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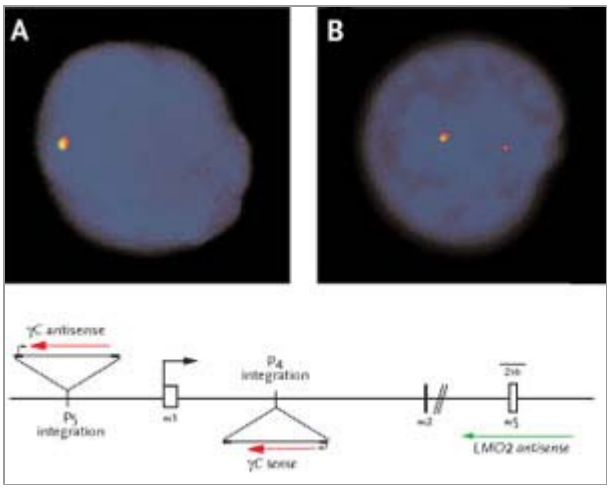
FEATURE | HOT PAPERS

Insertional Mutagenesis from a Viral Vector

Findings demonstrate the possibility and the reality

By **Josh P. Roberts**



FINDING LMO:
RNA Fluorescence In Situ Hybridization reveals transcription at the *LMO2* promoter for patients P4 (a) and P5 (b). In each case, an antisense LMO2 probe and an antisense c probe were hybridized to T cells recovered from the patients. (From S. Hacein-Bey-Abina et al., *Science*, 302:415–9, 2003.)

Integrated viral sequences can dysregulate genes. Thus, the specter of insertional oncogenesis had loomed in the background of every gene therapy trial that used an integrating viral vector. "The original calculations were that the risk would be very small because the genome is big," says Jennifer Puck, a gene therapist at the National Human Genome Research Institute (NHGRI). "No one recognized any reason why retroviruses should be more often inserted into any one place than any other."

Yet the first two of this issue's Hot Papers, from Frederic Bushman's lab at the Salk Institute in La Jolla, Calif.,[1] and Shawn Burgess' lab at the NHGRI,[2] show that retroviruses do preferentially integrate in or near active genes. And as the third Hot Paper shows, insertional oncogenesis is no longer just a theoretical possibility, as evidenced by two children in a clinical trial for X-linked severe combined immunodeficiency (X-SCID) who developed T-cell leukemia.[3] One of these children died last fall, and a third was diagnosed with a lymphoproliferative disorder in January. Yet nearly all in the field are quick to point out that most of the 18 X-SCID gene-therapy patients, many of whom now live a normal life, could have died without this experimental treatment.

NONRANDOM INTEGRATION

Donald Kohn, director of the program for gene, immune, and stem cell therapy at Childrens Hospital Los Angeles, says that before people could look in a detailed way, "the thought was that insertion was relatively random." Bushman's was the first study to show that HIV demonstrated a bias, Kohn recalls, and Burgess shortly thereafter showed that the Moloney murine leukemia virus (MLV) was also biased in its preference for

Data derived from the Science Watch/Hot Papers database and the Web of Science (Thomson Scientific, Philadelphia) show

integration sites. "That has been very, very important work."

Bushman's group had a long-standing interest in HIV virology. To better understand the mechanisms of integration, they infected a human T-cell line with HIV. They then located and cloned 524 distinct junctions between HIV and its host by adding a linker sequence to digested DNA ends, followed by PCR amplification between the viral long terminal repeats (LTRs) and the linker. They mapped insertion sites onto a recently-released draft of the human genome and found that genes – in particular active genes, and especially those active following HIV infection – were the most likely targets, indicating unexpected specificity.

At the same time, Burgess' group was refining a technology to use MLV as a mutagento help locate and clone zebrafish genes. They tested it on human cells, because the human genome was the most thoroughly elucidated vertebrate sequence at the time. "In the process of developing a high-throughput method for mapping where these integrations landed, we realized immediately that we could answer a question that had been out there in retroviruses for a long time," Burgess says, "which is, do these things really integrate randomly or not?"

The group showed that MLV tended to integrate near the start of transcriptional units both upstream and downstream. HIV-1 preferred integration sites within transcriptional units but not upstream of the start site.

Several labs have since confirmed, expanded, and refined these results to include a wider array of cell lines and primary cells. And in a paper published last summer, Bushman, who had since moved to the University of Pennsylvania, and his colleagues examined integration of a third retrovirus, the avian sarcoma-leukosis virus. ASLV showed only a modest preference for active genes, and did not show any preference for transcription start regions.[4] (The authors do, however, leave open the possibility that ASLV might be more selective in its native chicken target.) The implication, says Burgess, is that each retrovirus is likely to have its own mechanism for site selection.

that Hot Papers are cited 50 to 100 times more often than the average paper of the same type and age.

AR Schröder et al, "HIV-1 integration in the human genome favors active genes and local hotspots,"
Cell 2002, **110:** 521-9
(Cited in 159 papers, Hist Cite Analysis)

X Wu et al, "Transcription start regions in the human genome are favored targets for MLV integration,"
Science 2003, **300:** 1749-51
(Cited in 101 papers, Hist Cite Analysis)

S Hacein-Bey-Abina et al, "LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1,"
Science 2003, **302:** 415-9
(Cited in 182 papers, Hist Cite Analysis)

INSERTIONAL MUTAGENESIS

Burgess' goal in zebrafish was to induce mutation, but this is hardly desired in gene therapy. Just months after the initial MLV work appeared, it was revealed that a T-cell leukemia-like syndrome had developed in two patients following insertion of an MLV vector near the *LMO2* gene, a known human T-cell oncogene.[5]

Alain Fischer, the INSERM group leader that headed the X-SCID trials, says he's unaware of any insertional mutagenesis cases among other gene-therapy trials, "which actually suggests that there is something specific in our trial, something relevant to the disease itself which makes this particular setting more risky for the

development of that complication."

LMO2 may not be the sole malefactor. The gene's inappropriate activation likely contributed to the first two leukemias, but a third case of T-cell leukemia found this past January is not due to *LMO2*, according to Fischer.

X-SCID is the result of a mutation of the γ chain (γc) cytokine-receptor subunit gene – the common γ chain, so-called because of its role as part of several interleukin receptors – which results in a lack of mature T or NK cells. Fischer's group (and later a group led by Adrian Thrasher at the Institute for Child Health, University College London) introduced the γ*c* gene into autologous bone marrow stem cells by retroviral transduction before returning them to the patients.

LMO2 is a "common insertion site" in the Mouse Retroviral Tagged Cancer Gene Database, a database of retroviral integration sites cloned from retrovirally induced hematopoietic tumors, created by Neal Copeland at the National Cancer Institute in Frederick, Maryland.[6] That did not surprise Utpal Davé, a fellow in Copeland and Nancy Jenkins' laboratory, because many of these are known human oncogenes. But Davé was surprised to find insertions in γ*c* as well: "That implies that the common gamma chain is oncogenic," he says, "and there was no such previous data to suggest it."

Davé and colleagues found a tumor with insertions in both *LMO2* and γ*c*. Because "the probability of finding a leukemia with clonal integrations at *LMO2*and [γc] by random chance is exceedingly small," says Davé, theyspeculated that the two dysregulated genes acted cooperatively to produce the tumor.[7] They went on to suggest that a similar cooperativity might have occurred in the X-SCID leukemias. "The gene therapy vector was also delivering a hit," explains Davé.

If this explanation is correct, it could answer why such cancers have not developed in similar trials, as well as why, despite no evidence of disruption in *LMO2*, a third X-SCID leukemia has been reported: " γ*c* is providing survival as well as proliferative signals to cells that express it," Fischer says. Adenosine deaminase, on the other hand, "just encodes an enzyme that doesn't really have any signaling or proliferative effect," says Kohn, who is conducting an ADA-SCID trial in which the defective *ADA* gene is introduced by MLV into bone marrow cells.

WHAT NOW, WHAT NEXT?

MULTIPLE INTEGRATION: Human chromosomes are shown as numbered with HIV-integration sites from various datasets shown as blue "lollipops." MLV integration sites are shown in lavender, and ASLV integration

After the third case of leukemia was reported, Fischer's trials were put on hold, and he does not expect to resume treating patients with the same vector. Thrashers' trial is continuing. The work of Bushman and Burgess, Fischer says, is "very important, because they provided us with new information we didn't have at the time we initiated our trials."

In light of that work, Fischer anticipates replacing MLV with an HIV-based vector that has a self-inactivating LTR. Such a vector should be less likely to land in the regulatory region of an oncogene, and less likely to promote that gene's inappropriate activation if it does. Other laboratories have been exploring different ways to deliver genes as well, including retroviral variations, nonintegrating viruses, transposon-like sequences, nonviral vectors, and naked DNA.

sites are shown in green. Transcriptional activity is shown by the red shading on each of the chromosomes. (from R.S. Mitchell et al., *PLoS Biology*, 2:1127–37 (e234), 2004.)

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Some of this work involves mapping integration preferences of native and engineered viruses. Indeed, a session was devoted to the topic at last summer's meeting of the American Society for Gene Therapy, notes Kohn, ASGT's past president. Bushman predicts: "We'll probably soon see large collections of integration maps." Other work involves swapping or modifying integrases. "We should ultimately and optimally be able to modulate where the integration takes place," Bushman says, "by choosing which integrating systems we use."

References

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2. X Wu et al, "Transcription start regions in the human genome are favored targets for MLV integration," *Science* 2003, 300: 1749-51. [PubMed Abstract][Publisher Full Text] (Cited in 101 papers, Hist Cite Analysis)

3. S Hacein-Bey-Abina et al, "LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1," *Science* 2003, 302: 415-9. [PubMed Abstract][Publisher Full Text] (Cited in 182 papers, Hist Cite Analysis)

4. RS Mitchell et al, "Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences," *PloS Biology* 2004, 2: 1127-37. [Publisher Full Text]

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7. U Davé et al, "Gene therapy insertional mutagenesis insights," *Science* 303: Jan. 16, 2004

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