doi:10.1006/mthe.2002.0724, available online at http://www.idealibrary.com on IDEAL

Targeting Retroviral Integration?

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Can retroviral integration be targeted to preselected locations in the human genome? If so, targeting might improve the safety of retroviral vectors for use in gene therapy. Directing integration of vectors to benign locations in the human genome, for example, might reduce the risk of transformation by insertional activation of oncogenes. This issue has become a lot less theoretical with the recent report from Li and coworkers describing transformation by a gene therapy vector [1]. Interpreting this experiment is not straightforward, however, in part because the marker gene transduced by the vector probably contributed to oncogenesis. However, this result does increase interest in possible means for directing retroviral integration to preselected locations in the human genome.

The first efforts to target retroviral integration involved fusing retroviral integrase enzymes to sequence-specific DNA-binding domains [2–5]. The integrase enzyme carries out the initial DNA breaking and joining reactions that connect the reverse-transcribed retroviral DNA to the host chromosome [6]. Normally integration takes place with little target sequence specificity [6]. Tests with *in vitro* integration reactions using preintegration complexes isolated from virus-infected cells showed relatively slight target sequence preferences [7,8]. More recently some biases have been detected in integration *in vivo*, but these are of a more global nature and are not dependent on local primary sequence [9].

Fusions of integrase to DNA-binding domains were made by several groups and the modified integrase enzymes were tested for targeting *in vitro*. The first report described a fusion of HIV-1 integrase to the sequence-specific DNA binding domain of phage lambda repressor [2,3]. Repressor normally needs to form a dimer to bind DNA, and initial binding studies showed that the repressor–integrase fusion was indeed capable of proper dimerization and binding to lambda operators (repressor binding sites). In optimized reactions *in vitro*, integration was found to take place preferentially near lambda operators in target DNA. Favored sites were found to lie on the same face of the DNA helix as the operator sites, suggesting that the bound repressor–integrase fusion captured target DNA by looping out the intervening sequences.

Later studies demonstrated that the LexA DNA-binding domain could also be used to direct integration targeting *in vitro* [4,5], and other integrases could be used as fusion partners [5].

Efforts to introduce such fusions into retroviruses or retroviral vectors, however, have not gone smoothly. Two studies found that added DNA binding domains were not well tolerated in replication-competent genomes [5,10]. However, it did turn out to be possible to form viral stocks containing integrase fusions by expressing both wild-type and fusion integrases together in the same cell, thereby forming viral particles with both types of integrase proteins [10].

Recent studies of integrase fusions have used the zinc-finger DNA binding domain zif268 [11] instead of prokaryotic helix-turn-helix proteins. One favorable feature of zif268 is that it acts as a monomer, not requiring correct dimerization in the context of a preintegration complex *in vivo*. Another exciting feature is that large libraries have been made of zif268 mutants with diverse DNA binding specificities [12–14]. Ideally, if integration could be targeted using this domain, zif268 variants with different binding specificities could be used to direct integration to diverse positions in the human genome.

Preintegration complexes containing zif268 domains did target integration detectably, but the reprogramming of specificity was incomplete [10]. Viruses containing the zif domain were used to infect target cells, preintegration complexes were isolated, and targeting was assayed in integration reactions *in vitro*. New hotspots for integration were found specifically in reactions containing preintegration complexes with the zif domain and target DNA bearing a zif recognition site. Considerable integration was also seen, however, at points distant from the zif binding site in the target DNA. DNAs lacking zif binding sites also worked well as target DNAs. Thus the alteration of specificity was only partial. No more specific targeting systems have been reported so far for retroviruses.

Chow and coworkers described another approach to delivering integrase–zif268 fusions to preintegration complexes [15]. The HIV vpr protein is also incorporated into viral particles and preintegration complexes, and fusions of vpr to other proteins have been used to target the fusions to virions. Chow and coworkers targeted IN–zif268 fusions to preintegration complexes by forming vpr-IN-zif268 fusions. This has the potential to bypass possible adverse effects of adding the zif268 protein to the normal gag-pol polyprotein precursor. These workers have not yet shown that integration can be targeted *in vivo* with these vpr fusions, but the approach seems promising. Perhaps even vpr–zif268 fusions could

be added to normal particles, thereby tethering the DNA-binding domain to an altogether different protein.

Targeting retroviral integration to specific locations in the human genome remains an unmet challenge. However, there are reasons to be optimistic, as there are naturally occurring systems that target integration quite specifically [16–18]. The yeast retrotransposons replicate via cycles of transcription, reverse transcription, and integration, and do so using enzymes clearly homologous to the retroviral reverse transcriptases and integrases. These retrotransposons maintain stable, long-term relationships with their hosts, so damaging the host genome by indiscriminant integration would be suicidal for the element. Probably as a consequence, retrotransposon integration is tightly targeted to benign regions in the host chromosome. Perhaps in the long run a deep understanding of integration by these elements will suggest means of engineering the capacity for site-specific integration into retroviral vectors.

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