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as viral vectors are used for gene therapies that are engineered to affect a broader range of cells, gene-therapy developers are likely to find more cases where they must use a vector that has the ability to replicate. this may include efforts to treat systemic diseases such as hypertension, diabetes, and obesity. the use of self-replicating vectors would enable the therapy to persist over time and would not require reinjections. however, the risks associated with self-replication must be carefully weighed. the development of synthetic biology, or the use of dna-encoded information to create biological entities, has enabled the construction of artificial viruses. recently, synthetic biology has been used to create self-replicating viral vectors, including aav 22 22. yongjoo baek et al., a recombinant adeno-associated virus vector induces and maintains transgene expression in the mouse brain and retina, nature biotechnology, march 2018, volume 36, pp. 667-672, journals.nature.com. and recombinant adeno-associated virus (raav) 23 23. kaoru sawai et al., optimized human immunodeficiency virus type 1 (hiv-1) vector based on an adeno-associated virus vector: rapid production, purification, and infection of cos-7 cells, gene therapy, april 2018, volume 15, number 4, pp. 584-593, liebertpub. one potential use of such vectors would be to develop immune-system modulators. for this reason, strategies to limit transgene expression to the cells of interest are gaining traction. these strategies include using promoters and regulatory elements that are selectively active only in cells of interest, using cis-regulatory elements such as splice variants that are only expressed in cells of interest, using small molecules that can be used to control the expression of gene therapy vectors, and using transgene codon optimizers that can limit transgene expression in order to reduce unintended off-target expression. the latter strategy, which is employed by the use of transgene codon optimizers, is the approach that is most frequently used to produce safe and efficacious gene therapies today.

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aav vectors have been used to deliver a broad range of genes to the brain, eye, and muscle. in addition, scientists have achieved an efficient aav vector-mediated gene transfer in the liver by pretargeting approaches.39 targeted aav vectors have also been successfully used in animals to achieve widespread gene transfer across multiple tissues, such as the heart, liver, muscle, and pancreas.41 most people who arent exposed to a viral vector vaccine receive their first dose of vaccine at the time of immunization with a non-human adenovirus vector. this so-called homologous prime-boost strategy is optimal in that the priming vaccine is believed to provide anamnestic immunity (i.e., the ability to generate an immune response when a second, second vaccine dose is administered months later) in the majority of people. the subsequent boost vaccine provides extra antigenic stimulation, leading to a stronger immune response to the antigen. however, in people who have been exposed to a non-human adenovirus vaccine, the immune system tends to mount an immune response to the vector, resulting in immune interference and poor immune responses. while this is a minor issue for most gene-therapy applications, the limited efficacy of adenovirus-based gene therapy vaccines has been highlighted as a major barrier. the second challenge is long-term durability. the majority of viral vectors used in current gene-therapy trials are derived from human adenovirus (ad) serotypes. these are best known for their ability to infect the epithelial cells of the respiratory tract, but they also can infect dendritic cells, hepatocytes, and neurons, which can lead to adverse events. the third challenge is manufacturing. in recent years, we have seen a number of manufacturing setbacks, including in manufacturing of the aav vector. while many of these are highlighted in previous chapters of this book, the recent use of chinese-manufactured recombinant aav (raav) vectors to treat patients in the united states with hemophilia b is a recent example of this issue. 5ec8ef588b