Expression of genomic and cDNA transgenes after co-integration in transgenic mice

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In general, genomic transgenes are expressed efficiently in mice, while their cDNA-based transgenes are frequently silent. Clark et al. (1992) have shown that silent cDNA transgenes under the control of the sheep β-lactoglobulin promoter can be activated after co-injecting them with a genomic sheep β-lactoglobulin transgene. We have tested the general utility of this concept using mouse whey acidic protein (WAP) transgenes. Here we show that WAP cDNA transgenes are virtually silent in transgenic mice. In contrast, WAP transgenes containing all introns are expressed in approximately 50% of the lines at levels ranging from 1% to more than 100% of the endogenous RNA (McKnight et al., 1992). When a WAP-genomic transgene was co-injected with a WAP-cDNA, basal activation of the cDNA was possible. However, the activity of the WAP-cDNA transgene did not exceed 1% of the WAP-genomic transgene. This suggests that a permissive integration site capable of supporting basal level transcription can be established, but further events are required for full activation of the transgene.

Introduction

Transgenic animals are useful tools for the study of genes and their functions (Jaenisch, 1988). Researchers interested in using transgenic animals often have only a cDNA at their disposal, or work with genes too large for practical manipulation. However, transgenes without intronic sequences are expressed poorly, if at all, whereas genomic transgenes are, in general, expressed efficiently (Palmiter and Brinster, 1986). Attempts have been made to enhance the expression of cDNA-based transgenes through the inclusion of homologous (Brinster et al. 1988; Whitelaw et al., 1991) and heterologous (Choi et al. 1991; Palmiter et al., 1991) introns. While the presence of some introns enhanced transgene expression, others had no effect, suggesting that splicing per se was not sufficient for efficient transgene expression.

The poor performance of cDNA derived transgenes could, in part, be caused by increased sensitivity to silencing by neighbouring genomic sequences. Clark and coworkers (Clark et al., 1992) have reasoned that increased expression of cDNA-derived transgenes may be achieved through the manipulation of the integration site. They have shown in transgenic mice that an actively expressed sheep β-lactoglobulin gene created an 'environment' in which juxtaposed cDNA transgenes were expressed. However, the α1-antitrypsin and Factor IX cDNA transgenes appeared to respond differently, suggesting that the degree of activation may depend on the cDNA transgene used. To test further whether the concept of activating cDNA transgenes through their physical proximity to intron-containing genes is of general utility, we investigated the effect of a mouse WAP-genomic transgene on the expression of a juxtaposed WAP-cDNA transgene.

Materials and methods

Generation of transgenes

Three different WAP-based transgenes were constructed (Fig. 1). The first was a 7 kb genomic clone containing a
2.4 kb promoter, four exons, three introns and 1.6 kb of 3' flanking sequence. In order to distinguish this transgene from the endogenous WAP gene, a Hin dIII linker was cloned into the Kpn I site located at +24 in the gene (Burdon et al., 1991). The second WAP transgene (WAP-delta 3) was made by deleting intron 3. The Sal–Bam HI fragment from the genomic clone, which spans sequences from the Sal I site within the third exon and the Bam HI site within the fourth exon, had been replaced with a Sal I–Bam HI fragment from the cloned cDNA, yielding a final length of 6 kb. An Eco RV linker was inserted in the Kpn I site to identify the transgene. The third WAP transgene had all introns deleted resulting in a fragment 4.5 kb in length. It was generated by restricting the WAP-genomic clone with Kpn I and Bam HI, which cut within exon 1 and exon 4, respectively, and replacing this fragment with a Kpn I–Bam HI cDNA fragment. This cDNA transgene was tagged with a Not I linker in the Kpn I site. The introduction of Hin dIII, Eco RV and Not I linker sequences into the blunt Kpn I site does not interfere with the expression of WAP transgenes (unpublished results; Burdon et al., 1991).

**Generation of transgenic mice**

The transgene constructs were restricted from the vector by Eco RI digestion, separated on a 0.8% agarose TBE gel, and isolated from the gel using the Quiagen Qiax DNA purification kit. The purified fragments were then phenol extracted 1x, chloroform extracted 1x, ethanol precipitated, resuspended in 0.1 × TE pH 7.5 and spun through a Millipore Ultrafree-M 0.22 μm filter unit.

A solution containing approximately 5 μg per ml of the linear WAP-cDNA transgene was injected into 1031 pronuclear stage zygotes harvested from superovulated C57BL/6 × SJL F1 females. Then, 753 microinjected zygotes were transferred into 28 CB6 oestrus stage females that had been mated with vasectomized males the day before.

For the co-injection, the WAP-genomic, the WAP-delta 3 and the WAP-cDNA transgenes were mixed in equimolar amounts and microinjected into the male pronuclei of 1185 zygotes. Of these, 881 zygotes survived the injection process and were transferred into 35 oestrus stage recipient females. Fifteen litters were produced, resulting in the live birth of 121 pups.

**Analysis of transgenic mice**

DNA was prepared from mouse tails and screened by PCR for the presence of transgenes. Primers P1 (5’ TAGAGCTTGTCGCCACCTCCTC 3’) and P2 (5’ GTTCTCCACAGGCACACCCGG 3’) were used to amplify a 250 bp fragment spanning the first exon of the endogenous WAP gene and the WAP-genomic and WAP-delta 3 transgenes (Fig. 1). Primers P1 and P3 (5’ CTGAGATT-GAAGACTTTGTTCCCTC 3’) were used to amplify the first exon of the cDNA transgene. Aliquots were restricted either with Kpn I (to identify the endogenous WAP gene), with Eco RV (for WAP-delta 3), with Hin dIII (for the WAP-genomic) or with Not I (for the WAP-cDNA), and the fragments were separated on an agarose gel. Transgene-positive mice were confirmed by Southern blotting. From the cDNA injections, 22 transgenic founder mice were generated and twelve of the founders were bred to generate females for this study. From the co-injection, 19 transgenic founders were identified, of which eight contained all three transgenes.

The introduction of specific oligonucleotides into the transcribed region of the transgenes permitted us to distinguish endogenous WAP transcripts from transgene transcripts. Total RNA was isolated (Chomczynski et al., 1987) and duplicate samples of 10 μg were electrophoresed in 1.5% agarose formaldehyde gels and blotted onto GeneScreenPlus. Each blot was used for two oligonucleotide probes with stripping in between. Oligonucleotides were labelled using γ32P ATP and T4 polynucleotide kinase. To detect endogenous WAP RNA, blots were probed with oligo 101 (5’ CAAACGATGTTACCCGTGTCAC 3’) at 55°C. Transcripts from the genomic transgene was detected with oligo 102b (5’ GGCACGCAACAGTCTGCTCAGGC 3’) at 60°C. WAP-delta 3 transcripts were detected by hybridizing at 55°C with oligo 190 (5’ CGCATGGGAATCCCGGTGCAGGCA 3’) at 60°C. WAP-delta 3 transcripts were detected by hybridizing at 55°C with oligo 191 (5’ CGCAGAGGTGCACGCCACGTTAG 3’). WAP-cDNA transcripts were detected by hybridizing at 55°C with oligo 192 (5’ CGCAGAGGTGCACGCCACGTTAG 3’). Hybridization signals on northern blots were quantitated using a Betagen BetaScope 603. As a control for hybridization efficiency, a synthetic RNA template was constructed, which contained

![Fig. 1. Diagram of WAP constructs injected into fertilized mouse eggs.](image-url)