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Neonatal Lethality and Lymphopenia in Mice with a Homozygous Disruption of the *c-abl* Proto-Oncogene

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throughout gestation (Muller et al., 1982) and in all tissues surveyed, but its levels are highest in thymus and spleen (Ben-Neriah et al., 1986; Renshaw et al., 1988; Wang and Baltimore, 1983). Thus, *c-abl* may play an important role in lymphopoiesis or hematopoiesis. This is circumstantially supported by the observation that activated *c-abl* oncogenes have generally been associated with tumors of the hematopoietic system (al-Rushdi et al., 1988; Bartram et al., 1985; Clark et al., 1987; Grosveld et al., 1986; Shtivelman et al., 1985). In addition, transgenic mice expressing *bcr/abl* under the control of the non-tissue-specific metallothienin promoter develop acute leukemia (myeloid or lymphoid) in spite of the presumed expression of the *bcr/abl* protein in all tissues (Heisterkamp et al., 1990). *c-abl*

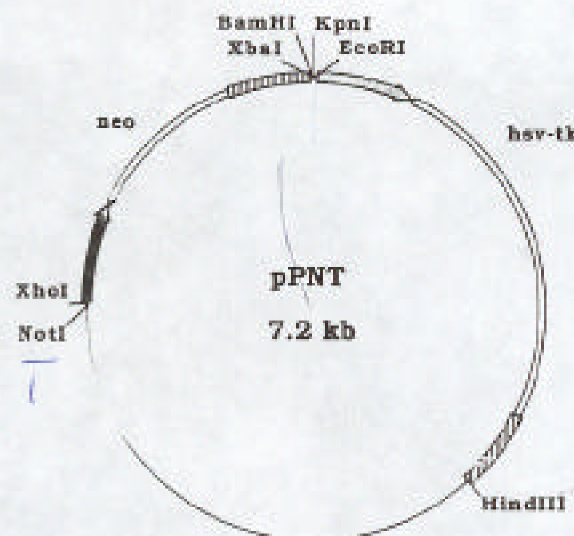

Figure 1. Structure of pPNT

The shaded arrows represent the PGK-1 promoter, the hatched boxes represent the PGK-1 poly(A) addition sequences, the open boxes are the *neo* and *HSV-tk* genes as labeled, and the line represents the plasmid backbone. Unique restriction sites are indicated. The precise nature of each of the fragments is described in Experimental Procedures.

mouse phosphoglycerate kinase-1 (PGK-1) gene gives 13-fold more *neo* colonies than MC1neopA (M. Rudnicki and R. Jaenisch, personal communication; V. L. J Tybulewicz and R. C. Mulligan, unpublished data). In light of these results, the PGK promoter, rather than the MC1 promoter, was chosen to drive expression of both the *neo* and the HSV-*tk* genes, in order to reduce the possibility that the expression of targeting constructs might be sensitive to specific chromosomal sites of integration and in some cases insufficient for the generation of G418ʳ transformants.

Initially, a vector, pPNT, was constructed containing PGKneo and PGKtk cassettes separated and flanked by a number of unique cloning sites (Figure 1). A 4.1 kb BglII fragment of *c-abl* from within intron 4 (M. Paskind, P. K. Jackson, and D. Baltimore, unpublished data) was inserted into the BamHI site of pPNT, and a 2.5 kb SmaI fragment extending from exon 6 to intron 7 was inserted into the XhoI site to generate pPNT-A2 (Figure 2). The predicted targeted recombination event with this vector would replace all of exon 5 and the first part of exon 6 (amino acids 275–307 in *c-abl*(I); Oppi et al., 1987) with PGKneo sequences. The deleted sequences lie within the N-terminal part of the tyrosine kinase domain of *c-abl*, a region highly conserved between all tyrosine (and serine/threonine) kinases, which contains the nucleotide- and ATP-binding sites (Hanks et al., 1988). Thus, the disruption would be predicted to abolish the kinase activity of *c-abl* and should result in a null allele.

pPNT-A2 was linearized at a unique NotI site and transfected on three separate occasions into CCE ES cells (Robertson et al., 1986) by electroporation. A small fraction of every electroporation was selected only with G418 and the remainder doubly selected with G418 and gancyclovir. Table 1 shows the results of these three experiments. The