Journal of Cellular Biochemistry Supplements 32/33:1–14 (1999)

Eukaryotic DNA Replication

Maria Zannis-Hadjopoulos* and Gerald B. Price

McGill Cancer Centre, McGill University, Montreal, Quebec H3G 1Y6, Canada

Abstract One of the fundamental characteristics of life is the ability of an entity to reproduce itself, which stems from the ability of the DNA molecule to replicate itself. The initiation step of DNA replication, where control over the timing and frequency of replication is exerted, is poorly understood in eukaryotes in general, and in mammalian cells in particular. The *cis*-acting DNA element defining the position and providing control over initiation is the replication origin. The activation of replication origins seems to be dependent on the presence of both a particular sequence and of structural determinants. In the past few years, the development of new methods for identification and mapping of origins of DNA replication has allowed some understanding of the fundamental elements that control the replication process. This review summarizes some of the major findings of this century, regarding the mechanism of DNA replication, emphasizing what is known about the replication of mammalian DNA. J. Cell. Biochem. Suppls. 32/33:1–14, 1999. © 1999 Wiley-Liss, Inc.

Key words: eukaryotic; DNA replication; origins; regulation

DNA REPLICATION

Among the landmark discoveries of the twentieth century was the demonstration by Emmons and Hollander [1939] that DNA is the critical component essential for cell survival, by showing that the ultraviolet (UV) activation spectra for cell survival was the same as the UV absorption spectra of DNA and RNA. Later, another landmark discovery was the description of the structure of DNA by Watson and Crick [1953]. This structure, a right-handed double helix (or B-DNA helix), enables the DNA to replicate itself. Numerous laboratories around the world have devoted their research activities to deciphering the mechanism of replication of this fascinating molecule that constitutes the very basis of life. This review summarizes the major findings regarding the mechanism of mammalian DNA replication.

MODELS OF DNA REPLICATION

According to the bacterial replicon model [Jacob et al., 1963], a *cis*-acting replicator element is bound by a *trans*-acting initiator protein (IP), permitting the initiation of replication. The replicon was defined as a genetic element that replicates as a unit, with a unique origin of replication (replicator) serving as the target of a positive-acting IP. Eukaryotic chromosomes, because of their increased complexity, initiate replication at multiple sites, estimated at 10^4–10^6 [Hand, 1978; Martin, 1981]. As in prokaryotes, control over the timing and frequency of initiation are exerted at these sites [Hand, 1978; Kornberg and Baker, 1992; Gavin et al., 1995]. Eukaryotic DNA replication occurs in a spatial and temporal order from replication units (replicons).

Most of our current knowledge about DNA replication has come from the study of model organisms, both prokaryotic (bacteria, bacteriophage, and plasmids) and eukaryotic (virus, yeast, protists, and mammalian cultured cells). Several general principles have emerged from work dissecting the replication process in these and other model systems [Kornberg and Baker, 1992].

GENERAL RULES OF REPLICATION

The intensive research activity aimed at understanding the mechanism of DNA replication resulted in the development of new technical strategies, allowing researchers to take a closer look at replicating DNA and dissect its various components. The most notable of the early techniques that were employed for this purpose and

*Correspondence to: Maria Zannis-Hadjopoulos, McGill Cancer Centre, 3655 Drummond Street, Montreal, Quebec H3G 1Y6, Canada. E-mail: mzannis@med.mcgill.ca

Received 29 September 1999; Accepted 30 September 1999

© 1999 Wiley-Liss, Inc.

that provided a great deal of information about the mechanism of DNA replication is DNA fiber autoradiography, first employed in prokaryotes [Cairns, 1966] and then in mammalian cells [Huberman and Riggs, 1968]. A major outcome of the early studies was the realization that DNA replication proceeds according to universal basic rules, from the simplest genomes to the most complex [reviewed in Kornberg and Baker, 1992].

DNA replication is semiconservative. The overall rate of replication is determined by two factors, the frequency of initiation and the rate of elongation or fork progression. The latter is the better characterized of the two processes, due mostly to the development of cell-free (in vitro) replication systems, such as those based on plasmids containing the *Escherichia coli* chromosomal origin of DNA replication, *oriC* [Fuller et al., 1981], or the SV40 viral replication origin [Li and Kelly, 1984]. The basic enzymatic activities required for DNA replication are the *helicase*, which unwinds the double-stranded template and defines the growing point of the replication fork; topoisomerase, which relieves torsional strain that builds up ahead of the growing fork; SSB (single-stranded binding proteins), which stabilize the unwound, single-stranded template DNA; and DNA polymerase, which carries out the polymerization step, adding new nucleotides to the growing nascent strand in the 5′ to 3′ direction, that are complementary to those of the template strand. New bases can only be added by polymerases to free 3′-OH ends of the growing chain (in part, to facilitate associated exonuclease proofreading functions that ensure the fidelity of the process). This requirement for 5′-3′ replication polarity, coupled with the fact that the two strands of the double helix are of opposite polarity and that replication is bidirectional, necessitates a different mode of replication for each of the two unwound template strands. The *leading strand*, which is unwound in the 3′-5′ direction, can act directly as a template for 5′-3′ elongation of the nascent strand, in the same direction as growing fork progression. The *lagging strand*, on the other hand, due to its antiparallel direction, must be regularly reinitiated by *primase* activity, which lays down RNA primers (initiator RNA) for polymerase extension, as new tracts of template strand DNA are unwound. Extension of these RNA primers by DNA polymerase produces Okazaki fragments, which are variable in size, but generally 2,000–3,000 bp long in prokaryotes and 100–300 bp long in eukaryotes. Okazaki fragments are ligated together by the action of *DNA ligase*, after the initiator RNA has been replaced with DNA [reviewed in Kornberg and Baker, 1992].

MAMMALIAN DNA REPLICATION

For the purpose of replication, the eukaryotic genome is divided into multiple, tandemly arranged, replication units (replicons) [reviewed by Hand, 1978]. Each unit contains a centrally placed origin of replication and is replicated by two diverging replication forks. DNA fiber autoradiography experiments indicated this arrangement and suggested that replication initiates at distinct sites and then proceeds bidirectionally outward [Cairns, 1966; Huberman and Riggs, 1968]. The experimental design permitted measurements of the rate of replication fork movement, replicon size, estimates of the replication initiation frequency, and timing of initiation events on adjacent replicons. Such measurements suggested that in a typical mammalian nucleus there were approximately 10^4–10^5 initiations, serving replicons whose size varied from 50 to 250 kb [Hand and Tamm, 1974; reviewed in Edenberg and Huberman, 1975]. Replication was semidiscontinuous, i.e., continuous on the leading (5′-3′) strand and discontinuous on the lagging (3′-5′), on which Okazaki fragments of approximately 100–300 bp were synthesized [Hay et al., 1984; Hendrickson et al., 1987]. Termination of genomic replication is thought to occur when two adjacent replication forks meet and fuse. Pulse-labeling of synchronized mammalian cells and DNA fiber autoradiography provided evidence of site-specific initiation [Amaldi et al., 1973].

Groups of adjacent replication units are activated coordinately in clusters [Huberman and Riggs, 1968]. Estimates of the size of clusters vary from 23 to 100 replication units, depending on the method of measurement [reviewed in Hand, 1978]. The replicating clusters are associated with the nuclear matrix. Clusters of replication origins are activated at different times throughout S phase [Hand, 1978; Taylor, 1960], and their respective replicons are replicated in a defined spatial and temporal order [Spector, 1992 and references therein]. Origins activated simultaneously might either share a common DNA sequence [Zannis-Hadjopoulos et al., 1984], or secondary structure [Stinchcomb et

al., 1979; Struhl et al., 1979: Small et al., 1982; Zannis-Hadjopoulos et al., 1984, 1988; Schroth and Ho, 1995] recognized by cognate regulatory proteins, or they may occupy a nuclear position favorable to activation at a given time [Edenberg and Huberman, 1975; DePamphilis, 1993b, and references therein]. Under normal growth conditions, each origin initiates replication only once per cell cycle. Immunofluorescent staining of cells showed that replication is confined to a few hundred discrete loci or replication centers [Nakamura et al., 1986; Laskey et al., 1989; Hozak et al., 1993]. Confocal analysis of human (HeLa) X chromosomes, visualized using fluorescence in situ hybridization (FISH) with a specific painting probe, showed that in S phase the chromosomes occupy distinct nuclear territories and their apparent size does not differ from that in non-S phase cells [Solovjeva et al., 1998].

There are more potential initiation sites than those activated during a normal S phase. Thus, more origins are activated during embryonic development [Kriegstein and Hogness, 1974], following a fluorodeoxyuridine block [Taylor, 1977] or infection by polyoma [Cheevers et al., 1972] or SV40 [Martin and Oppenheim, 1977]. Specificity of origin selection may also vary during embryogenesis or tissue development [Carminati and Orr-Weaver, 1996].

TEMPORAL ORDER OF DNA REPLICATION

The principles that govern the spatial and temporal organization of cellular replicons are not fully understood. The eukaryotic nucleus provides a confined and highly organized system of structural and functional activities, in which DNA is replicated. The replication of DNA follows a dynamic order with specific regions of DNA replicating at defined times and in defined locations of the nucleus during S-phase [Spector, 1992 and references therein].

The replication of mammalian DNA during S phase is a highly ordered and regulated process. Chromosomal subsections replicate at defined times [Latt, 1974], and the DNA replicated at a particular time during S phase is likely to be replicated at the same time in subsequent S phases [Mueller and Kajiwara, 1966; Adegoke and Taylor, 1977; Taylor, 1977]. Different subclasses of DNA, such as satellites are replicated at defined times during S phase [May and Bello, 1974; Tapiero et al., 1974]. For example, DNA with a high content of A and T bases (e.g., α-satellite DNA) tends to be late replicating [Bostock and Prescott, 1971a,b; Comings, 1972]. However, the order of replication of the large chromosomal subsections is not fixed, and cell and tissue variation in the late-replicating regions of the chromosome has been reported [Willard and Latt, 1976; Willard, 1977]. Synthesis of late-replicating DNA depends on the structural integrity and completion of synthesis of early-replicating DNA [Hamlin and Pardee, 1978]. The temporal control of DNA replication became apparent from earlier work involving cell fusion experiments [Rao and Johnson, 1970; Yanishevsky and Prescott, 1978]. Thus, when a cell in late S phase was fused to a cell in G1 phase, the G1-derived nucleus was induced to initiate DNA replication [Yanishevsky and Prescott, 1978], whereas cells in G2 phase could not induce G1 nuclei into DNA synthesis, nor could G2 nuclei initiate DNA replication when fused to S-phase cells. The cell fusion experiments provided strong evidence for a stringent program for DNA synthesis and indicated that entry of cells into S phase requires a *trans*-acting factor(s), as only S phase cells can activate initiation of DNA replication in competent (G1) cells. They also revealed that only G1 cell DNA is competent to initiate replication, and that G2 nuclei do not re-replicate DNA until they pass through mitosis [Stillman, 1996]. More recent experiments, using the *Xenopus* egg extract replication system and the transient permeabilization of the nuclear membrane, support the cell fusion data [Blow and Laskey, 1988; Blow et al., 1989; Blow, 1993; Laskey et al., 1989] and led to the proposal of the licensing factor model [Blow and Laskey, 1988].

Two parameters that establish the order of replication are the proximity of the replication site to certain DNA sequences such as, centromeres, telomeres [Ten Hagen et al., 1990; Ferguson and Fangman, 1992], or unusual DNA sequences that may cause alterations in chromatin structure [Weaver and DePamphilis, 1984; Baran et al., 1991; Thoma, 1992], and transcriptional activity [Michaelson et al., 1997]. Most, but not all, actively transcribed genes are replicated early in S phase, whereas quiescent genes are replicated later in S phase [Goldman et al., 1984; Gilbert, 1986; Schmidt and Migeon, 1990; Benard et al., 1992]. Recent studies suggest a direct role for transcription factors in activating eukaryotic origins of DNA

replication [reviewed in DePamphilis, 1993a, 1999]. A putative origin of DNA replication, p82, was identified in the γ-aminobutyric acid (GABA) receptor subunit β3 and α5 gene cluster on chromosome 15q11q13, a region that has been associated with establishing the allele-specific replication timing domains in the $GABA_A$ receptor subunit gene cluster [Sinnett et al., 1996]. p82 was recently confirmed as a differentially active origin by polymerase chain reaction (PCR) mapping [Strehl et al., 1997].

ORIGINS OF DNA REPLICATION

The DNA sequence within which DNA replication initiates is called the origin of DNA replication. Its location is genetically defined by *cis*-acting DNA sequences (replicators) that are recognized and bound by the initiator proteins. Origins can also refer to the actual initiation site (functional origin), where unwinding of the template DNA and synthesis begin [DePamphilis, 1993b, 1999]. Replication origins in simple genomes such as bacteria, eukaryotic virus, and the yeast *Saccharomyces cerevisiae*, have been well characterized and consist of specific *cis*-acting sequences (replicators), that interact with specific IPs. In these systems, both the genetic and the functional origin map within a small region of a few hundred base pairs [DePamphilis, 1993b,c,d, 1999].

Identification and characterization of replicators in the budding yeast, *Saccharomyces cerevisiae*, were facilitated by the discovery that DNA sequences serving as replicators in chromosomes can also serve as replicators in plasmids [reviewed in Zannis-Hadjopoulos and Price, 1998]. These sequences, called autonomously replicating sequence (ARS) elements, when cloned into plasmids, permit them to replicate autonomously in yeast cells. In complex genomes, new technology has permitted mapping of initiation sites for DNA replication in single-copy sequences in the chromosomes of metazoa [DePamphilis, 1993b; Zannis-Hadjopoulos and Price, 1998 and references therein]. In these systems, DNA replication also begins at specific sites (origins of bidirectional replication or OBR) using the same mechanism that is prevalent in simple genomes [reviewed in Zannis-Hadjopoulos and Price, 1998; DePamphilis, 1999]. This supports the hypothesis that specific sequences designate the DNA replication initiation site in all genomes. In metazoa, however, putative *cis*-acting replicator elements may be distributed over large distances [DePamphilis, 1993b, 1999]. Mapping of replication initiation sites by two-dimensional replicon mapping methods indicates that replication initiates at multiple sites over a region of up to 55 kb in the hamster DHFR locus [Dijkwel and Hamlin, 1992], the human c-*myc* locus [Waltz et al., 1996; Trivedi et al., 1998], and in other systems [Benard et al., 1995; Delidakis and Kafatos, 1989; Little et al., 1993; Shinomiya and Ina, 1994; Wang et al., 1998]. Nonetheless, initiation in these large zones may occur nonrandomly at multiple sites within a replicon [Waltz et al., 1996; Trivedi et al., 1998; Kobayashi et al., 1998; Pelletier et al., 1999; reviewed in DePamphilis, 1999].

MAMMALIAN ORIGINS OF DNA REPLICATION

Although origins of replication in mammalian cells are still poorly defined, a large body of biochemical, molecular biological, and electron microscopic evidence, including studies of chromosomal loci in *Tetrahymena*, *Physarum*, *Drosophila,* chicken, mouse, hamster, monkey, and human cell systems, suggests that initiation of mammalian DNA replication is site-specific [reviewed in Zannis-Hadjopoulos and Price, 1998; DePamphilis, 1999]. Furthermore, there is a growing body of evidence indicating that metazoan origins are genetically determined. Thus, they are the same in subsequent cell divisions and replicate at specific times during S-phase [Simon and Cedar, 1996]; in some amplified loci such as, the DHFR gene locus in CHOC 400 cells [Vaughn et al., 1990; Dijkwel et al., 1991, 1994; Dijkwel and Hamlin, 1995] and the ADA gene locus in mouse cells [Virta-Pearlman et al., 1993], the same OBR is used in both the amplified and nonamplified lines, suggesting that specific *cis*-acting sequences designate origin use; upon translocation of the DHFR *ori*β [Handeli et al., 1989; Kobayashi et al., 1998] or the chorion gene amplification origin [Orr-Weaver, 1991] to other chromosomal sites, initiation of replication still occurred at that origin; a natural deletion of the β-globin OBR, included in a 135-kb region in a thalassemic cell line, resulted in the passive replication of the locus by forks emanating from another nearby origin [Kitsberg et al., 1993]; and transfer of the c-*myc* replication origin to other genomic locations by genomic recombination resulted in origin activity at the integration sites

[Malott and Leffak, 1999]. Such evidence indicates that mammalian origins of replication are genetically determined, supporting the replicator model [Stillman, 1993].

STRUCTURES ASSOCIATED WITH ORIGIN FUNCTION

The activation of origins seems to be dependent not only on the presence of a particular sequence, but of structural determinants as well, which either facilitate the local unwinding of DNA or serve as recognition signals for the initiation of DNA replication. Certain types of sequences and structures have been found to be common to most replication origins of prokaryotic, lower eukaryotic, and mammalian organisms [reviewed in DePamphilis, 1993a; Pearson et al., 1996; Zannis-Hadjopoulos and Price, 1998]. Such sequences include bent DNA, DNA unwinding elements (DUEs), matrix attachment regions (MARs) and inverted repeats that are capable of forming cruciform DNA [reviewed in Pearson et al., 1996]. Some or all of them may constitute an origin and/or mediate the proper origin function and regulation in concert with the cell cycle.

Bent DNA

Bent DNA, a sequence-directed curvature of the DNA helix [Selsing et al., 1979; Koo et al., 1986], is associated with origins of prokaryotes [Zahn and Blattner, 1987], SV40 [Deb et al., 1986], yeast [Snyder et al., 1986] [Williams et al., 1988], and the DHFR amplicon [Caddle et al., 1990]. Although bent DNA may be important in origins, its precise role is unclear; it may act as an enhancer-like element, participating in the transcriptional activation of the origins [Brand et al., 1987; Inokuchi et al., 1988], or it may participate in the melting of the double helix at the origin, facilitating priming of initiation.

DUE

The DUE is an easily unwound DNA region. It is determined by base-stacking interactions and, therefore, depends on nucleotide sequence and not simply on A/T content [Natale et al., 1992]. The DUE was first demonstrated as a component of yeast [Umek and Kowalski, 1988] and bacterial [Bramhill and Kornberg, 1988] origins, and was suggested as the site where DNA unwinding and synthesis begins [Umek and Kowalski, 1988].

Cruciform DNA

Cruciform DNA can arise through intrastrand base pairing of palindromic (inverted repeat; IR) DNA sequences, which are widely distributed in the chromosomal DNA of many eukaryotes [Wilson and Thomas, 1974; Schmid et al., 1975]. They may affect the supercoiling degree of DNA, the positioning of nucleosomes, the formation of other secondary structures of DNA, or directly interact with proteins [reviewed in Pearson et al., 1996]. It has been suggested that, under certain physiological conditions, palindromic sequences can switch from the linear to the cruciform conformation and serve as recognition signals for specific regulatory proteins of DNA replication and transcription [Zannis-Hadjopoulos et al., 1984; Bell et al., 1991; Pearson et al., 1996; Sinden, 1994]. Thus, palindromes have been identified in operator and transcription termination regions in prokaryotes as well as in DNA replication origins of many prokaryotes and mammalian virus [reviewed in Sinden, 1994; Pearson et al., 1996; Zannis-Hadjopoulos and Price, 1998]. Inverted repeats and cruciforms are present at the replication origins of phage, plasmids, mitochondria, eukaryotic virus, and mammalian cells and have been associated with amplified genes [reviewed in Boulikas and Kong, 1993; Sinden, 1994; Pearson et al., 1996; Zannis-Hadjopoulos and Price, 1998]. They are functionally important for the initiation of DNA replication in plasmids, prokaryotes, and eukaryotic virus [Pearson et al., 1996 and references therein]. The formation of cruciforms in vivo has been demonstrated for prokaryotic [Panayotatos and Fontaine, 1987; Zheng et al., 1991; Dayn et al., 1992], mammalian [Ward et al., 1990, 1991] and viral DNA [Pearson et al., 1996, and references therein]. The regulation of replication may be controlled by cruciform-specific binding proteins [Zannis-Hadjopoulos et al., 1988; Hiasa et al., 1990; Pearson et al., 1996, and references therein].

Monoclonal antibodies (MAbs) have been raised with unique specificity to cruciform DNA structures [Frappier et al., 1987, 1989]. Using these MAbs, it was shown that there is a dynamic distribution of DNA cruciforms in mammalian nuclei, their numbers being at a maximum at the G1/S boundary [Ward et al., 1990,

1991]. Introduction of the MAb into a permeablized cell system capable of carrying out DNA replication resulted in an enhancement of DNA replication. It was hypothesized that this was presumably through the interaction of the MAb with cruciforms present at or near replication origins, which resulted in their stabilization and the increase of DNA synthesis through multiple initiations at the same site [Zannis-Hadjopoulos et al., 1988]. Furthermore, these anti-cruciform antibodies were used to affinity-purify active mammalian origins of replication [Bell et al., 1991; Nielsen et al., 1994a]. These observations support the hypothesis that certain IRs, giving rise to cruciforms, may be part of initiation sites for DNA replication [Edenberg and Huberman, 1975; Boulikas and Kong, 1993; Pearson et al., 1996] and serve as the attachment site for protein(s) involved in this process [Hand, 1978; Sinden, 1994]. Proteins that interact with specific structural recognition elements in DNA are thought to contribute to the temporal and spatial regulation of important cellular processes such as replication and transcription.

A novel human cruciform binding protein (CBP) with binding specificity for the cruciform-containing DNA, was recently purified [Pearson et al., 1994]. Hydroxyl radical footprinting analysis localized the CBP-DNA interaction to the four-way junction at the base of the cruciform DNAs [Pearson et al., 1995]. Upon CBP binding, associated structural distortions were observed at the cruciform stems and at a DNA region adjacent to the junction. Microsequence analysis identified CBP as a member of the 14–3-3 family of proteins [Todd et al., 1998]. The interaction of 14–3-3 with cruciform DNA may be of particular significance, since, among the plethora of functions that have been ascribed to 14–3-3 proteins are signal transduction pathways and cell cycle regulation [Aitken, 1995].

SITE-SPECIFICITY OF MAMMALIAN REPLICATION ORIGINS

Several experimental strategies have been used in the effort to isolate mammalian replication origins [reviewed in Vassilev and DePamphilis, 1992], which can be classified into two groups. The first group consists of methods for physical mapping and isolation of replication origins. These methods can be subdivided into analyses of (1) nascent DNA strands, and (2) replication structures. The second group consists of functional assays for *cis*-acting DNA sequences conferring autonomous plasmid replication. A combination of the two strategies has also been used, whereby (1) nascent sequences containing replication origins were isolated by induced extrusion of early replicating DNA strands from active replication bubbles [Zannis-Hadjopoulos et al., 1981, 1983, 1985; Kaufmann et al., 1985], or (2) replication origins were isolated by taking advantage of the presence of replication structures (specifically, DNA cruciforms) in active replication origins [Bell et al., 1991; Nielsen et al., 1994a]. The use of these strategies for mapping eukaryotic origins of DNA replication has identified at least 23 specific sites in the chromosomes of flies, frogs, and mammals [reviewed in DePamphilis, 1999].

AUTONOMOUS REPLICATION ASSAYS

Eukaryotic genomic fragments cloned into prokaryotic vectors have been assayed for autonomous replication, in assays analogous to those that identified yeast autonomously replicating sequences (ARS). These assays have yielded contradictory results, with some studies showing that only large fragments (>10 kb) could be observed to support autonomous DNA replication in human [Heinzel et al., 1991; Krysan et al., 1993] or rodent [Krysan and Calos, 1993; Caddle and Calos, 1992] cells. Other studies indicate that small (approximately 100 bp–2 kb), selected sequences can successfully support autonomous replication [Zannis-Hadjopoulos et al., 1985; Frappier and Zannis-Hadjopoulos, 1987; McWhinney and Leffak, 1990; Bell et al., 1991; Landry and Zannis-Hadjopoulos, 1991; Iguchi-Ariga et al., 1993; Virta-Pearlman et al., 1993; Wu et al., 1993a; Nielsen et al.,1994a,b, 1999; McWhinney et al., 1995; Sinnett et al., 1996]. Furthermore, plasmids carrying the OBR (*ori*β) of the Chinese hamster *dhfr* gene replicated autonomously in vivo and in vitro regardless of plasmid size, whereas plasmids of equivalent size carrying random sequences did not [Zannis-Hadjopoulos et al., 1994]. In several cases, the ability of certain specific sequences to support autonomous replication of plasmids has also been correlated with the chromosomal mapping of the same sequences as initiation sites for DNA replication [Iguchi-Ariga et al., 1993; Virta-Pearlman et al., 1993; Wu et al., 1993a,b; Zannis-Hadjopoulos et al., 1994; McWhinney et al., 1995; Pelletier et al., 1999]. Finally, Malott

and Leffak [1999] demonstrated the activity of the c-*myc* replicator at an ectopic chromosomal location. The control used in these experiments was non-*myc* DNA, comprising a bacterial transcription unit. The data obtained with this bacterial DNA suggested that this particular unit was capable of changing the replication pattern near the integration locus, although the abundance of newly replicated strands in equivalent regions was lower than that near the neighboring c-*myc* DNA. In these experiments, Malott and Leffak [1990] argue that the results are indicative of previous work by Calos and coworkers [Krysan et al., 1993] regarding the ability of bacterial sequences to affect initiation of replication in mammalian cells.

EUKARYOTIC INITIATOR PROTEINS (IP) AND MULTIPROTEIN REPLICATION COMPLEXES

The mechanism of initiation of chromosomal DNA replication appears to be conserved in eukaryotes. In *Saccharomyces cerevisiae*, an origin recognition complex (ORC), consisting of six protein subunits was identified [Bell and Stillman, 1992]. ORC binds to the yeast ARS in an ATP-dependent manner and is required for initiation of replication [Bell and Stillman, 1992; Dutta and Bell, 1997; Dillin and Rine, 1998]. Although ORC is bound to the ARS site throughout the yeast cell cycle [Diffley and Cocker, 1992], it is involved in the process of initiation through its ability to form complexes with proteins that control initiation [Dillin and Rine, 1998]. Thus, in vivo and in vitro footprinting studies demonstrated the presence of two types of protein-DNA complexes at the yeast replication origin during the cell cycle: a postreplicative complex (post-RC) present during the S, G2, and M phases, and a larger prereplicative complex (pre-RC) present at late M and G1 [Diffley et al., 1994]. Genetic and molecular evidence suggests that the pre-RC contains ORC, Cdc6p, and the MCM family of proteins [Loo et al., 1995; Santocanale and Diffley, 1996; Aparicio et al., 1997]. This pattern of binding fits the "licensing" model of replication, whereby initiation events are limited to once per cell cycle [Blow and Laskey, 1988; Laskey et al., 1989]. Two different signals are required for activation of chromosomal replication: the replication licensing factor (RLF) "licenses" replication origins before 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 Chevalier and Blow, 1996]. Evidence from yeast and *Drosophila* strongly suggests that Cdks play also a role in preventing re-replication of DNA in a single cell cycle [Chevalier and Blow, 1996 and references therein].

Evidence for multiprotein replication complexes (MRCs) has been accumulating over the past few years [reviewed in Hickey and Malkas, 1997; Zannis-Hadjopoulos and Price, 1998]. Recently, a model of DNA replication was proposed, to represent a multiprotein DNA replication complex isolated from a wide variety of mammalian cell types and tissues [Malkas et al., 1990; Wu et al., 1994; Applegren et al., 1995], termed the "DNA synthesome" [Hickey and Malkas, 1997; Lin et al., 1997]. According to this model, the synthesome "core" comprises proteins involved in the elongation phase of DNA replication (e.g., 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 (e.g., PCNA, RPA, and topoisomerase I) are more loosely associated with it [Hickey and Malkas, 1997]. Such studies have demonstrated that many proteins involved in DNA replication (e.g., RPA and PCNA) are also involved in DNA repair.

Recently, an activity was purified on the basis of its ability to bind to a putative replication origin, *ors8* [Ruiz et al., 1995]. OBA was identified by microsequence analysis as the 86-kDa subunit of Ku (Ku86) antigen, a heterodimeric (p70/p86) DNA-binding protein [Ruiz et al., 1999 and references therein]. Ku had been previously purified from HeLa cells as part of a 21S multiprotein complex competent for DNA synthesis [Vishwanatha and Baril, 1990], was reported to interact with a human DNA region (B48) containing a replication origin [Tóth et al., 1993], and to have DNA helicase activity [Tuteja et al., 1994; Ochem et al., 1997]. Ku is the DNA binding subunit of the DNA-dependent protein kinase (DNA-PK) holoenzyme [Gottlieb and Jackson, 1993; Suwa et al., 1994], a nuclear component that phosphorylates a number of DNA binding, regulatory proteins, including transcription factors (Sp1, p53), RNA polymerase II, topoisomerases I and II, Ku antigen, and SV-40 large T antigen [Anderson, 1993, and references therein]. Although Ku has been characterized as a DNA-end binding protein, it was recently shown to be a sequence specific

DNA-binding protein, recruiting DNA-PK directly to specific DNA sequences [Giffin et al., 1996, 1997]. Ku also binds in a sequence-specific manner to a 36-bp sequence (A3/4) deduced from mammalian replication origins and is involved in mammalian DNA replication [Ruiz et al., 1999]. A Ku-like protein from *Saccharomyces cerevisiae* was reported to be required for the in vitro assembly of a multiprotein complex at the *ARS121* origin of replication [Shakibai et al, 1996].

ORIGIN USAGE

A comparison of origin activity among four origins of DNA replication in human HeLa and normal skin fibroblast (NSF) cells has demonstrated approximately a 2-fold difference between these cell lines in the activation of the c-*myc* origin and NOA3 [Wu et al., 1993], an origin associated with the 3′ region of the eta subunit of 14–3-3; the other two (S14 and 343) exhibited no significant difference in activation between the two cell lines [Tao et al., 1997 and references therein]. The most plausible explanation for this differential activity is the use of different initiation sites for replication of certain loci, some of which may be differentially preferred in different cell types or states of cell maturation. In another study, it was reported that a 400 bp replication enhancer within the *ura4* origin region of *S. pombe* defines the relative activities of the three replication origins located in this region [Kim and Huberman, 1999], suggesting that enhancers may affect the relative activities in the origin clusters of animal cells, thus influencing origin efficiency and usage.

METHYLATION

In addition to genetic elements, epigenetic chromosomal components, such as modification of the DNA by methylation and chromatin structure, have been postulated as determinants of replication origins [Tasheva and Roufa, 1994; Delgado et al., 1998; Rein et al., 1997]. DNA methylation at CpG dinucleotides is an important mechanism of epigenetic regulation of genomic function and of processes such as transcription, recombination, imprinting, development, carcinogenesis, and replication timing [reviewed in Zannis-Hadjopoulos and Price, 1998]. It was recently postulated that methylated CpG clusters mark specific origins for replication through changes in chromatin structure [Rein et al., 1997]. Another recent study showed that replication origins are differentially methylated, with some origins (e.g., lamin B2) partially methylated, some (e.g., c-*myc*) not methylated, and others (e.g., the previously reported DHFR *ori*β and *ori-RPS14*) heavily methylated [Araujo et al., 1998]. It has been suggested that DNA methylation at mammalian origins could play a role in delaying the reassembly of prereplication complexes (pre-RCs) [Rein et al., 1999], in a manner analogous to the role of DNA methylation at the *E. coli* replication origin, *oriC*, where a delay in the reassembly of pre-RCs is observed due to the maintenance of *oriC* in a hemimethylated state [Boye and Lobner-Olesen, 1990; Campbell and Kleckner, 1990]. Recent evidence, however, suggests that this is not the case in mammalian cells, but, rather, changes in the concentrations of initiator proteins determine the number and location of replication origins [Rein et al., 1999]. Finally, it was recently shown that the human *dnmt1* (DNA methyltransferase) locus contains an OBA/Ku86 binding site and initiation sites for DNA replication [Araujo et al., 1999]. DNA methyltransferase is the enzyme responsible for propagating the DNA methylation pattern and the epigenetic information that it encodes during replication.

CHROMATIN STRUCTURE AND DNA REPLICATION

During eukaryotic DNA replication, the newly synthesized DNA must be assembled into chromatin. A number of studies have established a general effect of chromatin structure on processes such as replication and transcription [Wolffe, 1996]. Chromatin structure can inhibit both replication origin and transcriptional promoter activities by blocking access of initiation factors to specific sites [reviewed by DePamphilis, 1999]. Biochemical analysis of chromatin assembly has led to the identification of core histone-binding proteins that may be involved in both replication and transcription. These include, among others, nucleosome assembly proteins (NAP) [Rodriguez et al., 1997 and references therein; Wolffe, 1995] and chromatin assembly factors [reviewed in Krude, 1999]. Chromatin assembly factor 1 (CAF-1) has a key role in assembling nucleosomes onto replicating DNA. Proliferating cell nuclear antigen (PCNA), a DNA polymerase clamp, interacts directly with the largest subunit (p150) of

CAF-1, and the two proteins co-localize at sites of DNA replication [Shibahara and Stillman, 1999]. Since PCNA also interacts with the mammalian DNA-methyltransferase at replication sites [Chuang et al., 1997], it was suggested that it may provide a common platform for both chromatin assembly and DNA methylation [Shibahara and Stillman, 1999].

FUTURE DIRECTIONS

In order to understand how DNA replication is regulated, the nature of replication origins (*cis*-acting sequences for origin function or replicators) and the proteins that interact with them must first be determined. More information about the molecular features, structure, function, distribution, and nuclear location of individual and groups of replication origins will be essential for resolving the mechanisms of normal and abnormal mammalian DNA replication.

The foundation built during the 1960s–1980s has provided a burst of activity that has led to rapid accumulation of key information about eukaryotic DNA replication this last decade before the millennium. In the short term (next decade), there is sufficient technical know-how and indications from viral, prokaryotic, and yeast models that the discovery of *cis*-replicator sequences, *cis*-elements of essential secondary structures for origin activity, and the identification and characterization of the *trans*-regulatory initiation proteins should be anticipated. DNA replication as a field of study will also include more intensive studies of signaling between molecules as the machinery for replication is assembled, functions, and then becomes quiescent. As better definition of the regulation of initiation of higher eukaryotic DNA replication is accomplished, application of this knowledge to the development of new therapies (e.g. specifically targeted therapies in cancer and other dysfunctions of replication; improvement in wound healing and progress in organ regeneration; and better gene therapy, including new all-homologous vectors and artificial chromosome) will be included in medical practice in the next century.

In the coming years, the relationships that exist between DNA repair, recombination, gene expression, and DNA replication will become more clearly understood. As we understand the nature of these relationships and the control of their interdigitation, a more complete picture of the most fundamental processes effecting biologic life will be revealed. Already, researchers are turning to focus upon higher order structures of chromatin, nucleus, and cell, to begin to add to our knowledge of the coordinated processes that precede and follow the duplication of DNA. The control of DNA replication throughout the life span of a cell and organism is recognized to include these higher-order structures, which also play roles in establishing the behavior of a cell. The hierarchy of control of cell proliferation, the most fundamental life process, will be facilitated through comparative studies of models (ontogeny demonstrated by phylogenetic models) and then the application of technology and expertise to experimentation in higher eukaryotic cells.

REFERENCES

Adegoke JA, Taylor JH. 1977. Sequence programming of DNA replication over the S phase of Chinese hamster cells. Exp Cell Res 104:47–54.

Aitken A. 1995. 14-3-3 proteins on the map. Trends Biochem Sci 20:95–97.

Amaldi F, Buongiorno-Nardelli M, Carnevali F, Leoni L, Mariotti D, Pomponi M. 1973. Replicon origins in Chinese hamster cell DNA. Exp Cell Res 80:79–87.

Andersen CW. 1993. DNA damage and the DNA-activated protein kinase. Trends Biochem Sci 18:433–437.

Aparicio OM, Weinstein DM, Bell SP. 1997. Components and dynamics of DNA replication complexes in *S. cerevisiae*: redistribution of mcm proteins and cdc45p during S phase. Cell 91:59–69.

Applegren N, Hickey RJ, Kleinschmidt AM, Zhou QQ, Cell J, Wills P, Swaby R, Wei YT, Quan JY, Lee MYWT, Malkas LH. 1995. Further characterization of the human cell multiprotein DNA replication complex. J Cell Biochem 59:91–107.

Araujo FD, Knox JD, Szyf M, Price GB, Zannis-Hadjopoulos M. 1998. Concurrent replication and methylation at mammalian origins of replication. Mol Cell Biol 18:3475–3482.

Araujo FD, Knox JD, Ramchandani S, Pelletier R, Bigey P, Price G, Szyf M, Zannis-Hadjopoulos M. 1999. Identification of initiation sites for DNA replication in the human *dnmt1* (DNA-methyltransferase) locus. J Biol Chem 274:9335–9341.

Baran N, Lappidot A, Manor H. 1991. Formation of DNA triplexes accounts for arrests of DNA synthesis at d(tC)n and d(GA)n. Proc Natl Acad Sci USA 88:507–511.

Bell D, Sabloff M, Zannis-Hadjopoulos M, Price GB. 1991. Anti-cruciform DNA affinity purification of active mammalian origins of replication. Biochim Biophys Acta 1089:299–308.

Bell SP, Stillman B. 1992. ATP-dependent recognition of eukaryotic recognition of origins of DNA replication by multiprotein complex. Nature 357:128–134.

Benard M, Pallotta D, Pierron G. 1992. Structure and identity of a late-replicating and transcriptionally active gene. Exp Cell Res 201:506–513.

Benard M, Lagnel C, Pierron G. 1995. Site-specific initiation of DNA replication within the non-transcribed spacer of *Physarum* rDNA. Nucleic Acids Res 23:1447–1453.
Blow JJ. 1993. Preventing re-replication of DNA in a single cell cycle: evidence for a replication licensing factor. J Cell Biol 122:993–1002.
Blow JJ, Laskey RA. 1988. A role for the nuclear envelope in controlling DNA replication within the cell cycle. Nature 332:546–548.
Blow JJ, Sheehan MA, Watson JV, Laskey RA. 1989. Nuclear structure and the control of DNA replication in the *Xenopus* embryo. J Cell Sci 12:183–195.
Bostock CJ, Prescott DM. 1971a. Shift in buoyant density of DNA during the synthetic period and its relation to euchromatin and heterochromatin in mammalian cells. J Mol Biol 60:151–162.
Bostock DJ, Prescott DM. 1971b, Bouyant density of DNA synthesized at different stages of the S phase of mouse L cells. Exp Cell Res 64:267–274.
Boulikas T, Kong CF. 1993. Multitude of inverted repeats characterizes a class of anchorage sites of chromatin loops to the nuclear matrix. J Cell Biochem 53:1–12.
Boye E, Lobner-Olesen A. 1990. The role of dam methyltransferase in the control of DNA replication in *E. coli*. Cell 62:981–989.
Bramhill D, Kornberg A. 1988. Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell 52:743–755.
Brand AH, Micklem G, Nasmyth K. 1987. A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell 51: 709–719.
Caddle MS, Calos MP. 1992. Analysis of the autonomous replication behavior in human cells of the dihydrofolate reductase putative chromosomal origin of replication. Nucleic Acids Res 20:5971–5978.
Caddle MS, Lussier RH, Heintz NH. 1990. Intramolecular DNA triplexes, bent DNA and DNA unwinding elements in the initiation region of an amplified dihydrofolate reductase replicon. J Mol Biol 211:19–33.
Cairns J. 1966. Autoradiography of HeLa cell DNA. J Mol Biol 15:372–373.
Campbell JL, Kleckner N. 1990. *E. coli* oriC and the dnaA gene promoter are sequestered from the dam methyltransferase following passage of the chromosomal replication fork. Cell 62:967–979.
Carminati JL, Orr-Weaver TL. 1996. Changes in DNA replication during animal development. In: DePamphilis ML, editor. DNA replication in eukarotic cells. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 409–434.
Cheevers WP, Kowalski J, Yu KK-Y. 1972. Synthesis of high molecular weight cellular DNA in productive polyoma virus infection. J Mol Biol 65:347–364.
Chevalier S, Blow JJ. 1996. Cell cycle control of replication initiation in eukaryotes. Curr Opin Cell Biol 8:815–821.
Chung LS-H, Ian H-I, Koh T-W, Ng H-H, Xu G, Li BFL. 1997. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21^{WAF1}. Science 277:1996–2000.
Comings DE. 1972. Replicative heterogeneity of mammalian DNA. Exp Cell Res 71:106–112.
Coverley D, Laskey RA. 1994. Regulation of eukaryotic DNA replication. Annu Rev Biochem 63:745–776.
Dayn A, Malkhosyan S, Mirkin SM. 1992. Transcriptionally driven cruciform formation in vivo. Nucleic Acids Res 20:5991–5997.
Deb S, DeLucia AL, Koff A, Tsui S, Tegtmeyer P. 1986. The adenine-thymine domain of the simian virus 40 core origin directs DNA bending and coordinately regulates DNA replication. Mol Cell Biol 6:4578–4584.
Delidakis C, Kafatos FC. 1989. Amplification enhancers and replication origins in the autosomal chorion gene cluster of *Drosophila*. EMBO J 8:891–901.
Delgado S, Gomez M, Bird A, Antequera F. 1998. Initiation of DNA replication at CpG islands in mammalian chromosomes. EMBO J 17:2426–2435.
DePamphilis M. 1993a. How transcription factor regulate origins of DNA replication in eukaryotic cells. Trends Cell Biol 3:161–167.
DePamphilis ML. 1993b. Eukaryotic DNA replication: Anatomy of an origin. Annu Rev Biochem 62:29–63.
DePamphilis ML. 1993c. Origins of DNA replication in metazoan chromosomes. J Biol Chem 268:1–4.
DePamphilis ML. 1993d. Origins of DNA replication that function in eukaryotic cells. Curr Opin Cell Biol 5:434–441.
DePamphilis M. 1996. Origins of DNA replication. In: DePamphilis M, editor. DNA replication in eukaryotic cells. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 45–86.
DePamphilis ML. 1999. Replication origins in metazoan chromosomes: fact or fiction? BioEssays 21:5–16.
Diffley JFX, Cocker JH. 1992. Protein-DNA interactions at a yeast replication origin. Nature 357:169–172.
Diffley JFX, Cocker JH, Dowell SJ, Rowley A. 1994. Two steps in the assembly of complexes at yeast replication origins in vivo. Cell 78:303–316.
Dijkwel PA, Hamlin JL. 1992. Initiation of DNA replication in the dihydrofolate reductase locus is confined to the early S period in CHO cells synchronized with the plant amino acid mimosine. Mol Cell Biol 12:3715–3722.
Dijkwel PA, Hamlin JL. 1995. Origins of replication and the nuclear matrix: the DHFR domain as a paradigm. Int Rev Cytol 162A:455–484.
Dijkwel PA, Vaughn JP, Hamlin JL. 1991. Mapping of replication initiation sites in mammalian genomes by two-dimensional gel analysis: stabilization and enrichment of replication intermediates by isolation on the nuclear matrix. Mol Cell Biol 11:3850–3859.
Dijkwel PA, Vaughn JP, Hamlin JL. 1994. Replication initiation sites are distributed widely in the amplified CHO dihydrofolate reductase domain. Nucleic Acids Res 22: 4989–4996.
Dillin A, Rine J. 1998. Roles for ORC in M phase and S phase. Science 279:1733–1737.
Dutta A, Bell SP. 1997. Initiation of DNA replication in eukaryotic cells. Annu Rev Cell Dev Biol 13:293–332.
Edenberg HJ, Huberman JA. 1975. Eukaryotic chromosome replication. Annu Rev Genet 9:245–284.
Emmons CW, Hollander A. 1939. The action of ultraviolet light on dermatophytes. II. Mutation induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. Am J Bot 26:467–454.
Ferguson BM, Fangman WL. 1992. A position effect on the time of replication origin activation in yeast. Cell 68:333–339.

Frappier L, Zannis-Hadjopoulos M. 1987. Autonomous replication of plasmids bearing monkey DNA origin-enriched sequences. Proc Natl Acad Sci USA 84:6668–6672.
Frappier L, Price GB, Martin RG, Zannis-Hadjopoulos M. 1987. Monoclonal antibodies to cruciform DNA structures. J Mol Biol 193:751–758.
Frappier L, Price GB, Martin RG, Zannis-Hadjopoulos M. 1989. Characterization of the binding specificity of two anticruciform DNA monoclonal antibodies. J Biol Chem 264:334–341.
Fuller RS, Kaguni JM, Kornberg A. 1981. Enzymatic replication of the origin of the *Escherichia coli* chromosome. Proc Natl Acad Sci USA 78:7370–7374.
Gavin KA, Hidaka M, Stillman B. 1995. Conserved initiator proteins in eukaryotes. Science 270:1667–1671.
Giffin W, Torrance H, Rodda DJ, Préfontaine GG, Pope L, Haché RJG. 1996. Sequence-specific DNA binding by Ku autoantigen and its effects on transcription. Nature 380: 265–268.
Giffin W, Kwast-Welfeld J, Rodda DJ, Préfontaine GG, Traykova-Andonova M, Zhang Y, Weigel NL, Lefebvre YA, Haché RJG. 1997. Sequence-specific DNA binding and transcription factor phosphorylation by Ku autoantigen? DNA-dependent protein kinase. J Biol Chem 272: 5647–5658.
Gilbert DM. 1986. Temporal order of replication of *Xenopus laevis* 5S ribosomal RNA genes in somatic cells. Proc Natl Acad Sci USA 83:2924–2928.
Gilbert DM. 1998. Replication origins in yeast versus metazoa: separation of the haves and the have nots. Curr Opin Genet Dev 8:194–199.
Gilbert DM, Miyazawa H, Depamphilis ML. 1995. Site-specific initiation of DNA replication in *Xenopus* egg extract requires nuclear structure. Mol Cell Biol 15:2942–2954.
Goldman MA, Holmquist GP, Gray MC, Gaston LA, Nag A. 1984. Replication timing of genes and middle repetitive sequences. Science 224:686–692.
Gottlieb TM, Jackson SP. 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell 72:131–142.
Hamlin JL, Pardee AB. 1978. Control of DNA synthesis in tissue culture cells. In Vitro 14:119–127.
Hand R. 1978. Eucaryotic DNA: organization of the genome for replication. Cell 15:317–325.
Hand R, Tamm I. 1974. Initiation of DNA replication in mammalian cells and its inhibition by reovirus infection. J Mol Biol 82:175–183.
Handeli S, Klar A, Meuth M, Cedar H. 1989. Mapping replication units in animal cells. Cell 57:909–920.
Hay RT, Hendrickson EA, DePamphilis ML. 1984. Sequence specificity for the initiation of RNA-primed simian virus 40 DNA synthesis in vivo. J Mol Biol 175:131–57.
Heinzel SS, Krysan PJ, Tran CT, Calos MP. 1991. Autonomous DNA replication in human cells is affected by the size and the source of DNA. Mol Cell Biol 11:2263–2273.
Hendrickson EA, Fritze EE, Folk WR, DePamphilis ML. 1987. Polyoma virus DNA replication is semi-discontinuous. Nucleic Acids Res 15:6369–6385.
Hiasa H, Sakai H, Komano T, Godson N. 1990. Structural features of the priming signal recognized by primase: mutational analysis of the phage G4 origin of complementary DNA strand synthesis. Nucleic Acids Res 18:4825–4831.
Hickey RJ, Malkas LH. 1997. Mammalian cell DNA replication. Crit. Rev. Eukaryot Gene Expr 7:125–157.
Hozak P, Hassan AB, Jackson DA, Cook PR. 1993. Visualization of replication factories attached to a nucleoskeleton. Cell 73:361–373.
Huberman JA, Riggs AD. 1968. On the mechanism of DNA replication in mammalian chromosomes. J Mol Biol 32: 327–341.
Iguchi-Ariga SMM, Ogawa N, Ariga H. 1993. Identification of the initiation region of DNA replication in the murine immunoglobulin heavy chain gene and possible function of the octamer motif as a putative DNA replication origin in mammalian cells. Biochim Biophys Acta 1172:73–81.
Inokuchi K, Nakayama A, Hishinuma F. 1988. Sequence-directed bends of DNA helix axis at the upstream activation sites of alpha-cell-specific genes in yeast. Nucleic Acids Res 16:6693–6711.
Jacob F, Brenner S, Cuzin F. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp Quant Biol 28:329–348.
Kaufmann G, Zannis-Hadjopoulos M, Martin RG. 1985. Cloning of nascent monkey DNA synthesized early in the cell cycle. Mol Cell Biol 5:721–727.
Kim SM, Huberman JA. 1999. Influence of a replication enhancer on the hierarchy of origin efficiencies within a cluster of DNA replication origins. J Mol Biol 288:867–882.
Kitsberg D, Selig S, Keshet I, Cedar H. 1993. Replication structure of the human β-globin gene domain. Nature 366:588–590.
Kobayashi T, Rein T, DePamphilis M. 1998. Identification of primary initiation sites for DNA replication in the hamster dihydrofolate reductase gene initiation zone. Mol Cell Biol 18:3266–3277.
Koo HS, Wu HM, Crothers DM. 1986. DNA bending at adenine.thymine tracts. Nature 320:501–506.
Kornberg A, Baker TA. 1992. DNA replication. New York: WH Freeman.
Kriegstein HJ, Hogness DS. 1974. Mechanism of DNA replication in *Drosophila* chromosomes: structure of replication forks and evidence for bidirectionality. Proc Natl Acad Sci USA 71:135–139.
Krude T. 1999. Chromatin replication: finding the right connection. Curr Biol 9:R394–R396.
Krysan PJ, Calos MP. 1993. Epstein-Barr virus-based vectors that replicate in rodent cells. Gene 136:137–143.
Krysan PJ, Smith JG, Calos MP. 1993. Autonomous replication in human cells of multimers of specific human and bacterial DNA sequences. Mol Cell Biol 13:2688–2696.
Landry S, Zannis-Hadjopoulos M. 1991. Classes of autonomously replicating sequences are found among early-replicating monkey DNA. Biochim Biophys Acta 1088: 234–244.
Laskey RA, Fairman MP, Blow JJ. 1989. S phase of the cell cycle. Science 246:609–614.
Latt D. 1974. Microfluorometric analysis of deoxyribonucleic acid replication kinetics and sister chromatid exchanges in human chromosomes. J Histochem Cytochem 22:478–491.
Li JJ, Kelly TJ. 1984. Simian virus 40 DNA replication in vitro. Proc. Natl Acad Sci USA 86:6973–6977.
Lin SK, Hickey RJ, Malkas LH. 1997. The isolation of a DNA synthesome from human leukemia cells. Leuk Res 21:501–512.

Little RD, Platt TH, Schildkraut CL. 1993. Initiation and termination of DNA replication in human rRNA genes. Mol Cell Biol 13:6600–6613.
Loo S, Fox CA, Rine J, Kobayashi R, Stillman B, Bell S. 1995. The origin recognition complex in silencing, cell cycle progression, and DNA replication. Mol Biol Cell 6:741–756.
Malkas LH, Hickey RJ, Li C, Pedersen N, Baril EF. 1990. A 21S enzyme complex from HeLa cells that functions in simian virus 40 DNA replication in vitro. Biochemistry 29:6362–6374.
Malott M, Leffak M. 1999. Activity of the c-*myc* replicator at an ectopic chromosomal location. Mol Cell Biol 19:5685–5695.
Martin R. 1981. The transformation of cell growth and transmogrification of DNA synthesis by simian virus 40. Adv Cancer Res 34:1–68.
Martin RG, Oppenheim A. 1977. Initiation points for DNA replication in nontransformed and simian virus 40-transformed Chinese hamster lung cells. Cell 11:859–869.
May MS, Bello LJ. 1974. Replication of repeated DNA in human cells. Exp Cell Res 83:79–86.
McWhinney C, Leffak M. 1990. Autonomous replication of a DNA fragment containing the chromosomal replication origin of the human c-*myc* gene. Nucleic Acids Res 18:1233–1242.
McWhinney C, Waltz SE, Leffak M. 1995. *Cis*-acting effects of sequences within 2.4-kb upstream of the human c-*myc* gene on autonomous plasmid replication in HeLa cells. DNA Cell Biol 14:565–579.
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.
Mueller GC, Kajiwara K. 1966. Early- and late-replicating deoxyribonucleic acid complexes in HeLa nuclei. Biochim Biophys Acta 114:108–115.
Nakamura H, Morita T, Sato C. 1986. Structural organisation of replicon domains during DNA synthetic phase in the mammalian nucleus. Exp Cell Res 165:291–297.
Natale DA, Schubert AE, Kowalski D. 1992. DNA helical stability accounts for mutational defects in a yeast replication origin. Proc Natl Acad Sci USA 89:2654–2658.
Natale DA, Umek RM, Kowalski D. 1993. Ease of DNA unwinding is a conserved property of yeast replication origins. Nucleic Acids Res 21:555–560.
Nielsen TO, Cossons, NH, Zannis-Hadjopoulos M, Price G. 1999. Circular YAC vectors containing short mammalian origin sequences are maintained under selection as HeLa episomes. J Cell Biochem (in press).
Nielsen TO, Bell D, Lamoureux C, Zannis-Hadjopoulos M, Price GB. 1994a. A reproducible method for identification of human genomic DNA autonomously replicating sequences. Mol Gen Genet 242:280–288.
Nielsen TO, Piatyszek MA, Shay JW, Pearson CE, Zannis-Hadjopoulos M, Price GB. 1994b. Autonomous replication activity of a human mitochondrial DNA sequence inserted into genomic DNA. Int J Oncol 5:1003–1008.
Ochem AE, Skopac D, Costa M, Rabilloud T, Vuillard L, Simoncsits A, Giacca M, Falaschi A. 1997. Functional properties of the separate subunits of human DNA helicase II/Ku autoantigen. J Biol Chem 272:29919–29926.
Orr-Weaver TL. 1991. *Drosophila* chorion genes: cracking the eggshell's secrets. BioEssays 13:97–105.
Panayotatos N, Fontaine A. 1987. A native cruciform DNA structure probed in bacteria by recombinant T7 endonuclease. J Biol Chem 262:11364–11368.
Pearson CE, Ruiz MT, Price GB, Zannis-Hadjopoulos M. 1994. Cruciform DNA binding protein in HeLa cell extracts. Biochemistry 33:14185–14196.
Pearson CE, Zannis-Hadjopoulos M, Price GB, Zorbas H. 1995. A novel type of interaction between cruciform DNA and a cruciform binding protein from HeLa cells. EMBO J 14:1571–1580.
Pearson CE, Zorbas H, Price GB, Zannis-Hadjopoulos M. 1996. Inverted repeats, stem-loops, and cruciforms: significance for initiation of DNA replication. J Cell Biochem 63:1–22.
Pelletier R, Price GB, Zannis-Hadjopoulos M. 1999. Functional genomic mapping of an early-activated centromeric mammalian origin of DNA replication. J Cell Biochem 74:562–575.
Rao PN, Johnson RT. 1970. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. Nature 225:159–164.
Rein T, Kobayashi T, Malott M, Leffak M, DePamphilis M. 1999. DNA methylation at mammalian replication origins. J Biol Chem 274:25792–25800.
Rein T, Zorbas H, Depamphilis ML. 1997. Active mammalian replication origins are associated with a high-density cluster of ^{m}CpG dinucleotides. Mol Cell Biol 17:416–426.
Rodriguez P, Munroe D, Prawitt D, Chu LL, Bric E, Kim J, Reid LH, Davies C, Nakagama H, Loebbert R, Winterpacht A, Petruzzi MJ, Higgins MJ, Nowak N, Evans G, Shows T, Weissman BE, Zabel B, Housman DE, Pelletier J. 1997. Functional characterization of human nucleosome assembly protein-2 (NAP1L4) suggests a role as a histone chaperone. Genomics 44:253–265.
Ruiz MT, Pearson CE, Nielsen T, Price GB, Zannis-Hadjopoulos M. 1995. Cofractionation of HeLa cell replication proteins with *ors*-binding activity. J Cell Biochem 58:221–236.
Ruiz MT, Matheos D, Price GB, Zannis-Hadjopoulos M. 1999. OBA/Ku86: DNA binding specificity and involvement in mammalian DNA replication. Mol Biol Cell 10:567–580.
Santocanale C, Diffley JFX. 1996. ORC- and Cdc6-dependent complexes at active and inactive chromosomal replication origins in *Saccharomyces cerevisiae*. EMBO J 15:6671–6679.
Schmid CW, Manning JE, Davidson N. 1975. Inverted repeat sequences in the *Drosophila* genome. Cell 5:159–172.
Schmidt M, Migeon BR. 1990. Asynchronous replication of homologous loci on human active and inactive X chromosomes. Proc Natl Acad Sci USA 87:3685–3689.
Schroth GP, Ho PS. 1995. Occurrence of potential cruciform and H-DNA forming sequences in genomic DNA. Nucleic Acids Res 23:1977–1983.
Selsing E, Wells RD, Alden CJ, Arnott S. 1979. Bent DNA: visualization of a base-paired and stacked A-B conformational junction. J Biol Chem 254:5417–5422.

Shakibai N, Kumar V, Eisenberg S. 1996. The Ku-like protein from *Saccharomyces cerevisiae* is required in vitro for the assembly of a stable multiprotein complex at a eukaryotic origin of replication. Proc Natl Acad Sci USA 93:11569–11574.

Shibahara K-I, Stillman B. 1999. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. Cell 96:575–585.

Shinomiya T, Ina S. 1994. Mapping an initiation region of DNA replication at a single-copy chromosomal locus in *Drosophila melanogaster* cells by two-dimensional gel methods and PCR-mediated nascent-strand analysis: multiple replication origins in a broad zone. Mol Cell Biol 14:7394–7403.

Simon I, Cedar H. 1996. Temporal order of DNA replication. In: DePamphilis ML, editor. DNA replication in eukaryotic cells. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 271–293.

Sinden R. 1994. "DNA Structure and Function." San Diego: Academic Press, Inc.

Sinnett D, Woolf E, Xie W, Glatt K, Kirkness EF, Nielsen TO, Zannis-Hadjopoulos M, Price GB, Lalande M. 1996. Identification of a putative DNA replication origin in the gamma-aminobutyric acid receptor subunit β3 and α5 gene cluster on human chromosome 15q11–q13, a region associated with parental imprinting and allele-specific replication timing. Gene 173:171–177.

Small D, Nelkin B, Vogelstein B. 1982. Nonrandom distribution of repeated DNA sequences with respect to supercoiled loops and the nuclear matrix. Proc Natl Acad Sci USA 79:5911–5915.

Snyder M, Buchman AR, Davis RW. 1986. Bent DNA at a yeast autonomously replicating sequence. Nature 324:87–89.

Solovjeva L, Rozanov Y, Svetlova M, Hancock R, Zannis-Hadjopoulos M, Price GB, Tomilin N. 1998. Conformation of replicated segments of chromosome fibres in human S-phase nucleus. Chromosome Res 6:595–602.

Spector DH. 1992. Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific alpha-satellite DNA sequences. J Cell Biol 116:1095–1110.

Stillman B. 1993. Replicator renaissance. Nature 366:506–507.

Stillman B. 1996. Cell cycle control of DNA replication. Science 274:1659–1664.

Stinchcomb DT, Stuhl K, Davis RW. 1979. Isolation and characterisation of a yeast chromosomal replicator. Nature 282:39–43.

Strehl S, LaSalle JM, Lalande M. 1997. High-resolution analysis of DNA replication domain organization across an R/G-band boundary. Mol Cell Biol 17:6157–6166.

Struhl K, Stinchcomb DT, Scherer S, Davis RW. 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc Natl Acad Sci USA 76:1035–1039.

Tao L, Nielsen T, Friedlander P, Zannis-Hadjopoulos M, Price G. 1997. Differential DNA replication origin activities in human normal skin fibroblast and HeLa cell lines. J Mol Biol 273:509–518.

Tapiero H, Shaool D, Monier MP, Harel J. 1974. Replication of repetitious DNA in synchronized chick embryo fibroblast cells. Exp Cell Res 89:39–46.

Tasheva ES, Roufa DJ. 1994. Densely methylated DNA islands in mammalian chromosome origins. Mol Cell Biol 14:5636–5644.

Taylor JH. 1960. Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. J Biophys Biochem Cytol 7:455–464.

Taylor JH. 1977. Increase in DNA replication sites in cells held at the beginning of S phase. Chromosoma 62:291–300.

Ten Hagen KG, Gilbert DM, Willard HF, Cohen SN. 1990. Replication timing of DNA sequences associated with human centromeres and telomeres. Mol Cell Biol 10:6348–6355.

Thoma F. 1992. Nucleosome positioning. Biochim Biophys Acta 1130:1–19.

Todd A, Cossons N, Aitken A, Price GB, Zannis-Hadjopoulos M. 1998. Human cruciform binding protein belongs to the 14–3-3 family. Biochemistry 37:14317–14325.

Tóth EC, Marusic L, Ochem A, Patthy A, Pongor S, Giacca M, Falaschi A. 1993. Interactions of USF and Ku antigen with a human DNA region containing a replication origin. Nucleic Acids Res 21:3257–3263.

Tuteja N, Rahman R, Tuteja R, Ochem A, Skopac D, Falaschi A. 1994. Human DNA helicase II: a novel DNA unwinding enzyme identified as the autoantigen. EMBO J 13: 4991–5001.

Trivedi A, Waltz SE, Kamath S, Leffak M. 1998. Multiple initiations in the c-*myc* replication origin independent of chromosomal location. DNA Cell Biol 17:885–896.

Umek RM, Kowalski D. 1988. The ease of DNA unwinding as a determinant of initiation at yeast replication origins. Cell 52:559–567.

Vassilev LT, DePamphilis ML. 1992. Guide to identification of origins of DNA replication in eukaryotic cell chromosomes. Crit Rev Biochem Mol Biol :445–472.

Vaughn JP, Dijkwel PA, Hamlin JL. 1990. Replication initiates in a broad zone in the amplified CHO dihydrofolate reductase domain. Cell 61:1075–1087.

Virta-Pearlman VJ, Gunaratne P, Chinault C. 1993. Analysis of a replication initiation sequence from the adenosine deaminase region of the mouse genome. Mol Cell Biol 13:5931–5942.

Vishwanatha JK, Baril EF. 1990. Single-stranded DNA-dependent ATPase from HeLa cells that stimulates DNA polymerase α-primase activity: purification and characterization of the ATPase. Biochemistry 29:8753–8759.

Waltz SE, Trivedi AA, Leffak M. 1996. DNA replication initiates non-randomly at multiple sites near the c-*myc* gene in HeLa cells. Nucleic Acids Res 24:1887–1894.

Wang S, Dijkwel PA, Hamlin JL. 1998. Lagging-strand, early-labelling, and two dimensional gel assays suggest multiple potential initiation sites in the Chinese hamster dihydrofolate reductase origin. Mol Cell Biol 18: 39–50.

Ward GK, McKenzie R, Zannis-Hadjopoulos M, Price GB. 1990. Dynamic distribution and quantification of DNA cruciforms in eukaryotic nuclei. Exp Cell Res 188:235–246.

Ward GK, Shihab-El-Deen A, Zannis-Hadjopoulos M, Price GB. 1991. DNA cruciforms and the nuclear supporting structure. Exp Cell Res 195:92–98.

Weaver DT, DePamphilis ML. 1984. The role of palindromic and non-palindromic sequences in arresting DNA synthesis in vitro and in vivo. J Mol Biol 180:961–986.

Willard HF. 1977. Tissue-specific heterogeneity in DNA replication patterns of human X chromosomes. Chromosoma 61:61–73.

Willard HF, Latt SA. 1976. Analysis of deoxyribonucleic acid replication in human X chromosomes by fluorescence microscopy. Am J Hum Genet 28:213–227.

Williams JS, Eckdahl TT, Anderson JN. 1988. Bent DNA functions as a replication enhancer in *Saccharomyces cerevisiae*. Mol Cell Biol. 8:2763–2769.

Wilson DA, Thomas CA. 1974. Palindromes in chromosomes. J Mol Biol 84:115–144.

Wolffe A. 1995. Chromatin structure and function. San Diego: Academic Press.

Wolffe A. 1996. Chromatin structure and DNA replication: implications for transcriptional activity. In: DePamphilis ML, editor. DNA replication in eukaryotic cells. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 271–293.

Wu C, Friedlander P, Lamoureux C, Zannis-Hadjopoulos M, Price GB. 1993a. cDNA clones contain autonomous replication activity. Biochim Biophys Acta 1174:241–257.

Wu C, Zannis-Hadjopoulos M, Price GB. 1993b. In vivo activity for initiation of DNA replication resides in a transcribed region of the human genome. Biochim Biophys Acta 1174:258–266.

Wu Y, Hickey R, Lawlor K, Willis P, Yu F, Ozar H, Starr R, Quan JY, Lee M, Malkas L. 1994. A 17S multiprotein form of murine cell DNA polymerase mediates polyomavirus DNA replication in vitro. J Cell Biochem 54:32–46.

Yanishevsky RM, Prescott DM. 1978. Late S phase cells (Chinese hamster ovary) induce early S phase DNA labeling patterns in G1 phase nuclei. Proc Natl Acad Sci USA 75:3307–3311.

Zahn K, Blattner FR. 1987. Direct evidence for DNA bending at the lambda replication origin. Science 236:416–422.

Zannis-Hadjopoulos M, Price G. 1998. Regulatory parameters of DNA replication. Crit Rev Eukaryot Gene Expr 8:81–106.

Zannis-Hadjopoulos M, Persico M, Martin RG. 1981. The remarkable instability of replication loops provides a general method for the isolation of origins of DNA replication. Cell 27:155–163.

Zannis-Hadjopoulos M, Chepelinsky A-B, Martin RG. 1983. Mapping of the 3′-end positions of simian virus 40 nascent strands. J Mol Biol 165:599–607.

Zannis-Hadjopoulos M, Kaufmann G, Martin RG. 1984. Mammalian DNA enriched for replication origins is enriched for snap-back sequences. J Mol Biol 179:577–586.

Zannis-Hadjopoulos M, Kaufmann G, Wang S-S, Lechner RL, Karawya E, Hesse J, Martin RG. 1985. Properties of some monkey DNA sequences obtained by a procedure that enriches for DNA replication origins. Mol Cell Biol 5:1621–1629.

Zannis-Hadjopoulos M, Frappier L, Khoury M, Price GB. 1988. Effect of anti-cruciform DNA monoclonal antibodies on DNA replication. EMBO J 7:1837–1844.

Zannis-Hadjopoulos M, Pearson CE, Nielsen TO, Todd A, Price GB. 1994. Autonomous replication in vivo and in vitro of clones spanning the region of the DHFR origin of bidirectional replication (*ori*β). Gene 151:273–277.

Zheng G, Kochel T, Hoepfner RW, Timmons SE, Sinden RR. 1991. Torsionally tuned cruciform and Z-DNA probes for measuring unrestrained supercoiling at specific sites in DNA of living cells. J Mol Biol 221:107–129.