Simian adenovirus 7 (SAdV-7, originally designated SV25 as part of a sequential numbering of the viral agents) was one of a series of adenoviruses that was isolated following the observation of cytopathic effect in some of the cultures made from thousands of monkey kidneys used for the production and testing of poliomyelitis vaccine (Hull et al., 1958). Simian adenoviruses have been previously classified into four subgroups using haemagglutination characteristics that are analogous to those used to classify human adenoviruses, where SAdV-7 was found to belong to the same subgroup as SAdV-1 (Rapoza, 1967).

We have investigated the use of adenovirus gene transfer vectors based on adenoviruses derived from non-human sources because the prevalence of antibodies capable of neutralizing these viruses is probably low in humans (Roy et al., 2004, 2006). We screened for simian adenoviruses where the E1 deletions could be complemented in cell lines such as HEK 293 that are currently used to manufacture adenovirus vectors, i.e. their growth characteristics would not restrict them to grow in simian-derived cell lines. Both SAdV-1 and SAdV-7 were able to propagate in HEK 293 cells. In order to facilitate the construction of a plasmid molecular clone of SAdV-7, the complete viral genome was sequenced.

SAdV-7 (ATCC VR-201) was propagated and amplified in the African green monkey kidney cell line BS-C-1 (ATCC CCL-26). The virus was purified by caesium banding and the viral DNA was extracted by using standard procedures. This viral genomic DNA was sequenced (Qiagen Genomics Services) by generating a whole genome shotgun library. Complete sequencing was achieved with four- to sixfold coverage. The sequences of the viral left and right ends including the inverted terminal repeats (ITRs) were reconfirmed by sequencing of the clones obtained during molecular clone construction. The sequence was submitted to GenBank (accession no. DQ792570). The genome length of SAdV-7 was found to be 31045 bp. Sequence analysis revealed it to be very closely related to SAdV-1 (Kovács et al., 2005), and like SAdV-1 is most similar to the recently reported human adenovirus isolate HAdV-52 (Jones et al., 2007) and the species HAdV-F as exemplified by the enteric strains HAdV-40 and HAdV-41. HAdV-F members and HAdV-52 are unique among human adenoviruses in possessing two fiber coding sequences; SAdV-7 (and SAdV-1) also encodes two fiber proteins. The genome organization of SAdV-7 is shown in Fig. 1. The comparatively short genome length of SAdV-7 is due to two factors: truncation of the E1 region and absence of all but one of the E3 protein genes.

Analysis of the genome sequence showed that of the three major E1 products present in most adenoviruses, the SAdV-7 isolate that was sequenced encodes only one intact E1 gene product, the E1B 55K protein. This is in contrast to all other reported macaque adenovirus sequences (Kovács et al., 2004, 2005; Roy et al., 2009) as well as the genus Mastadenovirus as a whole, where an E1A protein coding sequence is always identifiable (Davison et al., 2003). The overall nucleotide sequence identity between SAdV-7 and SAdV-1 in the E1 region is 90% and when the two sequences are aligned, the extent of the deletion can be mapped as shown in Fig. 1. The approximately 850 bp

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Construction of gene transfer vectors based on simian adenovirus 7

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The complete nucleotide sequence of an isolate of simian adenovirus 7 (SAdV-7) was determined. The genome organization of this isolate was found to be similar to that of other primate adenoviruses with two principal notable points: severe truncation of the E1A and E1B 19K proteins and an E3 region encoding only the 12.5K homologue. The viral gene products of SAdV-7 are most closely related to simian adenovirus 1 (SAdV-1), and like SAdV-1, are related to the human adenovirus species HAdV-F, such as the enteric adenoviruses HAdV-40 and HAdV-41 and the recently defined HAdV-G (HAdV-52). Two kinds of gene transfer vectors were made: a replication-competent SAdV-7-based vector with no genomic deletion, and a standard replication-incompotent vector deleted for E1. Importantly, the E1-deleted vector could be propagated to high titre by trans-complementation in human HEK 293 cells.
deletion extends from within the coding region of the E1A protein to past the start of the E1B 19K protein. The TATA box and the start codon for E1A are preserved; however, the putative E1A ORF is only 153 nt long. Thus, it is likely that the only functional protein expressed from SAdV-7 E1 region is the E1B 55K protein, although the functionality of the putative 51 residue E1A product cannot be ruled out. The other important difference of the SAdV-7 genome compared with the genome of SAdV-1 was the absence of most of the E3 region. The only ORF found was that for the 105 residue E3 12.5K protein. The E3 12.5K protein in SAdV-7 is 96 % identical to the homologues present in both SAdV-1 and HAdV-52. It is interesting to note that all other macaque adenovirus sequences reported to date such as SAdV-1 (Kovács et al., 2005), SAdV-3 (Kovács et al., 2004), SAdV-49 and SAdV-50 (Roy et al., 2009) harbour a more extensive E3 region, as is present in most human and simian adenoviruses, i.e. including ORFs for at least one of the E3 CR1 proteins, as well as the anti-apoptosis proteins RID-α, RID-β and the 14.7K protein. In this respect, it is similar to reported porcine, murine and canine adenoviruses (Davison et al., 2003).

As discussed above, SAdV-7 possesses two fiber genes, one with a shaft length of 143 residues and a second with a shaft length of 335 residues. The highest degree of homology of the fiber genes are with the homologues in SAdV-1 and HAdV-52. Remarkably, the N-terminal region of the SAdV-7 short fiber ORF corresponding to the fiber tail (the domain considered to interact with the penton base) is not homologous to other fiber tail sequences, but is co-opted from the N-terminal region of an adenoviral E3 CR1-α protein, although no such protein was encoded in the E3 region of this isolate. It would be interesting to determine whether this short fiber is efficiently incorporated into the adenoviral vertex. Because the sequencing of the SAdV-7 isolate revealed a severely truncated E1 region, we wished to rule out the possibility that the E1A ‘deletion’ was being complemented by E1A protein expression from a minor component of the adenoviral preparation that had escaped detection. To do this, a plasmid was constructed (pSV25wt, Fig. 2, top) that harboured the complete SAdV-7 genome. This plasmid was digested with Swal to release the viral ends and transfected into the monkey kidney BS-C-1 cells. Virus rescue was
successful within a few days, establishing the viability of this SAdV-7 isolate harbouring the truncated E1 region.

We next tested whether SAdV-7 could be used to generate a conventional E1-deleted replication-incompetent adeno viral vector that could be complemented by the HAdV-5 E1 genes in the HEK 293 cell line. A plasmid was constructed where a 1479 bp (552–2030 bp) deletion was created encompassing the vestigial E1A- and most of the E1B-encoding region. In its place an expression cassette [where the expression of a green fluorescent protein (eGFP) is driven by the cytomegalovirus immediate-early promoter] was inserted (pSV25eGFP, Fig. 2, middle). This plasmid was transfected into HEK 293 cells (that provide HAdV-5 E1 functions in trans) as well as BS-C-1 cells. Virus could only be rescued from HEK 293 cells, suggesting that deletion of E1B 55K may have rendered the adenovirus replication defective in non-complementing cell lines. Finally, a replication-competent vector was constructed by inserting the transgene cassette between the right ITR and the E4 region (Chanda et al., 1990) and transfecting the plasmid pSAdV-7(RC)eGFP (Fig. 2, bottom) into BS-C-1 cells.

One important aspect in the successful complementation of E1-defective adenoviruses is the functional interaction of the E1B 55K protein (produced by the trans-complementing cell line) with the E4 orf6 protein encoded by the virus. Therefore, E1-deleted HAdV-B adenoviruses such as HAdV-35 can be propagated in HEK 293 or PER.C6 cells (which express the HAdV-5 E1 genes) only if the cognate HAdV-5 E4 orf6 product is also present (Abrahamsen et al., 1997; Gao et al., 2003; Holterman et al., 2004; Seshidhar Reddy et al., 2003; Sirena et al., 2005; Stone et al., 2005; Vogels et al., 2003). The implication of this observation is that the HAdV-35 E4 orf6 protein interacts poorly with the HAdV-5 E1B 55K protein. The sequence similarity between HAdV-5 and the HAdV-35 E1B 55K proteins is about 69%; the similarity between the HAdV-5 and SAdV-7 E1B 55K proteins is a little lower (about 64%). Therefore, it was interesting that we were able to successfully complement the SAdV-7 E1 defect in HEK 293 cells.

Although rescue of the replication-deficient E1-deleted SAdV-7 vector was achieved in HEK 293 cells (i.e. without the HAdV-5 E4 orf6 protein), it was of interest to determine whether the successful complementation of E1-deleted SAdV-7 in HEK 293 cells was accompanied by delayed or impaired replication. Accordingly, the growth curves of the E1-deleted and the replication competent SAdV-7 vectors were determined in non-complementing BS-C-1 cells and in HEK 293 cells (Fig. 3). The E1-deleted SAdV-7 vector (SV25eGFP) was found to be non-replicating in BS-C-1 cells, whilst replication of the SAdV-7(RC)eGFP vector (that harbours the wild-type E1 region) steadily increased over time. In contrast, in the complementing HEK 293 cells, both vectors reached this peak of replication at about the same time, i.e. within 48 h of infection. These results indicate that HEK 293 cells are able to sustain rapid and high levels of the

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**Fig. 2.** The maps of the plasmid molecular clones harbouring the complete SAdV-7 genome – pSV25wt (top), the E1-deleted SAdV-7 genome where the E1 deletion (extended to eliminate all of E1 including E1B 55K) has been replaced by an expression cassette – pSV25eGFP (middle) and the replication competent SAdV-7 genome harbouring an eGFP transgene insert adjacent to the right ITR – pSAdV-7(RC)eGFP (bottom) are shown. Viruses were rescued by digesting the plasmids with Swal and transfecting into HEK 293 (pSV25eGFP) or BS-C-1 cells [pSV25wt and pSAdV-7(RC)eGFP].
replication-deficient vector, similar to those of the parental wild-type virus SAdV-7.

The E1A region of HAdV-C members express a transcript where differential splicing results in the expression of two principal protein products of 289 and 243 residues, respectively. Upon infection, these proteins bind to a number of cell-cycle regulators (such as Rb) and promote a transition to the S phase. The E1A proteins also bind to a number of transcription factors (such as the CREB-binding protein and p300) as well as members of the transcription complex (such as the TATA-binding protein). In the absence of the E1A gene products, transactivation of most of the other viral promoters does not occur, and as a result the other early region transcripts (those of the E2, E3 and E4 regions) are severely reduced, as is expression of the late genes. The domains of the E1 protein that have been identified as being responsible for these activities are the N-terminal region and the four conserved regions named CR1, CR2, CR3 and CR4 (Berk, 2005; Pelka et al., 2008). The SAdV-7 E1A ORF can encode a 51 residue protein (Fig. 1) that corresponds to the N-terminal region of the E1A protein as well as the first 13 residues of the CR1 region (which is natively about 40 aa long). The N-terminal and the CR1 together with the CR2 domains are responsible for the binding interactions that drive quiescent cells into the S phase in preparation for adenoviral replication: this function is thus not necessary for adenoviruses propagated in culture, although it is important in vivo. It is difficult to estimate whether this function may be preserved in the protein encoded by the 51 residue E1A ORF. However, the absence of the CR3 domain that is usually responsible for transactivating the other adenoviral early promoters is more striking. Absence of the CR3 domain in HAdV-C is associated with severe attenuation of viral growth properties (Schneider et al., 1987), which is clearly not the case with the SAdV-7 isolate. Also missing in the E1 region is the start codon for the E1B 19K protein (Fig. 2) that in large measure counteracts apoptosis induced by E1A (Berk, 2005).

Circulating antibodies to adenoviruses in humans has led to a search for adenovirus serotypes where pre-existing immunity is unlikely, thereby increasing the likelihood of successful administration. Rare human adenovirus serotypes or non-human adenoviruses have the potential to be developed into vaccine vectors, which may have widespread utility. However, development of cell lines to complement engineered E1 defects is onerous. Therefore, the availability of non-human serotypes that may be propagated in established cell lines such as HEK 293 is a great advantage. Adenoviruses isolated from monkeys are phylogenetically quite distant from HAdV-C members (Roy et al., 2009) that have been the basis for the majority of existing vectors; it was therefore surprising that a vector based on SAdV-7 is able to be complemented adequately in HEK 293 cells.

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References


