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Initiation of DNA Replication in Eukaryotic Chromosomes

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Abstract Our understanding of the process by which eukaryotes regulate initiation of DNA replication has made remarkable advances in the past few years, thanks in large part to the explosion of genetic and biochemical information on the budding yeast, *Saccharomyces cerevisiae*. At least three major concepts have emerged: 1) The sequence of molecular events that determines when replication begins and how frequently each replication site is used are conserved among most, if not all, eukaryotes; 2) specific replication origins are used in most, if not all, eukaryotes that consist of a flexible modular anatomy; and 3) epigenetic factors such as chromatin structure and nuclear organization determine which of many potential replication origins are used at different stages in animal development. Thus, the current state of our knowledge suggests a simple unifying concept—all eukaryotes utilize the same basic proteins and DNA sequences to initiate replication, but the metazoa can change both the number and locations of replication origins in response to the demands of animal development. J. Cell. Biochem. Suppls. 30/31:8–17, 1998. Published 1998 Wiley-Liss, Inc.†

Key words: eukaryote; DNA replication; replication origin; pre-replication complex; initiation proteins; origin recognition complex; DNA unwinding; nuclear structure; chromatin structure; DNA methylation; animal development; metazoa; mammal; frog; fly; yeast; *Xenopus*; *Drosophila*; *Sciara*

Initiation of DNA replication in most, if not all, DNA genomes that replicate in either prokaryotic or eukaryotic cells occurs in three steps [DePamphilis, 1993a, 1996]. First, one or more "origin recognition proteins" bind to specific cis-acting DNA sequences referred to as the "origin of replication." Replication origins are defined in two ways. The "replicator" is a sequence that is genetically required to initiate replication; it binds initiation proteins and functions as an autonomously replicating sequence (ARS) when transferred to a foreign DNA molecule or to an ectopic chromosomal location. The "initiation site" is a specific genomic locus where DNA replication begins. So far, both definitions map to the same locus ("replication origin") in all prokaryotic and eukaryotic genomes, as well as in all episomes, bacteriophage, viruses, and organelle genomes characterized so far. Second, DNA unwinding begins at an easily unwound site within the replicator. In some cases, this step is carried out by a helicase activity inherent in the origin recognition protein (e.g., papovavirus T-antigen, papillomavirus E1 protein, adenovirus pre-terminal protein/DNA polymerase). In other cases, the origin recognition protein may contribute to the unwinding process by distorting the DNA, but it must recruit other proteins to complete the job (e.g., Epstein-Barr virus EBNA-1 protein, yeast origin recognition complex, bacteriophage-lO protein, and *E. coli* dnaA protein). Finally, DNA synthesis is initiated on one or both strands of the origin using either an RNA primer (papovaviruses, cellular genomes), a protein-dNTP primer (adenoviruses, F29 bacteriophage), a DNA primer (parvoviruses, geminiviruses), or a cellular RNA molecule (mitochondria, retroviruses). Replication is usually bidirectional, but it can be unidirectional (e.g., F29 bacteriophages, adeno, parvo, and geminiviruses). A unique feature of eukaryotic cell genomes is that they replicate once and only once per cell division cycle, although specific regions may undergo endoreduplication (i.e., gene amplification) in specialized cells during animal develop-

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ment. In contrast, viral genomes (with the apparent exception of latent Epstein-Barr virus) replicate multiple times during a single S-phase, or in the case of mitochondrial genomes, throughout the cell cycle.

Replication origins, like transcription promoters, make the initiation process efficient by directing the appropriate proteins to specific sites and thus facilitating their interactions. These initiation sites must be uniformly distributed throughout the entire genome in order to replicate it within a limited time period, a task most easily accomplished using replication origins. During animal development, the length of S-phase can be as short as a few minutes in embryos undergoing rapid nuclear division or as long as a few hours in adult tissues, and these changes are determined by the frequency of initiation rather than by the rate of fork movement. Replication origins also can regulate the temporal order in which genomic regions replicate during S-phase. In yeast, simply binding initiation proteins to DNA does not always result in replication, because neighboring sequences and the proteins that bind to them can delay the activity of some origins until late S-phase. In fact, the efficiency and timing of replication origins in yeast varies dramatically at different sites along the chromosome. Finally, replication origins can prevent DNA replication from interfering with DNA transcription by directing initiation events to nontranscribed regions. Replication forks that enter genes in the 5' to 3' direction do not interfere with gene transcription, while replication forks moving in the opposite direction can be arrested before entering the 3'-end of the gene by replication fork barriers.

Initiation Proteins

At least 21 different proteins that are required to initiate and regulate origin activity in *Saccharomyces cerevisiae* chromosomes (Fig. 1) [reviewed in Dutta and Bell, 1997; Hua and Newport, 1998; Newlon, 1997; Rowles and Blow, 1997]. Remarkably, homologues for most of these proteins have been identified in many other eukaryotes including other yeast, molds, flies, frogs, and mammals [reviewed in DePamphilis, 1998]. Genetic mutations, microinjection of specific antibodies, or immunodepletion of cell extracts using specific antibodies demonstrate that the same proteins required to initiate DNA replication in *S. cerevisiae* are also required to initiate DNA replication in most, perhaps all, eukaryotes Moreover, the sequence of events leading to DNA replication in *S. cerevisiae* have, for the most part, been demonstrated in *Xenopus* egg extracts as well.

In *S. cerevisiae*, the origin recognition complex (ORC) is an assembly of six different proteins that, in the presence of ATP, bind to specific DNA sequences that function as replication origins. Once formed, this ORC/DNA complex appears to be stable throughout the cell division cycle. At the beginning of G1-phase, Cdc6 binds to the ORC/DNA complex, which then allows an aggregate of six mini-chromosome maintenance (Mcm) proteins to bind to the chromatin at or close to the replication origin. Cdc6 is then replaced by Cdc45, a step requiring cyclin-dependent protein kinase activity [Cdk1)/Ckb5, 6 [Zou and Stillman, 1998]] to form a pre-initiation complex that is activated by the protein kinase Cdc7/Dbf4 to initiate DNA replication. Mcm proteins are released from the chromosome shortly after DNA replication begins, apparently by a mechanism that involves Mcm phosphorylation. In yeast, some Mcm proteins and Cdc45 that initially associate with chromatin at ORC/Cdc6 sites appear to migrate with replication forks during S-phase. Since a mammalian Mcm4/6/7 complex exhibits DNA helicase activity, Mcm proteins may be involved in DNA unwinding at replication forks.

Three differences appear to exist between yeast and the metazoa. Once ORC is bound to DNA in yeast, it appears to remain bound throughout the cell cycle, but in *Xenopus* and mammals, ORC appears to be displaced from chromatin during mitosis and reassociates with chromatin during G1-phase [Abdurashidova et al., 1998; Coleman et al., 1996; Hua and Newport, 1998; Romanowski et al., 1996]. In *Xenopus*, binding of Mcm proteins to chromatin requires "replication licensing factor B" (RLF-B), a step that is sensitive to protein kinase inhibitors. Finally, metazoan pre-replication complexes are activated by Cdk2/Cyclins A, E instead of Cdk1/Clb5, 6.

At least four mechanisms insure that initiation events in eukaryotes are limited to once per origin per S-phase. First, the same cyclin-dependent protein kinase activity required for mitosis (Cdk1/Cyclin B) also prevents assembly of pre-replication complexes, thus insuring that DNA replication cannot begin until mitosis is complete. Second, the same cyclin dependent

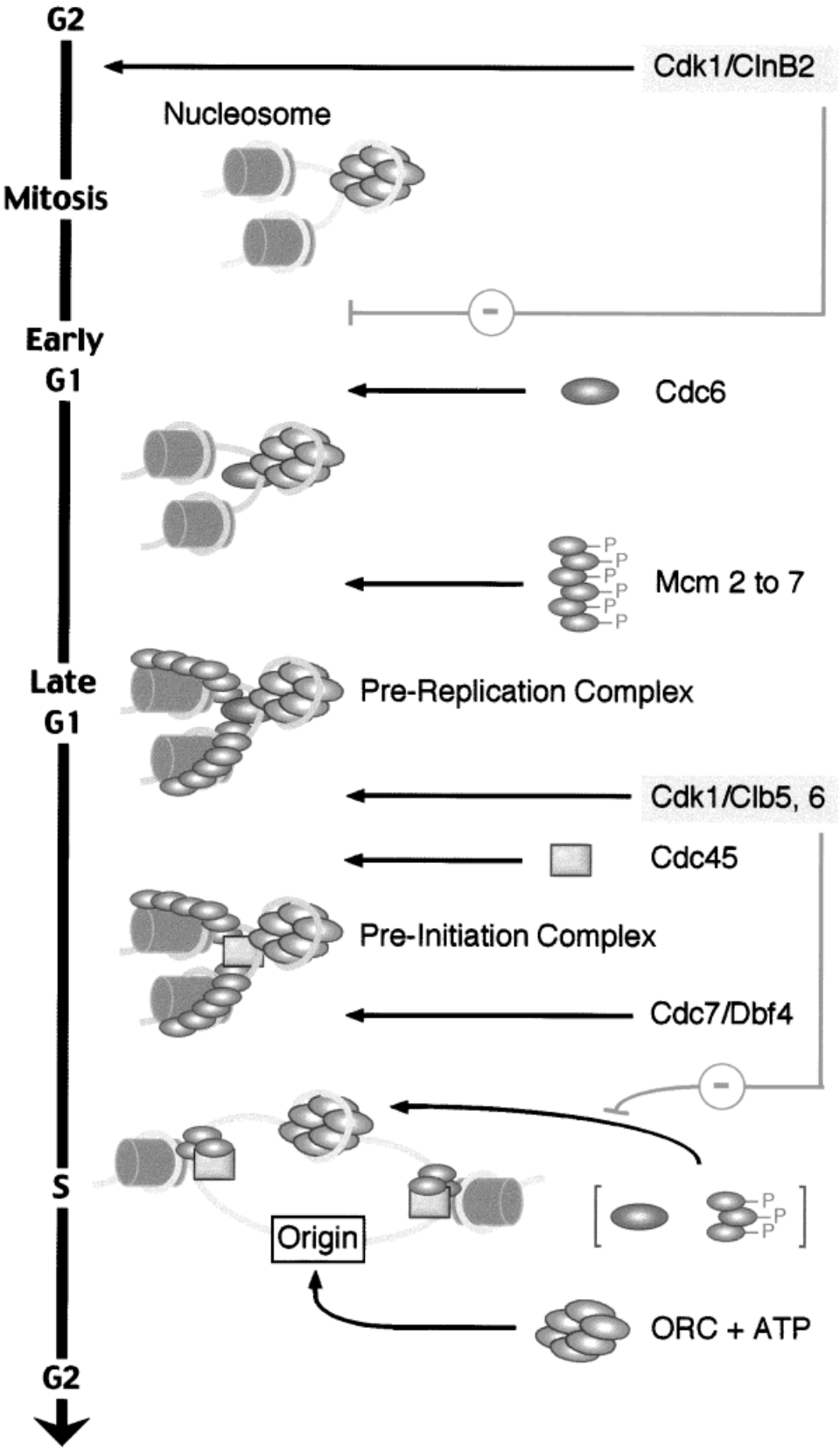

Fig. 1. Assembly and activation of pre-replication complexes in the budding yeast *S. cerevisiae*. See text for discussion. **Color plate on page 313.**

protein kinase activity required to activate pre-replication complexes also prevents reassociation of Mcm proteins with chromatin during S-phase. Third, at the onset of S-phase, and prior to DNA replication, Cdc6 is lost from chromosomes and does not reappear until early-G1 phase, thus insuring that pre-RCs cannot be reassembled until the next G1-phase. Finally, yeast Mcm proteins are released into the cytoplasm during S-phase, and thus not available for pre-RC assembly. In the metazoa, Mcm proteins remain within the nucleus during S-phase, but RLF-B is absent and new RLF-B cannot reenter nuclei unless they are permeabilized (e.g., during mitosis).

Replication Origins

Simple genomes. The essential features of eukaryotic replication origins are found in animal viruses that replicate in mammalian nuclei [reviewed in DePamphilis, 1993a, 1996, 1998] for which the paradigm is simian virus 40 (SV40; Fig. 2). These origins generally consist of a core component that contains an origin recognition element (ORE) to bind a specific origin recognition protein (e.g., T-antigen), an easily unwound DNA sequence [DNA unwinding element (DUE)] that is the initial target for a DNA helicase (e.g., T-antigen hexamer), and an A:T-rich element with thymines on one strand and adenines on the other. A:T-elements are either part of the origin recognition element or they facilitate DNA unwinding. The origin of bidirectional DNA replication (OBR) is the site where the two replication forks are born, and is identified by the transition from continuous to discontinuous DNA synthesis that occurs within a few base pairs on each template. Most origins contain auxiliary components that are not required, but that facilitate replication when the ratio of initiation proteins to DNA is low. Auxiliary components bind specific transcription factors (e.g., Sp1 and T-antigen dimer) that can facilitate either binding of the origin recognition complex (e.g., papillomavirus) or DNA unwinding (e.g., SV40), or that alleviate chromatin mediated repression of origin activity (e.g., polyomavirus) [reviewed in DePamphilis, 1993b]. Viral origins, like yeast origins, exhibit ARS activity. However, viral origins differ from yeast origins in that viral origins initiate replication many times per cell cycle, exhibit a rigid

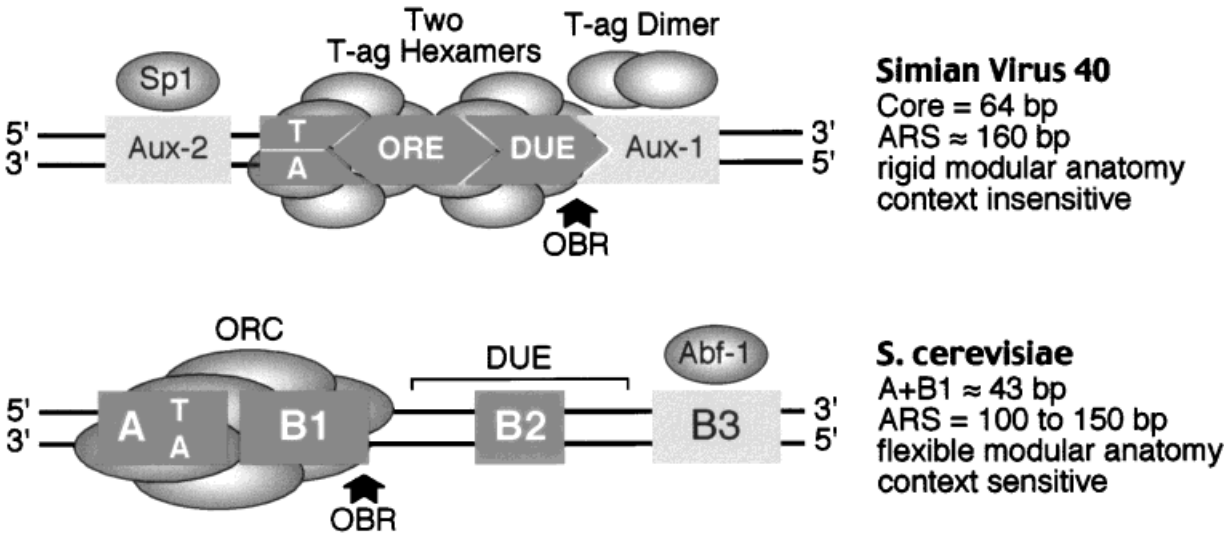

Fig. 2. Replication origins in simian virus 40 (SV40) and *Saccharomyces cerevisiae*. Red indicates sequence elements that are required under all conditions (core components), while yellow indicates sequence elements (transcription factor binding sites) that facilitate replication under some conditions (auxiliary components). Components include an origin recognition element (ORE) that is required for T-antigen binding, a DNA unwinding element (DUE) where DNA unwinding begins, and an A/T-rich element containing adenines on one strand and thymines on the other. The origin of bidirectional replication (OBR) is the site from which replication forks move out in opposite directions. Auxiliary elements binds a T-antigen dimer (aux-1) and transcription factor Sp1 (aux-2). The spacing and orientation of these elements are critical. The budding yeast *S. cerevisiae* origins consist of two core elements (A and B1) that constitute the binding site for the six protein origin recognition complex (ORC), and a DUE that generally contains a genetically defined B2 element. Some origins contain an auxiliary element (B3) that binds transcription factor Abf-1. Each element is interchangeable with homologous elements from other *S. cerevisiae* origins. **Color plate on page 314.**

modular anatomy in which the sequence elements require a specific spacing and orientation with respect to one another, and function independently of their DNA context.

The most well-characterized eukaryotic replication origins are found in the budding yeast, *S. cerevisiae* [reviewed in Marahrens and Stillman, 1996] (Fig. 2). Replication begins at specific DNA sequences consisting of 100 to 150 bp that exhibit ARS activity. The core component consists of an ORC binding site composed of elements A (an A:T-rich element corresponding to the 11 bp ARS consensus sequence) and B1, as well as a DUE (~50 to 100 bp) that usually encompasses a genetically identifiable element, B2. The B2 element exhibits some of the characteristics of a DUE, and a DUE that contains a B2 element can be replaced by a DUE from an origin that does not [Lin and Kowalski, 1997]. Some origins also contain a binding site for transcription factor Abf-1 (~22 bp). The OBR (~18 bp) overlaps the B1 element, and therefore lies adjacent to the ORC binding site and the DUE [Bielinsky and Gerbi, 1998]. Both the size of this OBR and its location relative to origin recognition proteins is remarkably similar to the situation in SV40 and polyomavirus.

Despite the fact that only ~15% of the sequences in *S. cerevisiae* origins are shared in common (parts of elements A and B1), they exhibit a flexible modular anatomy in which homologous elements from different origins are interchangeable. Yeast origins vary considerably in the frequency at which they are activated during cell proliferation and in the temporal order they are activated during S-phase. Sequence context is critical in determining the temporal order of origin activation [Friedman et al., 1996], and this order is determined between mitosis and early G1-phase through the action of Cdk1 [Donaldson et al., 1998]. Either Cdk1/Clb5 or Cdk1/Clb6 can activate early origins, but only Cdk1/Clb5 can activate late origins.

Metazoan genomes. Given the remarkable conservation in eukaryotes of the proteins used to initiate replication, and the fundamental need for replication origins, one would expect initiation of DNA replication in metazoa, like initiation in unicellular organisms, to occur at specific DNA sequences. The conserved ATP binding motif in ORC1 implies that metazoan ORC will exhibit an ATP-dependent, sequence specific affinity for specific DNA sites. In differentiated cells of flies, frogs, and mammals, it is now clear that DNA replication begins at specific genomic loci [reviewed in DePamphilis, 1996, in press]. For example, a 200 kb region at the human β-globin gene [Aladjem et al., 1995] and a 500 kb region at the mouse Igh gene [Michaelson et al., 1997] are both replicated from a single initiation locus. What has been difficult to nail down is the size of initiation loci, and the parameters that determine where replication begins.

While all the data are consistent with bidirectional replication involving classical replication bubbles and forks, a complex and sometimes contradictory view of replication origins has emerged [reviewed in DePamphilis, 1993a, 1996, in press]. When cellular DNA is first fractionated by two-D gel electrophoresis in order to identify replication bubble and fork structures, and then hybridized with sequence specific radio-labeled probes in order to identify their genomic location ("two-D gel" mapping protocols), initiation events often appear to be distributed throughout large (up to 55 kb) DNA regions ("initiation zones") with no clear preference for one site over another. In contrast, when newly synthesized mammalian DNA is labeled with nucleotide precursors during its biosynthesis and then its origin mapped by various strategies ("nascent strand analysis" mapping protocols), most initiation events (85% to >95%) appear to occur at specific sites of 0.5 to 2 kb ["origins of bidirectional replication" (OBRs)], analogous to those found in single cell eukaryotes such as yeast, *Tetrahymena* and *Physarum*. Thus, while some mammalian replication origins consist of a single high frequency initiation site (1.7 kb) that can be detected both by two-D gel and nascent DNA strand analyses [Toledo et al., 1998], other mammalian replication origins appear to contain multiple replication origins that give rise to the appearance of an initiation zone. The 55 kb initiation zone between the DHFR and 2BE2121 genes in hamster cells contains at least three primary initiation sites (ori-β, β', and γ) within a 28 kb locus that could account for most of the initiation events in this region, because the intensity of replication bubble structures in the DHFR gene initiation zone appears to be greatest within a 12 kb region containing ori-β and β' [Kobayashi et al., 1998]. Similarly, the fraction of replication bubbles observed in the 31 kb human rRNA intergenic region was greatest in the same re-

gion that contained a primary initiation site, a locus upstream of the rRNA gene promoter. This locus is >10-fold more active than distal sites, and it appears in rRNA genes of all eukaryotes examined so far [Sanchez et al., 1998].

The inability of two-D gel analyses to detect the primary initiation sites in metazoan chromosomes appears to result primarily from an absence of data quantification, a strong dependence on selective enrichment for replicating DNA, and an inability to resolve closely spaced origins [Kobayashi et al., 1998]. A relevant paradigm is provided by yeast. Yeast replication origins generally consist of a single ARS element, but two-D gel analyses of the ura4 region in *S. pombe* initially suggested that initiation events were distributed throughout a ~5 kb initiation zone. However, when combined with genetic analyses, it became clear that the replication bubbles were coincident with three separate ARS elements. Thus, what at first appeared to be a continuous initiation zone is actually a cluster of three replication origins. This phenomenon can be understood from observations that origins in close proximity (~6 kb) function at reduced efficiency, and only one of the origins is activated in each cell during each S-phase. Dominance of one origin over another most likely results from the fact that origin activity is strongly affected by its surrounding sequences ("sequence context").

Replicators in metazoan genomes. Although DNA sequence is clearly a determinant of origin activity in yeast, its role in metazoan cells has been more elusive. The simple fact that mammalian origins map to specific sites that replicate at specific times during S-phase demonstrates that origins of replication are inherited from one cell division to the next. Moreover, the same OBR identified in cells containing two copies of the origin region per diploid genome are also identified in cells containing many copies, and the same replication origin is used by different cell lines derived from the same species. Therefore, OBRs are specified by DNA sequences.

Direct evidence for "replicator" sequences comes from several reports that sequences where replication occurs in mammalian chromosomes can also confer ARS activity on plasmids either in mammalian cells or cell extracts [DePamphilis, 1996]. However, not all mammalian replication origins have yielded ARS activity and the activities that have been reported are frequently modest relative to experimental background. A second approach has been genetic analysis of origin activity within the context of real chromosomes. For example, in *Drosophila*, 1.2 kb fragment containing the major initiation site for chorion gene amplification is sufficient for high level gene amplification [Lu and Tower, 1997, personal communication]. Similarly, in mammalian cells [Aladjem et al., 1998], DNA fragments as small as 4 kb containing the human β-globin OBR exhibit origin activity when transferred to ectopic chromosomal locations, while fragments that lack origin activity in situ exhibit little if any ectopic activity. Deletions as small as 2.7 kb at the human β-globin OBR eliminates the only initiation site detected within a 200 kb region. The activity of this origin also is regulated from a Locus Control Region located about 50 kb distal to the OBR. Locus control regions affect the accessibility of initiation sites for transcription and replication to initiation proteins. Thus, metazoan origins of replication are determined by specific sequences encompassed within 1 to 4 kb, comparable in size to those identified in *S. pombe* and *Tetrahymena*. These sequences appear to direct assembly of replication complexes, because cell cycle dependent changes in a footprint at the lamin B2 origin in human cells are consistent with assembly of a pre-replication complex during G1-phase that is modified to a post-replicative state during S-phase and then lost completely from DNA during mitosis [Abdurashidova et al., 1998].

Parameters that determine initiation sites. Initiation of DNA replication in metazoan chromosomes follows the Jesuit dictum that "many are called, but few are chosen" [reviewed in DePamphilis, 1993a, 1996, in press] (Fig. 3). DNA contains many potential initiation sites, because the rapidly cleaving embryos of frogs, flies, and sea urchin can initiate replication throughout their genomes, apparently independent of DNA sequence. During animal development, specific initiation sites first appear in *Xenopus* [Hyrien et al., 1995] and *Drosophila* [Sasaki et al., 1998] after zygotic gene expression begins, suggesting that changes occur during animal development that result in selection of specific initiation sites. In mammals, auxiliary origin sequences such as enhancers are not required to activate replication origins until the two- to four-cell stage when zygotic gene expression begins and

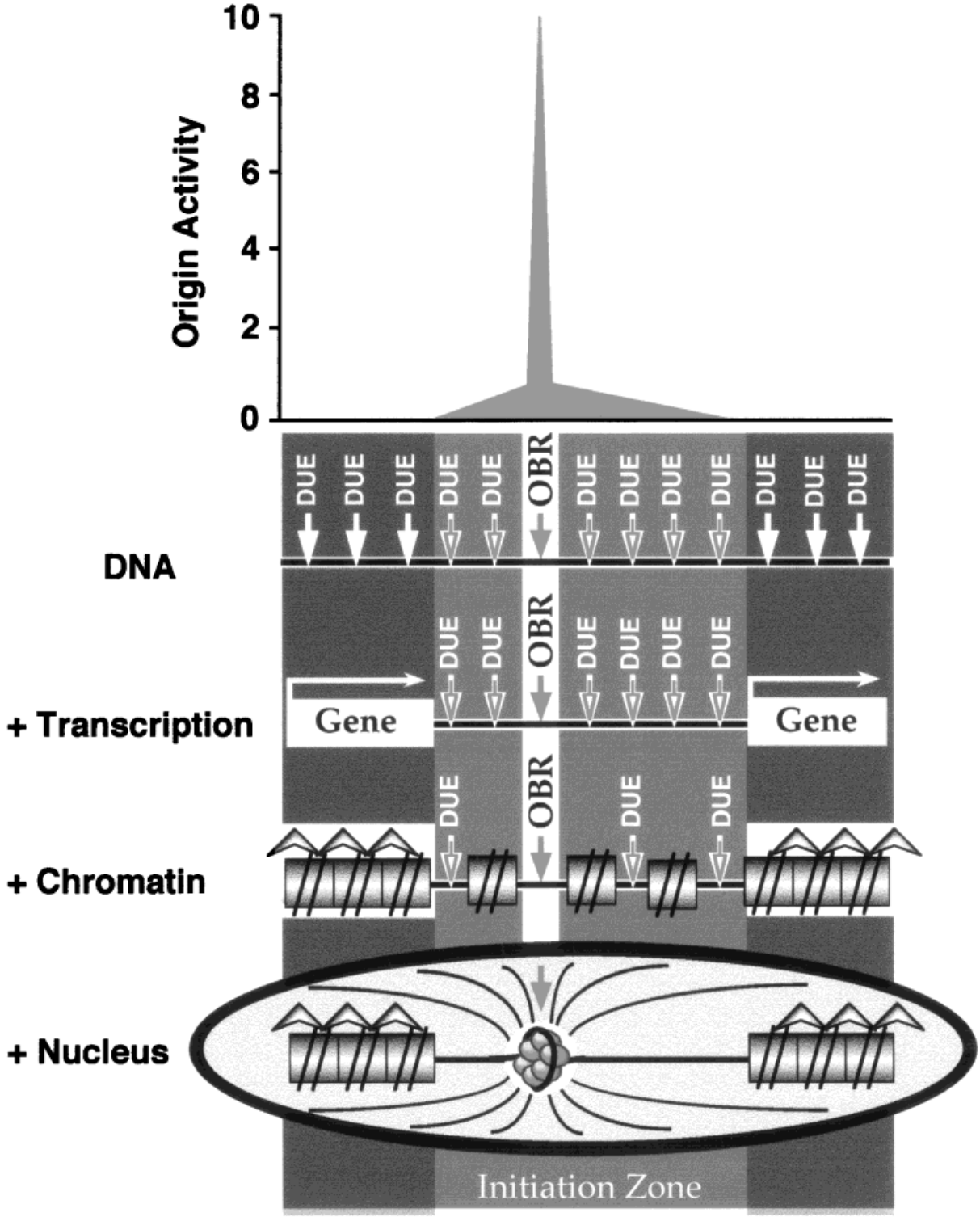

Fig. 3. The "Jesuit Model" for site specific initiation in the chromosomes of metazoan cells. While DNA contains many potential replication origins that may correspond to easily unwound DNA sequences called DNA unwinding elements (DUE), gene transcription, chromatin structure, and nuclear organization may represses initiation at some sites while selectively activating initiation at other sites. Thus, one or more strong origins of bidirectional replication (OBR) are found among non-specific initiation sites that together comprise an initiation zone. **Color plate on page 315.**

changes in chromatin structure repress origin and promoter activity [Majumder and De-Pamphilis, 1995]. Thus, in embryos, a high ratio of initiation factors to DNA together with a more accessible chromatin structure could allow initiation to occur at many sites along the genome. As development progresses, changes in gene transcription, chromatin structure, and nuclear organization could prevent access of initiation proteins to some DNA sites, while making other sites either more accessible or easier to unwind. This could create initiation zones consisting of one or more high frequency initiation sites (OBRs) and perhaps several low frequency initiation sites that escape detection by bio-labeling methods but not by two-D gel methods. The low frequency sites may represent accessible DUEs, while the high frequency sites may represent strong ORC binding sites adjacent to a DUE. As the ratio of initiation factors to DNA is reduced, the frequency of initiation is reduced [Walter and Newport, 1997], and those sites with the greatest affinity for ORC and the greatest accessibility to initiation factors will be selected. This process provides animals with the flexibility to change the rate of cell division by changing the number of active origins, while at the same time selecting initiation sites that do not interfere with gene expression as cells undergo differentiation.

Chromatin structure can inhibit both promoter and origin activity by blocking access of transcription factors to specific DNA sites. Histone H1 can reduce the frequency of initiation in *Xenopus* egg extract by limiting the assembly of prereplication complexes on sperm chromatin [Lu et al., 1998], and changes in chromosome structure that occur during a cell cycle can create new initiation sites while eliminating others, presumably by affecting their accessibility to initiation factors [Lawlis et al., 1996]. DNA elements that organize transcriptionally active domains can also protect replication origins from chromatin-mediated repression [Lu and Tower, 1997]. Thus, weaker origins could be more accessible to higher concentrations of initiation proteins in rapidly cleaving embryos, and less accessible to lower concentrations of initiation proteins in adult tissues.

Nuclear structure plays one or more critical roles in regulating eukaryotic DNA replication. One function is to concentrate replication factors required to initiate replication (e.g., Cdk2/Cyclin A, E) and exclude those that would interfere with replication (e.g., Cdk1/Cyclin B) [Walter et al., 1998]. Preventing RLF-B entry into the nucleus until mitosis would be another example [Rowles and Blow, 1997]. Another function is to establish specific initiation sites along the genome. Newly replicated DNA is preferentially associated with nuclear structure to form replication foci throughout the nucleus, and formation of these foci as well as DNA replication depends on assembly of a nuclear lamina [Ellis et al., 1997; Spann et al., 1997]. Site specific initiation of DNA replication in either frog or yeast extracts has only been observed when intact nuclei isolated from cells in late G1-phase are used as the substrate [Pasero et al., 1997; Wu et al., 1997; Wu and Gilbert, 1997]. Nuclei from early G1-phase can also initiate DNA replication under these conditions, but initiation occurs "randomly" throughout the genome. Therefore, specific initiation sites are established during G1-phase of each cell division cycle.

What Constitutes a Metazoan Replication Origin?

The results of origin mapping and genetic analyses on S-phase origins in mammals and amplification origins in flies suggests that replication origins in metazoan chromosomes are defined by 0.5 to 2 kb of DNA, exclusive of locus control regions. Based on analysis of replication origins in simpler genomes, metazoan origins will likely contain at least one ORC binding site in close proximity to a DUE, and one or more A:T-rich elements. However, the specificity of ORC binding to metazoan origins may be less than observed in *S. cerevisiae*. The larger size of *S. pombe* ARS elements would allow for multiple weak ORC binding sites to act in concert to produce a strong origin, just as multiple copies of the *S. cerevisiae* A-element alone can produce strong origin activity [Marahrens and Stillman, 1996]. The OBR that can be mapped to specific locations in mammalian chromosomes should lie between the ORC binding site and the DUE. The OBR in simple origins spans from 2 to 20 bp, but if several ORC-DUE motifs lie in close proximity, then the transition from discontinuous to continuous DNA synthesis will span the distance between the two most outlying OBRs, and interference between closely spaced replication origins will make it more difficult to resolve individual OBRs. Auxiliary sequences may regulate origin activity by affecting either its accessibility to initiation factors

or the ability of its DNA to unwind. Thus, while weak ORC binding sites may be adequate when high concentrations of initiation proteins are present (e.g., embryos undergoing rapid cleavage events), additional sequences such as transcription factor binding sites or methylated clusters of CpG dinucleotides may facilitate ORC binding or DNA unwinding at later stages in development.

The dependence of origin activity in metazoan chromosomes on chromatin and nuclear structure is likely related to the facts that the metazoan ORC appears to be displaced from chromatin during mitosis and reassociates with chromatin during G1-phase and that pre-RCs are not detected at specific initiation sites until late G1-phase. Thus, the cell appears to reprogram its replication origins during each cell division cycle. This would allow an animal to change the length of S-phase during development, to change the temporal order in which its genome is replicated in response to cell differentiation, and to change the locations of active replication origins in response to changes in the locations of active genes.

Over the past decade, our understanding of metazoan replication origins and their potential roles in biology have progressed from enigmatic to complex to resolvable. The next decade will witness a complete characterization of replication origins, the proteins that interact with them, and the construction of artificial metazoan chromosomes.

REFERENCES

Abdurashidova G, Riva S, Biamonti G, Giacca M, Falaschi A (1998): Cell cycle modulation of protein-DNA interactions at a human replication origin. EMBO J 17:2961–2969.

Aladjem MI, Groudine M, Brody LL, Dieken ES, Fournier RE, Wahl GM, Epner EM (1995): Participation of the human beta-globin locus control region in initiation of DNA replication. Science 270:815–819.

Aladjem MI, Rodewald LW, Kolman JL, Wahl GM (1998): Genetic dissection of a mammalian replicator in the human beta-globin locus. Science 281:1005–1009.

Bielinsky AK, Gerbi SA (1998): Discrete start sites for DNA synthesis in the yeast ARS1 origin. Science 279:95–98.

Coleman TR, Carpenter PB, Dunphy WG (1996): The Xenopus Cdc6 protein is essential for the initiation of a single round of DNA replication in cell-free extracts. Cell 87: 53–63.

DePamphilis ML (1993a): Eukaryotic DNA replication: anatomy of an origin. Annu Rev Biochem 62:29–63.

DePamphilis ML (1993b): How transcription factors regulate origins of DNA replication in eukaryotic cells. Trends Cell Biol 3:161–167.

DePamphilis ML (1996): Origins of DNA replication. In: DePamphilis ML (ed) DNA replication in eukaryotic cells. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 45–86.

DePamphilis ML. in press. DNA replication in eukaryotic cells (1996–1998): In: DePamphilis ML (ed.) Concepts in eukaryotic DNA replication. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

DePamphilis ML. in press. Replication origins in metazoan chromosomes: Fact or fiction? BioEssays.

Donaldson AD, Raghuraman MK, Friedman KL, Cross FR, Brewer BJ, Fangman WL (1998): CLB5-Dependent activation of late replication origins in *S. cerevisiae*. Mol Cell 2:173–182.

Dutta A, Bell SP (1997): Initiation of DNA replication in eukaryotic cells. Annu Rev Cell Dev Biol 13:293–332.

Ellis DJ, Jenkins H, Whitfield WG, Hutchison CJ (1997): GST-lamin fusion proteins act as dominant negative mutants in Xenopus egg extract and reveal the function of the lamina in DNA replication. J Cell Sci 110:2507–2518.

Friedman KL, Diller JD, Ferguson BM, Nyland SV, Brewer BJ, Fangman WL (1996): Multiple determinants controlling activation of yeast replication origins late in S phase. Genes Dev 10:1595–1607.

Hua XH, Newport J (1998): Identification of a preinitiation step in DNA replication that is independent of origin recognition complex and cdc6, but dependent on cdk2. J Cell Biol 140:271–281.

Hyrien O, Maric C, Mechali M (1995): Transition in specification of embryonic metazoan DNA replication origins. Science 270:994–997.

Kobayashi T, Rein T, DePamphilis ML (1998): Identification of primary initiation sites for DNA replication in the hamster dihydrofolate reductase gene initiation zone. Mol Cell Biol 18:3266–3277.

Lawlis SJ, Keezer SM, Wu J-R, Gilbert DM (1996): Chromosome architecture can dictate site-specific initiation of DNA replication in *Xenopus* egg extracts. J Cell Biol 135:1207–1218.

Lin S, Kowalski D (1997): Functional equivalency and diversity of cis-acting elements among yeast replication origins. Mol Cell Biol 17:5473–5484.

Lu L, Tower J (1997): A transcriptional insulator element, the su(Hw) binding site, protects a chromosomal DNA replication origin from position effects. Mol Cell Biol 17:2202–2206.

Lu ZH, Sittman DB, Romanowski P, Leno GH (1998): Histone H1 reduces the frequency of initiation in *Xenopus* egg extract by limiting the assembly of prereplication complexes on sperm chromatin. Mol Biol Cell 9:1163–1176.

Majumder, S, DePamphilis, ML (1995): A unique role for enhancers is revealed during early mouse development. BioEssays 17:879–889.

Marahrens Y, Stillman B (1996): Initiation of DNA replication in the yeast *Saccharomyces cerevisiae*. In: Blow JJ, (ed) Eukaryotic DNA replication. IRL Press, New York. pp 66–95.

Michaelson JS, Ermakova O, Birshtein BK, Ashouian N, Chevillard C, Riblet R, Schildkraut CL (1997): Regulation of the replication of the murine immunoglobulin heavy chain gene locus: Evaluation of the role of the 3' regulatory region. Mol Cell Biol 17:6167–6174.

Newlon CS (1997): Putting it all together: building a prereplicative complex. Cell 91:717–720.

Pasero P, Braguglia D, Gasser SM (1997): ORC-dependent and origin-specific initiation of DNA replication at defined foci in isolated yeast nuclei. Genes Dev 11:1504–1518.

Romanowski P, Madine MA, Rowles A, Blow JJ, Laskey RA (1996): The *Xenopus* origin recognition complex is essential for DNA replication and MCM binding to chromatin. Curr Biol 6:1416–1425.

Rowles A, Blow JJ (1997): Chromatin proteins involved in the initiation of DNA replication. Curr Opin Genet Dev 7:152–157.

Sanchez JA, Kim SM, Huberman JA (1998): Ribosomal DNA replication in the fission yeast, *Schizosaccharomyces pombe*. Exp Cell Res 238:220–230.

Sasaki T, Sawado T, Yamaguchi M, Shinomiya T. in press. Specification of initiation regons of DNA replication during embryogenesis in the 65 kb DNApola-dE2F locus of *Drosophila melanogaster*. Mol Cell Biol

Spann TP, Moir RD, Goldman AE, Stick R, Goldman RD (1997): Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. J Cell Biol 136:1201–1212.

Toledo F, Baron B, Fernandez MA, Lachages AM, Mayau V, Buttin G, Debatisse M (1998): oriGNAI3: A narrow zone of preferential replication initiation in mammalian cells identified by 2D gel and competitive PCR replicon mapping techniques. Nucl Acids Res 26:2313–2321.

Walter J, Newport JW (1997): Regulation of replicon size in Xenopus egg extracts. Science 275:993–995.

Walter J, Sun L, Newport J (1998): Regulated chromosomal DNA replication in the absence of a nucleus. Mol Cell 1:519–529.

Wu J-R, Yu G, Gilbert DM (1997): Origin-specific initiation of mammalian nuclear DNA replication in a *Xenopus* cell-free system. Methods 13:313–324.

Wu JR, Gilbert DM (1997): The replication origin decision point is a mitogen-independent, 2- aminopurine-sensitive, G1-phase event that precedes restriction point control. Mol Cell Biol 17:4312–4321.

Zou L, Stillman B (1998): Formation of a preinitiation complex by S-phase cyclin CDK-dependent loading of Cdc45p onto chromatin. Science 280:593–596.