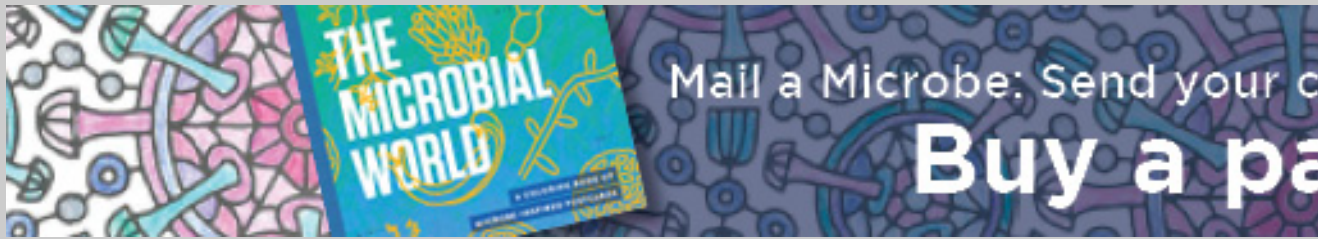


Ping-pong amplification of a retroviral vector achieves high-level gene expression: human growth hormone production.

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Ping-pong amplification of a retroviral vector achieves high-level gene expression: human growth hormone production.



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ABSTRACT

Retroviral vectors offer major advantages for gene transfer studies but have not been useful for producing proteins in large quantities. This deficiency has resulted in part from interference to superinfection, which limits the numbers of active proviruses in cells. Recently, we found that these vectors amplify when they are added as calcium phosphate precipitates to cocultures of cells that package retroviruses into ecotropic and amphotropic host range envelopes. Helper-free virions from either cell type can infect the other without interference, resulting in theoretically limitless back-and-forth (ping-pong) vector replication. In initial studies, however, amplifications of a vector that contained the human growth hormone gene ceased when the hormone produced was 0.3% or less of cellular protein synthesis. This limit was caused by two factors. First, recombinant shutoff viruses that are replication defective and encode envelope glycoproteins form at a low probability during any round of the vector replication cycle and these spread in cocultures, thereby establishing interference. Single cells in shutoff cocultures therefore synthesize both ecotropic and amphotropic envelope glycoproteins, and they release promiscuous (presumably hybrid) virions. The

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probability of forming shutoff viruses before the vector had amplified to a high multiplicity was reduced by using small cocultures. Second, cells with large numbers of proviruses are unhealthy and their proviral expression can be unstable. Stable expresser cell clones were obtained by selection. Thereby, cell lines were readily obtained that stably produce human growth hormone as 4 to 6% of the total protein synthesis. A ping-pong retroviral vector can be used for high-level protein production in vertebrate cells.

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