EUCARYOTIC REPLICATION ORIGIN BINDING PROTEINS

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1. ABSTRACT

Initiation of eukaryotic DNA replication is a tightly controlled process. Replication initiates at multiple specific sites (replication origins) that have been licensed for replication, following the cell cycle-dependent, multi-step assembly of specific factors. Thus, replication origins occur in two chromatin states: a replication-competent pre-replicative (pre-RC) state, when a number of replication proteins assemble on the origin in a stepwise fashion, and a replication-incompetent post-replicative (post-RC) state, in which the origin (or elements of it) is bound only by the origin recognition complex (ORC) (or subunits of it). This review summarizes the origin binding proteins that have been have been identified to date.

2. INTRODUCTION

Eukaryotic DNA replication is a stringently regulated process. Multiple pathways of signal transduction and cell growth regulatory checkpoints converge upon the DNA to permit replication only when all the prerequisites have been met and the conditions are appropriate. DNA replication initiates at the origin of DNA replication, which is defined both functionally and genetically. The "functional" origin refers to the actual site of initiation of DNA replication, as determined biochemically, while the "genetic" origin (called replicator) refers to sequences that dictate the site of initiation, as determined genetically by mutations in a locus required for replication initiation (1, reviewed in 2-4). In simple genomes, the functional and the genetic *ori* may coincide. The DNA replication process involves many protein-protein and protein-DNA interactions, occurring in multiple stages (5-7). As in prokaryotes, control of DNA replication occurs predominantly at the initiation step. The cell cycle-dependent changes occurring at replication origins control the timing and frequency of initiation of DNA replication (reviewed in 2, 7). Origin activation starts with the binding of an initiator protein (IP) to origin-specific recognition sequences. Binding of the IP results in melting at the origin and triggers the ordered assembly of multiprotein complexes, leading to the formation of a stable pre-replication complex (pre-RC) and the initiation of bi-directional replication (reviewed in (8-10). The timing and frequency of initiation may be regulated by the availability of the IP or by cell cycle dependent topological changes in the DNA that affect the IP's ability to interact with the origin (5).

3. ORIGINS OF DNA REPLICATION

In simple genomes (prokaryotes, plasmids and bacteriophage, lower eukaryotes such as protozoa, yeast and slime mold, animal viruses and mitochondria of higher eukaryotes), replication origins consist of well-characterized specific *cis*-acting sequences (replicators), which interact with specific initiator proteins. In contrast, the multiple sequence and protein components present at the chromosomal origins of higher eukaryotes (metazoa) have not yet been fully defined (reviewed in 2, 3, 7).

3.1. Mammalian origins of DNA replication

Identification of origins in higher eukaryotes has been complicated due to their genomic complexity and the large number of origins (3). Mammalian DNA replication is

initiated at 10^4-10^6 sites (replication origins) (11-13) and proceeds bidirectionally (14, reviewed in 15). A large body of biochemical, molecular biological and electron microscopic evidence, suggests that mammalian DNA replication is initiated at specific sites (reviewed in 2, 3, 7, 16, 17). Other studies, however, using two-dimensional gel electrophoresis mapping methodologies, have detected multiple replication origins that are dispersed over large intergenic regions (initiation zones) (18-20). With regard to the Chinese hamster ovary DHFR gene locus, in particular, different mapping methodologies have detected both specific (21, 22) and multiple initiation sites (19, 20). This discrepancy might be reconciled by the findings of a recent study, showing that origin specificity or site selection can be modulated by the size of nucleotide pools within cells (23, reviewed in 4).

Clusters of origins and the DNA segments (replicons or replication units), whose replication they control, are activated at different times throughout S phase (reviewed in 15), and are replicated in a defined spatial and temporal order (reviewed in 2, 7). Replication origins that are activated synchronously might either share a common DNA sequence (24) or secondary structure (reviewed in (25) recognized by specific regulatory proteins. Furthermore, their subnuclear localization might be such that it facilitates their activation at a given time (15, 26 and refs. therein). Under normal growth conditions, each eukaryotic origin initiates replication only once per cell cycle. However, the decrease in replicon size in embryonic and transformed cells as well as the higher initiation frequency of origins at certain loci suggest the presence of more potential initiation sites than those activated during a normal S phase (reviewed in 2, 7). The increase in the frequency of initiation in virally transformed cells is estimated to be of the order of 2-10-fold (13, 27). New sites of initiation of DNA replication are activated and others, like the *c-myc* origins, that are already active display an increase in their activity induced upon cell immortalization (28, 29). In addition to the activation of a new set of origins, there may also be changes in the regulatory signals of the normal set of origins.

3.2. Origin Activation

Origin activation depends upon the presence of both specific sequence and structural determinants that facilitate the local unwinding of DNA and serve as recognition signals for initiation of replication. Multiple functional elements, including a DNA unwinding element (DUE) and transcription factor binding sites, are essential for activity of the human *c-myc* origin (1). Also, DNA cruciforms are among structural signals forming transiently at replication origins at the onset of S phase, their numbers being maximal at the G1/S boundary (30, 31). The evidence implicating cruciforms in the regulation of DNA replication supports the hypothesis that certain inverted repeats (IRs), giving rise to cruciforms, are included in potential initiation sites for DNA replication (11, 15), serving as the attachment site for initiator protein(s) (7, 32). Thus, cruciform-specific binding proteins may be involved in the regulation of replication (2, 7, 25, 33, 34).

Recently, a 36-bp origin consensus sequence was identified, which controls the autonomous DNA replication of a plasmid after its transfection in eukaryotic (mammalian, chicken, and *Drosophila*) cells (35). Versions of the consensus sequence that are found in association with some CpG island sequences support autonomous replication. Also, homologues were identified at replication origins, including the well characterized replication origin of the lamin B2 locus. The consensus sequence will permit, under selective pressure, the persistence of genes it carries into cells as episomes for >170 cell doublings. Mutagenesis analyses identified a putative 20 bp minimal sequence within the 36 bp (35).

4. ORIGIN BINDING PROTEINS

Origin recognition proteins bind specifically to their cognate site within the replication origin and initiate replication, participating either directly by unwinding the DNA (helicase activity, e.g., as in papilloma, SV40 and polyoma virus), or indirectly through their association with other replication proteins (e.g., ORC, in the budding yeast, *Saccharomyces cerevisiae*) (26). Although much is known about prokaryotic initiator proteins, comparatively little is known about their eukaryotic counterparts. *S. cerevisiae* has been a very useful system for the identification of origin-associated proteins and the study of the controlled activation of origins.

4.1. ORC (Origin Recognition Complex) (see recent reviews (9, 36))

S. cerevisiae autonomously replicating sequences (ARS) contain a 15-35-bp origin recognition element (ORE) that includes a conserved 11-bp A/T-rich element (ARS consensus sequence or ACS or A element) as the binding site for its origin recognition proteins (37, 38). In addition to ORE, flanking A/T-rich sequences situated 3' to the A domain, known as domain B, comprise multiple 10-15-bp B elements and contribute to ARS activity (reviewed in 9). The discovery of the *S. cerevisiae* origin recognition complex, ORC (39), was a breakthrough for the study of regulation of initiation of eukaryotic DNA replication. ORC consists of six polypeptide subunits, ranging from 50 to 120 kD, is essential for initiation of DNA replication and is, most likely, the initiator protein for yeast replication (reviewed in 8, 9). ORC homologues have been identified in human, worms (*Caenorabditis elegans*), amphibia (X*enopus laevis*), plant (*Arabidopsis thaliana*), yeast (*Schizosaccharomyces pombe*) (40), and insects (*Drosophila melanogaster)* (41). In yeast and frog (X*enopus)*, ORC is bound to DNA throughout the cell cycle and recruits other proteins, in a cell cycle regulated manner (42). ORC mutants arrest as large, budded cells with a single nucleus, indicating ORC function in yeast DNA replication (43). In *Saccharomyces cerevisiae*, although ORC is bound to the ARS throughout the cell cycle (42), it is involved in the process of initiation through its ability to form complexes with cell cycle regulated proteins that control initiation (44). Thus, *in vivo* and *in vitro* footprinting studies revealed two types of protein-DNA complexes present at the yeast replication origin during the cell cycle: a post-replicative complex (post-RC) present during the S, G2 and M

phases and a larger pre-replicative complex (pre-RC) present at late M and G1 (45).

The six genes for the yeast (46) and human ORC subunits have been cloned, but the role of the hORC in DNA replication has not yet been fully defined. It was recently reported that hORC binds the well characterized human lamin B2 origin (47). Furthermore, association of the hOrc1 and hOrc2 proteins with chromatin revealed a novel replication origin located in the intergenic region between the divergently transcribed human genes MCM4 and PRKDC (the gene encoding the catalytic subunit [DNA-PKcs] of the human DNA-dependent protein kinase, DNA-PK) (48). Small interfering RNAs (siRNA) directed against hOrc6 indicated its involvement in DNA replication, chromosome segregation and cytokinesis (49). Finally, hORC has also been implicated in cancer cell proliferation (50). Recent reports suggested dynamic changes in hORC during the cell cycle, such that pre-RC formation in human cells may be regulated by the temporal accumulation of Orc1 in G1 nuclei (51).

4.2. ORC binding to replication origins

Although ORC binding to the replication origins of *S. cerevisiae* is directed mostly by the ACS and its adjacent B1 element, it is unclear how it is directed to bind to other eukaryotic origins (9). There is differential binding of ORC subunits to origin DNA in different organisms: thus, in *S. cerevisiae*, protein-DNA crosslinking studies identified four ORC subunits (Orc1p, Orc2p, Orc4p, and Orc5p) as binding to the ACS and B1 elements (52), while in *S. pombe*, ORC binding to its cognate origins involves nine repeats of an AT-hook motif present at the N-terminus of the *S. pombe* Orc4 subunit (53, 56), and in *Drosophila*, all six ORC subunits are binding to replication origins (ACE3 and oriβ) of the chorion amplification locus (54). Human Orc2 specifically associates with the Epstein-Barr virus replication origin, oriP, most likely through its interaction with the viral protein EBNA1 (50). The fact that Geminin, an inhibitor of the mammalian replication initiation complex, inhibits replication from oriP, suggests that ORC and the human replication initiation machinery are necessary for replication from oriP (50). ORC also binds to a specific region of the amplification origin II/9A of the fly, *Sciara coprophila*, at a sequence that is adjacent to the replication start site (57). Such studies implicated ORC binding as a determinant of the position of replication origins in eukaryotes. Based on this premise, two studies in *S. cerevisiae*, one using high-density oligonucleotide microarrays (58) and the other using a chromatin immunoprecipitation (ChIP) assay that relied on ORC and MCM binding as a molecular landmark for the identification of origins (59), identified a total of 332 and 429 replication initiation sites, respectively. Furthermore, 75 initiation sites were bound by MCM but not by ORC, while 12 sites were bound by ORC, but not by MCM (59). The discrepancy in the number of initiation sites detected by the two methods suggested that initiation proteins might be also involved in non-replication events (60). Furthermore, the data suggested that ORC binding to a sequence does not necessarily indicate that it is an active replication origin. Indeed, in addition to its involvement in

DNA replication, ORC has been implicated in the regulation of chromatin structure (61, 62) and transcriptional silencing (63).

Comparison of the origin binding proteins between yeast and humans suggests that replication initiation mechanisms are different; e.g., in contrast to yeast ORC, where the 6 subunits are in a tight complex, the hORC subunits are not (50). Furthermore, several of the subunits are associated with non-ORC proteins and are also expressed in non-proliferating cells, suggesting that hORC subunits may have additional activities (64). Interestingly, although ORC protein binds to it, the 11-bp yeast ARS consensus sequence (ACS) is not essential for origin function in monkey and human origin enriched sequences (*ors*) (65-67), indicating the presence of other features that distinguish replication initiation in simple and complex genomes, in support of the current thinking (2, 7, 68). The present evidence indicates that another protein may be involved in sequence-specific origin activation in human cells (9), (36), especially since origin activity was not found to be altered in Orc2$^{Δ/-}$ hypomorphic cells (50). Recent studies have identified OBA/Ku86 as a novel potential initiator protein, participating in site-specific origin activation (69-72) and CBP/14-3-3 as a structure-specific initiator (73-76).

4.3. OBA/Ku

Based on its ability to bind to a putative mammalian (monkey) replication origin, *ors8*, an <u>O</u>rs <u>B</u>inding <u>A</u>ctivity (OBA) was purified (77), which was subsequently identified by peptide sequence analysis as the 86 kDa subunit of Ku antigen (78). The heterodimeric Ku protein (79) consists of 70 kDa and 80 (or 86) kDa subunits and is present in bacteriophages (Mu), prokaryotic (several bacterial and one archaeal genome; 80) and all eukaryotic organisms, suggesting conservation of function. It is predominantly a nuclear protein that was originally identified as an autoantigen in sera of patients with autoimmune diseases (81). As the DNA-binding subunit of the DNA-dependent protein kinase, DNA-PK, Ku allosterically activates the catalytic subunit (DNA-PKcs) (81, 82). This trimeric complex (DNA-PK-Ku70/Ku80) is implicated in the non-homologous end joining (NHEJ) of DNA double strand break (DSB) repair, site-specific V(D)J recombination (83, 84), transcription (85, 86), telomeric maintenance (reviewed in 87), replicative senescence (88), DNA replication (69, 70, 78, 89), and anti-apoptotic activity in developing neurons (90). Ku80 is also involved in the maintenance of genomic integrity, suppression of chromosomal aberrations and malignant transformation (91). Ku also interacts with the Werner protein (WRN), which is defective in the premature aging disorder, Werner syndrome, both *in vivo* and *in vitro* (92). Aging human fibroblasts were found to harbor elevated levels of a Ku80 proteolytic product (93). It was recently suggested that the induction of a Ku86-specific protease in non-damaged human cells may play a role in the cellular regulation of Ku function (e.g., protease induction correlated negatively with apoptosis, necrotic cell death or general proteolysis (94). Moreover, the nuclear localization of the Ku70 and Ku80

subunits was proposed as a regulator of the multiple physiological functions of the Ku protein (95).

4.4. Ku binding to DNA

Ku is a versatile DNA binding protein. Its DNA substrates include free DNA ends, structured DNA, as well as specific DNA sequences such as those related to DNA transcription or replication (72 and refs. therein). Regarding DNA-end interactions, X-ray crystallography studies using portions of the Ku heterodimer showed that DNA ends pass through a preformed ring in a dyad symmetrical heterodimer, such that the central region of both subunits mediate multiple contacts with the sugar phosphate backbone (96). Ku also binds to specific regulatory DNA sequence elements, influencing gene transcription and DNA replication (78), although the protein domains involved in these interactions have not been defined.

4.5. Ku knockouts

Genetic knockout of the budding yeast Ku homologue, YKu80, resulted in a thermosensitive phenotype (97), while that of the mouse severely affected growth and caused early onset of senescence (98). Unlike yeast and mice, however, the targeted disruption of the human Ku80 locus in HCT116 colon cancer cells has shown that the Ku80$^{-/-}$ cells are nonviable, while the heterozygotes (Ku80$^{+/-}$) exhibit a growth defect (99). Thus, the Ku86 locus is essential in human somatic cells, unlike their yeast and murine counterparts. The viability of the Ku86$^{-/-}$ murine cells (98) suggests the existence of a redundant, alternative pathway in mice, which may be limiting in humans (99).

4.6. Ku involvement in DNA replication

There is increasing evidence, implicating Ku in DNA replication (69-71, 78, 99). Ku is identical to the DNA-dependent ATPase purified from HeLa cells (100) which co-fractionated with a 21S multiprotein complex that is able to support SV40 *in vitro* DNA replication (101). Ku binds to several replication origins, including the adenovirus type 2 origin (102), the B48 human origin (103), a mammalian replication origin consensus sequence, A3/4, comprised in part in the minimal origin of the monkey *ors*8 (35, 78), the Chinese hamster dihydrofolate reductase (DHFR) replication origin, *oriβ* (78), and the human *dnmt*1 (DNA-methyltransferase 1) origin (104). OBA/Ku binds to A3/4 in a sequence-specific manner (72, 78). By chromatin immunoprecipitation (ChIP) assays, Ku70/86 was shown to bind *in vivo* to replication origins (mouse ADA, hamster DHFR *oriβ*, monkey *ors8* and *ors12*) in a cell cycle dependent manner (69, 71), while co-immunoprecipitation assays identified known replication proteins in Ku-containing complexes (DNA polymerases α, δ and ε, PCNA, topoisomerase II, RF-C, RP-A, ORC-2, and Oct-1) as well as DNA-PKcs (70). Some of these proteins (e.g., PCNA and RPA) have a dual role in replication and repair, but, thus far, ORC proteins have been associated only with DNA replication and transcriptional silencing (105), and, possibly, chromatin structure (62). A Ku-like protein from *S. cerevisiae*, OBF2, consisting of a 65 and 80 kDa subunit, binds to the yeast ARS121 replication origin and supports the *in vitro* assembly of a multiprotein complex at this origin (106). Although the 65 kDa subunit of OBF2 is identical to HDF1, a yeast homologue of the mammalian Ku70, the assumption is that the 80 kDa subunit of OBF2 is identical to YKu80 (97) and a homologue of the mammalian Ku80 subunit. Furthermore, Ku may be involved in DNA replication through its association with DNA-PK, which phosphorylates several DNA-binding proteins (107), some of which are involved in replication, e.g. RPA, topoisomerases I and II, SV40 large T antigen, Oct-1 and Ku (108).

The phenotypes of the Ku80 knockout mice (i.e., the small size of the Ku-deficient mice, their prolonged doubling time, and their premature senescence (83), are compatible with Ku's involvement in DNA replication. Recent studies of the Ku86-mutant Chinese hamster ovary (CHO) cell line, xrs-5, again showed that Ku plays a direct role in DNA replication, as the ability of the xrs-5 cells to support DNA replication of a mammalian origin-containing plasmid (p186) was drastically reduced both *in vivo* and *in vitro* (71). Since the knockout of the yeast Ku80 homologue is viable (albeit thermosensitive), as is the Ku80 knockout of mouse, while the human Ku80 knockout is lethal, it appears that the replication mechanisms operating in yeast, mouse and human differ. It is also apparent that a salvage Ku-like replication protein is present in mice, which is absent or not as effective in humans (69), (71).

It was recently shown that the differential binding of Ku to DNA is a determining factor in its involvement in DNA replication (72). Thus, Ku binding to the A3/4 DNA sequence is distinct from its binding to DNA ends and at least two other internal DNA sequences, IAP and HSE (72). [IAP includes an enhancer core sequence from the intracisternal A-particle long terminal repeat of the mouse c-*mos*, over which Ku accumulates and from which Ku has been reported to activate transcription (109), and HSE contains a heat shock response element previously defined as a site of Ku accumulation and which has been implicated in the regulation of heat responses (110)]. Footprinting analyses revealed a specific association of purified OBA/Ku and recombinant Ku with A3/4, showing direct contact of Ku on A3/4 and over the region of *ors8* that is homologous to A3/4 (72). Ku binding to DNA has been classified into eight categories, based on bandshift and UV-crosslinking analyses (72).

4.7. CBP/14-3-3

A human cruciform DNA binding protein (CBP; 66 kDa) was purified from HeLa cells (73), and footprinting analysis showed that it binds DNA cruciforms at the four-way junction at the base of the cruciform stem (74). CBP was identified by peptide sequence analysis, as a member of the 14-3-3 protein family, consisting of isoforms β, ε, γ ζ and σ (75, 76). CBP/14-3-3 was recently shown to be a mammalian replication origin binding protein, binding to origins in a cell cycle dependent manner; it acts at the initiation step of DNA replication by binding to cruciform-containing molecules, and dissociates after origin firing (111). It was also recently shown that the
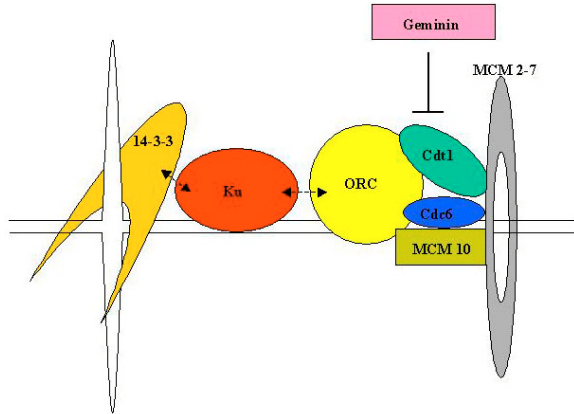
Figure 1. The mammalian pre-replication complex at an origin of DNA replication. Ku, 14-3-3 and ORC bind to their respective locations at the origin, and are followed by Cdc6, Cdt1, MCM 10 to form the pre-replication complex. This leads to recruitement of the MCM2-7 helicase and initiation of DNA replication. Geminin, an inhibitor of Cdt1, can prevent initiation of DNA replication. The double headed dashed arrows indicate interactions as detected by co-immunoprecipitation experiments.

Saccharomyces cerevisiae homologues of 14-3-3, Bmh1p and Bmh2p, bind specifically to cruciform-containing DNA *in vitro*, and they associate *in vivo* with the *S. cerevisiae* autonomous replication sequence ARS307. Furthermore, the presence of a cruciform structure at the ARS307 was revealed by anti-cruciform DNA immunoprecipitation and conventional PCR (34). The monoclonal anti-cruciform DNA antibodies (112, 113) bind to DNA cruciforms in a similar manner as CBP, as shown by footprinting analysis (114).

4.8. MCM proteins/Licensing

The minichromosome maintenance (MCM) proteins, a family of proteins that were discovered in yeast for their role in plasmid replication (115-117) and cell cycle control (118, 119), are one of the components required for replication licensing (120; reviewed in 117, 121). MCM proteins have also been cloned from several other species, including *S. pombe*, *Xenopus*, mouse and human (122). The vertebrate homologues of MCM proteins are not excluded from nuclei during G2 like their yeast counterparts, but their association with chromatin is under cell cycle control. The ORC complex (39) serves as a 'landing pad' at replication origins for the recruitment of replication proteins, among them the MCM complex (MCM2-7), and the formation of the pre-replication complex (pre-RC) (45). The pre-RC, which consists of ORC1-6p, Cdc6p, Cdt1, and the MCM proteins, assembles during G1 in preparation for initiation of DNA replication at the origin (123-125). Then, activation of cell-cycle-regulated protein kinases (cdk's) ushers the 'licensed' origin into S phase. The pre-RC gradually dissociates by releasing Cdc6p and MCM proteins; the remaining post-RC persists until the next G1 phase. MCM10, a recently identified protein, interacts with the MCM complex to participate in the initiation of DNA replication (126). Cdc6 and Cdt1, two origin binding proteins that interact with

ORC, also facilitate the loading of the MCM complex onto the origin (127, 128). This step is inhibited by Geminin, an inhibitor of Cdt1 (129-131). Another MCM protein, Mcm1, which is a general transcription factor for many diverse genes, including several proteins involved in the initiation of DNA replication, was recently identified by ChIP analysis as an ARS binding protein (132). Furthermore, a new member of the MCM protein family, hMcm8, was recently cloned from human cells; hMcm8 does not participate in the MCM complex, is evolutionarily conserved, and is potentially involved in the control of cell proliferation (133).

The pattern of binding observed in the pre-RC fits the "licensing" model of replication, whereby initiation events are limited to once per cell cycle (134, 135). Two different signals are required for activation of chromosomal replication: the replication licensing factor (RLF) 'licenses' replication origins prior to S phase, and the S phase promoting factor (SPF), a cyclin-dependent kinase (Cdk), allows the initiation of replication forks at the licensed origins (reviewed in 136). Evidence from yeast and *Drosophila* strongly suggests that Cdks also play a role in preventing re-replication of DNA in a single cell cycle (136 and refs. therein).

5. MULTIPROTEIN REPLICATION COMPLEXES (DNA SYNTHESOME)

Evidence for the existence of multiprotein replication complexes (MRCs) has been accumulating over the past few years (reviewed in 2, 7, 137). Using an *in vitro* replication system that is based on the simian virus 40 (SV40), a multiprotein replication complex was identified and isolated from a wide variety of mammalian cell types and tissues (138-140). On the basis of this complex that was termed the 'DNA synthesome' (137, 141), a model of DNA replication has been proposed, in which the synthesome 'core' comprises proteins involved in the elongation phase of DNA replication (such as DNA polymerases α and δ, primase, RF-C, DNA helicase, ligase I and topoisomerase II), whereas the proteins involved in the initiation phase of DNA replication (such as RPA, PCNA and topoisomerase I) are more loosely associated with it (reviewed in 137). Such studies have demonstrated that many proteins involved in DNA replication (e.g., RPA and PCNA) are also involved in DNA repair. Interestingly, the DNA synthesome from breast cancer cells has a significantly decreased replication fidelity compared to that of non-malignant breast cells, and was found to contain a novel form of proliferating cell nuclear antigen (PCNA) (142).

6. PERSPECTIVE

Here, we have reviewed the origin binding proteins that have been identified to date and summarized their activities (Table 1 and Figure 1). Significant progress has been made in the past ten years towards understanding the multiple protein-DNA and protein-protein interactions that occur in a cell cycle regulated manner and lead to the initiation of DNA replication. Although ORC binds at

Origin binding proteins

Table 1. Mammalian Origin Binding Proteins

Proteins	Replication Function	Reference
ORC	Acts as Landing pad; designates location of potential origins; directs pre-RC formation; regulates chromatin structure; S. cerevisiae initiator	45, 62
OBA/Ku	Origin binding protein (Initiator?)	78, 70, 69
CBP/14-3-3	CBP/14-3-3 Cruciform binding protein at replication origins (Initiator?)	111, 73, 25
MCM/ Licensing	MCM complex (Mcm2-7p): participates in pre-RC formation	143, 144, 145
	MCM1: Transcription factor, ARS binding protein	132
	MCM10: interacts with MCM complex	126
DNA synthesome	Involved in DNA replication initiation and elongation	137

specific sites in the budding yeast *S. cerevisiae* and flies (*Drosophila* and *Sciara coprophila*), several of which coincide with replication origins, its binding sites and manner of binding in metazoan DNA as well as its role in the process of initiation of DNA replication remain to be determined. The present evidence points to the involvement of another, yet unspecified, protein in sequence-specific origin activation in human cells. The cell cycle regulated binding of OBA/Ku and CBP/14-3-3 to replication origins, including A3/4, a version of a mammalian origin consensus sequence, suggests that these proteins are potentially directing pre-RC formation on at least a subset of origins. The relationship between these proteins and ORC as well as the order of protein loading on human origins remains to be determined. Ku's helicase activity may be important for initiation of DNA replication and its role in the interaction with other replication proteins. Finally, DNA and chromatin structure are clearly important players in the regulation of initiation of DNA replication. The question of how all these parameters come together to maintain a stringent control of DNA replication and allow origin activation once every cell cycle remains to be answered.

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Abbreviations: RC, Replication complex, ORC, Origin replication complex, IP, Initiator protein, DUE, DNA unwinding element, IR, Inverted repeat, ARS, Autonomously replicating sequences, ORE, Origin recognition element, ACS, ARS consensus sequence, Ors, Origin enriched sequence, OBA, Ors binding activity, NHEJ, Non-homologous end joining, DSB, DNA double strand break, IAP, Intracisternal A-particle, HSE, Heat shock element, RLF, Replication licensing factor, SPF, S phase promoting factor, Cdk, Cyclin-dependent kinase, MRC, Multiprotein replication complex, PCNA, Proliferating cell nuclear antigen

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