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The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice

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Transgenic mice provide one of the best experimental systems to study gene function *in vivo*. Although transgenic mice carry the additional genetic material in every cell of the body, expression of the transgenes under the control of constitutive promoter/enhancer units, such as the murine major histocompatibility complex promoter H2-K^b, the CMV enhancer/promoter, the murine *Pgk-1* promoter or the chicken cytoplasmatic β-*actin* promoter is often restricted to a limited number of tissues (1–5). However, for most experimental dominant gain-of-function approaches addressing the role of a given protein in a multicellular organism an ubiquitous expression of the transgene might be highly desired. Here we report the application of a powerful expression vector using the 5′-flanking region of the human ubiquitin C gene that allows very efficient expression of a given transgene in a broad range of tissues.

The structure of the ubiquitin proteins is highly conserved during evolution. In human, there exist several ubiquitin proteins which are encoded by a multigene family (6). According to the general requirement of these proteins for ATP-dependent, non-lysosomal intracellular protein degradation ubiquitin proteins have been found in all eukaryotic cells examined so far (for review see 7,8).

Previous work has demonstrated that the human ubiquitin C promoter is very active in conferring expression of exogenous genes following transient transfection of the appropriate expression vectors in various cell lines (9; Schorpp and Angel, unpublished). In view of its powerful and ubiquitous activity in tissue culture cells we have used the human ubiquitin C promoter as regulatory unit to drive overexpression of two cellular genes, junB and bcl-2α, in transgenic mice.

As shown in Figure 1A, Ubi-JunB and Ubi-Bcl-2α carry 1.2 kb of the human ubiquitin C promoter region (position –1225 to –6) fused to either the coding region of the mouse junB gene (position +240 to +1485; 10) or the human bcl-2α cDNA (11) respectively. 3′ of the coding sequences, 150 bp of 3′ nontranslated sequences of the human c-jun gene (12) and the splice and poly(A) sequences of SV40 were inserted.

These gene constructs were used to generate four independent Ubi-junB and two bcl-2α transgenic mouse lines. Two out of four Ubi-junB lines (1598 and 1605) and both bcl-2α lines (2266 and 2272) showed high level expression of the transgene in all tissues which were examined (Fig. 1B and C). Efficient expression from the Ubi-JunB construct could be detected at early stages of development (10.5, 11.5, 12.5, 14.5 and 15.5 d.p.c.; data not shown) and in tissues of adult animals (Fig. 1B). The levels of expression of junB and bcl-2α transgenes in some tissues were up to 20-fold higher than of the endogenous transcript (e.g. liver and brain, and brain; Fig. 1B and C respectively). The ubiquitous presence of the bcl-2α transgene product was also confirmed by western blot analysis (data not shown).

The human ubiquitin C sequences described here allow expression of transgenes in an even wider range of tissues compared to the promoter/enhancer units that are routinely used. Transgenes driven by H2-K^b, CMV or *Pgk-1* regulatory sequences are highly expressed in spleen, lung and salivary glands (H2-K^b; 1), in spleen, stomach and heart (CMV; 2,3), or in heart, kidney and brain (*Pgk-1*; 4). Only very low expression has been found for each regulatory unit in certain tissues, such as stomach and brain (H2-K^b), liver (CMV and *Pgk-1*) and spleen *(Pgk-1)*. Most importantly, the human ubiquitin C promoter is able to confer high expression also in those tissues in which the other promoter/enhancer sequences are hardly active. Therefore, the human ubiquitin C 5′-flanking sequences appear to be a powerful transcriptional control unit useful for experiments in which transgene expression in a very broad range of tissues is required.

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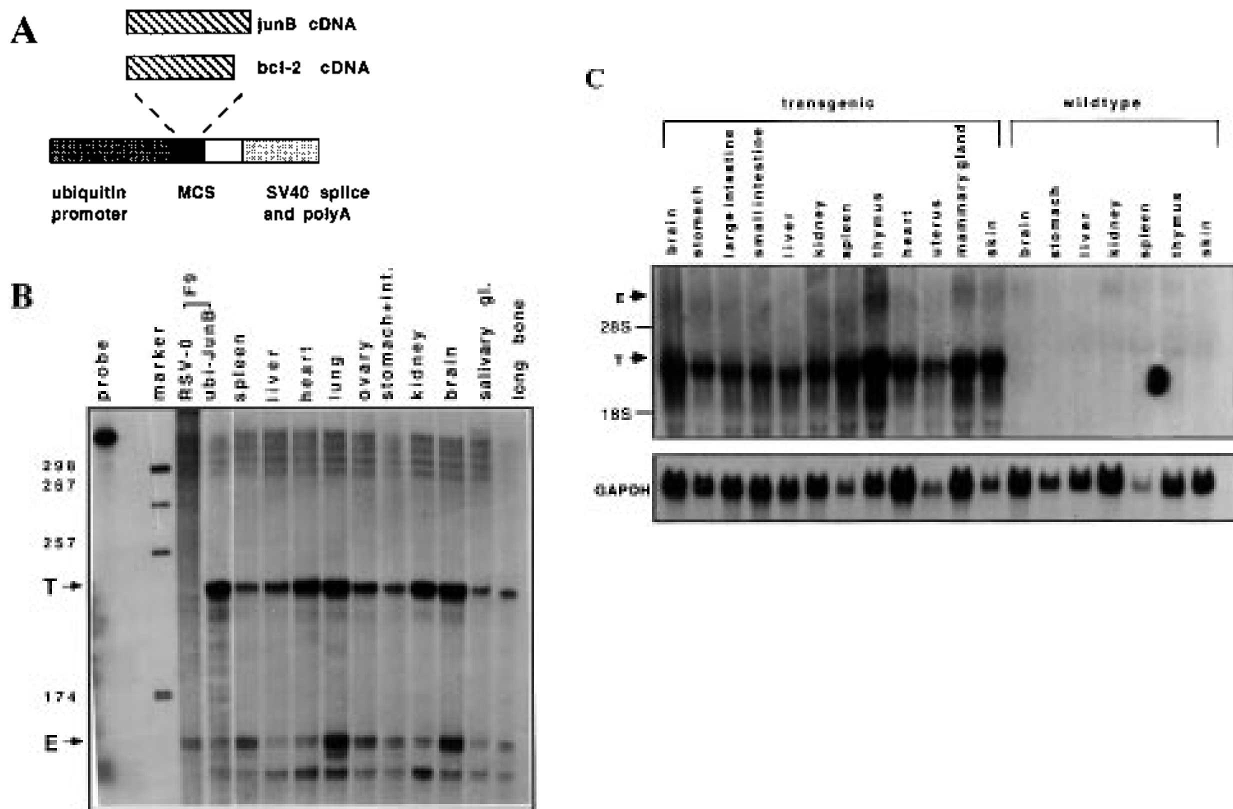

Figure 1. (**A**) The structure of the transgene constructs. Dashed boxes represent the junB cDNA and the bcl-2α cDNA that have been cloned into the multiple cloning site of the vector (MCS, solid box). The 5′ end of either one of the transgene consists of the human ubiquitin C promoter from position –1225 to –6 (9). The 3′ end is composed of 150 bp from the human c-jun (12) and SV40 splice and poly(A) sequences. (B and C) Expression of the transgenes in various mouse tissues. (**B**) Transcripts derived from the Ubi-JunB transgene were monitored by RNase protection mapping (12) using a junB-specific antisense RNA probe and 20μg total RNA from 10 different organs of an adult transgenic mouse (line 1598). The protected fragments specific for the transcript from the transgene (T) as well as from the endogenous gene (E) are indicated by arrows. As a control for the transcripts derived from the Ubi-junB transgene, RNA from F9 cells that had been transiently transfected with either the Ubi-JunB expression vector, or with an empty expression vector (RSV-O) was analyzed in parallel. (**C**) Northern Blot analysis of total RNA from various organs of wild-type or transgenic animals (line 2272) was performed to analyze the expression of the bcl-2α transgene. Fifteen μg (or 5 μg from skin) total RNA were electrophoresed, blotted and probed for human bcl-2α expression. The transcripts originated from the transgene are marked by an arrow (T). The endogenous mouse bcl-2α transcript that crosshybridizes with the human probe is shown as well (E: brain, thymus). Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as an internal control for the quality of the RNA.

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