

Construction of the full coding information for murine J-chain protein from cDNA and its homologous genomic clone

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The cDNA for murine J-chain (MJ-cDNA) (1) contains a short complementary inversion at its 5'-end (2). Replacing this segment by the appropriate genomic fragment was achieved through the HinI restriction site immediately downstream the inversion, where no intron exists (2). The nearby single KpnI recognition site circumvented the complication caused by 10 additional HinI sites on the pBR322 vector carrying the genomic clone (pPE_S). The 5'-end PstI-HinI fragment of pPE_S and the appropriate HinI fragment of MJ-cDNA were introduced into the PstI site of pUC9 (3) after removing a 15 bp PstI-SmaI fragment, sequentially and in the correct orientation because the nucleotide sequences of the two HinI sites flanking this fragment (H₁ and H₂) are not identical (Figure). The remaining HinI sticky end was filled-in, and the blunt end thus formed (H₂^{*}) ligated to the SmaI open end.

The resultant 585 bp fragment (Jcf) contains most coding information for J-chain and 122 bp upstream, but lacks the coding information for the five C-terminal amino acids. To obtain the complete coding information for J-chain (Jci), the BamHI-EcoRI large fragment from Jcf-containing pUC9 [EcoRI overlapping the original SmaI site of the polylinker (3)] was ligated to the BamHI-EcoRI fragment from MJ-cDNA (1).

- (1) Cann, G.M., Zaritsky, A. & Koshland M.E. (1982) Proc. natl. Acad. Sci. USA **79**, 6656-6660.
- (2) Matsuuchi, L., Cann, G.M. & Koshland, M.E. (1986) Proc. natl. Acad. Sci. USA **83**, 456-460.
- (3) Vieira, J. & Messing, J. (1982) Gene **19**, 259-268.

FIGURE LEGEND

Recognition sites for the following enzymes drawn to scale: B, BamHI; K, KpnI; P, PstI; E, EcoRI; S, SmaI; H₁ and H₂, two different sites for HinI; H₂^{*}, the filled-in H₂. lacPOZ, promoter, operator and structural genes for beta-galactosidase, including a polylinker (3).

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