SUPPLEMENTARY INFORMATION

Plasmid constructions

For yeast two-hybrid screening, a Krox20 bait was prepared by cloning a BamHI fragment encoding amino acids 184 to 470 from plasmid pSCT-Krox20 (Vesque, unpublished) into the MscI site of pDBLeu (Invitrogen). Other constructs were generated as follows: for plasmids encoding amino acids 184 to 332 and 332 to 470, BamHI-ApaLI and ApaLI-XbaI fragments obtained from plasmid pSCT-Krox20 were cloned into the NotI and StuI sites of pDBLeu, respectively; for plasmids encoding amino acids 329 to 425 and 422 to 470, SmaI-SpeI fragments to be cloned in pDBLeu were obtained by digestion of PCR fragments obtained with the primers 5’-CAGCCCCGGCAAAACGCCAGTGCACG-3’ and 5’-AGACTAGTCTGCTCTCCGTTCCCTTC-3’ or 5’-CCTCCCGGGGAAGGAACGGGAAGGCA-3’ and 5’-TGACTAGTAGAGCTTCATCTCAACGTACG-3’, respectively; for plasmids containing the R409W and S382RD383Y mutations, the PshAI-SfiI fragment of the wild-type construct was substituted by mutant fragments from plasmids pAdRSVKrox20R409W (Giudicelli et. al. 2001) and pAdRSVKrox20S382R/D383Y (see below), respectively.

For Krox20 in vitro coupled transcription and translation (TNT), the cDNA was cloned in plasmid pBluescriptII KS+ (Stratagene) and transcribed from the T7 promoter. The GST-Krox20 fusion was obtained by cloning the StyI-XbaI fragment from Krox20 cDNA into the SmaI site of pGEX-4T-1 (Amersham Biosciences, Inc.). TNT Krox20R409W and GST-Krox20R409W constructs were derived from TNT Krox20 and GST-Krox20 constructs by substituting the wild-type PshAI-SfiI fragment for that of plasmid pAdRSVKrox20R409W. The expression constructs for electroporation
pAdRSVp, pAdRSVKrox20 and pAdRSVKrox20R409W were previously described (Giudicelli et. al. 2001). The pAdRSVKrox20S382R/D383Y was derived from pAdRSVKrox20 by site directed mutagenesis using the oligonucleotide 5’AACTTCAGCCGAAGATACCACCTTACTACTAC-3’ (the mutated residues are in bold) using the Transformer Site-Directed Mutagenesis Kit (Clontech). Construction of the PIASxβ TNT construct involved the transfer of the two-hybrid clone into pBluescriptII SK+ (Stratagene) and substitution of the 5’ end by a PCR fragment obtained with the primers 5’-GACCATGGCGGATTTCGAGGAG-3’ and 5’-GAAGGCAACGAGTGGATCCCAG-3’. The other TNT PIASxβ constructs were prepared as follows: 1-489, 1-339 and 1-101 were C-terminal deletions from NsiI, SpeI and BamHI sites, respectively; 486-621 and 336-621 were N-terminal deletions from BamHI and SpeI sites, respectively; 1-286 was a 3’ replacement by a PCR fragment obtained with primers 5’-CATCACCCCGTAGAACCTGACT-3’ and 5’-AGTAACTAGTTCCAATTTCAGATGC-3’; 132-621 was a 5’ replacement by a PCR fragment obtained with primers 5’-AAGCCCACGCTAGCCATGCAGCAGCCGT-3’ and 5’-AGACTAGTTGTAGCAATTTCACTA-3’(SPE); 101-286 was a N-terminal deletion to the BamHI site of the construct 1-286. Two-hybrid constructs of PIASxβ were as follows: Δ102-162 and Δ102-131 were substitutions of the BamHI-SpeI fragment of the original clone by the PCR fragments obtained with primers 5’-TTCCGATCCAGCCCACGAGTTTAGTTCA-3’ and SPE or 5’-CCAGGATCCAGATGCAGCAGCCGTCTCCG-3’ and SPE, respectively; Δ133-162 was a substitution of the SalI-SpeI fragment of the original clone by a PCR fragment obtained with primers 5’-TATAACGCGTTTGGGAATCCT-3’ and 5’-GGACTAGTGGGCGCCTCATCTAAAGGTGGGCTTA-3’, followed by cloning of a PCR fragment obtained with primers 5’-
TTGGCGCGCCCCACGAGTTTAGTCAA-3’ and 5’-GCACATCAAGGACACTCGAAG-3’ in the BssHII-SpeI sites. The PIASy two-hybrid construct was obtained by cloning a XhoI fragment from plasmid CMV-T7-PIASy (Sachdev et. al. 2001) into the SmaI site of pPC86. PIASxβ expression constructs were cloned into plasmid pAdRSVSp-HA (Garcia-Dominguez et al. 2003) to generate N-terminal HA-tags. For expression of full-length PIASxβ, a NcoI fragment from the TNT construct was cloned into the PmlI site of pAdRSVSp-HA. Expression constructs 1-489 and 132-621 were derived from the corresponding TNT constructs. Expression construct of Δ102-162 was derived from the corresponding two-hybrid construct. Expression construct of Δ340-396 was generated by substitution of the SpeI-NsiI fragment of the full-length construct by a PCR fragment obtained with primers 5’-TGCCTATACTAGTCTGATACTAGA-3’ and 5’-TTGGACTGCTTTGTGTTTCTGACA-3’. The GST-PIASxβ fusion construct was obtained by cloning the NcoI fragment from the full-length TNT construct in the SmaI site of pGEX-4T-1. The Par4 positive clone isolated in the two-hybrid screening encoded the 158 C-terminal amino acids. The two electroporation reporter constructs consisted of the lacZ gene driven by a minimal β-globin promoter associated with the following enhancer elements: a 470 bp SacI-BglIII fragment carrying the mouse EphA4-r3/r5 enhancer (construct#10 of Theil et al., 1998) or a PstI-HindIII 2130 bp fragment carrying the mouse Hoxb1-r4 enhancer (Studer et. al. 1994).

References


