support the idea of different stoichiometries of the Cdt1-geminin complex (Lee et al, 2004), (Okorokov et al, 2004), (De Marco et al, 2009).

MCM8 and MCM9:

Besides the heterohexameric MCM2-7 complex, the family of MCM-domain containing DNA helicases also includes MCM8 and MCM9. In contrast to the MCM2-7 complex, MCM8 and MCM9 do not exist in all eukaryotic genomes, but with the only exception of Drosophila melanogaster, both are either together present or absent in a given genome, suggesting that the two proteins have interdependent roles (Liu et al, 2009). Yeast and C.elegans do not have MCM8 and MCM9, but several fungi possess open reading frames coding for shortened versions of MCM8 and MCM9. However, a long version of MCM9 with a C-terminal extension of about 500 amino acids is found only in vertebrates (Lutzmann et al, 2005).

Whereas one report on MCM8 employing overexpression of the protein places MCM8 on the pre-RC (Volkening & Hoffmann, 2005), another report depleting the endogeneous protein using Xenopus egg extracts shows that pre-RC formation and the initiation of DNA

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replication is not affected by the absence of MCM8, but that elongation of replication forks is hindered after depletion of MCM8 (Maiorano et al, 2005).

Recently, single nucleotide polymorphisms (SNPs) in human MCM8 exons were shown to affect the fertile life span in women. However, the biological mechanism of these results is at present unknown (He et al, 2009), (Stolk et al, 2009).

Using Xenopus egg extracts, which recapitulate the early embryonic cell cycles during embryonic development, it was shown that MCM9 is necessary for pre-RC assembly by binding to Cdt1 and so limiting the amount of geminin that can associate to Cdt1 during the period where Cdt1 has to be active in recruiting the MCM2-7 complex in the presence of geminin (Lutzmann & Mechali, 2008). However, whether the functions of MCM9 (and MCM8) are similar in these early embryonic cell cycles and in normal somatic cycles has still to be investigated.

HBO1:

A highly active field in investigating the regulation of DNA replication origin analyses the chromatin environment at origins. The identification of the HBO1 protein, a MYST-family histone acetyltransferase that is necessary for normal origin licensing and MCM2-7 recruitment was an important step in deciphering chromatin remodeling during the licensing reaction. HBO1 (**H**uman acetylase **B**inding to **O**RC1) was originally identified as an ORC-interacting acetylase in a yeast two hybrid-screen (Iizuka & Stillman, 1999). By affinity purification, HBO1 is also found in a large chromatin-remodeling complex containing ING5 and this complex was shown to be essential for normal replication and S-phase progression (Doyon et al, 2006).

In such different systems as cultured cells from humans, Drosophila or Xenopus egg extracts, the depletion or inactivation of HBO1 affects the chromatin recruitment of the MCM2-7 complex and leads to replication defects. However, the effects on pre-RC formation vary. For example, whereas in Xenopus egg extract, depletion of the putative Xenopus HBO1 leads not only to a defect in MCM2-7 loading and replication but also to a recruitment defect of Cdt1 (Iizuka et al, 2006), knock-down of HBO1 in human cells does not affect the chromatin association of Cdt1 (Miotto & Struhl, 2008). HBO1 is found in its soluble, non-chromatin bound form in complex with Cdt1 and seems to enhance Cdt1's MCM2-7 recruiting activity once bound to chromatin.

Using ChIP, it was shown that HBO1 bound to Cdt1 is specifically recruited to origins, and acetylates at these sites histone H4 tails in order to facilitate MCM2-7 loading. Interestingly, it was reported further that geminin binding to Cdt1 does not physically affect the association of HBO1 with Cdt1 or its targeting to origins, but inhibits HBO1's acetylase activity specifically toward H4 at origins (Miotto & Struhl, 2010). Consistent with this, HBO1 is absent in organisms that also do not possess geminin, including yeast and possibly worms.

HOX proteins:

Several studies reported that geminin binds not only to the promoter regions of several HOX genes, but also directly to a subset of HOX proteins. One study investigated the binding of HOX proteins to replication origins and reported binding of several HOX-members, especially HOXD13 to origin DNA (Salsi et al, 2009). HOXD13 was found in this study to interact further with CDC6, in order to enhance the recruitment of pre-RC proteins to origins. When overexpressed, HOXD13 seems to accelerate DNA replication. The authors of this study claim further that geminin binding to HOXD13 blocks the interaction with CDC6 and the positive effect of HOXD13 to origin licensing.

ORCA:

Up to now, the most upstream event of pre-RC formation known is the recruitment of the ORC complex to chromatin. Since besides yeast, no clear consensus site of ORC binding could be found on DNA, it remains unclear which factors specify ORC-binding sites. Chromatin context, influenced by multiple factors such as histone modifications, transcription and the presence of other chromatin binding proteins seem to play crucial roles.

Very recently, Vermeulen et al. (Vermeulen et al, 2010) and Shen et al. (Shen et al, 2010) identified in human cells a protein that was called ORCA (**ORC A**ssociated) that stabilizes ORC binding to chromatin.

ORCA was identified by mass spectrometric analysis of ORC2-immunoprecipitates from human cells and homologous proteins were identified in higher eukaryotes from human to flies, but the protein seems to be absent in yeast and worms. Interestingly, when the reciprocal ORCA-IP was analyzed, not only ORC subunits were found to interact with ORCA, but also Cdt1 and geminin (Shen et al, 2010). ORCA has about 650 amino acids and contains leucin-rich repeats and a WD-repeat containing domain. It is this WD-repeat containing domain that confers both chromatin association of ORCA and also binding to ORC. ORCA was shown to associate predominantly with chromatin during the G1 phase and to colocalize partially with HP1, mainly at heterochromatic regions.

Knockdown of ORCA leads in all cell lines analyzed to a reduction of ORC binding to chromatin. Interestingly, whereas cancer cell lines do not show a concomitant reduction in MCM2-7 recruitment or cell cycle alterations, primary cells indeed recruit less MCM2-7 and are enriched in G1 after knock-down of ORCA.

Also remarkable is the different colocalization of ORCA and ORC on centromeres and telomeres in several different immortalized cell lines. Telomerase-positive cells show ORCA and ORC localization at the centromeres whereas cells that use alternative lengthening of telomeres (ALT) through recombination recruit ORCA and ORC to telomeres. These findings indicate that ORCA and ORC might also have a function in recombination and / or DNA repair.

GEMC1:

GEMC1 was identified by database mining for ORFs containing sequence motifs of known replication proteins (Balestrini et al, 2010). The protein was named GEMC1 since its sequence contains a central coiled-coil region that shares similarities with geminin's coiled-coil region (**Gem**inin coiled-coil **C**ontaining protein **1**). The presence of GEMC1 was reported so far only in the genomes of vertebrates. However, despite its partial homology to geminin, GEMC1 does not exert a blocking function in DNA replication, but an activating one.

GEMC1 is necessary for the recruitment of CDC45, an essential factor that plays a key initiating role in turning the pre-RC into the pre-initiation complex (pre-IC) at S-phase onset. CDC45 binds to chromatin downstream of pre-RC formation, when S-phase kinase activity is already high, but upstream of the recruitment of replisome-factors such as MCM10, GINS and polymerase α. GemC1 is highly phosphorylated by S-phase kinase activities and is recruited to chromatin through its association with the TopBP1 protein. This association is strengthened by GEMC1 phosphorylation. In Xenopus egg extracts, GemC1 and TopBP1 however bind to chromatin already during pre-RC formation. Whereas Balestrini et al. present data showing that binding of GEMC1 is indeed ORC-dependent, it is still debated whether the chromatin association of TopBP1 is equally ORC-dependent.

However, the question of whether GEMC1 is a true pre-RC protein is not clearly answered by this fact since its absence (by depletion from Xenopus egg extract) does not inhibit the final step of pre-RC assembly, the recruitment of the MCM2-7 helicase. Thus, pre-RC formation and origin licensing are not dependent on GEMC1.

Maybe, somehow similar to the ORCA protein, GEMC1 might be a factor that does not fit entirely into the textbook model of pre-RC assembly, were the chromatin-binding of one factor is strictly and entirely dependent on the presence of its upstream predecessor on chromatin. Once ORC and / or ORCA are bound to chromatin at a potential origin of replication, several, and from there on independent, branches of protein-machinery assembly might take place. One is surely the loading of the replicative helicase, the MCM2-7 complex, and another might be the recruitment of factors that permit the activation of this helicase later at S-phase onset. If helicase loading is blocked, at least a part of the machinery that would be needed further downstream of MCM2-7 loading to activate an origin might still be assembled: GemC1 binding to chromatin is dependent on ORC, but GemC1 is not necessary to load the MCM2-7 helicase, and if MCM2-7 chromatin loading is blocked (by addition of exogenous geminin), GEMC1 binds to chromatin nevertheless.

GEMC1 might be an interesting factor that forces us to question and maybe re-think the formerly-presumed strict dependences and hierarchies of licensing and replisome assembly.

4. Controlled re-replication

Before I finish the chapter by trying to interpret the increasing number of factors that are required for either pre-RC assembly or its regulation in multicellular organisms that have a more and more complex embryonic development and cell differentiation program, I want to mention situations in which controlled re-replication is required and physiological normal.

Whereas during aberrant re-replication cells re-replicate parts of the genome in an uncontrolled number, in most physiological situations of re-replication, either the whole genome (in so called *endocycles*) or only very particular loci (so called *gene amplifications*) are precisely re-replicated multiple times. Controlled re-replication is normally programmed in cells that have a very high metabolic turnover or have to produce a certain (sub-) set of proteins in extremely large amounts. Not surprisingly, in most of the known cases of gene amplification, ribosomal genes are amplified. Another prominent example is the amplification of chorion genes in Drosophila ovary cells that encode the proteins forming the eggshell.

Endocycles, in which cells re-replicate their entire genome (albeit often less accurately than in normal cycles) are executed when cells pass directly from a G2 phase into a new G1 phase (without passing through mitosis) or if only a partial mitosis that lacks cytokinesis is interposed. Endocycles are common in plants and flies and also in some vertebrates (most often in fish and reptiles) but are rare in mammals. Very generally, endocycles require that Cdk1 activity is inhibited in order to block mitosis and that the APC(Cdh1) activity is high to degrade mitotic cyclins and - in higher eukaryotes - also geminin at moments when Cdk activity is low.

In addition, another important change in cell cycle control mechanisms has to take place: Normally, in order to block the propagation of damaged DNA or of extra copies of DNA to daughter cells, the DNA damage checkpoint becomes activated when re-replication occurs and this response activates either cell senescence or cellular death via apoptosis. Therefore, another distinctive mark of cells undergoing scheduled endoreplication is the inactivation of

the DNA damage checkpoint during re-replication of the genome: Even if artificially (by overexpression of the Drosophila Cdt1 homologue Dup1) in endocycling Drosophila cells DNA damage is caused, the DNA damage checkpoint is not activated in these cell cycles (Mehrotra et al, 2008).

The best described situation of mammalian cells that undergo endocycles is found during the differentiation of trophoblast stem cells into trophoblast giant cells that are produced to facilitate the implantation of the early embryo into the epithelium of the uterus. Importantly, the licensing machinery is essential and active also in endocycling cells. Therefore, like in normal mitotic cycles, gap phases are required where Cdk2 activity is low and pre-RC assembly is allowed to take place.

5. Origin choice and pre-RC complexity

In this final part of the chapter, I will resume the ideas in the field why multicellular eukaryotes with a complex developmental biology and cell differentiation program obviously need more factors to regulate replication origins as do single-cell eukaryotes. As mentioned in the beginning, the yeast *S. cerevisiae* possesses a simple licensing apparatus, maybe the most *sized-down* of all eukaryotes. It is surely not a coincidence that this organism specifies all possible replication origins through clearly defined DNA sequence motifs, the autonomous replication sequences (ARS), which work when placed on plasmids outside the chromosomal context as well as in their natural genomic environment (even though if not all ARS sequences in the yeast genome lead to active replication origins in each cell cycle). Furthermore, besides a meiotic cell cycle, yeast does not have a developmental- or cell differentiation program.

For multicellular organisms (and for most unicellular ones as well), no consensus sequence or sequences could be found that specify pre-RC assembly sites or origins of replication. In contrast, the positions of assembled and finally also used replication origins can change due to gene expression, growth conditions and chromatin structure (for review see (Mechali, 2010)).

It seems that during evolution, epigenetic factors took at least a part of origin determination from the pure genetic influence (the sequence) in order to cope with the replication of an always similar sequence that is present in very different chromatin states depending on cell identity and transcriptional programs.

As pointed out, in higher eukaryotes a number of pre-RC factors interact with chromatin-shaping proteins and chromatin remodelers. The ORC complex interacts with HP1 and seems to be dependent on ORCA for its normal localization, especially at heterochromatic sites. ORC and MCM2 further interact with the histone acetylase HBO1. Cdt1 is dependent on HBO1 and its histone acetylation activity to exert its function in recruiting the MCM2-7 complex.

Furthermore, geminin, likely not an essential pre-RC factor itself, but essential for its proper regulation, has complex roles in origin regulation. The protein interacts not only with crucial developmental transcription factors but also with the chromatin remodeler Brg1 (Seo et al, 2005). Thus, several chromatin-remodeling activities seem to be required to assemble and regulate pre-RCs.

It was shown that origin positioning changes with the transcriptional status of a chromatin domain (Gregoire et al, 2006) and further that binding of a transcription factor to a plasmid specifies the site where replication of the plasmid starts when incubated in Xenopus egg extract that otherwise does not show sequence specificity of DNA templates (Danis et al, 2004). Interestingly, the moment during G1 when origins are chosen that will be activated later in S-phase is precisely defined and called the **O**rigin **D**ecision **P**oint (ODP) (Wu & Gilbert,

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1996), (Li et al, 2003). Once this program is established, cells have to go through mitosis in order to "reset" this programming. Incubation of differentiated nuclei (having large origin distances) in mitotic egg extract (resembling the undifferentiated state with short inter-origin distances) is sufficient to switch from large to small replicons (Lemaitre et al, 2005).

Mostly, cell differentiation has to go along with a block in proliferation. In organisms composed of billions of cells, which are found at all possible positions between the extremes of completely undifferentiated, fast proliferating stem cells and on the other side fully differentiated cells that dropped completely out of the cell cycle, the correct tuning between differentiation and proliferation is a complex and vital task. Several data suggest that also here, the pre-RC and its regulation play an important role.

Since its discovery, two roles of geminin have been described: Its regulating (blocking) function on the licensing system (McGarry & Kirschner, 1998) and its involvement in neuronal differentiation (Kroll et al, 1998). Several situations were described where geminin is placed on the crossroads between proliferation and differentiation.

Geminin binds the homeobox-containing transcription factor Six3 that is necessary for proliferation of retinal precursor cells. Six3 can displace Cdt1 from geminin. By this means, even without affecting transcription, Six3 can specifically activate the proliferation of retinal precursor cells by sequestering geminin from Cdt1 (Del Bene et al, 2004).

Geminin also interacts with HOX proteins and blocks their binding to their promoter targets. In addition, it also binds to polycomb group proteins that repress HOX transcription and function. Like in the case of Six3, HOX proteins seem to be able to displace geminin from Cdt1. Therefore, a model arises in that geminin can block differentiation by binding and blocking HOX proteins and at the same time releasing Cdt1 to favor proliferation. Or, in contrast, geminin can release HOX proteins and block Cdt1, which leads the cell to take the road away from proliferation towards differentiation (Luo et al, 2004).

In yet another study, geminin was shown to bind the catalytic subunit of the SWI/SNF chromatin-remodeling complex, Brg1 (Seo et al, 2005). This complex is necessary for the transcription of neuronal genes that lead to neuronal differentiation in neuronal progenitor tissue. Thus, in this case, more geminin means a block of Brg1 activity, no transcription of neuronal genes and ongoing proliferation of neuronal progenitor tissue. Less geminin keeps Brg1 active, neuronal genes are transcribed and differentiation wins over proliferation, even if less geminin (which means more free Cdt1) is present.

Finally, in Xenopus egg extracts recapitulating the very early embryonic cell cycles in which geminin is not degraded but present in G1, the MCM9 protein limits the binding of geminin to Cdt1, keeping Cdt1 active in recruiting the MCM2-7 complex to chromatin (Lutzmann & Mechali, 2008).

Clearly, most of the principal, and obviously all of the fine-tuning mechanisms that use the licensing reaction to balance proliferation and differentiation have still to be discovered and characterized. Likely, in the coming years we will learn much more about how replication and its regulation are connected to and interdependent with cell differentiation and development. Pre-RC assembly and origin choice might play crucial roles in these processes.

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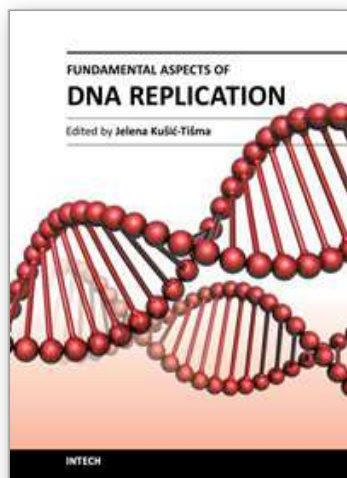
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DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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