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TRANSCRIPTION FACTORS IN DNA REPLICATION

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1. ABSTRACTS

Accumulating evidence suggests the involvement of transcription factors in the regulation of DNA replication in eukaryotic cells. Almost all eukaryotic DNA viruses contain binding sites for transcription factors which function as auxiliary elements for DNA replication initiation at replication origins, and, indeed, the binding of transcription factors to these elements has been shown to stimulate DNA replication. Transcription factors also regulate some of the chromosome DNA replication origins of budding yeast, indicating that transcription factor involvement in DNA replication is not restricted to viruses. Consistent with this notion, recently determined replication origins of higher eukaryotes have been found occasionally to associate with transcription factor binding sites, although there is no direct evidence for the involvement of the factors that bind to these sequences in DNA replication. Analyses using viral and yeast systems have suggested that transcription factors stimulate the formation of the replication initiation complex by engaging in specific interactions with proteins of the initiation complex and/or by modulating the repressive chromatin structure around origins of replication. These mechanisms are analogous to those advanced to explain stimulation of transcription by transcription factors. The accumulated data suggests that transcription factors play a general role in the formation of functional complexes on chromosomes.

2. INTRODUCTION

A number of events occurring on chromosomes such as DNA replication, transcription and site specific recombination require the prior formation of active complexes at defined positions on the chromosome. In transcription, the best characterized nuclear event, specific initiation complexes for transcription containing RNA polymerase are formed at certain gene promoters. This process is regulated by sequence-specific DNA binding transcription factors, which bind to the regulatory region of each gene. Recently, *in vitro* and *in vivo* analyses have revealed how transcription factors activate transcription. Basically, transcription factors enhance the formation of the initiation complex at a promoter by two mechanisms. The first one is recruitment of the initiation complex to the promoter through specific protein-protein interaction between transcription factors and components of the complex (82). The second one is modulation of chromatin structure around the promoter sequence, which serves to counteract the inhibitory effect of chromatin on transcription. In this case, transcription factors interact with protein complexes that modulate chromatin structure (103). An important feature of certain transcription factors is the regulation of their activity by signal transduction pathways which transmit signals that reflect the physiological context of the cell. In other words, transcription factors are nuclear targets of extra- and inner-cellular stimuli.

Recently, an increasing body of evidence indicates that some transcription factors are involved in the regulation of DNA replication. This was first suggested by the study of the replication of certain eukaryotic DNA viruses such as adenovirus, papovaviruses and papillomaviruses, and later by the analyses of the DNA

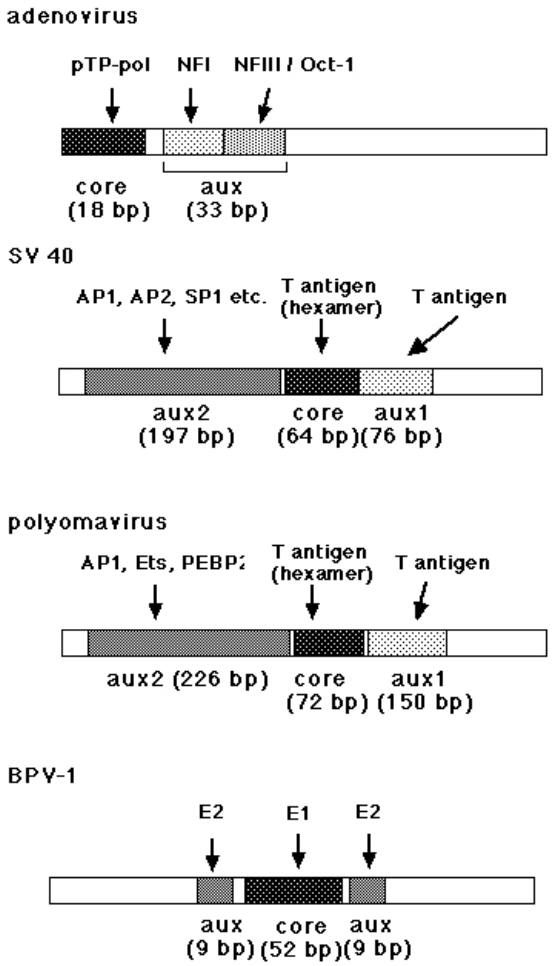

Figure 1. Structure of replication origin of various eukaryotic DNA viruses. Proteins which binds to the each elements are also indicated. For detail, see text. core: core origin, aux: auxiliary elements.

replication origins of the budding yeast, *Saccharomyces cerevisiae*. The recently determined origins of higher eukaryotes also contain binding sites for transcription factors, indicating possible involvement of transcription factors in the regulation of their DNA replication activity (23, 24).

In this review, we first provide an overview of the structure of eukaryotic replication origins and of the transcription factors involved in their replication activity. And then we focus on the mechanisms by which transcription factors stimulate DNA replication. Finally, we discuss the general role of transcription factors in various nuclear processes.

3. STRUCTURE OF DNA REPLICATION ORIGINS AND TRANSCRIPTION FACTORS

3.1. Viral origins

Eukaryotic DNA viruses have compact and simple replication origins composed of a core sequence which is recognized by viral coded initiator proteins and auxiliary sequences which enhance the core origin activity. In almost all cases, the auxiliary sequences contain binding sites for various transcription factors. The involvement of transcription factors in DNA replication has been clearly indicated in adenovirus, papovaviruses including simian virus 40 (SV40) and polyomavirus (Py), and papillomaviruses.

3.1.1. Adenovirus

Human adenoviruses have linear genomes and initiate DNA replication through a protein priming mechanism in which the 3'-OH group of dCMP bound to the viral precursor terminal protein (pTP) serves as a primer for DNA synthesis (85). Adenovirus was the first eukaryotic virus for which an *in vitro* replication system was developed (1). The *in vitro* system enabled the identification of viral and cellular proteins involved in DNA replication. Three viral coded proteins, pTP, viral DNA polymerase (pol) and DNA binding protein, form an initiation complex on the 18 bp long core origin which is located at the end of the genome (figure 1) (93). In addition to the three viral proteins, at least two host proteins, nuclear factor I (NFI) (53, 71, 84) and nuclear factor III (76, 80), which is identical to octamer-binding factor Oct-1 (76, 81), bind to sequences adjacent to the core origin. NFI binds as a dimer to the partially symmetric sequence located at nucleotides 25-38 on adenovirus type 2 and type 5 origins (22, 72). Oct-1 binds immediately next to the NFI site to a sequence known as the "octamer" sequence (18, 67). NFI and Oct-1 independently stimulate adenovirus replication about 60-fold and 3-7 fold, respectively, *in vitro* and together stimulate by up to 200-fold (18, 67, 68). The DNA binding domain of either protein is sufficient for stimulation (30, 63, 99).

3.1.2. SV40

The SV40 genome is a 5 kb long circular DNA that carries a single replication origin. The core sequence of the origin is 72 bp long and serves as a binding site for the viral coded large T antigen (T antigen). T antigen is the only viral protein required for DNA replication, as all the other replication factors are supplied by the host cell (13, 40, 91). T antigen acts as a initiation complex for DNA replication by forming a double hexamer on the core origin in the presence of ATP (10). There are two auxiliary sequences located adjacent to the origin (figure 1) which synergistically activate initiation of DNA replication by up to 100 fold, depending on the conditions and the methods used to measure DNA replication (32, 34). Aux1 is located at the early side of the core sequence and contains T antigen binding sites (33). Aux2, which is located on the late side of the core origin, contains binding sites for various transcription factors including SP1, AP1, AP2, AP4 and p53. The Aux2 sequence enhances SV40 DNA replication between 10 and 100 fold *in vivo*, depending on the assay conditions (31). Aux2 can be replaced with tandem multimers of single transcription factor binding sites such as those of SP1, AP1, ATF and NFI (16, 31, 36, 51). However, binding sites for the transcription factor Gal4 cannot substitute for the native binding sites, even when fusion proteins containing the Gal4 DNA binding domain fused to strong transcription activation domains

such as those of VP16 or c-Jun are exogeneously expressed (31).

3.1.3. Polyomavirus

Polyomavirus (Py) is closely related to SV40 and its origin structure is similar to that of SV40 (figure 1). Like SV40, a 68 bp-long core origin, which provides the binding sites for T antigen, is associated with two auxiliary sequences (figure 1). Aux1 contains the T antigen binding sites and Aux2 comprises the transcriptional enhancer which is made up of various transcription factor binding sites (31, 79). The requirement for Aux2 in Py DNA replication is more strict than in SV40. In the absence of the enhancer, Py replicates very poorly and the enhancer stimulates replication activity by more than 200-fold (21). Interestingly, the enhancer determines the tissue specificity of Py DNA replication (21). Py with the wild type enhancer cannot replicate in hematopoietic cell lines, even when large amounts of T antigen are supplied exogeneously. However, Py is able to replicate in hematopoietic cells when the native enhancer is replaced with the enhancer of the immunoglobulin gene. Like SV40, the enhancer of polyomavirus can be replaced by a single kind of transcription factor binding site and many kinds of transcription factors including AP1, Ets, c-Rel, p53, Gal4 , BPV E2 and Gal4-VP16 have been shown to stimulate Py DNA replication (6, 8, 31, 41, 46, 66, 69, 70, 102). Except for c-Rel (41), these transcription factors stimulate DNA replication through their transcription activation domains which are distinct from their DNA binding domains. The activation domain for DNA replication usually overlaps with that for transcription. In some cases, however, both activities cannot be delimited to the same domain (41, 46). The transcription factor binding sites must be located close to the late side of the core origin for efficient stimulation. Increasing the distance between the core origin and the binding sites results in a sharp decrease in replication activity. When the AP1 binding site is located 120 bp from the late side of the core origin, AP1 no longer stimulates DNA replication (70). This feature distinguishes replication enhancers from transcription enhancers which can activate transcription when located even more than 2 kb away from the promoter.

3.1.4. Papillomaviruses

The papillomaviruse genome comprises a circular DNA that is slightly larger than that of papovaviruses. Two viral coded early proteins, E1 and E2, are required for DNA replication (95). E1 is an initiator protein that has equivalent activities to those of T antigen of SV40 and Py. Namely, E1 also possesses origin binding and helicase activities (60, 89, 90, 95, 104, 105). On the other hand, E2 is a viral coded transcription factor. The basic structure of the origins of papillomaviruses show significant similarity to those of SV40 and Py: the core sequence contains the recognition sequence for the initiator protein (E1BS) and AT rich sequences and transcription factor (E2) binding sites (E2BS) are also present (96) (figure 1). All papillomavirus origins contain one or more E2BS that are essential for origin activity *in vivo* except for human papillomavirus-1 (HPV-1) which can replicate without E2BS and E2, albeit with low efficiency (29). On the contrary, in HPV-11 and HPV-18, significant replication activity can be observed with an fragment containing only two high-affinity E2BS, although E1 is still required for DNA replication (50, 59, 92). This suggests that E2 determines the specificity of DNA binding and recruits E1 to the origin (see Section 2.1)

3.2. Cellular origins

3.2.1. *Saccharomyces cerevisiae*

The Budding yeast, *Saccharomyces cerevisiae*, has compact origins similar to those of the viral origins described above. Each origin is 100-120 bp long and consists of an essential A element and auxiliary B elements (12, 62). The A element contains an 11-bp-long ARS consensus sequence which is recognized by a six-subunit complex, ORC (origin recognition complex) (9). ORC functions as a landing pad for other factors required for the initiation of DNA replication such as CDC6 and MCMs (25). In contrast, B elements are not conserved among ARS sequences, although some of them are interchangeable between different ARS elements (39, 83, 94). ARS1 is the best characterized ARS. One of the B elements of ARS1, B3, serves as a binding site for a transcription factor, Abf1, and mutations in B3 that prevent the binding of Abf1 cause significant loss of ARS activity, indicating that Abf1 activates ARS activity (62). In addition, the B3 element can be replaced with the binding site of the GAL4 transcription factor, which acts as a strong transcriptional activator. Moreover, when the GAL4 DNA binding domain is fused to the acidic transcriptional activation domains of other activators, such as those of p53 or VP16, these fusion proteins can also activate ARS activity, indicating that acidic activation domains have the ability to enhance the initiation of DNA replication as well as that of transcription (57, 62). All the experiments described above used the assay (so called "Stability assay") which measured the mitotic stability of plasmids carrying the ARS. However, it was also confirmed that the requirement of B3 element of ARS1 on the chromosome (61) and moreover, the Gal4-fused to p53 activation domain was shown to activate ARS1 on the chromosome (57).

ARS121 also has Abf1 binding sites in addition to the essential A element and these sites enhance ARS activity. In this case, the Abf1 binding site can be placed more than 2 kb from the A element, showing that Abf1 can stimulate the initiation of DNA replication from a distance (100). On the chromosome, origins are surrounded by the genes each of which has transcription factor binding sites. Moreover, the plasmids used in the stability assay carry a selectable marker gene and a centromere in addition to an ARS. Therefore, it is very likely that the function of transcription factors in the ARS or the origin is influenced by the transcription factors binding to the outside of the ARS or the origin. Indeed, when the replication activity of ARS by itself, devoid of these extraneous transcription elements, was measured directly using the *Dpn*I assay, which relies on the fact that *Dpn*I only cuts unreplicated DNA and measures the accumulation of DNA in the cells during a given period, results different from those of the stability assay were obtained. Under these conditions, B3

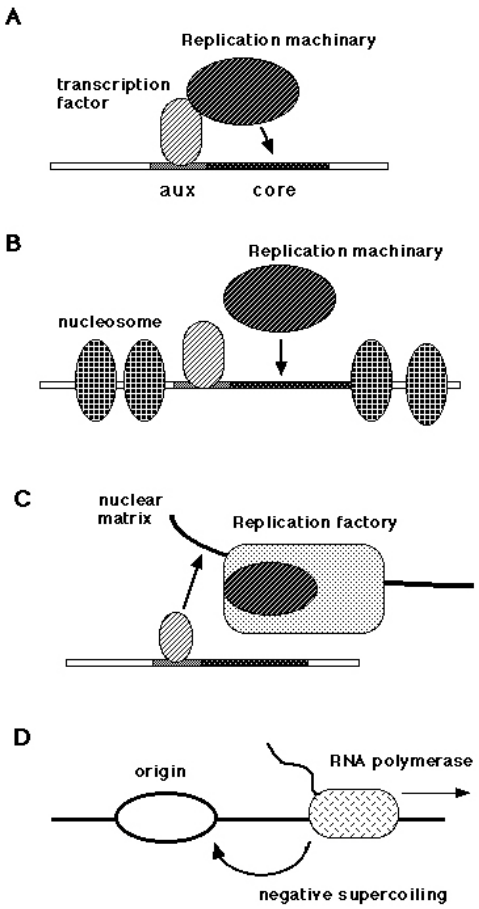

Figure 2. Schematic indication of mechanisms by which transcription factors stimulate DNA replication. A. Recruitment of the replication machinery to the core origin through protein-protein interaction. B. Modulation of chromatin structure to allow the binding of replication proteins to the core origin. C. Recruitment of origin to the replication factory formed on the nuclear matrix. D. Transcription activation of DNA replication.

element no longer stimulated the replication of ARS1. Exogenous expression of the strong transcriptional activators Gal4 and Gal4-VP16 inhibited the replication activity of ARS1 when B3 was replaced by a Gal4 binding site. In addition, a chromosomally inactive ARS, ARS301, which was active by itself on a plasmid, was inactivated by placing an Abf1 binding site in its vicinity. These results suggest that the sequences surrounding the ARS as well as properties of the ARS element itself determine its response to transcription factors (48).

3.2.2. Higher eukaryotes

In contrast to eukaryotic DNA viruses and budding yeast, the replication origins of multicellular organisms are larger and more complicated (23). However, various mapping techniques have enabled the localization of initiation sites of DNA replication on the chromosomes of multi-cellular organisms. Interestingly, many of the origins so far analyzed have mapped to the regulatory or putatively regulatory regions of a number of genes, including those of *c-myc* (54, 98), *ppv1* , which locates at the 3' region of lamin B2 gene and is provisionally named, (28), beta-globin (47) and rat aldorase B genes (107). In other words these initiation sites comprise potential binding sites for transcription factors. However, the functional importance of these transcription factor binding sites for the initiation of DNA replication is still not known because no mutational analysis has been performed. One exception was the isolation of an ARS sequence from human cells using the nuclear retention sequence of Epstein Bar Virus 1 to keep the plasmids in the nucleus. An approximately 20 kb DNA fragment derived from the human genome showed significant ARS activity in the 293 human cell line. Deletion analysis revealed a 25 bp long element that enhanced the replication activity of the ARS element about 2-fold. Interestingly, this element showed silencing activity when placed upstream of the promoter. Mutational analysis indicated that the sequences required for the repression of transcription were the same as those required for the enhancement of replication. The sequence contains the consensus sequence for the binding of transcription factor YY-1. Therefore, it seems that YY-1 enhances ARS activity in human cells (77).

4. HOW DO TRANSCRIPTION FACTORS STIMULATE INITIATION OF DNA REPLICATION?

The mechanistic aspects of transcription factor stimulation of DNA replication were mainly uncovered using viral systems because *in vitro* assays for the replication of a number of viral DNAs were readily available. These studies revealed similar mechanisms to those involved in the stimulation of transcription, namely recruitment of the replication machinery through protein-protein interactions and modulation of chromatin structure. In addition, a recent study has suggested the importance of nuclear substructure and transcription in the stimulation of DNA replication by transcription factors. These various mechanisms may function cooperatively rather than exclusively

4.1. Recruitment of the replication machinery through protein-protein interaction.

As in transcription activation, the recruitment of the replication machinery appears to be an important mechanism for transcription factor stimulation of DNA replication in various systems (figure 2A).

In adenovirus DNA replication, transcription factor NFI stimulates initiation in a pTP-pol complex concentration-dependent manner, suggesting that both proteins interact (67). Indeed NFI interacts directly with polymerase in the pTP-pol complex through its DNA binding domain (11, 15, 67) and a mutational study indicates that this interaction is required for stimulation (5). Detailed analysis *in vitro* indicated that NFI stabilizes the pTP-pol complex on the core origin about 10-fold (68). Another transcription factor, Oct-1, also interacts with the pTP-pol complex, but this interaction is mediated by the pTP moiety rather than by the polymerase itself through the

DNA binding domain of Oct-1 (POU domain) (18). Like NF1, the protein-protein interaction leads to increased binding of pTP-pol to the core origin, reflecting the reduced off-rate of pTP-pol (97) The synergistic stimulation of DNA replication by these factors may reflect the fact that NFI and Oct-1 can interact with different subunits of the pTP-pol complex.

The initiation steps of Py DNA replication are very similar to those of SV40 (58, 101): T antigen forms a double hexamer on the core origin in a ATP-dependent manner and then each T antigen hexamer starts unwinding the origin in opposite directions in the presence of the single stranded DNA binding protein, RP-A. Transcription factor, AP1, which consists of a c-Jun/c-Fos heterodimer or a c-Jun homodimer, stimulates the unwinding reaction about 5-fold *in vitro* when its binding site is located on the late side of the core origin in place of Aux2. Efficient stimulation is observed at limiting concentrations of T antigen for unwinding, suggesting an interaction between c-Jun and T antigen. Under similar conditions, Py DNA replication is stimulated to a comparable extent by AP1 in a purified *in vitro* replication assay. c-Jun stimulates the ATP-dependent complex formation of T-antigen on the core origin. The activities of c-Jun that stimulate unwinding and origin binding of T antigen are harbored within the N-terminal region of c-Jun, which is distinct from the DNA binding domain. Furthermore, c-Jun interacts directly with T antigen, suggesting that c-Jun enhances T-antigen complex formation on the core origin through this interaction (43). In contrast, some transcription factors including p53, BPV E2, VP16 and c-Fos, which are known to stimulate Py DNA replication, interact with RP-A through their activation domains (26, 35, 56). Therefore, it is plausible that RP-A recruitment to the origin may be the mechanism through which these factors stimulate Py DNA replication, although direct evidence for this is still lacking.

Among papillomaviruses, bovine papillomavirus type-1 (BPV) has been the most extensively studied. The recent development of an *in vitro* replication system for BPV has enabled extensive study of the role of E2 protein in BPV DNA replication. In contrast to *in vivo*, requirement for E2 is not strict and DNA replication can take place even in the absence of E2 *in vitro*. However, addition of E2 to the reaction increased replication activity and origin specificity (105). E1 binds to the origin with low sequence specificity, but in the presence of E2, E1 and E2 bind to the core origin cooperatively with high affinity and specificity (60, 65, 87, 88, 90). As a result, unwinding from the core origin by E1 is enhanced by E2 (90). E1 and E2 physically associate with each other and the interaction is required for the stimulation of BPV DNA replication *in vivo* and for the cooperative binding of E1 and E2 to the origin *in vitro*. A more detailed analysis performed by Sanders and Stenlund revealed that E2 acts to assemble E1 into an active complex on the origin in two steps. First, E1 and E2 cooperatively bind to the origin, generating a sequence-specific origin recognition complex. In the second step, E2 is displaced and additional E1 molecules are incorporated in a ATP-dependent manner. As a result, an active complex with low-sequence specificity is deposited on the specific origin sequence by transcription factor E2 (86) Therefore, like DnaC protein, which loads DnaB helicase onto the chromosomal origin (*oriC*) of *Escherichia coli*., E2 works as a loading factor for E1. This mechanism explains well the observation that a high affinity E2 binding site can serve as a replication origin in HPV-11 and -18 as described earlier in a previous section (1.1.4).

4.2. Modulation of chromatin structure

Transcription is generally repressed by chromatin structure, and transcription factors are also thought to release transcription from the repressive effect of chromatin by modulating chromatin structure (103). There is some evidence showing that a similar mechanism may be working for the enhancement of DNA replication by transcription factors (figure 2B).

Pre-assembly of template DNA into chromatin inhibits SV40 DNA replication *in vitro*. Transcription factor NFI prevents the repression of DNA replication when the Aux2 is replaced by an NF1 binding site and NFI is included in the chromatin assembly reaction (16). A similar anti-repression effect is observed for Gal4-VP16 (17). In BPV DNA replication, E2 or Gal4-VP16 could also alleviate nucleosome repression (55). However, there are some arguments against the model when anti-repression is proposed to be the primary cause of the enhancement of DNA replication. For example, Gal4-VP16, which can alleviate repression by nucleosomes *in vitro*, did not stimulate SV40 DNA replication *in vivo* (31) and pre-binding of T antigen to origin DNA is sufficient to overcome chromatin repression (42). Thus, more detailed *in vivo* studies are required to establish the anti-repression effect as one of the prime roles of transcription factors in viral DNA replication.

In contrast, the remodeling of chromatin was recently shown to be involved in the activation of cellular origins by transcription factors in *S. cerevisiae* (38). The acidic transcriptional activation domain of breast cancer protein BRCA1 can activate the replication of ARS1 when fused to the Gal4 DNA binding domain and Gal4 binding sites are inserted into the region proximal to ARS1. At the same time, this fusion protein altered the chromatin structure around the replication origin. Mutations in BRCA1 that abolish transcriptional activation also prevent chromatin remodeling and activation of replication, suggesting that chromatin modulation is important for the stimulation of DNA replication. In native ARS1, transcription factor Abf1, which has a positive role in DNA replication, was shown to modulate chromatin structure. Therefore, increasing chromatin accessibility by modulating chromatin structure may be an important mechanism for transcription factor stimulation of DNA replication at cellular origins.

A recent study indicated that a transcriptional co-activator, which bound to a transcriptional activation domain of some transcription factors, had histone acetyltransferase activity and that the activity was required for activation of transcription. Nucleosomes containing acetylated histones possess a more "accessible" structure

compared to weakly acetylated nucleosomes. On the other hand, there are a number of "chromatin remodeling factors" in eukaryotic cells, which function as multi-subunit complexes to alter chromatin structure in a ATP-dependent manner. Chromatin remodeling factors are also thought to participate in transcriptional regulation. Interestingly, it was shown that chromatin remodeling by chromatin accessibility complex (CHRAC) at the SV40 origin facilitates the binding of T antigen to the origin covered by nucleosome *in vitro* (2). Furthermore, it was shown that E1 of HPV-18 interacts with one of the members of the chromatin remodeling factor family and that the interaction was required for HPV DNA replication (52). These results suggest that chromatin remodeling factors play a role in DNA replication. Therefore, it is attractive to speculate that transcription factors recruit co-activators harboring histone acetyltransferase activity and/or chromatin remodeling factors to origins in order to provide a more suitable chromatin structure for the initiation of DNA replication.

4.3. Nuclear matrix and replication factory

Some evidence exists to show that eukaryotic DNA replication takes place in nuclear compartments termed "replication foci", which can be visualized by immunolabeling of the incorporated analog, 5-bromodeoxyuridine (BrdU) (20, 27, 64, 73-75). The foci are attached to the ribonucleic protein structure, the nuclear matrix (also called nuclear scaffold or skeleton), which have been shown to contain proteins involving in DNA replication, such as DNA polymerase alpha, PCNA, and RP-A (37). Such foci have been referred to as "replication factories". Viral DNA replication also seems to take place within similar sub-nuclear structures. For example, adenovirus and herpes simplex virus were shown to replicate within such nuclear substructures (49, 106). Recently, it was shown that the nuclear matrix associated transcription factor PEBP2alphaB1(alphaB1) can stimulate Py DNA replication through PEBP2 binding sites. PEBP2 is an heterodimeric transcription facotr consisting of PEBP2alpha and beta subunits. Interestingly, one of the members of PEBP2alpha family, alphaB1, which are involved in the differentiation of hematopoietic cells, exclusively localizes to the nuclear matrix (14, 44). For the stimulation of Py DNA replication, alphaB1 needed to be attached to the nuclear matrix (14). Py T antigen was also found to be localized to the nuclear matrix, but was not co-localized with alphaB1. However, in the presence of a plasmid containing the Py origin and PEBP2 binding sites, T antigen was co-localized with alphaB1 and formed distinct foci. Staining with BrdU revealed that the foci contained newly synthesized DNA, indicating that the foci represent replication factories for Py (L.-F. Chen, Y. Murakami and Y. Ito unpublished results). These results suggest that alphaB1 recruits the origin of Py DNA to the nuclear matrix and promotes formation of the replication factory (figure 2C). The attachment of cell factors to the nuclear matrix is not limited to alphaB1. Other transcription factors such as Gal4-VP16 also bind to the nuclear matrix (14). In addition, the region containing the replication origin was shown to bind to the nuclear scaffold in both budding yeast and fission yeast (3, 4). Therefore, the nuclear matrix may have an important role in the regulation of DNA replication and the recruitment of origins to the nuclear matrix may furnish the necessary conditions for transcription factor enhancement of viral and cellular DNA replication.

4.4. Transcriptional activation of DNA replication

In lambda phage and *oriC* replication in *E. coli*, transcription of the template DNA in the vicinity of the origin activates DNA replication (7). Therefore, transcription plays some regulatory role in DNA replication in prokaryotic systems. In polyomavirus and papillomaviruses, replication origins are located upstream of the early promoter. However, deletion of the promoter did not affect replication *in vivo* or *in vitro* (79). Although the replication origin of SV40 contains the early promoter, it was shown that inhibition of transcription does not affect replication *in vitro*. Thus, transcription *per se* is unlikely to provide a valid explanation for transcription factor involvement in the replication of these DNA viruses. Some cellular origins in higher eukaryotes are mapped in regions containing promoters for such genes as human c-myc, beta-globin and the rat aldolase. Ihsimi and colleagues indicated that transcription can induce replication from replication origins located near the promoter region of the human c-myc gene in a model replication system (78). In this model system, DNA replication was induced by the unwinding of the duplex DNA at the origin sequence as a result of the negative supercoiling introduced by transcription (figure 2D). It remains to be determined whether transcriptional activation of DNA replication can take place through this mechanism in vivo.

5. PERSPECTIVES

As discussed in this review, transcription factors regulate DNA replication as well as transcription. In addition, transcription factors are also involved in the initiation of meiotic recombination in *S. cerevisiae* and *S. pombe*. From these findings and considering the necessity for the formation of protein complexes at specific sites on chromosomes as a first step in many nuclear events, we suggest that transcription factor activity is not limited to only transcription, but plays a more general role in the formation of multi-functional complexes on the chromosome. To achieve this role, transcription factors must possess polyvalent functions as discussed in the previous sections. One of these functions, chromatin remodeling, may be a common mechanism for many, if not all, nuclear events requiring transcription factors. The importance of chromatin remodeling in the regulation of transcription has recently been firmly established (103). Chromatin remodeling by transcription factors was also shown to be important for hot spot activity in meiotic recombination. In addition to chromatin remodeling, recruitment of transcription factors to sub-nuclear compartments on the nuclear matrix may play a general role in the control of a number of nuclear processes. Interesting in this respect, transcription has also been suggested occur in a sub-nuclear compartment on the nuclear matrix (19, 45).

In contrast, the recruitment or stabilization of an initiation complex by virtue of its interaction with a transcription factor may be a highly specific process for

each nuclear event. Transcription factors most likely contribute to this specificity by interacting with only specific sites on the chromosome and by engaging in privileged interactions with specific components of each type of nuclear machinery.

Since the activity of many transcription factors is regulated by signal transduction pathways and finely tuned to the physiological state of the cell, the involvement of transcription factors in multiple chromosomal processes is attractive from the point of view of the integration of the cell's regulatory machinery. Further analysis of the role of transcription factors in DNA replication and comparison of their roles in other processes should shed light on their general role in cell regulation.

6. ACKNOWLEDGMENTS

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