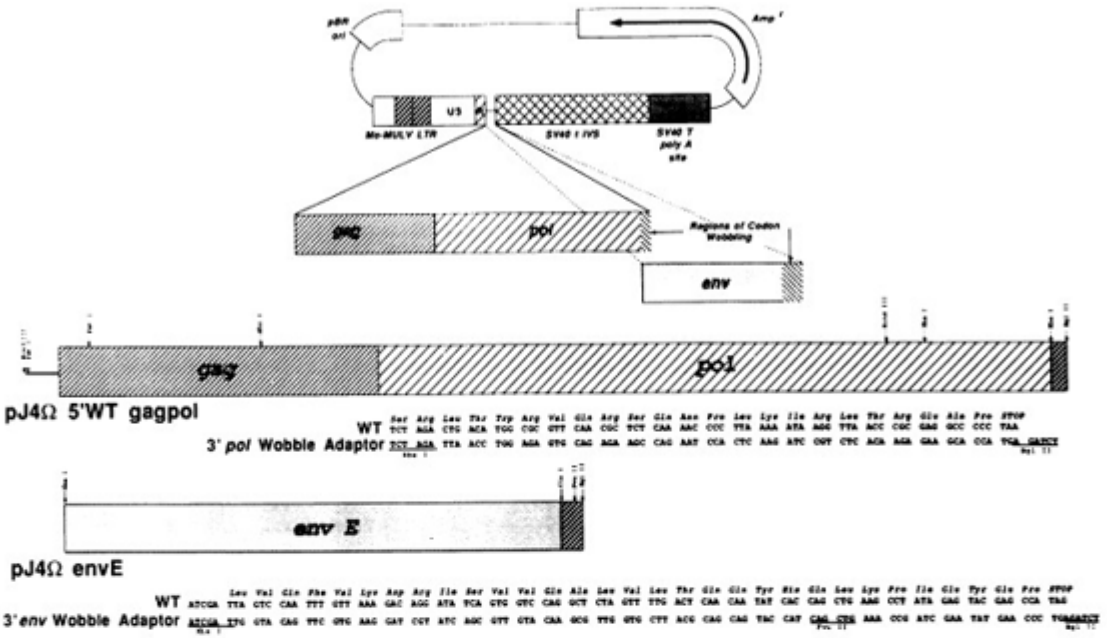

Figure 8. Schematic representation of ΩE third generation packaging *gagpol* and *env* constructs in pJ4Ω (47). The exact derivation of pJ4Ω gagpol and pJ4Ω envE, including a comparison of wild type Mo MuLV coding sequence and codon 'wobble' adaptors used to decrease nucleotide sequence homology between the constructs is indicated. The wobble adaptors were subcloned into pJ4Ω, after which the *gagpol* orf was inserted as a fragment from a Pst I to Xba I partial digest of the NB-tropic proviral clone pMov 9-2 (48) (positions 739 to 5325 of Mo MuLV) and the ecotropic *env* coding region was inserted as an Xba I to Cla fragment (positions 5766 to 7674 of pMo Mu LV).

frame (orf), but decreases the primary nucleotide sequence homology over the 61 bp region of *pol/env* overlap in pJ4Ω 5' WT gagpol and pJ4Ω envE to 55%. At the 3' end of the ecotropic *env* expression construct pJ4Ω envE the final 31 codons were wobbled (decreasing homology with the ecotropic Mo MuLV to 62%), and all sequences 3' of the *env* stop codon (polypurine tract and 3' LTR) were deleted (Figure 8B).

Both constructs were co-transfected with the pSV2 gpt marker (30) into NIH 3T3 cells, and 14 days later 192 mycophenolic acid resistant colonies were picked and assayed for reverse transcriptase activity in their supernatants (31). Twelve clones with the highest reverse transcriptase levels (as well as Ψ-2 cells as a positive control) were infected with an amphotropic stock of pBN cat. Subsequently, G418 resistant colonies were pooled, and their supernatants used in titrations on NIH 3T3 cells. The above procedure was repeated for the three packaging clones yielding the highest titres in the intial experiment. Clone 2E1, which gave titres 50% as high as Ψ-2 (15) or the 'third generation' GP+E (27) packaging cells, was designated as the ΩE packaging cell line.

As a preliminary test of the ΩE cell line's propensity for generating wild type virus, a polyclonal population of ΩE cells with pBN cat proviruses from the above titrations were passaged for four weeks and their undiluted supernatant used to infect 7.5×10^5 NIH 3T3 cells. In order to promote any potential helper virus to spread, the infected NIH 3T3 cells were not selected in G418 and were passaged for two weeks in the presence of 2 μg/ml of polybrene. Undiluted supernatant from the infected/passaged NIH 3T3 cells was used to infect 7.5×10^5 fresh NIH 3T3 cells, all of which were split and selected in G418. After 14 days no $G418^r$ colonies, indicative of contaminating helper virus in the initial ΩE pBN cat producer supernatants, were detected.

DISCUSSION

By exploring the role of coding sequences of the Mo MuLV genome as *cis* acting signals in the retroviral life cycle, we have been able to develop a highly efficient retroviral based gene transfer system for stable expression within mammalian cells. While a number of laboratories have reported either high titre retroviral vectors or safe helper free packaging cell lines, we have extended their initial observations and designed a series of highly efficient vectors in conjunction with a packaging cell line.

Initial work with DO retroviral vectors revealed that alterations in the splice donor region of Mo MuLV can have deleterious effects on vector titre. A similar effect was noted by another group, however they failed to observe a significant effect of *gag* sequences on vector titre (32). In order to avoid splicing artifacts, a splice donor point mutant which did not adversely affect vector titre was made and demonstrated to inhibit splicing (as infered by a 10^3-fold decrease in $G418^r$ titre) when introduced into the vector pZipNeo SV(X). A similar inhibition of splicing was obtained when the identical splice donor mutation from Ha MuSV was introduced into the splicing vector N2 (33).