Stable and safe HIV provirus clones

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Much of the work currently being carried out on Human Immunodeficiency Virus (HIV) involves the use of infectious molecular clones of the DNA provirus. However, working with such clones has two serious disadvantages. First, these clones tend to be unstable and can be very difficult to propagate successfully, and secondly, they are potentially infectious and therefore pose a low-level risk of infection to laboratory personnel. Here, I propose a method involving circularly permuted provirus DNA which overcomes both of these drawbacks.

There appear to be two main reasons for the instability of HIV (and other retrovirus) proviral clones. 1) Certain parts of the HIV genome appear to be toxic to bacterial host strains, resulting in poor growth and poor yields of plasmid DNA. 2) Homologous recombination between the long terminal repeat (LTR) regions of the provirus results in deletion of the intervening region at high frequencies. The instability varies considerably between different isolates of the virus and it is very difficult to work with certain biologically interesting isolates (unpublished observations and ref. 1). As to the issue of safe handling of cloned DNA, whereas infectious HIV particles are classified as a dangerous pathogen and require the use of high level containment conditions, cloned HIV DNA is generally not handled under such stringent conditions. I now describe the use of a technique to improve the stability of an infectious proviral clone of an isolate of HIV_{JR-CSF} (2). The method described here can potentially be used to construct clones of any infectious retrovirus genome, some of which have proved to be very unstable and difficult to propagate (3).

Starting with an infectious proviral clone in a plasmid or lambda phage vector, the clone is digested with a restriction enzyme which cuts within the LTR regions of the virus genome (R_1) and is then ligated to circularize this internal fragment (Figure 1). The circles are then linearized by digestion at any convenient unique internal restriction site (R_2) and cloned into a plasmid vector. To rescue infectious virus particles from the re-arranged clone, the viral DNA is excised from the plasmid with restriction enzyme R_2, ligated to form high molecular weight concatemers and introduced into a suitable target cell by transfection or electroporation. The formation of DNA concatemers, some of which will have the same configuration as the original provirus, enables infectious particles to be produced (Figure 2).

Provirus clones with this permuted configuration have proved to be more stable on propagation than linear clones, and in addition, possess several other advantages. 1) Cellular DNA sequences flanking the integrated provirus are precisely removed. 2) The creation of a single LTR can be useful for mutagenesis experiments, since any alterations will be incorporated into both LTRs of the rescued virus, and passed on to subsequent generations. 3) Perhaps most importantly, the permuted form inserted into its plasmid vector is inherently non-infectious and can only regain infectivity by precise excision of the viral DNA at the restriction site used for cloning and ligation to form concatemers.

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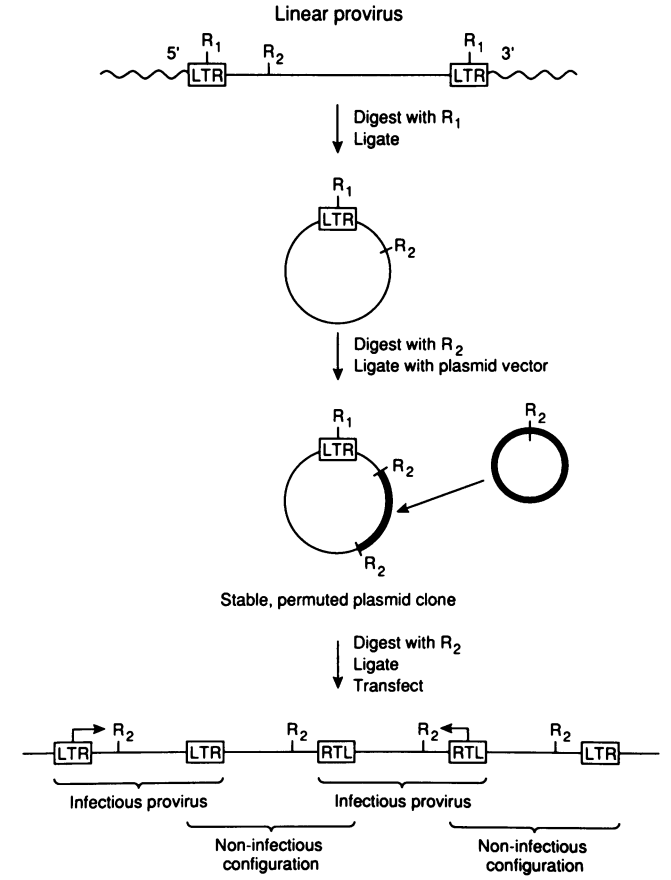

Figure 1. Construction of circularly permuted provirus clones and rescue of infectious virus.

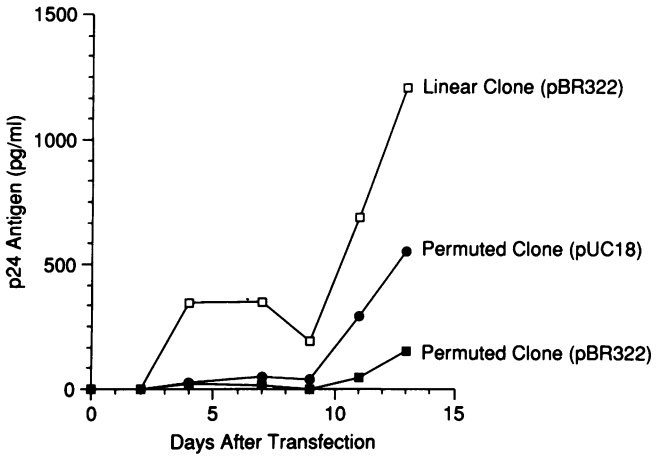

Figure 2. Virus rescue from linear and circularly permuted HIV provirus clones. COS cells were transfected with cloned DNA of linear or circularly permuted HIV proviral clones, which were co-cultured with PHA-stimulated primary human lymphocytes (PBL) on day 2 after transfection. Rescue of infectious virus is shown by rising p24 antigen titres in the cultures.

REFERENCES

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