Activity of JC virus archetype and PML-type regulatory regions in glial cells

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Sequence variations are seen in the JC virus promoter/enhancer in virus taken from progressive multifocal leukoencephalopathy (PML) brains and it has been hypothesized that the variations arise in the host at some point in the development of PML. These rearrangements may be adaptations for enhanced growth in glial cells; if so, transcription or replication levels should differ between archetypal and rearranged PML-type promoters. The archetype and four PML-type promoters were analysed in human glial cells for early and late transcriptional activity in the absence or presence of virus T antigen, and for DNA replication. CAT reporter expression differed within a fivefold range and the archetype was intermediate in strength to the PML-type regulatory regions. The archetype differed from rearranged promoters in that the late promoter was less responsive to T antigen and the shift from early to late activity with T antigen was less pronounced. All five regulatory regions demonstrated similar levels of DNA replicating activity. Rearrangement of the archetype was not required for activity in glial cells, but the potential for differences in the regulation of the late capsid genes was found.

Introduction

The human polyomavirus JC virus (JCV) infects the majority of the population worldwide and is retained in a latent state in the urinary tract and other organs (Padgett & Walker, 1973; Arthur & Shah, 1989; Houff et al., 1988; Tornatore et al., 1992). JCV is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), a lytic infection of oligodendrocytes (Tornatore et al., 1994; Major & Ault, 1995). PML arises during prolonged immunosuppression and, most frequently, in AIDS (Major & Ault, 1995). The reactivation of the urinary tract infection to viruria occurs under several conditions (Coleman et al., 1980; Kitamura et al., 1990; Hogan et al., 1991), but probably not in response to immunosuppression (Markowitz et al., 1991; Flaegstad et al., 1991; Tominaga et al., 1992). This is likely to be the means by which the virus is transmitted, but there is no knowledge of the site of initial infection. There is evidence that JCV also establishes latency in cells of the bone marrow and haematopoietic system, and is associated with cells of the B lineage (Houff et al., 1988; Atwood et al., 1992). The latent infection in this tissue is the likely source of the reactivation leading to PML and it has been proposed that the virus gains access to the brain in infected B cells (Major & Ault, 1995).

The non-coding regulatory region contains a bidirectional promoter/enhancer which directs early transcription of large and small T antigens from one strand. After DNA replication begins it directs late transcription of the capsid proteins from the opposite strand. The nucleotide sequence of the promoter/enhancer is highly variable between isolates taken from different PML brains. In contrast, the promoters of isolates from urine and kidneys have an identical sequence that lacks repeats and contains additional elements, termed the archetype (Yogo et al., 1990; Markowitz et al., 1991; Flaegstad et al., 1991; Tominaga et al., 1992). The sequences of the PML-type regulatory regions can be derived from the archetype by a small number of deletions and duplications. We have shown previously that a different, unique sequence arrangement is obtained from each PML brain examined (Ault & Stoner, 1993), all of which are type 1 or 2 genomes. Also, genomes with identical point mutation patterns throughout the coding region have been found with an archetypal regulatory region in the kidney and a unique rearrangement of the regulatory region in the brain (Loeber & Dörries, 1988; Ault & Stoner, 1994). It is hypothesized that virus with the archetypal regulatory region is the transmitted form and that rearrangement occurs within the host.
In tissue culture cells, as in the host, JCV with a PML-type promoter has a strong preference for multiplication in glial cells (Kenney et al., 1984; Feigenbaum et al., 1987). Transcription factor availability is the main difference between permissive and non-permissive tissues (Tada et al., 1989; Feigenbaum et al., 1987; Krebs et al., 1995). It has been suggested that rearrangement of the archetype promoter/enhancer adapts it to growth in brain tissue (Loeb & Dorries, 1988; Frisque & White, 1992; Iida et al., 1993), which implies that the archetype would have low activity in glial cells and regulatory regions obtained from brain tissue would be more active in directing transcription and/or replication. However, a basis for the inactivity of the archetype in glial cells is not apparent. While certain sequence elements of the archetype promoter/enhancer are consistently retained in naturally occurring PML-type promoters, and others are deleted in some isolates, there is no sequence which is consistently either deleted from or duplicated in promoters of virus from PML brains. The majority (at least 50%) of sequences obtained from PML brains show a moderate degree of rearrangement compared with the archetype, while the rest are either highly rearranged with triplications and more sequences missing, or are minimally rearranged with only small alterations, in about equal proportion. This was described previously (Ault & Stoner, 1993) and has been consistently observed in other work (Yogo et al., 1990; Henson et al., 1992).

In this report, three questions are specifically asked: whether or not the archetype has transcriptional and replicational activity in glial cells that is comparable to PML-type promoters; whether the three patterns of PML-type sequence arrangements differ in transcriptional or replicational activity relative to each other; and whether any feature distinguishes the function of the archetype regulatory region from the rearranged sequences.

Methods

Oligonucleotides and plasmids. To construct the reporter plasmids, the entire non-coding regulatory regions cloned from PML brain (Ault & Stoner, 1993) or from kidney (Ault & Stoner, 1994) were amplified with primers which disrupt the ATG codon in the virus sequence, then cloned into the Xbal–SalI site of pCAT-basic. This reporter plasmid expresses chloramphenicol acetyl transferase (CAT; Promega), with both early and late orientations to the CAT gene. The Mad-1 regulatory region was amplified from a cloned virus genome (ATCC). Primers used were: ECX (5′ GACACTCTAGATCCATTTTCCCATTTTGGCT 3′) and ECS (5′ GACACTCTAGATCCATTTTCCCATTTTGGCT 3′) for early; and LCX (5′ GACACTCTAGATCCATTTTCCCATTTTGGCT 3′) and LCS (5′ GACACTCTAGATCCATTTTCCCATTTTGGCT 3′) for late. A total of 11 plasmid pJC-T, containing the full repertoire of JCV promoter and enhancer elements was used. For this study, pJC-T was co-transfected and a β-galactosidase assay performed by a diffusion scintillation method as described (Atwood et al., 1995). Radioactivity (c.p.m.) was measured at 20 min intervals for 3 h (background was subtracted at each time point) and CAT activity values were reported as the change in c.p.m. per h per mg of protein. This assay measures conversion rate in the linear phase of the reaction, giving more accuracy than single time-point determination methods. When cell culture and transfection conditions were precisely duplicated, equivalent µg of protein gave CAT values with ± 10% between experiments of approximately 10% in addition to checking protein concentration to normalize results between samples, transfection efficiency was monitored by dot blot hybridization to detect transfected DNA in the extracts (Nakshatri et al., 1990). Filters were hybridized with a random primer-labelled 679 bp fragment containing the CAT gene, then cut into segments and counted in scintillation fluid. Also, in one experiment, the plasmid pSV-β-gal was co-transfected and a β-galactosidase assay (Promega) was performed. Although CAT readings were proportionally the same between promoters, without pJC-T, as with standardization by the two external control methods, the overall readings were lower in the presence of the SV40 promoter. This control was therefore not used in the reported values.

Replication assay. Transfection of CAT plasmids plus pJC-T was performed as for CAT assays, and cells were grown for 48 or 96 h before harvesting low molecular mass DNA (Hirt, 1967). The presence of DNA replicated in the cells was detected as described (Scock et al., 1991) after linearization with XhoI, digestion of unmethylated DNA with DpnI and hybridization with the 679 bp CAT gene probe.

Results

Transcription of the archetype and four PML-type promoters

The aim of this study was to determine what, if any, change in function occurs in the different arrangements of promoter/enhancer regions and whether the archetype differs in any way from the promoters taken from PML brain. The majority of data on JCV transcription and replication have been generated in a human glioblastoma line (Chowdhury et al., 1993; Renner et al., 1994; Tada et al., 1990; Lashgari et al., 1989; Wegner et al., 1993). Therefore we used the same cell line (U87) to provide the best direct comparison with existing knowledge of promoter activity. The archetype regulatory region, three regulatory regions from PML brain tissues and one from the prototype strain Mad-1 were compared for their ability to drive early and late transcription and replication. The archetype contains the full repertoire of JCV promoter and enhancer elements, while 104, 203 and 102 have minimally, moderately and highly rearranged promoter sequences, respectively (Ault & Stoner, 1993). Mad-1 is the prototype strain initially derived from serial passage in culture (Frisque et al., 1984), which has been used in most JCV studies. These regulatory regions are
Fig. 1. (a) Alignment of PML-type promoter/enhancers with archetype sequence (top) between the origin of replication (at nucleotide 1 in genome map) and the late ATG codon. Dashes indicate identity to the archetype sequence, gaps are deletions relative to archetype, repeats begin on the next line. Transcription factor-binding sites or homologies are indicated above the sequence. Regions A–F in the sequence are alternately underlined beneath the sequence. A/B and C are sites discussed in Amemiya et al. (1989). (b) Diagram of archetype and prototype regulatory regions between the ATG codons for early and late coding sequences, indicating relative orientation of sequence elements.

Illustrated in Fig. 1. The entire non-coding region between the ATG codons was placed in both the early and late orientations with respect to the CAT reporter gene. Early and late direction transcriptional activity was tested first at the constitutive level, i.e. without T antigen. The prototype Mad-1 was the strongest promoter and its early activity was counted as 100% (Fig. 2). The early promoter activity of the archetype and the moderately rearranged 203 was nearly equal. The highly rearranged 102 early activity was lower and the minimally rearranged 104 was the lowest. Expression from the SV40 early promoter was 5–9-fold higher than from the Mad-1 early and was not included in the graph. Late promoter strength ranked in the same order, with the exception that the archetype late was lower than 203, in the
The transcriptional strength of the archetype promoter/enhancer in glial cells fell within the same range as that of the PML-type promoters. However, the archetype differed in the ability of the late promoter to respond to T antigen, and this property was shared by the four rearranged promoters with different sequence patterns and absolute levels of transcription.

T antigen stimulation and relative late and early expression

Next, the plasmid pJC-T was co-transfected with the promoter–CAT vectors. pJC-T expresses T antigen in U87 cells, as seen by fluorescent antibody detection of T antigen-positive cells 48 h after transfection (data not shown). An increased activity in the late direction and a decrease in early resulted in each case, albeit to different degrees (Fig. 2). The late promoter of the archetype was less responsive to T antigen stimulation, showing an increase in CAT activity of 59%, whereas the rearranged promoters showed an increase of 140 to 240%. The repression of early transcription and the stimulation of late brought on by T antigen resulted in late promoter output exceeding early in the four PML-type promoters but not in the archetype. This change was reflected in the ratio of early:late activity, which dropped to less than 1 upon stimulation by T antigen for each of the PML-type promoters but was 2-4 for the archetype. This property was shared by the four rearranged promoters with different sequence patterns and absolute levels of transcription.

Replication

Promoter/enhancer elements in JCV, especially the NF-1 site in region C, also have a role in genome replication (Lynch & Frisque, 1990; Sock et al., 1991, 1993). We next determined whether the PML-type rearrangements serve to stimulate the origin of replication. The ability of these five regulatory regions to replicate the CAT plasmid when co-transfected with pJC-T into U87 cells was compared. Neither at 48 h nor at 96 h was there an apparent difference between the five regulatory regions in the amount of newly replicated DNA that accumulated; no advantage over the archetype structure was conferred by rearrangement. Replication could not have accounted for the differences in transcription between the five plasmids, since the level was indistinguishable between them. Relative to the input plasmid Dpln fragment, there had not been extensive replication by 48 h, the time-point at which transcription was measured. Late transcription more than doubled while early transcription decreased, indicating that replication did not account for the transcriptional change in response to T antigen that was measured by CAT activity.

Discussion

The transcriptional strength of the archetype promoter/enhancer in glial cells fell within the same range as that of the PML-type promoters. However, the archetype differed in the ability of the late promoter to respond to T antigen, and...
therefore differed in relative early and late direction strength when T antigen was present. It was previously reported that deletions introduced into the BK virus promoter can shift relative early and late transcription (Cassill & Subramani, 1989), and here such a shift was observed in naturally occurring promoters. No indication was seen that the rearrangements enhanced replication. Interestingly, some of the promoters obtained from PML brains had lower transcriptional output than the archetype.

This paper is the first report on transcription of JCV promoters that takes into account the hypothesis that virus with the archetype regulatory region is the transmitted form of JCV and rearrangement occurs in the host along with the development of PML. This point is not proven, as the rearrangement of archetype has not been experimentally observed. However, it is indicated by a large body of circumstantial evidence, as described in the Introduction, and no other theory explains all the observations. In the context of this hypothesis, a set of promoters representing the different degrees to which the sequence is rearranged were compared with the archetype to determine if a functional change can be detected because of sequence alteration. Using this design, a functional difference between archetype and all the other sequences (the late promoter’s ability to be activated by the presence of T antigen) was found. The results also show that a simple increase in overall level of transcription did not always result from promoter rearrangement, nor is it likely to be the driving force behind rearrangement. In a recent report (Sock et al., 1996), three clones of archetype were compared to each other, apparently to make the point that the investigators did not acknowledge that the archetype is a general phenomenon. In that paper, the other promoters used for comparison and the presentation of the results do not allow a conclusion to be drawn as to whether or not a qualitatively different response had occurred between archetype and the PML-type promoters as a group.

Because the entire non-coding region was included in the plasmids, it was possible to observe the effects of changes in spatial relationships between enhancer elements created by the rearrangements. For maximum activity, an optimum balance of elements appeared to be reached in the moderately rearranged pattern seen in 203 and Mad-1, which consists mainly of loss of the 66 bp region D and duplication of regions C and E. The majority of genomes amplified from PML brains had this pattern (Ault & Stoner, 1993). It is unlikely that genomes with strong, ‘optimally rearranged’ promoters occurred along with the less active arrangements to complement their growth. In 14 PML brains from which multiple clones were sequenced, usually only a single sequence was found rather than a mixture of sequences (Ault & Stoner, 1993, 1994), and 99 clones from a single patient had only two sequences (Yogo et al., 1994). CNS symptoms did not differ in duration or severity in the PML cases from which 102, 104 and others like them were isolated, nor did the presence of human immunodeficiency virus appear to confer viability on otherwise inactive promoters (Ault & Stoner, 1992). This suggests that quantitative transcriptional output within this range is not the determining factor in glial cell infection.

The activities of these promoter permutations can show which sequence elements to test for early and late function. An NF-1 site in region C, adjacent to an Ap1/cre site, appears to be central to JCV promoter activity (Amemiya et al., 1989; Tada et al., 1989; Ahmed et al., 1990; Tamura et al., 1988; Ault & Stoner, 1993). Mad-1 and 203 have two copies of this region while the archetype has only one, yet the archetype early promoter was as active as 203. Promoter 102, with three copies of the site, had a lower activity, and 104, which differs from the archetype only in sequences near the late side, was still lower. A possible explanation for these results comes from observing an NF-1 element in the first part of region F, which also binds NF-1 (Amemiya et al., 1989; Tamura et al., 1988). Adjacent to this site is a region with similarity to a binding site for p53 (Raj & Khalili, 1995), which has the potential to regulate the promoter significantly and is known to be involved with polyomavirus replication cycles (Iida et al., 1993). The lower activity of 104, in which this site is deleted, suggests that the site should be reexamined for function in some promoters. Promoter 102 has three copies of region C, which suggests that positional or spatial effects, or an unidentified element in the second half of region C, may affect early transcription.

A late transcription initiation site found in Mad-1 exists in region E (Kenney et al., 1986; Daniel & Frisque, 1993), which is consistently retained in viable rearranged promoters (Ault & Stoner, 1993). Although the constitutive late activity of the archetype was higher than 104 and 102, which have two and four copies of region E, respectively, the archetype produced a weaker stimulation of late activity by T antigen. This suggests that region E may be a significant feature of the late promoter and important for T antigen stimulation.

No evidence of an inhibitory transcriptional element was shown in these experiments. Region D is usually deleted when rearrangement occurs, but when it is retained it does not prevent the virus from multiplying in brain (Ault & Stoner, 1993; Henson et al., 1992). It was demonstrated here that the archetype is an active promoter in glial cells, indicating that region D does not contain an inhibitory element. A new report (Daniel et al., 1996) suggested that regions B and D are inhibitory because a genome with the Mad-1 promoter was the most active in producing virions, even though the data also showed that deletion of either region B or region D from the archetype promoter resulted in a large decrease in transcription. However, as noted before, all published sequences obtained directly from brain retain region B, while the prototype Mad-1 and the closely related Mad-4, both derived from passage in culture (Frisque et al., 1984), are unique in their deletion of region B. Taken together with the results presented here, there is nothing to suggest that regions B and D contain inhibitory elements.
Do sequence rearrangement and oligodendrocyte infection simply coincide as two separate consequences of reactivation in the immune-compromised host, or does rearrangement enhance the ability of JCV to reach the brain and multiply there? Virus genomes with an archetype promoter predominate in the kidneys of PML patients, but a very minor amount of rearranged promoter sequence can be detected as well that is identical to the unique sequence found in the brain of the patient (Ault & Stoner, 1994). This finding is consistent with an infrequent rearrangement event (not presumed to occur in the urinary tract) which produces a promoter with enhanced activity, especially in brain, which then becomes widely disseminated. The present observation that the late promoter of all the rearranged regulatory regions had enhanced responsiveness to T antigen, relative to archetype, may be significant in the switch to the late phase of the lytic cycle in which capsid proteins are produced. The changes discussed above in the spatial relationships of promoter or enhancer elements may mean that rearranged promoters respond differently to the stimuli received within the oligodendrocytes or the tissues in which JCV is activated prior to entry into the brain. The ubiquitous appearance of the archetype in the urine and kidneys of patients indicates that it plays a role in some phase of virus infection. It may be preferred for maintaining persistent infection or for growth in non-glial cells. The changes seen in PML-type regulatory regions are likely to arise from the release of pressure to maintain the archetype sequence when conditions of immune suppression or introduction into the very permissive oligodendrocytes occur. The results presented here suggest that an attenuated response to T antigen stimulation, or proportional early/late expression, may be the significant feature of the archetype form of the JCV promoter/enhancer.

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References


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