Sequence-specific recognition of DNA by proteins is essential for both transcription and DNA replication. Transcription factors form a growing family of regulatory proteins that can positively or negatively influence transcription by binding to regulatory elements in DNA contacting components of the basal transcription machinery. In recent years, it has become clear that several transcription factors are multifunctional and also directly influence initiation of DNA replication. This was first detected during studies of the replication of eukaryotic viruses, such as adenovirus and papovaviruses, but it may well be a more general phenomenon. Many viral origins consist of a core origin and auxiliary regions that contribute to the initiation of replication and are required for optimal viral growth (for review, see DePamphilis 1988, 1993a; Challberg and Kelly 1989; Stillman 1989). These auxiliary regions contain transcription factor-binding sites. In polyomavirus, presence of the transcriptional enhancer stimulated DNA replication up to 1000-fold, and presence of the enhancer increased SV40 replication approximately 100-fold. The auxiliary region of adenovirus stimulates initiation of replication up to 200-fold, although this region is not directly involved in transcription. The transcription factors used for enhancement of replication can be cellular as well as viral. Cellular transcription factors are involved in adenovirus, SV40, and polyomavirus replication. For papillomavirus and Epstein-Barr virus, the viral proteins (BPV-E2, EBNA1) combine functions in transcription and replication, as is the case for T antigen of SV40 and polyomavirus.

The availability of initiation systems that can be reconstituted with purified proteins for adenovirus and papovaviruses, combined with effi-
cient transfection assays with origin-containing plasmids, has facilitated studies of the mechanism by which transcription factors enhance initiation. A number of distinct modes of action have been discovered. As a general rule, transcription factors enhance replication by facilitating rate-limiting steps in the initiation process. As summarized in Table 1 and Figure 1, these steps include:

1. Recruiting initiation proteins such as adenovirus DNA polymerase, the single-strand binding protein RP-A, or BPV-E1 helicase to the origin. By binding these proteins and targeting them to the origin employing their DNA-binding domains, transcription factors can facilitate the assembly of a multiprotein preinitiation complex and also stabilize such a complex. This method of action requires, understandably, a strict positioning of the transcription factors relative to the core origin.

2. Changing the activity of initiation proteins. Several examples exist in which the helicase activities of SV40 T antigen or BPV-E1 are influenced by transcription factors.

3. Induction of structural changes in origin DNA. This could lead to facilitated formation of a preinitiation complex by bringing initiation proteins closer together or by winding DNA on the surface of a multiprotein complex. Many transcription factors such as ABF-1, NFI, and Oct-1 can bend DNA. Bending could also be instrumental in origin opening.

4. Global changes in the chromatin structure surrounding the origin. Opening up the nucleosome structure could prevent the inhibitory effect of histones and nucleosomes on initiation (antirepressor effect) and could lead to a destabilization of the origin region, which could facilitate binding of initiation proteins. For this mode of action, the position of the transcription factor-binding site relative to the core origin is less fixed.

Of course, these various mechanisms are not mutually exclusive and may operate together to optimize the initiation process. The contribution of each individual mechanism depends on the system and the conditions. No evidence has been obtained so far that transcription factors also are involved in elongation or even origin clearance. On the contrary, several transcription factors seem to contact their targets only during a limited time span, being released soon after initiation or even before the actual initiation reaction has taken place.

Whether transcription factors employ the same mechanisms to enhance both processes, DNA replication and transcription, is not yet clear.
Global changes of chromatin structure could be instrumental in both processes. Such changes require, in general, the transcription activation domains of the transcription factors. On the contrary, interactions with initiation proteins are specific and use protein interfaces that are different from the ones involved in contacting the transcription machinery.

In most cases, the role of transcription factors in DNA replication is independent of the transcription process itself. Bound transcription factors do not appear to activate DNA replication by increasing transcription from, or through, the origin of DNA replication. An exception is mitochondrial DNA replication (Clayton 1991). Similar to prokaryotic systems (Baker and Kornberg 1992), the role of transcription factors is to regulate the synthesis of transcripts that act as a primer or a regulator. This is beyond the scope of this chapter; mitochondrial DNA replication is discussed in detail in Clayton (this volume). Below, I focus on recent results obtained in several viral systems as well as in yeast. Several reviews on the role of transcription factors in DNA replication have appeared previously (DePamphilis 1988, 1993b; Van der Vliet 1991; Heintz 1992).

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### Table 1: How can transcription factors enhance replication?

<table>
<thead>
<tr>
<th>System</th>
<th>Transcription factor</th>
<th>Target protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment of replication proteins (leading to assembly and stabilization of a multimeric initiation complex)</td>
<td>Ad</td>
<td>NFI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct-1</td>
</tr>
<tr>
<td>polyoma</td>
<td>T-ag</td>
<td>T-ag, pol-α, RP-A</td>
</tr>
<tr>
<td></td>
<td>AP1</td>
<td>RP-A</td>
</tr>
<tr>
<td>SV40</td>
<td>T-ag</td>
<td>T-ag, pol-α, RP-A</td>
</tr>
<tr>
<td></td>
<td>SP1</td>
<td>?</td>
</tr>
<tr>
<td>BPV</td>
<td>E2</td>
<td>E1</td>
</tr>
<tr>
<td>EBV oriP</td>
<td>EBNA1</td>
<td>EBNA1</td>
</tr>
<tr>
<td>oriLyt</td>
<td>BLTF-1</td>
<td>?</td>
</tr>
<tr>
<td>Changing the activity of an initiator protein</td>
<td>SV40</td>
<td>T-ag</td>
</tr>
<tr>
<td>BPV</td>
<td>E2</td>
<td>E2 helicase</td>
</tr>
<tr>
<td>Changing the global architecture or nucleosome structure of the origin, e.g., by bending or looping</td>
<td>Ad</td>
<td>Oct-1</td>
</tr>
<tr>
<td>BPV</td>
<td>E2</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>ABF-1</td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>EBNA1</td>
<td></td>
</tr>
</tbody>
</table>

Only naturally bound transcription factors are mentioned. For substitution of binding sites by synthetic ones, see Table 2. (T-ag) Large T antigen.
Figure 1  Ways in which transcription factors can stimulate initiation of DNA replication. (A) Direct contacts: (1) A core origin binding protein (CoBP) is recruited by a transcription factor (TF) binding to the auxiliary origin region (aux). Formation of a CoBP-TF complex may occur in solution or on the DNA. In this way a transcription factor stabilizes binding of CoBP to the core origin and may also influence the activity of CoBP. (2) A transcription factor may also recruit auxiliary initiation proteins, e.g., RP-A, that could help unwind the origin. (B) Structural changes in DNA or chromatin. (1) Bending of DNA by a transcription factor could bring two essential components, CoBP and X, another protein of the initiation complex, in close proximity. (2) Transcription factors disrupt the nucleosome structure, thereby enabling the initiation proteins to bind to the core origin (antirepressor effect).
VIRAL SYSTEMS

Adenovirus

The 36-kbp linear genome of human adenoviruses replicates via protein-primed initiation followed by elongation via a displacement mechanism (see Hay, this volume). The development of an in vitro initiation system has led to the purification of novel viral and cellular proteins that could be used to reconstitute efficient replication in vitro. Genetic and biochemical analysis indicates that three viral proteins are required for replication. These are the precursor terminal protein (pTP) and the viral DNA polymerase (pol), forming a pTP-pol heterodimer, and the DNA-binding protein (DBP).

The viral proteins can only sustain a very limited level of initiation, and two nuclear transcription factors stimulate initiation considerably. These are nuclear factor 1 (NFI, Nagata et al. 1982; Leegwater et al. 1985; Rosenfeld and Kelly 1986) and nuclear factor III (Pruijn et al. 1986; O’Neill and Kelly 1988). Nuclear factor III is identical to the octamer-binding transcription factor Oct-1 (O’Neill et al. 1988; Pruijn et al. 1989). NFI and Oct-1 function by binding to the origins of replication located at each molecular end. These consist, for Ad2 and Ad5, of a conserved core region, located at nucleotides 9–18 from the termini, two less well conserved space regions (1–9 and 18–24), and an auxiliary region (25–55). The core region binds pTP-pol, and the auxiliary region represents the binding site for NFI and Oct-1. The various domains within the origin are closely spaced, and enhancement of replication by NFI and Oct-1 depends critically on the spatial arrangement of their binding sites (Adhya et al. 1986; Wides et al. 1987; Bosher et al. 1990; Coenjaerts et al. 1991), suggesting essential protein-protein interactions.

How Does NFI Stimulate Initiation?

Human NFI is the collective name of a family of transcription factors (52–66 kD) generated by differential splicing from a single gene. They contain a highly conserved DNA-binding and dimerization domain (NFI-BD) located within the 220–240 amino-terminal amino acids (Paonessa et al. 1988; Santoro et al. 1988; Meisterernst et al. 1989). This region suffices for stimulation of replication (Mermod et al. 1989; Gounari et al. 1990). The NFI-BD dimer binds to positions 25–39 by contacting two successive regions in the major groove of the DNA, with a consensus TGGCA-N5-GCCAA (Nagata et al. 1983; Gronostajski 1986; De Vries et al. 1987). Interestingly, binding of NFI or NFI-BD to the origin is en-
hanced by the viral DBP (Cleat and Hay 1989; Stuiver and Van der Vliet 1990; Bosher et al. 1991), presumably through structural changes that DBP induces in origin DNA (Stuiver et al. 1992).

The level of stimulation by NFI is strongly dependent on the pTP-pol concentration and drops from 60-fold at low concentrations to approximately 2-fold at high concentrations of pTP-pol (Mul et al. 1990). This suggests that NFI recruits pTP-pol to the origin. In agreement with this, a direct DNA-independent interaction between NFI and pTP-pol has been reported (Bosher et al. 1990; Chen et al. 1990; Mul et al. 1990). The domain interacting with polymerase is located in NFI-BD between amino acids 68 and 150 (Chen et al. 1990) and is genetically distinguishable from the DNA-binding domain. Mutations in NFI that fail to bind pTP-pol are also defective for replication stimulation (Armentero et al. 1994). NFI not only recruits pTP-pol, but also stabilizes its interaction with the origin, as shown by template competition experiments and gel retardation (Mul and Van der Vliet 1992). Kinetic considerations are in accord with an increase in the amount of active preinitiation complex rather than an effect on the activity of pTP-pol, since NFI increases the $V_{\text{max}}$ without changing the $K_m$ (Mul and Van der Vliet 1993). The stabilizing effect of NFI, as measured by the half-life of a pTP-pol-DNA complex, was only approximately 10-fold compared to a 60-fold enhancement of initiation. This may indicate that NFI acts in multiple ways, possibly involving also structural changes within the origin (Zorbas et al. 1989). A role for NFI in origin unwinding is less likely, however, since NFI is still required when the template strand in the core origin is already unwound (Kenny and Hurwitz 1988).

Since NFI does not bind efficiently to single-stranded DNA, it can be anticipated that unwinding of the NFI site by the passing DNA polymerase leads to dissociation of NFI from DNA. Unexpectedly, dissociation of NFI occurs very early in initiation as soon as the DNA polymerase encounters dNTPs (Coenjaerts and Van der Vliet 1994). This result may be explained by conformational changes in the DNA polymerase and in origin DNA during initiation that could lead to disruption of both the NFI-pTP-pol interaction and the interaction between NFI and DNA. Apparently NFI delivers pTP-pol to the template but is released and recycled after initiation. This is reminiscent of similar effects observed in the BPV E1-E2 complex, where E2 dissociates as soon as E1 starts its helicase action (Lusky et al. 1994) and to the recruitment of DnaB helicase by DnaC or by $\lambda$P protein (Baker and Kornberg 1992). In the latter case, $\lambda$P protein must be removed by heat shock proteins before replication can start.
How Does Oct-1 Stimulate Initiation?

Oct-1 is a 91-kD ubiquitous transcription factor belonging to the POU-protein family (Herr et al. 1988; Verrijzer and Van der Vliet 1993). It binds to the sequence $^{40}\text{ATGATAATGA}^{49}$ in the Ad2 origin and stimulates initiation approximately 7-fold, independently of NFI (Mul et al. 1990; Coenjaerts et al. 1994). Like the NFI site (Hay 1985), the Oct-1 site is important for optimal replication in vivo. If other compensating DNA sequences are present, removal of the Oct-1 site is without consequence (Hatfield and Hearing 1993).

Deletion analysis indicated that the DNA-binding POU domain suffices for stimulation of replication (Verrijzer et al. 1990). This domain is highly conserved, and POU domain transcription factors from different subclasses all enhance replication, albeit to slightly different extents (Verrijzer et al. 1992).

In several aspects, similarities between enhancement of replication by Oct-1 and NFI exist. Like NFI, Oct-1 enhances the $V_{\text{max}}$ of the initiation reaction and still stimulates initiation when the origin is partially unwound. Stimulation by Oct-1 is also dependent on the pTP-pol concentration, although less sharply than observed for NFI (Mul and Van der Vliet 1992, 1993; Coenjaerts et al. 1994). In contrast to NFI, direct contacts between pTP-pol and Oct-1 or the POU domain were not observed in solution, but recently an interaction could be found employing a GST-POU fusion protein immobilized on glutathione-agarose beads (Coenjaerts et al. 1994). Previous attempts to detect this interaction in solution might have been hampered by a low affinity. Binding was also observed with the POU$_{\text{HD}}$, although more weakly than with the intact POU domain, indicating that additional contacts with POUs cannot be excluded. Interestingly, the POU-pTP-pol interaction could not be competed away by NFI, suggesting that the regions contacted on pTP-pol are different for NFI and Oct-1 (Coenjaerts et al. 1994). This also explains the independent stimulatory effects observed with these two transcription factors.

DNA bending by the POU domain (Verrijzer et al. 1991; Johansen et al. 1993) may contribute to the stimulation, but the way in which this occurs is not well understood. Bending may facilitate the interaction between the various proteins and thus have an architectural role in the formation and assembly of a multiprotein initiation complex. It is not likely that bending per se is sufficient for enhancement, since substitution of the Oct-1-binding site by an AP1 site did not lead to stimulation in the presence of Fos-Jun combinations that are able to bend the DNA to a similar extent as Oct-1 (Coenjaerts et al. 1994). This stresses the impor-
tance of specific protein-protein interactions between Oct-1 and pTP-pol and explains the strict positioning of its binding site.

In conclusion, NFI and Oct-1 stimulate initiation mainly by a direct interaction with different, so far undefined, domains in pTP-pol. Small distortions of the origin structure induced by these transcription factors may further facilitate the assembly of a preinitiation complex. \textit{POU}_{\text{HD}} and the NFI dimer bind to the same side of the DNA helix and, together with pTP-pol, the preinitiation complex spans five helical turns requiring considerable bending of the DNA to allow the necessary surface contacts. In addition to NFI and Oct-1, the terminal protein and DBP are required for optimal initiation. The presence of the terminal protein in the viral DNA leads to a modest increase in binding of pTP-pol to the origin (Pronk and Van der Vliet 1993), whereas bound DBP enhances binding of NFI. Together, these five proteins optimize formation and stability of a preinitiation complex. NFI and Oct-1 seem to function only in assembly of the preinitiation complex. Evidence for a function of NFI or Oct-1 in elongation or any other event during initiation is lacking. Unwinding of the origin or changing the kinetics of initiation appears to be mainly a function of the viral DBP (Mul and Van der Vliet 1993; Zijderveld and Van der Vliet 1994; Monaghan et al. 1994). A nucleosome antirepression effect, as suggested for papovaviruses, is unlikely since optimal stimulation is detected in the absence of nucleosomes, and adenovirus DNA is not constrained in a regular nucleosome structure in infected cells. Apparently, the viral origin and pTP-pol complex have evolved to allow interactions with conserved DNA-binding domains of transcription factors. Why these particular transcription factors are employed is not clear, and the adaptation process varies between serotypes, since Ad4 does not have an NFI-binding site. Presumably, the Ad4 pTP-pol complex has a stronger interaction with the core origin, thereby bypassing the need for NFI recruitment (Harris and Hay 1988). This is reminiscent of the T antigens of polyomavirus and SV40, which also differ in their affinity for the core origin, leading to an increased need for transcription factors in polyomavirus, which encodes a T antigen that binds more weakly to the origin than SV40 T antigen.

\textbf{SV40}

The 5243-bp circular SV40 DNA replicates bidirectionally from a fixed origin composed of a 64-bp core and adjacent auxiliary regions, Aux-1 and Aux-2, which each span approximately 40 bp. The core origin contains a 24-bp central region that forms the binding site for the SV40 T-antigen double hexamer flanked on one side by a 17-bp AT-rich element
and on the other by a 10-bp DNA unwinding element in which the origin of bidirectional replication is located (see also Hassell and Brinton, this volume).

Although the core origin suffices for initiation, the flanking auxiliary sequences function synergistically to increase the frequency of initiation up to 100-fold, depending on the conditions and methods used to measure DNA replication (Guo et al. 1989; Gutierrez et al. 1990). Aux-1 contains binding sites for T antigen, and precise positioning of Aux-1 relative to the core origin is required for its enhancing effect. Deletion of Aux-1 had only a very slight (1.6-fold) effect on binding of T antigen to the core origin or on the bidirectionality of initiation, even under conditions of limiting T antigen. In contrast, a strong effect on T-antigen-dependent DNA unwinding was observed (Guo et al. 1991). This suggests that any interaction between T-antigen molecules bound at Aux-1 and at the core origin influences the helicase activity rather than increasing the binding of T antigen. The level of stimulation by Aux-1 depends on the type of papovavirus. JC virus relies on Aux-1 to the greatest extent, then SV40, and BK virus the least (Sock et al. 1993). This may be related to small differences in the interaction between the various T antigens, leading to different strengths of the intramolecular interactions.

Aux-2 also contains binding sites for T antigen, albeit weak ones, and is recognized by several cellular transcription factors, including SP1, LSF, AP1, AP2, AP4, GT-1B, and p53 (Table 2). Due to the complexity of this region, it has been difficult to establish, by mutational analysis, which transcription factor-binding sites are essential for stimulation of initiation. Therefore, another approach was taken. Aux-2 was replaced by multimers of one site or by nonnatural binding sites for various transcription factors, and their effects were studied by transfection (see Table 2). Substitution of Aux-2 by two synthetic SP1-binding sites restored replication to wild-type levels (Guo and DePamphilis 1992; Lednicky and Folk 1992), whereas one copy was less effective. Such a copy-number-dependent stimulation was also found upon substitution with NFI or ATF sites (Hoang et al. 1992) and is reminiscent of the results obtained with transcriptional enhancers.

In a more extensive study using a number of different transcription factors, AP1 and, to a lesser extent, T antigen, were shown to be active. However, other DNA-binding proteins like Gal4 could not substitute, even when fused to the strong trans-activation domains of VP16 or c-Jun (Guo and DePamphilis 1992). The same was true for binding of Escherichia coli lac repressor adjacent to the origin (Cheng et al. 1992). This shows that binding of proteins to Aux-2 is insufficient and indicates
Table 2  Natural auxiliary regions can sometimes be substituted by synthetic auxiliary regions

<table>
<thead>
<tr>
<th>System</th>
<th>Natural sites</th>
<th>Substituting sites and proteins</th>
<th>Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Oct-1</td>
<td>AP1, (SP1)3, (AP1)3, (Tag)3, VP16, c-Jun, Gal4, NFI</td>
<td>in vivo, in vitro</td>
<td>1</td>
</tr>
<tr>
<td>SV40 Aux-2</td>
<td>SP1, T-ag, LSF, p53, AP1, AP2, AP4, GI-IB</td>
<td>(AP1)3, (Tag)3, VP16, c-Jun, Gal4, NFI</td>
<td>-</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Polyoma Aux-2</td>
<td>AP1 (PEA1), AP3 (EBP-20), T-ag, cETS (PEA3), PEA2</td>
<td>(SP)3, (AP1)3, (Tag)3, VP16, c-Jun, v-Jun, Gal4, CREB, Rel, E1A, Fos</td>
<td>+, +, +, +</td>
<td>2, 2, 2, 4, 5, 6, 7, 8, 10, 11</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>ABF-1</td>
<td>RAP-1, Gal4</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>ARS-1 B3</td>
<td></td>
<td>Gal4</td>
<td>+</td>
<td>13</td>
</tr>
</tbody>
</table>

Transcription activation domains were bound to different DNA-binding domains, with comparable results. The positions of the binding sites vary. The levels of activity differ considerably, but this is not indicated in this table. For details, see the references. Since the conditions for testing are different, a direct comparison of the results is not always possible. PEA3, PEA1, and EBP-20 are the murine homologs of human c-ETS, AP1, and AP3, respectively. References: (1) Coenjaerts et al. 1994; (2) Guo and DePamphilis 1992; (3) Lednicky and Folk 1992; (4) Hoang et al. 1992; (5) Cheng et al. 1992; (6) Cheng and Kelly 1989; (7) Bennett Cook and Hassell 1991; (8) Baru et al. 1991; (9) Morgan et al. 1993; (10) Murakami et al. 1991; (11) Wasylyk et al. 1990; (12) Ishikawa et al. 1993; (13) Marahrens and Stillman 1992.

specificity, presumably related to the possibility to interact with other replication proteins such as T antigen or RP-A.

Binding of the active transcription factors to T antigen has not been reported, but a direct interaction was observed between VP16 and the large subunit of RP-A, RPA-1 (He et al. 1993; Li and Botchan 1993). This has led to the interesting hypothesis that recruitment of RP-A could stabilize single-stranded DNA in the origin or assist in RP-A-dependent unwinding by the T-antigen helicase. RP-A can also specifically interact with DNA polymerase-α and SV40 T antigen (Dornreiter et al. 1992; Melendy and Stillman 1993), and these protein-protein interactions may facilitate primosome assembly. Therefore, RP-A may function to in-
fluence two rate-limiting steps, the formation of an initiation complex and the subsequent helicase activity. For recruitment of RP-A to be important, the intracellular or local concentrations of RP-A should be limiting. RP-A is a rather abundant protein involved in many different processes; within the nucleus, competition for the protein may be strong. Thus, it is of advantage for the virus to recruit as much RP-A as possible for its own replication. Nevertheless, some caution in the interpretation is needed. The VP16 trans-activation domain is notoriously promiscuous in its interactions and, in addition to RP-A, has been reported to bind to many other proteins, including TBP, TFIIIB, TFIIH, TAF40, and Oct-1. Moreover, Gal4-VP16 does not stimulate initiation in vivo (Guo and DePamphilis 1992; Hoang et al. 1992) despite its interaction with RP-A. This may be caused by sufficiently high concentrations of RP-A present in the cells used, or it may indicate that in vivo this mechanism is not important.

Another explanation of the effect of transcription factors on Aux-2 is based on replacement of Aux-2 by NFI-binding sites. In vivo, strong stimulation was observed, but the effect of adding NFI in a standard SV40 cell-free system was negligible. However, NFI specifically prevented the repression of DNA replication occurring when the template was preassembled into chromatin, suggesting a role as antirepressor (Cheng and Kelly 1989). A similar antirepressing effect was observed for Gal4-VP16 (Cheng et al. 1992). This agrees with results obtained with the naturally bound SP1, which can counteract histone H1-mediated inhibition of RNA pol II transcription (Croston et al. 1991; Laybourn and Kadonaga 1991). Several observations, however, argue against a model in which perturbation of the local nucleosome structure is the primary mechanism by which transcription factors stimulate SV40 replication. As mentioned above, Gal4-VP16 does not stimulate in vivo. Furthermore, a number of transcription factors, including T antigen itself, can inhibit chromatin assembly and prevent nucleosomes from repressing initiation (Ishimi 1992; Gruss et al. 1993). Finally, it has been reported that enhancement by SP1 is maintained when nucleosomes have been stripped (Lednicky and Folk 1992). Thus, although antirepression can be observed, it is not necessarily the primary reason for enhancement but, rather, could be a consequence of transcription factor binding. This explanation has also been put forward for similar effects observed upon binding of the BPV E1-E2 complex (see below).

Although binding of transcription factors to Aux-2 is the most likely way in which these proteins function, it may not be the only way. Recently, Oct-1 was shown to bind to the AT-rich element of the core
origin, and binding inhibits the T-antigen-dependent helicase activity (Kilwinski et al. 1995). This may provide another level of control by transcription factors.

**Polyomavirus**

The polyomavirus (Py) genome is closely related to SV40 but replicates only in murine cells. The origin consists also of a core region flanked by two auxiliary regions. Like SV40, Py encodes a large T antigen essential for replication that binds to the core origin as a hexamer, has DNA-dependent ATPase and helicase activities, and binds DNA polymerase-α:DNA primase. However, Py T antigen binds more weakly to DNA and thus may rely more heavily on auxiliary factors (see Hassell and Brinton, this volume).

Binding of T antigen to Aux-1 activates replication 5- to 10-fold and presumably serves the same role as in SV40. The 200-bp Aux-2 region, coinciding with the enhancer element (de Villiers et al. 1984), stimulates 200- to 1000-fold, which is more than in SV40. Within Aux-2, two elements have been recognized (α and β) that are functionally redundant. Proteins binding to these sites are known as polyoma enhancer binding proteins (PEBP) or polyoma enhancer activators (PEA) and are homologs of the AP1 and ETS families of transcription factors, respectively. Several experiments indicate that binding of these transcription factors is important for replication and that overexpression of AP1 leads to stimulation of DNA replication (Murakami et al. 1991). Fusion proteins of various DNA-binding domains and the trans-activation domains of Fos-Jun were active in a DNA-binding-dependent manner, indicating the need for trans-activation domains (Wasylyk et al. 1990; Morgan et al. 1993). Interestingly, stimulation of replication was under control of growth-promoting agents such as the phorbol ester TPA. This suggests that protein kinase C-dependent signal transduction pathways are coupled directly to DNA replication, possibly through phosphorylation of the trans-activation domain. This effect seemed to be specific for AP1, since overexpression of CREB, also recognizing the AP1 site, stimulated only transcription but not replication (Murakami et al. 1991). So far, specific interactions between AP1 and other replication proteins have not been reported.

To uncouple effects on transcription and replication and to determine if other transcription factors could substitute for AP1 or ETS, a similar approach as for SV40 was employed. Natural Aux-2 sequences were replaced by synthetic binding sites capable of binding just one of the transcription factors, either individually or in tandem. Also tested were sites
for well-characterized transcription factors such as AP1 and NF-κB (Guo and DePamphilis 1992; Ishikawa et al. 1993), and factors without a mammalian counterpart, such as Gal4 or fusion proteins between the Gal4 DNA-binding domain and the trans-activation domains of VP16, E1A, c-Jun, or BPV-E2 (Table 2) (Wasylyk et al. 1990; Baru et al. 1991; Murakami et al. 1991; Guo and DePamphilis 1992; Morgan et al. 1991). These binding sites, in the presence of appropriate proteins, could all substitute for Aux-2, but to a different extent and dependent on the positions of the sites.

In contrast to SV40, stimulation by VP16 was observed, although natural AP1 stimulates better than Gal4-VP16, Gal4, and c-Jun. Except for Rel oncoproteins (Ishikawa et al. 1993), trans-activation domains were essential. Synergistic activation was achieved by multimerization of the Gal4-binding sites (Baru et al. 1991; Bennett Cook and Hassell 1991), which should be juxtaposed to the core origin for optimal functioning. Gal4-VP16 stimulated replication of polyomavirus but not SV40 DNA in vivo, which may be related to the weak binding of Py T antigen and correspondingly higher requirement for auxiliary factors. Alternatively, there may be different levels of coactivators in the cells used for transfection.

The models put forward to explain the mechanisms by which these trans-activation domains work are the same as described above for SV40. A chromatin interference model is not excluded but is difficult to reconcile with the finding that one cluster of five identical Gal4-binding sites stimulates replication, whereas two such clusters are inhibitory (Baru et al. 1991). A strong argument for the importance of an interaction with RP-A comes from mutagenesis studies. Mutations in VP16 that reduce the ability of Gal4-VP16 to stimulate replication are also defective in RP-A binding (He et al. 1993). An extensive analysis (B.T. Brinton et al., in prep.; Hassell and Brinton, this volume) revealed that mutation of 6 of the 46 amino acids that make up the amino-terminal domain of VP16 coordinately reduced DNA replication, as well as binding to RPA-1. This provides strong evidence in favor of RP-A recruitment as the main mechanism for stimulation of DNA replication. Whether RP-A is a limiting factor is not known, but even if RP-A is present in excess, binding of VP16 could be essential to activate RP-A or to induce structural changes.

**Papillomaviruses**

The papillomavirus genome is slightly larger than that of papovaviruses and encodes two, rather than one, proteins required for replication (E1
and E2). E2 is a viral transcription factor. Much of the information on viral DNA replication has been derived from bovine papillomavirus type-1 (BPV-1) and has been extended recently for several human serotypes (HPV) (see Stenlund, this volume).

BPV-1 contains an origin of bidirectional replication spanning approximately 60 bp. A core origin consisting of an AT-rich region and an imperfect inverted repeat is flanked by two auxiliary regions (BS11 and BS12) that stimulate replication considerably (Ustav et al. 1991). The 68-kD E1 phosphoprotein (Sun et al. 1990) binds specifically to the core origin (Ustav et al. 1991). It possesses DNA-dependent ATPase and helicase activities (Seo et al. 1993a; Thorner et al. 1993; Yang et al. 1993) and distorts the origin structure severely upon binding in an ATP-dependent fashion, as shown by KMnO4 sensitivity assays (Gillette et al. 1994). Interestingly, E2 can also bind the p180 subunit of DNA polymerase-α:DNA primase (Park et al. 1994). Thus, in many respects E1 resembles T antigen and is the essential initiator protein, although only limited sequence homology with T antigen exists. Under in vitro conditions, E1 is sufficient for initiation if added in high amounts to the reaction (Li and Botchan 1993). In vivo the minimal origin includes an E2-binding site (Ustav et al. 1993).

E2 binds as a dimer to a 12-bp palindromic sequence, ACC N6 GGT, present in BS11 and BS12, and binding leads to severe bending of the DNA. The E2 open reading frame can encode three proteins by differential splicing: E2, E2-TR, and E8/E2. All three contain the 85-amino-acid carboxy-terminal DNA-binding domain, but only the full-length 48-kD E2 enhances replication and transcription. The other two proteins function as repressors of transcription and possibly also DNA replication. The regions of E2 required for enhancement of replication and transcription are not identical (Winokur and McBride 1992).

Enhancement of initiation by E2 is directly related to its capacity to bind the E1 helicase. The interaction between E1 and E2 is cooperative and leads to an increase in the affinity of E1 for its binding site (Mohr et al. 1990; Blitz and Laimins 1991; Lusky et al. 1993; Seo et al. 1993b). E2 can even rescue an E1 mutant defective in DNA binding (Spalholz et al. 1993; Thorner et al. 1993). Moreover, the amount of E1 required to generate structural changes in the origin is lowered by the presence of E2 (Gillette et al. 1994). E2 also enhances origin-dependent unwinding by E1 (Seo et al. 1993b). Thus, E2 stimulates replication by enhancing several properties of E1; in agreement with this, the highest level of stimulation by E2 in vitro occurs at low E1 concentrations (Müller et al. 1994). Interestingly, an intact DNA-binding domain is not a strict re-
requirement for E2 to enhance replication (Winokur and McBride 1992). This has led to the hypothesis that the cooperative nature of the interaction, accompanied by structural changes in E1 upon complex formation with E2, suffices for enhancement of E1 binding to DNA. This targeting role of E2 may be short-lived, as indicated by band-shift experiments (Lusky et al. 1994). The E1-E2 complex binds to the origin but is still replication-inactive. It may function as a focal point for attracting other replication proteins, leading to a replication-active complex that can start unwinding. As soon as this occurs, E2 is released, possibly through structural changes induced in E1 and the origin. This is reminiscent of the early release of NFI during initiation of adenovirus DNA replication (Coenjaerts and Van der Vliet 1994) and of the recruitment of DnaB helicase by DnaC or λP protein (Baker and Kornberg 1992).

The mechanism of action by E2 might well involve more than just interacting with E1. E2 can also recruit RP-A, and mutants defective in RP-A binding are less effective in enhancing BPV replication in vitro (Li and Botchan 1993). Moreover, E2 interferes with nucleosome binding, but this antirepressor action is not specific for E1 and E2 and may be a consequence of E1/E2 binding rather than a primary effect (Li and Botchan 1994).

The results obtained with BPV were largely confirmed for several HPV serotypes, although some differences were observed (Frattini and Laimins 1994; Gopalakrishnan and Khan 1994). Interestingly, mutational analysis of HPV-11 showed that the presence of an E1-binding site is less important than the presence of an E2-binding site (Russell and Botchan 1995), stressing the function of E2 as the major targeting protein for the E1 helicase, which binds with lower specificity. Moreover, E2 also suppressed nonspecific initiation by E1 in vitro (Kuo et al. 1994).

In conclusion, the combination of E1 and E2 has many similarities to T antigen. Not only does it have similar enzymatic activities, but it also attracts both RP-A and DNA polymerase-α:DNA primase. However, the binding of E1 to the core origin is not always strong enough, and the most important role of E2 appears to be to facilitate binding of limiting amounts of E1. The intracellular ratio of E1 to E2 may be an important factor in determining the replication efficiency and thus the copy number. Once they have been delivered by E2, possibly assisted by E2-induced DNA bending and by the cooperative nature of the E1-E2 interaction, E1 multimers may attract other replication proteins, and a preinitiation complex will be formed. The cooperative binding of E1 and E2 leads to ATP-induced structural changes that may release E2 and activate the helicase activity.
Herpesvirus

DNA replication has been most extensively studied in the α-herpesvirus HSV and the γ-herpesvirus Epstein-Barr virus (EBV). In the 150-kbp linear HSV-DNA, three distinct origins have been described, ori_L and two copies of ori_S, located in the inverted repeats of the S component of the DNA. Seven viral proteins required for replication have been described (see Challberg, this volume).

As in other DNA viruses, both ori_S and ori_L are flanked by cis-acting sequences containing transcriptional regulatory elements that have been postulated to function in DNA replication as shown by deletion analysis (Stow and McMonagle 1983; Wong and Schaffer 1991). Whether binding of transcription factors to these elements is essential and how these transcription factors contribute to replication efficiency will be difficult to establish in view of the lack of an origin-dependent in vitro system. Besides the mechanisms proposed for papovaviruses, even a role of transcription itself is not excluded since the elements are still active in transcription during replication. This transcription process could influence ori function by modifying the local DNA structure in a way similar to that described for E. coli oriC (Baker and Kornberg 1988).

EBV oriP Is Activated by Transcription Factor-induced DNA Looping

The 192-kbp linear genome of EBV is maintained as multiple copies of a plasmid in latently infected B lymphocytes. Latent replication originates from oriP, the plasmid origin of replication, and requires only one viral protein, EBNA1, which also functions as a transcription factor.

oriP contains two noncontiguous elements, DS (dyad symmetry) and FR (family of repeats), separated by approximately 1 kbp. DS forms the functional origin of replication and contains four imperfect binding sites for EBNA1, two within a 65-bp region of dyad symmetry and two flanking this sequence. FR contains 20 tandemly repeated copies of a 30-bp sequence, each containing a 12-bp palindrome core that forms the EBNA1-binding site. This region functions as an enhancer both for transcription and DNA replication and also governs stable segregation of oriP-containing plasmids.

Upon initiation, the EBNA1 dimers bind first to the sites in FR, then to sites in DS. EBNA1 complexes formed on the two elements interact, and this leads to looping-out of the DNA (Frappier and Donnell 1991; Su et al. 1991). Binding of EBNA1 to FR or DS alone is much weaker than to both elements, indicating that the intramolecular interactions between
EBNA1 bound to FR and DS stabilize the DNA/protein complex, as shown by competition with nonspecific DNA (Frappier and Donnell 1991; Su et al. 1991). Thus, looping enables site saturation at lower concentrations via cooperativity. These interactions take place in a part of the EBNA1 molecule separate from the normal dimerization domain or the DNA-binding domain (Chen et al. 1993) and are located in a small region between amino acids 350 and 361 (Frappier et al. 1994). Contact with FR-bound EBNA1 leads to stabilization of EBNA1 at the DS element and could well be a trigger to activate initiation of DNA synthesis, either by recruitment of other replication factors or by a change in DNA structure. In particular, the protein-mediated looping might facilitate unwinding by introducing topological stress. Just DS-DS-bound EBNA1 does not stabilize binding. Interestingly, looping is also an important mechanism for bacteriophage R6k replication, where origin-bound Rep protein stabilizes the other Rep proteins bound weakly to orica. This cooperativity leads also to efficient replication. In these cases, apparently specific interacting surfaces can only make optimal contacts when positioned on the DNA such that a loop is occurring. Only this position leads to optimal protein-protein interactions, thus imposing torsional strain on the DNA that can be used for origin opening.

**Lytic Origin of Replication**

EBV has both a latent state and a lytic replication cycle. The lytic phase can be induced by treating cells with phorbol esters or by introducing a vector expressing the BZLF1 gene encoding a viral transcription factor. Two copies of oriLyt separated from oriP have been found, one in DS-L and one in DS-R (Hammerschmidt and Sugden 1988). These origins contain multiple regions required for replication and additional sequences that increase replication and can be functionally substituted by a transcriptional enhancer. Similar to HSV-1, at least seven viral genes required for replication have been found (Fixman et al. 1992). The DS-L origin is quite large and complex and consists of a 225-bp AT-rich region that is presumed to be the site of initiation, flanked by two elements, 320 and 370 bp long, respectively (Schepers et al. 1993b). These flanking elements contain promoters of two divergently transcribed genes, BHLF1 and BHRF1, and also act as enhancers for replication. The BHLF1 promoter/replication enhancer is controlled by the viral transcription factor BZLF1 (synonyms: EBI, zta, ZEBRA, z) which belongs to the b-Zip class and is related to the AP1 family. Four binding sites are found in the promoter of BHLF1 and three in BHRF1, to which BZLF1 binds with
different affinities. Another trans-activator that binds to the BHRF1 promoter is Rta, which may also be involved in enhancement of replication.

Employing a transient replication and cotransfection protocol, it could be shown that for replication enhancement, the amino-terminal transcription activation domains of BZLF1 are required (Schepers et al. 1993a). In contrast to results in the papovavirus system, other transcription factors (c-Jun, BPV-E2, Myc, or VP16) could not substitute for BZLF1, suggesting the requirement for specific interactions, although the target proteins have not been identified.

Other Viral Systems
The need for transcription factors to optimize DNA replication is likely not confined to the systems described above. Evidence is also present for Geminivirus (see Bisaro, this volume) and paroviruses such as minute virus of mice (MVM). Here the active origin (50 bp) contains an ATF-binding site (Cotmore and Tattersall 1994). However, in these cases, the evidence for a function of these transcription factors is still circumstantial.

Cellular Origins
The use of transcription factors by viruses for the enhancement of their replication seems to be a rule rather than an exception. This is likely caused by adaptation of the viral genome to the presence of these proteins in their host cells. The need for economic use of genetic information due to packaging constraints may have forced the use of sequence information in cis-acting elements such as enhancers for dual purposes. This may also hold for the employment of viral transcription factors such as BPV-E2 and EBNA1 for multiple functions. Such sequence constraints do not exist in eukaryotic cells, and therefore an extrapolation of the need for transcription factors to cellular DNA synthesis is not a matter of fact. Nevertheless, recent results have indicated a strong link between transcription and replication also in cellular origins, and at least two proteins required for the initiation of DNA replication in Saccharomyces cerevisiae are also essential for control of transcription.

Origins in Yeast
In S. cerevisiae, replication origins have been well characterized, and although an in vitro system for initiation is not yet established, information
on the replication proteins has recently been obtained both by biochemical and by genetic means. Yeast origins coincide with ARS elements and are located in AT-rich regions of the genome with an average spacing of approximately 100 kbp (see Newlon, this volume). ARS elements are approximately 100 bp in length and contain two essential elements, A and B. The A element contains an 11-bp consensus sequence (ACS), which is the only region common to all *S. cerevisiae* origins. The B element is composed of three functional elements, B1, B2, and B3, which are also important for origin functions but are less conserved. The A element is bound in vitro by the multiprotein origin recognition complex (ORC) (Bell and Stillman 1992), which can also be observed in vivo (Diffley and Cocker 1992). ORC binds the ACS in an ATP-dependent manner and also covers part of the B1 element. The complex is composed of six different polypeptides, ORC1–ORC6, which are essential for yeast viability. Interestingly, the 72-kD ORC2 protein is also implicated in transcriptional silencing of the HMR-E locus (Rivier and Rine 1992; Bell et al. 1993; Foss et al. 1993; Micklem et al. 1993). In agreement with this, the cis-acting sequences required for silencing can function as ARS elements. These results provide strong evidence for a role in vivo of ORC in replication as well as in transcriptional control, in particular in silencing. Possibly the ORC2 protein can be present in different complexes, dependent on its function. Alternatively, DNA replication could be involved directly in transcriptional silencing by some form of mechanistic coupling in which ORC is involved (Rivier and Rine 1992). Due to the lack of an in vitro system, the exact role of ORC2 in replication remains obscure.

The ORC complex remains bound to the ACS throughout the cell cycle but can occur in two states differing in footprint pattern at different parts of the cycle (Diffley et al. 1994). Near the end of mitosis and through G1, an additional region of protection overlapping the ORC footprint is observed that is lost after entry into S phase. Diffley and coworkers suggest that this postreplicative state, which resembles the footprint pattern obtained in vitro with ORC and ABF-1, represents a ground state laid down immediately after S phase and remaining throughout the cell cycle even into G0. Thus, ORC and ABF-1, a transcription factor described below, may have to be activated to create a prereplicative state.

The B3 element contains the binding site for the multifunctional transcription factor ABF1 (Buchman et al. 1988; Diffley and Stillman 1988). ABF1-binding sites have been identified in several ARSs at variable positions up to 1.2 kbp from the A element, indicating that they can func-
tation in a position- and orientation-independent manner to stimulate replication, thereby resembling transcriptional enhancers. ABF-1 is an abundant 81-kD phosphoprotein. It binds in a sequence-specific manner to a consensus sequence GCAN₄,Y₂RCTR (R=purine, Y=pyrimidine). A detailed contact point analysis has indicated that ABF-1 is mainly oriented along one face of the helix (McBroom and Sadowski 1994a). Considerable bending (120°) of the DNA has been inferred from circular permutation and phasing analysis (McBroom and Sadowski 1994b). ABF-1 is involved in positive control of a number of genes, in silencing of the mating-type loci HML and HMR, and in DNA replication.

To understand its role in DNA replication in more detail, the B3 element from ARS-1 has been substituted by the binding sites of several other transcription factors including RAP-1, Gal4, and a LexA-Gal4 fusion protein (Marahrens and Stillman 1992). These binding sites substituted efficiently, in both orientations and in a DNA-binding-dependent manner in the presence of the appropriate transcription factors, indicating that the B3 element may be a rather nonspecific enhancer.

Two hypotheses, not mutually exclusive, have been put forward to explain the role of ABF-1. One is a direct contact between the ABF-1 activation domain and replication proteins, present at the core origin. A candidate is RP-A, which can be bound directly by Gal4 (He et al. 1993). Recruitment of RP-A followed by binding to the B1-B2 unwinding element and stabilizing single-stranded DNA or assisting in unwinding is an attractive hypothesis. However, a direct interaction between ABF-1 and RP-A has not been described, and the ABF-1 site can vary in position and orientation without loss of activity. Moreover, such a recruitment model is difficult to reconcile with the continuous presence of ABF-1 in vivo at the core origin (Diffley et al. 1994). As shown by in vivo footprinting of an ARS element in which the ABF-1 site is mutated, ABF-1 is not involved in recruitment of ORC (J. Cocker and J. Diffley, pers. comm.) or the ORC-bound factor Dbf4 (Dowell et al. 1994). An alternative is that the trans-activation domain of ABF-1 is an architectural component facilitating the interaction between multiprotein complexes and DNA or influencing the local chromatin structure, employing its capacity to bend DNA. Interestingly, RAP-1, which can substitute for ABF-1, can also strongly bend DNA (Gilson et al. 1993).

Another transcription factor that has been implicated in DNA replication is the TATA-box-binding protein TBP (Lue and Kornberg 1993). TBP can bind to the ACS, and TBP-binding sites can activate replication origins in vivo. Moreover, the ARS-1 B domain can act as a weak upstream activating sequence in transcription. However, this could just be
fortuitous; no example of an origin within a transcription unit in yeast chromosomes has been found. There is also no evidence that ARS elements have promoter activity (Fangman and Brewer 1991).

On the basis of genetic evidence, another class of proteins, Mcm (minichromosome maintenance), have been suggested to influence DNA replication (Christ and Tye 1991). One of these, Mcm-1, is a transcription factor that bridges the α1 and α2 regulatory homeobox proteins involved in mating-type control (Smith and Johnson 1992) and also interacts with other transcription factors. Its potential role in DNA replication is based on a plasmid maintenance defect in Mcm-1 mutants. As yet there is no evidence that Mcm-1 interacts directly with ARSs, and its role may be limited to transcriptional enhancement of genes coding for essential replication proteins.

In summary, the evidence for a link between DNA replication enhancement and transcriptional control, in particular silencing of HML-E, is compelling, but the mechanisms by which ABF-1 and ORC2 exert their multiple functions remain unclear. Most evidence points to an architectural role of ABF-1 rather than a direct interaction with other replication proteins.

Origins in Higher Eukaryotes

A number of potential eukaryotic origins in metazoa have been described recently (see DePamphilis, this volume). In those cases where sequence analysis of the potential origins is available, a large number of transcription factor-binding sites have been found. Since no mutational analysis has been performed, a functional analysis is not possible, and the significance of these sites is not clear. Although it is tempting to assume that they may act as enhancers of replication in a similar fashion as in yeast and the viral systems, they may just be localized close to origins by chance and represent promoter regions without any functional relationship to initiation of DNA replication. (Details of the binding sites in cellular origins can be found in DePamphilis, this volume.)

SUMMARIZING REMARKS

Why Are Transcription Factors Used for Replication?

Although at first sight the lack of a separate set of replication-enhancing proteins seems strange, it is understandable from an evolutionary point of view. Only a limited number of basic principles to recognize DNA have been found to date. This number may expand upon further structural
analysis, but nevertheless, it seems that Nature uses variations on successful binding modes such as helix-turn-helix, zinc fingers, and helix-loop-helix above development of completely novel modes. These conserved modes of DNA recognition can apparently be used for multiple purposes, in both DNA replication and transcription. A similar situation is found for RP-A, also multifunctional, and for TFIIH, active in transcription and repair (Schaeffer et al. 1993; Drapkin et al. 1994). An advantage of the use of transcription factors is that, in general, they open up chromatin structure. This may explain why transcriptionally active regions of chromosomal DNA replicate early in S phase. But why should transcription activation domains not have been adapted specifically to interact with replication proteins? Presumably, the flexible nature of activation domains increases the potential for different, specific interactions. Moreover, the specificity might be increased by the use of different coactivators. Transcription activation domains can be mimicked by reiteration of short peptide segments, revealing their modular organization (Tanaka et al. 1994). Their flexibility has so far hampered detailed structural analysis, but they may adopt a more fixed structure upon binding their target proteins functioning in the transcriptional machinery or in initiation of DNA replication. Such a flexible interaction exists also for major histocompatibility complex molecules in binding peptides (Young et al. 1994) and in the interaction between molecular chaperones and unfolded proteins (Braig et al. 1994). Thus, transcriptional activation domains may be very suitable to permit interactions with different molecules of the replication machinery.

A further advantage of the use of the transcription factors is that modification, in particular phosphorylation, could enable a subtle regulation of initiation. This has been described for transcription control by Fos-Jun (AP1), and in that respect it is interesting that a natural AP1 site in polyomaviruses plays such a prominent role and may be involved in replication timing.

Why would a virus not circumvent the need for transcription factors by enhancing the levels of rate-limiting factors directly? One reason may be that, when viral replication proteins and DNA are in low concentration early in infection, cellular proteins could enhance replication, thus enabling a quick start. However, this would just give a small advantage in time. More likely, for limiting factors such as E1 and pTP-pol, the ability to bind in a sequence-specific manner would be counterproductive. This holds in particular for DNA polymerase, which must clear the origin to enable elongation. Transcription factor guiding can also prevent nonproductive action outside the origin, which might occur if high levels
of the initiator are present (Kuo et al. 1994). Consistent with this notion, the BPV-E2 protein stimulated the origin-specific unwinding reaction catalyzed by E1, but had no stimulatory effect on nonspecific helicase activity (Seo et al. 1993b).

Are Similar Mechanisms Used to Activate Transcription and Replication?

As summarized in Table 3, the various mechanisms proposed for enhancement of replication apply to a great extent also for transcription. For the latter, various target proteins have been detected, like TBP, TAFs, or TFIIB, and even TFIIH or RNA polymerase itself. In one instance (TFIIB), a change in the structure is observed upon interaction with VP16 (Roberts and Green 1994). Transcription factors are involved in modulation of the global chromatin structure required for transcription, as exemplified by the MMTV-LTR promoter and NFI. These effects on chromatin structure might be similar in transcription and replication and therefore require the same domains of the transcription factors.

In most cases where direct protein-protein interactions have been observed, however, a high specificity is found, and the regions required for transcription activation and replication enhancement are different. This is most conspicuous when just a DNA-binding domain is required for repli-

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cation (adenovirus: NFI, Oct-1; polyoma: Rel oncoprotein). Despite their name, these DNA-binding domains can interact not only with DNA, but with other proteins as well. For instance, the Oct-1 POU homeodomain can interact with several other proteins besides the Ad DNA polymerase and employs different amino acids to contact these different targets (Coenjaerts et al. 1994).

Future experiments will doubtless reveal the details of interacting domains and the consequences for these interactions, employing reconstituted replication systems as well as structural studies. A major question will be whether these transcription factors only optimize viral replication systems, or whether they are also involved in cellular replication, in particular, timing and control of initiation of cell division. Potential candidates are transcription factors binding to the locus control region of the β-globin gene, which influences the time in S phase when the locus is replicated (Forrester et al. 1990; Kitsberg et al. 1993).

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