amounts of progeny virus that spread horizontally. Understanding the process of retroviral integration may help explain how the human genome has evolved and how retroviral infection may cause genomic damage. It will further help us assess potential risks of gene therapy using retroviral vectors.

In a recent study, published in *Cell*, Frederic Bushman and co-workers[2] mapped HIV type 1 (HIV-1) integration sites in the human genome. Bushman's group sequenced 524 integration junctions between viral and host DNA in an HIV-infected human cell line, and then located these in the human genome sequence. They then looked at the cDNA expression profile after viral infection to compare the locations of genes that are active after infection with the viral integration sites.

An impressive 67–86% of HIV-1 integration sites were found in transcribed regions of the human genome. Moreover, gene activity surrounding the integration hot spots increased by 2–3 fold in general after infection, suggesting a close association of viral and host gene expression.

As a control, the researchers also prepared naked DNA from the same T cell line as target DNA and HIV-1 preintegration complexes (PICs) from infected T cells so they could analyze *in vitro* integration events. They found that roughly 35% of the *in vitro* integration sites were in transcribed regions of the human genome. Since ~33% of the human genome is present in transcription units anyway, this is about what you would expect if integration was random.

The new data confirm previous observations that retroviral integration favors transcribed genes.[3,4] However, importantly, their analysis of host gene expression affected by HIV-1 infection showed, for the first time, that HIV-1 targets genes that are activated both before and after infection.

The pattern of HIV-1 integration clearly differs from those of the vast majority of human endogenous retrovirus (HERV) sequences. HERV sequences represent up to 1% of the human genome, but are located mostly outside of gene clusters or exons and thus probably do not affect gene function.[5] By contrast, these new findings clearly put the integrated HIV-1 provirus in the ''pest'' sequence category because targeting actively transcribed genes is likely to interfere with cellular function. Clinical evidence has now been reported. Following the successful treatment of X-linked severe combined immunodeficiency (SCID), one of the 10 children who had received murine leukemia virus (MLV) vector modified bone marrow cells has developed acute lymphoblastic leukemia, apparently caused by integration interruption of a possible oncogene located in chromosome 11.[6]

It is still not clear how HIV-1 PICs find the hot spots that are preferentially associated with the polII transcriptional units. Cellular components associated with chromatin structure and transcriptional machinery may affect retroviral integration directly or indirectly. For example, both MLV and HIV-1 favor sites of active chromatin assembly and DNA looping or bending.[7–9] This raises the possibility that physiological conditions that change chromatin structure and transcription profile (eg stress and hormone signaling) could affect HIV-1 infection and integration, and consequently disease progression.

Retroviral DNA contains two viral polyadenylation signals. When integrated in the same orientation as the targeted host gene, the viral polyadenylation signals may interrupt host RNA transcription and processing. HERV elements present in intragenic regions in the human genome are preferentially inserted in the opposite orientation from that of the host gene. However, the HIV-1 integration sites in the *Cell* study showed no orientation preference relative to that of the targeted genes.[2] The polyadenylation signal of retrovirus is known to be leaky and allows frequent transcriptional readthrough. However, the HIV-1 polyadenylation signal is not as leaky as that of the MLV.[10] Thus, HIV-1 integration into active genes may be more detrimental to the host cells than MLV. This is consistent with the observation that a high multiplicity of HIV-1 infection often causes cytotoxicity and apoptosis of the target cells.

In yeast, retrotransposons such as Ty elements shuttle freely in the host cell genome, but as respectful 'guests' they target specific sites with high precision, such as upstream of the polIII promoters or nonfunctional regions in the yeast genome.[11] This strategy prevents host genes from being interrupted. Regardless of the 'guest' or 'pest' nature, both Ty elements and HIV-1 must have evolved mechanisms to choose their preferred sites in the host chromosomes. The interaction of retroviral PICs with host cell factors, such as chromatin components or polII and polIII transcription factors, may help define the genome 'hot spots' for the transposable DNA.

It is still unclear which parts of the PICs interact with cellular factors to direct retroviral integration. Understanding the mechanisms by which retroviruses and retrotransposons are specifically integrated will shed light on additional strategies of HIV-1 intervention, as well as assist future development of targeted retroviral gene delivery systems.

When HIV-1-based vectors are used for gene therapy, a high multiplicity of infection is always associated with increased cell death. The new work by Bushman and his colleagues suggests that preferential integration of the vectors into hot spots surrounding active host genes could explain this. It might be possible to modify vector components and cell growth conditions to channel PICs into desired chromosomal locations. The ability to target retroviral integration will significantly improve safety and specificity of retroviral vectors for future gene therapy applications. ■

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Adeno-associated viruses

Monkey see, monkey do

JD Mountz

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Like many other gene therapy vectors, effectively transfecting target cells without provoking an antibody response that limits readministration has often proved to be a bridge too far for adeno-associated viruses (AAVs). Now James Wilson's group at the University of Pennsylvania has isolated two new AAV serotypes from chimps that may provide the solution to this tricky problem.[1]

AAVs seemed to be the ideal solution for problems that adenoviral vectors have in delivering long-term expression of transgenes in the target region. These problems result from the immune and inflammatory response adenoviral vectors provoke, which usually leads to rapid elimination of the transduced cells.[2–4]

Unlike adenoviruses, AAVs are well tolerated and do not cause a strong innate end response or cytotoxic T cell response. In addition, transgenes delivered with AAVs tend to be expressed for longer than adenoviral-delivered genes. However, AAVs still do provoke an antibody re-

sponse, and transfection efficiencies using AAVs are often low.

Antibody responses to one AAV serotype tend not to affect another, so to some extent the problem of readministration of the transgene could be overcome with judicious use of the six AAV serotypes previously available. However, for AAVs to be a really effective gene delivery system, an expanded set of AAVs with higher transfection efficiencies was needed. Now Wilson's group has isolated two new AAV serotypes from the chimpanzee and in doing so, it seems, has effectively achieved this goal.

The approach the authors used to isolate the new serotypes was unique and inventive. Genomes of latent AAVs (ie lacking helper viruses) were amplified using PCR primers designed to a conserved region of sequence in the gene encoding the capsid and flanking the variable regions of this gene. This method is more sensitive and more widely applicable than previous methods that required *in vivo* rescue of AAV by helper adenovirus. In future, this approach should allow quick and efficient isolation of new AAV variants and identification of previously isolated serotypes.

The targeting of chimpanzee AAVs was also a clever, and ultimately successful, strategy. The chimp AAVs are sufficiently similar to human AAVs that they should be able to deliver genes to human target cells and be propagated with human adenovirus. Conversely, the new AAVs (AAVs 7 and 8) are sufficiently different in the variable capsid area to AAVs 1–6 that there was no serological crossreactivity with these other types, or other human AAVs under development. Thus, these two new serotypes provide exciting new delivery options for gene therapists, which will be unhampered by any prior therapeutic attempts using human-derived AAVs.

There is no reason why further AAV serotypes cannot be isolated in the same way. Therefore, this new work paves the way to the isolation of an even larger set of AAVs that should circumvent a problem of repeated administration and production of neutralizing antibodies against a given AAV serotype.

Perhaps the most striking endorsement of the potential of these new serotypes as gene delivery vectors comes from the amazing efficiency that AAV8 demonstrates in transferring genes into liver cells. The Wilson group showed that this serotype was one or two orders of magnitude better than any other serotype previously used!

It will be important to determine why AAV 8 is expressed at high and sustained levels in the liver. One possibility might be that expression of the α-1 antitrypsin (A1AT) transgene under the control of the thyroid binding globulin (TBG) is optimal for long-term expression in the liver. This enabled long-term expression in the liver and in an almost unattenuated fashion for at least 50 days.

Another possibility is that AAVTBGA1AT elicits a low immune response in the liver, especially after delivery by the portal vein.

The third, and most likely, explanation is that modifications of the AAV 8 capsid protein enabled higher affinity binding and entry of the virus into the cell. Previously identified receptors include heparin sulfate proteoglycan,[5] alphaVbeta5 integrin[6] and fibroblast growth factor receptor 1 (FGFR1).[7] In addition to expression of receptors and coreceptors, impaired intracellular trafficking and escape from the endocytic pathway of AAV prior to processing in the nucleus is a rate-limiting step for AAV[8] (Figure 1).

Enabling binding, entry and final transcription of the AAV transgene, all without eliciting an immune response, is a challenging goal. The exact role that capsid proteins play in this process (including viral entry, endosomal escape and nuclear transport (Figure 1)) is not known.[8] Nevertheless, several investigators are investigating ways to engineer known AAV capsids and gen-

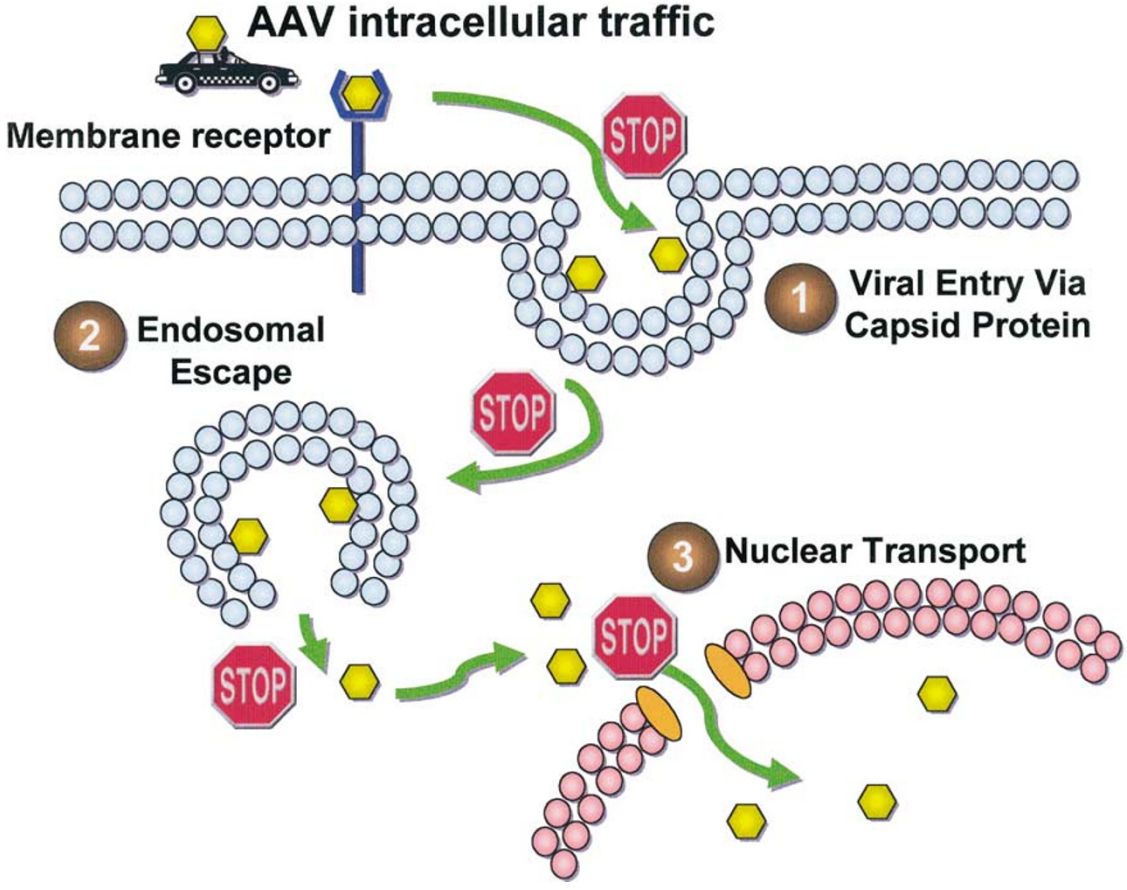

Figure 1 *AAV binds to cells by receptors and coreceptors including heparin sulfate glycoprotein, alphaVbeta5 integrin and fibroblast growth factor receptor. The AAVs are endocytosed by invagination of the plasma membrane. The AAVs must escape from this endocytic vesicle to travel to the nucleus where second-strand DNA synthesis occurs enabling expression of the transgene.*

omes in an attempt to achieve this goal for different cell types.

An equally attractive alternative to 'building your own' is to 'browse the catalog' of naturally available AAVs, in other words identifying AAVs that exhibit desired properties of high-affinity receptor binding, intracellular transportation, expression and evasion of the natural immune response.

The new results from Wilson's group demonstrate an effective method for identifying novel AAVs with different capsid variations. New AAVs potentially have different intracellular properties and provoke different types of immune responses. Thus, there is now real hope that naturally occurring AAVs can be identified that will transfer genes to target cells and allow them to be expressed for long enough and at high enough levels to be an effective genetic treatment. ■

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Obesity gene therapy

Slimming immature rats

RS Ahima

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The worldwide obesity epidemic has grave consequences because of increased risk of diabetes, cardiovascular disease, cancer, and other complications and reduced lifespan.[1] Diet and exercise are the cornerstones of treatment, but an increasing number of patients will require therapeutic intervention to decrease and maintain body weight. Now a new *in vivo* work by Satya Kalra's group at the University of Florida[2] shows that a gene therapy strategy has the potential to be tremendously effective as an obesity treatment in children.

To develop treatments for obesity, studies that help us understand the pathophysiology of body weight regulation are vital.[3] Such studies have shown that fat, rather than merely storing excess energy, also secretes substances that are actively involved in energy homeostasis as well as the complications of obesity.[3]

Leptin is the best known of these substances.[4] This hormone is secreted in proportion to body fat and regulates appetite and energy expenditure, mainly by influencing the brain.[3–5] Mutations of leptin or leptin receptor genes lead to overeating, impaired thermoregulation, massive weight gain, insulin resistance, diabetes, immune dysfunction, failure of sexual maturation and a variety of neuroendocrine abnormalities in rodents and humans.[3–5] Conversely, recombinant leptin reverses these abnormalities in leptin deficient animals.[3,4] Leptin has also been implicated in reproduction, angiogenesis, bone formation, brain development and regulation of the cardiovascular system.[3,4] These diverse effects appear to occur mainly through the long leptin receptor and JAK-STAT signal transduction pathway.[3,4]

The discovery of leptin created enormous excitement: surely here was a simple way of treating obesity. However, it turned out that normal animals are relatively insensitive to leptin.[3,4] In fact, 'common' (diet-induced) obesity is typically associated with high circulating leptin and diminished sensitivity to peripheral leptin administration.[3] Reduced transport of leptin to the brain and inhibition of leptin signal transduction are both possible causes of this reduction in sensitivity.[3] Regardless, we still do not know if reduced leptin sensitivity is a cause or a consequence of obesity in most humans.

Gene therapy has been used to deliver leptin in genetically obese and normal rodents.[6–8] Adeno-associated viruses (AAV) are ideal vehicles for leptin gene therapy as they are nonpathogenic, capable of infecting nondividing as well as dividing cells, and express the transgene over long periods.[7,8] Using this technology, Karla and co-workers[8] have previously demonstrated a prolonged reduction in body weight after injection of recombinant AAV virus encoding leptin (rAAV-leptin) in the brain (central leptin gene therapy). Presumably, central leptin gene therapy circumvents leptin resistance through a paracrine or autocrine process.[8]

In the new study published in *Paediatric Research*,[2] a single injection of rAAV-leptin into the cerebral ventricle of immature rats prevented weight gain during the 10-month duration of the experiment. The treatment reduced food consumption as well as serum leptin, insulin and fatty acids, but increased uncoupling protein (UCP)-1 in brown adipose tissue (BAT) and ghrelin. The changes in BAT UCP-1 and ghrelin were observed in younger but not older animals. The authors analysed mRNA levels of neuropeptides in the hypothalamus to understand the central actions of rAAV-leptin. NPY was decreased while proopiomelanocortin (precursor of α-MSH) was increased, suggesting that the reduction in appetite and body weight was mediated at least in part through hypothalamic neuropeptides. AGRP, a well-known leptin target that is colocalized in the arcuate nucleus with NPY, was not affected by central rAAV-leptin. Moreover, leptin gene therapy did not alter the timing of sexual maturation (vaginal opening) and duration of estrus cycles.[2]

These new data clearly show that single injection of rAAV-leptin can achieve sustained weight reduction. Moreover, they demonstrate that this strategy can be used on immature animals without harming sexual maturation or reproductive cyclicity.[2]

However, the mechanisms responsible for age-related differences in the response to rAAV-leptin, also found in other studies,[9,10] need to be investigated. For example: why does rAAV-leptin have only a prolonged effect on food intake in younger animals, but not older animals?[2,8] It seems that much of the long-term reduction in body weight is because of increased metabolic rate, although the effect of leptin is diminished in older animals.[9] In the latter case, activation of STAT-3 is normal despite the reduced physiologic response to central rAAV-leptin, suggesting that age-related leptin resistance occurs through a mechanism downstream of leptin receptors and JAK-STAT pathway.[10]

Unfortunately, for a number of reasons, it is unlikely that these encouraging results are immediately applicable to humans. First, intrathecal administration of rAAV-leptin is not a practical mode of treatment in large populations. Second, while it has been reported that leptin is synthesized *de novo* in the brain,[11] the long-term consequences of central rAAV-leptin on brain structure and function are not known. Third, the irreversibility of the rAAV-leptin and other gene therapy approaches raises safety and toxicity concerns.[2,6–10] There are no in-built controls for expression of the rAAV-leptin transgene, so in some circumstances (eg after continuous leptin infusion[12]) exposure to high leptin level can cause excessive weight loss with dire consequences in the long term. Theoretically, this obstacle may be overcome by placing the leptin gene under the control of a promoter responsive to signals involved in leptin regulation. Unfortunately, our understanding of how leptin is regulated in the brain is at best rudimentary.

Despite these issues, the Kalra group's new work is an important additional step towards the development of novel therapies for obesity. ■

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