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Editorial

Site-specific integration by adeno-associated virus: a basis for a potential gene therapy vector

Recently there has been considerable interest in the possible use of adeno-associated virus (AAV) as a vector for human gene therapy.[1] AAV has not been implicated as the cause of any disease; indeed, in cell cultures and animal model systems it is anti-oncogenic. Possibly the most striking property of the virus is its inability to productively infect healthy cells in culture in the absence of a co-infection by a helper virus (either adenovirus or herpesvirus).[2] Thus, AAV vectors are inherently replication defective. Additional features of the virus which enhance its desirability as a vector include its wide host range and the stability of the virion. Genetic analysis has been aided by the fact that the cloned viral DNA is infectious in the presence of helper virus, a feature that greatly simplifies production of recombinant virus particles. Infection in the absence of helper virus leads to integration of the AAV genome at a specific site on the q arm of chromosome 19 to establish a latent infection.[3–5] This is the only known example of site-specific integration by a mammalian virus and suggests the possibility of a vector which can be inserted at a known site in the human genome. In human cell culture there is little evidence of significant effect on the phenotype caused by latency. However, it should be noted that no cases have been detected in which both copies of an unoccupied preintegration site are disrupted by AAV integration. Thus, it is possible that integration at more than one preintegration site is lethal, albeit the likelihood of this happening in a significant number of cells in an intact host seems unlikely. An additional advantage of AAV as a vector is that a latent infection can be established in nondividing cells, although only at several per cent of the frequency seen in dividing cells. The target sequence for integration has been cloned and sequenced and is upstream from an open reading frame which is transcribed at a low level in several tissues. The critical sequences for site-specific integration have been determined to reside in a 33 nucleotide sequence which contains two signal sequences that play essential roles in AAV DNA replication.[6] These are a binding site for the AAV Rep protein and an appropriately positioned site which is specifically nicked by the Rep protein.[7] The genetic data are complementary to biochemical data showing Rep interaction with this sequence. A copy choice model involving limited Rep-dependent DNA synthesis has been developed to account for the data.[8,9] The model takes into account the recent observation that, in the absence of helper virus co-infection, the elongating strand in AAV DNA synthesis has a propensity to switch template strands.[9] Although we have gained a detailed knowledge of some of the molecular mechanisms underlying AAV site-specific integration, it is important to note that all of the information to date has been gained through studies in cell culture. Studies have been started in animal models with both wild-type virus and vectors, but detailed knowledge on tissue distribution and integration is not yet at hand. However, in several instances the vector DNA has persisted for many months and there has been evidence for continued expression of the transduced gene.[10]

All of the vectors which have been developed to date have foreign sequences inserted between the inverted terminal repeats (ITR) of the AAV genome. The ITR is required for both packaging of the genome and site-specific integration. However, as noted above, the AAV Rep protein is required to mediate site-specific integration. Vectors which lack the *rep* gene can integrate into the genome, but the frequency of such nonspecific integration is unknown. The *rep* gene has been deleted from the vectors for two reasons. The first is that the small size of the genome frequently precludes the inclusion of any AAV sequences beyond the ITR. The second is that detectable levels of *rep* expression are cytotoxic to many cells. Therefore, there is concern that a vector containing a *rep* gene might be toxic and thus unacceptable. Although this has not been observed with wild-type AAV, it is not certain that this would also be the case for a vector. This is particularly the case after integration of the vector. Will the *rep* gene in an integrated vector remain repressed? Of additional concern is that the presence of a *rep* gene in the integrated genome renders it potentially susceptible to rescue after superinfection by a helper or exposure of the cell to conditions which would render it permissive for AAV replication. In spite of these concerns, development of vectors which involve the presence of Rep would allow the vector to be preferentially inserted at a specific site in the cellular genome and thus, there would be a more controlled administration of the gene to be transduced. There are two approaches to resolution of the conundrum posed by the potential advantages of the presence of Rep and the possibly toxic consequences of its presence which have been considered. The first of these is to develop a hybrid vector which would contain both a vector composed of the foreign sequences between two copies of the ITR and a *rep* gene which would be sited outside the ITRs. Such a vector might either be a hybrid adenovirus vector or a plasmid. In either case the *rep* gene would be appropriately activated in the recipient cell, not enough to allow productive DNA

replication, but enough to rescue the integrated AAV vector from the backbone and allow it to integrate at a specific site in the cellular genome. In the former case (adenovirus–AAV hybrid), the challenge is to achieve exquisite control of the expression of both adenovirus and AAV gene expression in both the packaging cell and in the recipient cell. The alternative possibility is to construct a vector system which contains Rep protein. This presumes a knowledge of the appropriate form and amount, as well as intracellular localization, of the protein required to allow site-specific integration to occur. This type of approach would be greatly facilitated by the development of an *in vitro* assay for site-specific integration. Finally, we do not know if the site for wild-type AAV integration is optimal for the desired level of expression of the transduced gene. When we understand sufficiently the mechanisms involved in site-specific integration, it may be possible to modify the systems described above to direct site-specific integration at alternative sites in the human genome. In summary, current AAV vectors have already yielded intriguing and encouraging results. By making use of the mechanisms underlying site-specific integration by wild-type AAV, it is likely that even better vectors will be able to be designed.

R Michael Linden and Kenneth I Berns
Department of Microbiology
Hearst Microbiology Research Center
Cornell University Medical College
1300 York Avenue
New York, NY 10021, USA

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