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Guest Editorial

Coupling JC virus transcription and replication

Two papers in this issue of the *Journal of NeuroVirology* bring to mind an often ignored bridge between the fields of gene transcription and DNA replication (Sumner *et al*, 1996; Swenson *et al*, 1996). What is immediately common to the two papers is JC virus, a 5.1 kb circular double-stranded DNA virus that causes a fatal human demyelinating brain disease called progressive multifocal leukoencephalopathy (PML). The characteristic clinical and histopathological features of PML result from the specific infection of oligodendrocytes. This fact closely parallels the *in vivo* restriction of productive JC virus infection to cells of human glial origin. The closely related SV40 papovavirus can productively infect a wide variety of cells of primate origin, but produces no known disease in humans. Like SV40, the JC virus genome is divided into three regions, containing genes expressed early after infection (large and small T antigens), late after infection (viral coat proteins), and a 400 bp regulatory region between these transcriptional units. The regulatory region controls viral DNA replication from the replication origin (*ori*), and early and late gene transcription from a bi-directional promoter (see Figure 1). The glial tropism of JV virus is dependent on the fact that the JC virus early gene promoter is more active in glial cells than in cells of nonglial lineage. Thus, large T antigen expression is restricted to glial cells. Replication of JC virus DNA is T antigen dependent, but is much less host-range restricted than early gene transcription, in that replication can occur in numerous cell types of primate origin so long as JC virus T antigen is present (Feigenbaum *et al.*, 1987).

Genetic and biochemical analyses of simple eukaryotic genomes have shown that the regulatory mechanisms for gene transcription and DNA replication are remarkably similar. Transcription begins with the sequence-specific binding of TFIID to a TATA box sequence immediately upstream of the transcription initiation site (Figure 1). This is succeeded by the ordered assembly of a complex of proteins (not shown in Figure 1) in physical association with TFIID, ultimately leading to the recruitment of RNA polymerase II. Following local DNA unwinding, transcription initiation ensues with movement of RNA polymerase along the early gene coding strand. By comparison, SV40 replication (for which the most details are known) begins with the binding of two hexamers of T antigen to specific sequences in the origin recognition element (ORE) within the replication origin (DePamphilis, 1993). T antigen mediates local DNA unwinding through its helicase activity, and then recruits SSB protein, topoisomerase I, and ultimately a primase-polymerase complex to the ORE (Figure 1). RNA-primed DNA polymerization initiates with polymerase moving down the early gene coding strand. Bi-directional and discontinuous replication begin thereafter.

While this comparison ignores many dissimilarities, the mechanisms of transcription and replication are further linked by their mutual dependence on transcription factors. When bound to DNA sequences on the late side of the SV40 *ori*, for example, transcription factors stimulate DNA replication up to 100-fold. The binding sites for these transcription factors comprise the genetically-identified 'auxiliary components' of the replication origin, which in SV40 spans the well characterized 21 bp repeats (Sp1 binding sites and T antigen binding site III). Like the regulation of individual transcriptional promoters, SV40 replication is stimulated by some transcription factors, such as NF-1 and Sp1, but not by others, such as the acidic activation domain protein VP16 (Jones *et al*, 1987). Remarkably, activation of transcription and replication are often mediated by different domains of transcription factors (Mermod *et al*, 1989). The spacing and orientation of *cis*-acting auxiliary components (transcription factor binding sites) in relation to the replication ORE is tightly restricted, but elements in the upstream enhancer areas also exert a stimulatory effect. The mechanisms for activation of replication by transcription factors appear to be similar in theme to those that regulate transcription (DePamphilis, 1993). In the case of SV40, for instance, transcription factors stabilize the binding of T antigen to unwound *ori* DNA, thus enhancing the stability of the replication initiation complex. The role of transcription factors in the regulation of replication is not restricted to papovaviruses, as similar interactions have been observed with other DNA viruses such as adenoviruses, and with the *Saccharomyces* ORC multiprotein complex, which acts as both a transcriptional silencer and an ATP-dependent binding complex on autonomously replicating sequences (ARS).

An interesting issue raised by these similarities concerns why JC virus transcription is regulated in a cell-specific manner whereas viral replication is restricted only by a requirement for T antigen and cells of primate origin. A potential answer is suggested in the paper of Sumner *et al* (1996). The authors report differential expression of NF-1 family members in glial-derived cell lines compared to nonglial cells, and describe the cloning and analysis of a NF-1 class D protein, NF-1/AT1, that is highly and specifically expressed in glial cells. Nuclear factor-1 (NF-1/CTF) was originally identi-

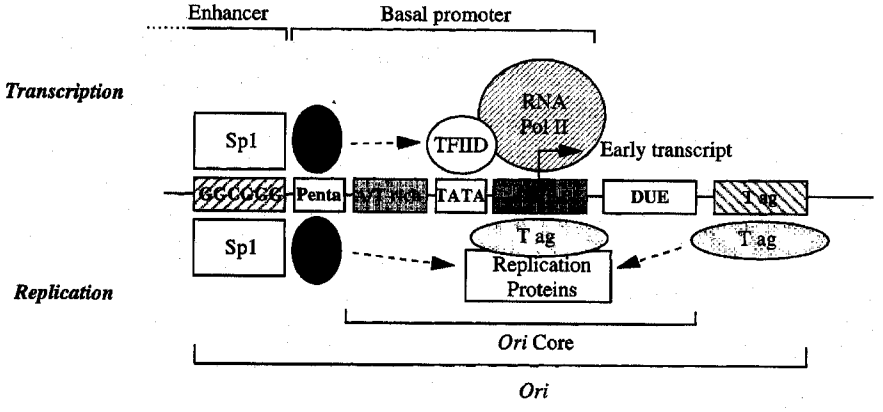

Figure 1 Schematic comparing regulatory mechanisms of early gene expression at the JC virus basal promoter and DNA replication at the *ori*. The JC virus sequence is that of the MH-1 form (Henson *et al*, 1995). Many of the features shown are extrapolated from data based on research with SV40, and have not yet been shown for the closely related JC virus. Dashed arrows indicate protein-protein interactions. ORE is origin recognition element, DUE is DNA unwinding element, T ag is large T antigen. The lower half of the Figure is adapted from DePamphilis (1993).

fied as a HeLa cell protein that stimulated adenovirus 2 replication, but it was subsequently found to activate transcription as well (Jones *et al*, 1987). Regulation of transcription and replication occurs through separate domains, with the amino-terminal end of NF-1/CTF mediating sequence-specific DNA binding and activation of replication, and the carboxyterminal end mediating transcriptional activation. That NF-1/AT1 diverges most significantly from NF-1/CTF at its carboxyterminal end suggests that any role in glial-specificity might arise through the unique carboxyterminal sequences, whereas the highly conserved aminoterminal portion of the protein might support replication in a non-specific fashion.

The specificity of JC virus T antigen in DNA replication is addressed in the paper by Swenson *et al* (1996), in which a mutational analysis of amino acids potentially involved in phosphorylation and secondary structure (T antigen zinc finger) is reported. The efficiency of JC virus DNA replication is substantially lower than that of SV40, an observation that is reflected in the 2 to 3 week intervals needed to detect substantial JC virus replication *in vivo*, and in the subacute nature of brain destruction in PML. The lower efficiency of JC virus could result from differences in the sequences of the JV virus and SV40 T antigens. For instance, the zinc finger of JC virus T antigen has different amino acids than SV40 T antigen at positions known to be crucial for the latter's function. Interestingly, mutations in the second half of the JC virus zinc finger had no effect on replication (Swenson *et al.*, 1996). Two of the four positions were changed to that of the SV40 amino acid sequence, and the lack of effect of these changes suggests that either the zinc finger is not the crucial feature limiting JC virus's restricted replication or that the sequence context of the changed residues is also important. Final interpretation of these results may await a three-dimensional picture of T antigen tertiary structure.

It should not go unnoticed that both NF-1 and T antigen are transcription factors *and* replication factors. Recent data showing that the JC virus basal promoter region can mediate glial-specific early gene expression (Krebs *et al*, 1995; Henson *et al*, 1995), and that the JC virus early promoter is strongly active in nonglial cells in the presence of T antigen suggests that JC virus replication and early gene transcription could be coupled by common mechanisms that co-operate to mediate cell-specificity. If that is so, clarification of these mechanisms could provide important insights into JC virus biology and more generally into biochemical mechanisms of cell-specificity.

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