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Tissue-specific and hormonal regulation of angiotensinogen minigenes in transgenic mice

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Angiotensinogen is the precursor of the potent vasoactive peptide angiotensin II, and is therefore an important determinant of blood pressure and electrolyte homeostasis. In order to map the tissue-specific and inducible enhancer elements governing angiotensinogen gene expression in transgenic mice, we constructed minigenes containing either 0.75 kb or 4 kb of 5′ flanking DNA from the BALB/c angiotensinogen gene. Sequences necessary and sufficient to mediate induction by glucocorticoids, oestrogen and bacterial endotoxin were contained on the minigene bearing 0.75 kb of DNA upstream of the cap-site. This construct was also able to confer tissue specificity in the majority of organs producing angiotensinogen. In the testis and salivary gland, differences between the donor (BALB/c) and recipient (Swiss) strains were responsible for the apparently aberrant expression of the minigene constructs. The genetic lesion responsible for these expression polymorphisms has been characterized using recombinant inbred mice. An *Eco*RI restriction fragment length polymorphism which co-segregates with the angiotensinogen expression phenotypes into many inbred mouse strains is also described.

Key words: angiotensinogen/minigenes/transgenic mice

Introduction

Angiotensinogen is the only known precursor of the biologically active peptide angiotensin II, which plays an essential role in blood pressure and electrolyte homeostasis (Campbell, 1987). Production of angiotensinogen in the liver is closely coupled to physiological stimuli, as the protein is constitutively secreted with minimal hepatic storage (Clauser *et al.*, 1983; Richoux *et al.*, 1983). In cultured cells the angiotensinogen gene is transcriptionally regulated by glucocorticoids (Brasier *et al.*, 1986). Angiotensinogen mRNA levels are induced by a variety of other stimuli including oestrogen, thyroxine, angiotensin II and bilateral nephrectomy (Campbell *et al.*, 1984; Klett *et al.*, 1988). This gene also responds to an acute phase stimulant, bacterial lipopolysaccharide (Kageyama *et al.*, 1985).

Recent interest has centred on the production of angiotensinogen mRNA in many extrahepatic tissues (Ohkubo *et al.*, 1986; Campbell and Habener, 1986; Dzau *et al.*, 1987), where it may participate in local renin-angiotensin systems (Dzau, 1988). Of particular relevance is the *de novo* synthesis of angiotensinogen in the brain (Lynch *et al.*, 1986; Buckley, 1988), because this 55 kd protein cannot cross the blood-brain barrier.

Whilst the angiotensinogen gene has now been cloned from rat (Tanaka *et al.*, 1984), mouse (Clouston *et al.*, 1988) and human (Gaillard *et al.*, 1989), there have been no functional studies of the enhancer elements controlling its expression. In the present study, we chose to map the tissue-specific and inducible enhancers of the angiotensinogen gene in transgenic mice using a minigene as the reporter of transcription. The use of a minigene reporter has been successfully exploited to map the developmentally-regulated enhancers governing expression of the alpha-fetoprotein gene in transgenic mice (Hammer *et al.*, 1987), and to study expression of the troponin T and dihydrofolate reductase genes in tissue culture (Breitbart and Nadal-Ginard, 1987; Crouse *et al.*, 1988). This approach has two main advantages. Firstly, by deleting the central portion of the coding region to create the minigene, complete homology of the 5′ and 3′ flanking regions is preserved. Secondly, the relative expression of the endogenous and mini-RNAs can be directly compared on a Northern blot using the same radiolabelled probe to determine whether all sequences necessary for reproduction of the endogenous mRNA level have been included in the construct. In contrast to *in vitro* transient expression assays in cultured cell lines, it is possible to use the minigene to survey expression in many organs from the one animal.

For the present study, we constructed two angiotensinogen minigenes containing different amounts of 5′ flanking region. The longer minigene contains 4 kb of DNA upstream of the cap-site, and was designated 4.0 A/2. This amount of 5′ flanking DNA was included to screen for far-upstream enhancer elements in an analogous fashion to those recently characterized from the mouse albumin and alpha-fetoprotein genes (Pinkert *et al.*, 1987; Hammer *et al.*, 1987). The shorter angiotensinogen minigene (0.75 A/2) contains 0.75 kb of 5′ flanking DNA and was designed to test the proximal promoter elements, which are sufficient to direct tissue-specific expression of genes such as elastase (Ornitz *et al.*, 1985) and atrial natriuretic peptide (Field, 1988).

We show that the shorter minigene (0.75 A/2) contains sufficient information to direct appropriate expression of the construct in the majority of organs examined, with the exception of the testis and salivary gland. We subsequently demonstrate that strain polymorphisms are responsible for aberrant expression of our BALB/c angiotensinogen minigenes in these latter two organs from Swiss mice. This observation again highlights the importance of genetic background in determining expression patterns of genes used for production of transgenic mice (Mullins *et al.*, 1988).

Results

Design of the minigenes

We placed several constraints on the design of the angiotensinogen minigenes (Figure 1). The normal initiation

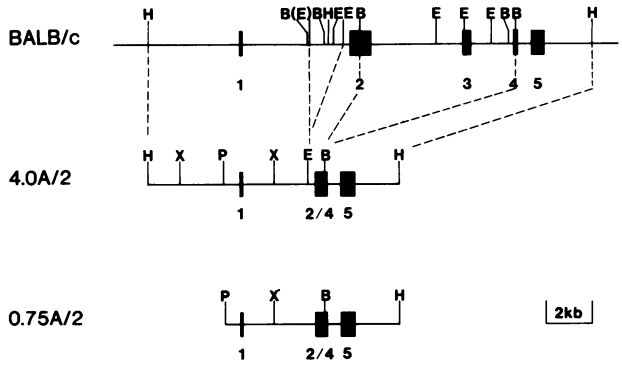

Fig. 1. Angiotensinogen minigene constructs. The top line shows a restriction map of the BALB/c angiotensinogen gene, with the exons numbered 1–5. The dotted lines indicate regions deleted from the endogenous gene to construct minigenes containing either 4 kb (4.0 A/2) or 0.75 kb (0.75 A/2) of 5′ flanking DNA. Restriction sites shown are *Hin*dIII (H), *Bam*HI (B), *Eco*RI (E), *Xba*I (X) and *Pst*I (P). The *Eco*RI site in brackets is derived from the vector pJB8. The *Pst*I site is not unique.

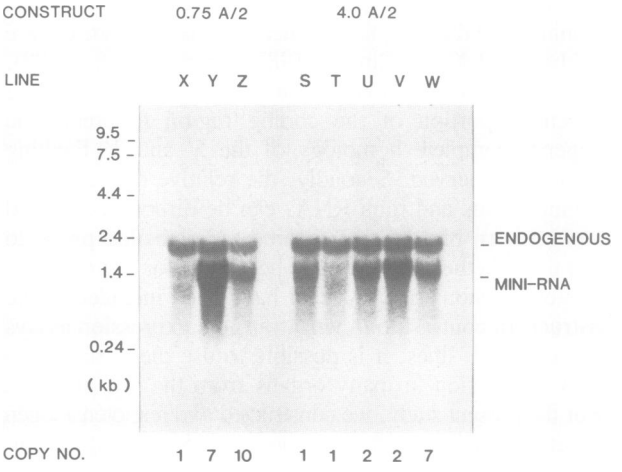

Fig. 2. Northern blot of total RNA (45 μg/track) extracted from livers of dexamethasone-treated transgenic mice. Expression of the endogenous and mini-angiotensinogen mRNAs from eight separate transgenic lines is shown, as is the copy number of the transgene in each line. Size markers are an *in vitro* synthesized RNA ladder (BRL). Autoradiographic exposure was for 5 h with an intensifying screen.

methionine and leader sequence were preserved in order to prevent the intracellular accumulation of a truncated protein. A deletion within the coding region was made to allow experimental discrimination on the basis of mol. wt between the endogenous and transgenic angiotensinogen mRNAs. This deletion within the coding region was made to preserve the translational reading frame so that the same termination signal would be used, thereby exposing identical 3′-untranslated regions on the mRNAs derived from both the endogenous angiotensinogen gene and the minigene. This should endow the mini-angiotensinogen RNA with similar stability to the normal RNA.

Liver expression

F1 animals from eight separate transgenic lines were analysed. Lines containing the 4.0 A/2 construct were designated S, T, U, V and W. Those containing the 0.75 A/2 minigene were named X, Y and Z. Expression of the transgene in each line was initially examined in the liver after dexamethasone treatment in order to enhance detection of

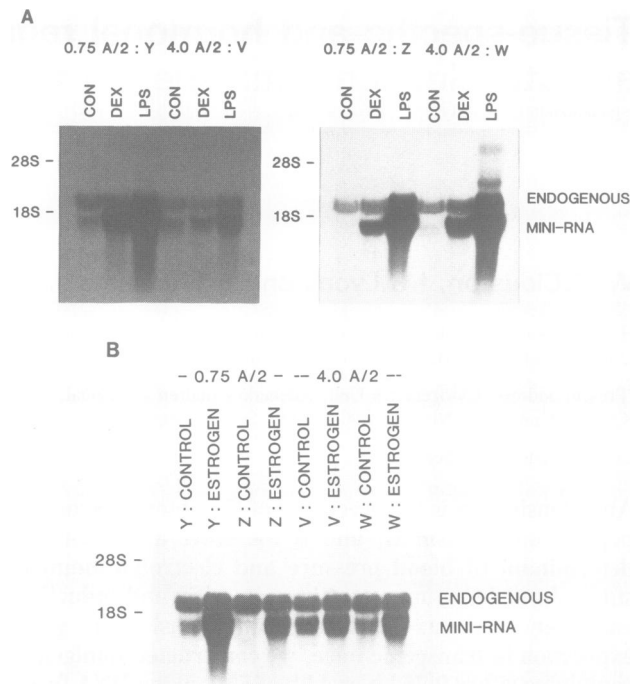

Fig. 3. Induction of endogenous and mini-angiotensinogen mRNA levels in livers of mice from four separate transgenic lines. (**A**) Reponse 6 h after a single i.p. injection of either dexamethasone (DEX; 7 mg/kg) or the acute phase stimulant *E.coli* lipopolysaccharide (LPS; 1 mg/kg) is compared to control levels (CON) is sex-matched littermates. (**B**). Induction by oestrogen (0.3 mg/kg) given s.c. for 2 days. Autoradiographic exposures were 2.5 h (**A**) or 5 h (**B**) with an intensifying screen.

both the endogenous and mini-angiotensinogen mRNAs (Figure 2). Six out of the eight lines showed high levels of expression of the minigenes in the liver, but the expression was not proportional to copy number. In other studies, this lack of copy number-dependent expression has been attributed to site of integration effects (Palmiter and Brinster, 1986).

Hormonal induction

In order to map the inducible enhancer elements controlling angiotensinogen expression in the liver, sex-matched littermates from several different transgenic lines were treated with agents known to induce the gene. Animals from two separate transgenic lines for each construct were studied to control for integration site effects on the degree of induction. The shorter construct (0.75 A/2) contains sufficient information to respond to dexamethasone, oestrogen, and the acute phase stimulant *Escherichia coli* lipopolysaccharide (Figure 3A and B). For each stimulus, the degree of induction was greater for the mini-RNA than the endogenous angiotensinogen mRNA. This excessive induction can be partially explained by transcription from multiple copies of the transgene. Because the response was not directly proportional to copy number, other factors such as the proximity of many copies of the putative enhancer elements, as well as integration site effects, may be contributing to the variation in response among the different transgenic lines.

Tissue-specific expression

The major objective of this study was to map the tissue-specific elements controlling angiotensinogen gene

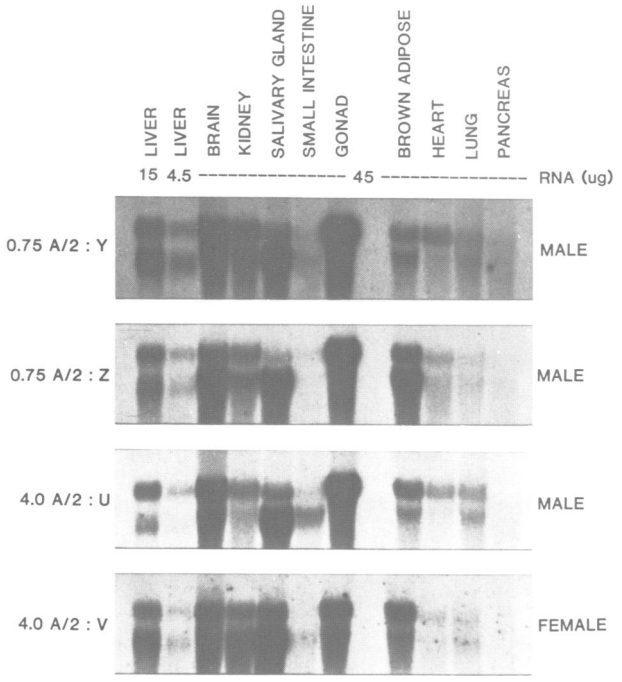

Fig. 4. Tissue-specific expression of angiotensinogen mRNA (upper band) and the mini-RNA (lower band) in four separate transgenic mouse lines. Animals were treated with dexamethasone for 2 days to enhance detection in tissues expressing low levels of angiotensinogen. Autoradiographic exposures ranged from 16 to 24 h with an intensifying screen.

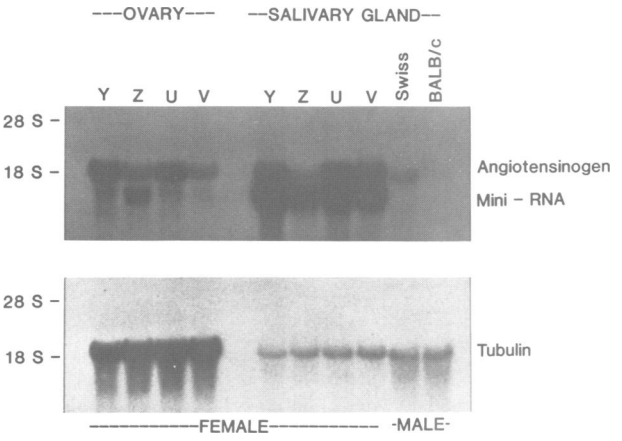

Fig. 5. Northern blot showing the relative expression of the endogenous and mini-angiotensinogen mRNAs in the ovary and salivary gland of untreated females from four transgenic lines. Salivary gland RNA from non-transgenic Swiss and BALB/c adult male mice is included for comparison. Autoradiographic exposure was for 12 h with an intensifying screen. The same blot was reprobed with a tubulin cDNA to control of the quantity (45 μg) and integrity of the RNA loaded in each track.

expression. Two separate lines from each construct were used to control for site of integration effects on expression. In order to compensate for lower levels of angiotensinogen gene expression in extrahepatic tissues (Campbell and Habener, 1986), more RNA was loaded from these tissues than from the liver. Animals were also pre-treated with dexamethasone to enhance detection of the endogenous and introduced mRNAs in organs expressing low levels of angiotensinogen.

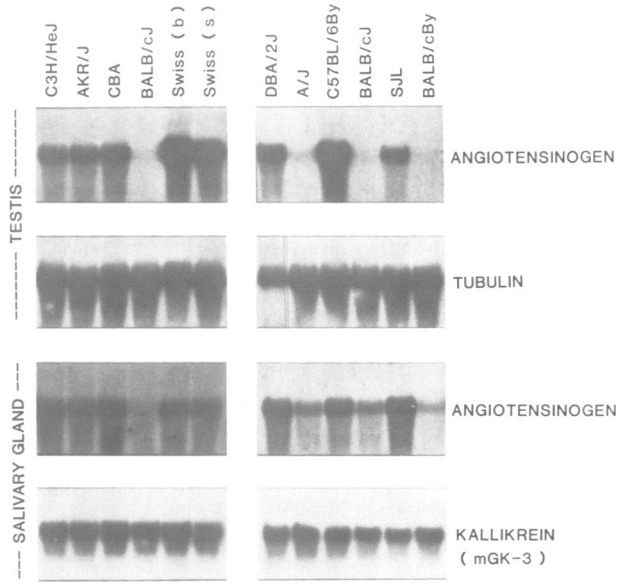

Fig. 6. Variability of angiotensinogen gene expression in testis and salivary gland from different inbred mouse strains. The Swiss mice are homozygous for either the Ang^s (s) or Ang^b (b) alleles of the angiotensinogen gene. BALB/c and A/J mice have low levels of angiotensinogen mRNA in both organs. Control probes are tubulin for the testis and the glandular kallikrein mGK-3 for the salivary gland.

With few exceptions, both the short (0.75 A/2) and long (4.0 A/2) minigenes were expressed at similar levels to the normal gene in liver, brain, kidney and brown adipose tissue (Figure 4; Campbell and Habener, 1987). There was no ectopic expression of the constructs in the pancreas, but two transgenic lines showed low levels of stray expression of the minigene in the small intestine. The normal angiotensinogen mRNA was present in heart and lung at low levels (~10% of liver), but two out of four lines did not show expression of the transgene in the heart (Figure 4).

Expression of the minigenes in the gonad differed between males and females. None of the lines examined showed expression of the transgene in the testis despite very high levels of endogenous angiotensinogen mRNA (Figure 4). Ovarian minigene expression was analysed in these same transgenic lines without dexamethasone pre-treatment (Figure 5). The minigene was expressed at detectable but low levels in ovaries from lines U, V and Y, and at higher than normal levels in line Z. These findings suggest that the BALB/c minigene constructs lack a testis-specific enhancer element.

The level of expression of the BALB/c minigenes was consistently higher than the endogenous Swiss angiotensinogen gene in the salivary gland of all lines examined, excluding the site of integration as the basis of this phenomenon (Figures 4 and 5). This over-expression of the minigene occurred at similar levels in both male and female transgenic mice (Figures 4 and 5). Endogenous angiotensinogen mRNA levels were variable in the salivary glands of the random bred Swiss animals, but were higher than in non-transgenic BALB/c mice (Figure 5). Therefore, the over-expression of the BALB/c minigene in the Swiss salivary gland is paradoxical. This finding, as well as the lack of expression of the BALB/c minigene in the testis, led us to systematically examine strain polymorphisms of angiotensinogen expression in testis and salivary gland.

Variability of angiotensinogen gene expression in different mouse strains

The salivary gland and testis represented the extremes of expression of the minigenes and were therefore the focus of the strain survey. The survey included two random bred Swiss mice which had been genotyped for a *Bam*HI allelic polymorphism of the angiotensinogen gene (Clouston and Richards, 1989). One animal was an Ang^s/Ang^s homozygote and the other was an Ang^b/Ang^b homozygote. BALB/c mice from three different sources were used to confirm that the low expression phenotypes of this strain were reproducible.

The strain survey of testicular angiotensinogen mRNA showed a spectrum of expression (Figure 6). In contrast to the high levels of angiotensinogen mRNA in testis of Swiss mice there were very low levels in the testis of BALB/c and A/J animals. This suggests that the absence of expression of the BALB/c minigenes in testis of Swiss mice is due to a difference in a *cis*-acting enhancer element between these strains. In this context, the *Bam*HI allelic polymorphism of the Swiss angiotensinogen gene did not appreciably affect testicular expression (Figure 6).

Expression of angiotensinogen in the male salivary gland was similar in all strains examined except BALB/c and A/J, where the mRNA was of very low abundance (Figure 6). This is in contrast to the over-expression of the BALB/c minigene when placed into a Swiss genetic background (Figures 4 and 5). There are two possible explanations for this phenomenon. The first is deletion of a repressor element from the BALB/c minigene, thereby causing over-expression in the Swiss salivary gland. An alternative hypothesis to explain the over-expression of the BALB/c minigene is the presence of a *trans*-acting factor in the salivary gland of Swiss but not BALB/c mice.

Genetic analysis of angiotensinogen expression phenotypes using recombinant inbred mice

We sought an alternative form of analysis because the gene transfer experiments could not definitively distinguish between *cis* and *trans* interactions as the basis of the salivary gland expression phenotypes. Recombinant inbred mice were used because they allow genetic discrimination between *cis* and *trans* interactions controlling expression phenotypes (Bailey, 1981). Based on the results of the strain survey (Figure 6), we chose the CXB recombinant inbred set for this analysis. The progenitor strain C57BL/6By has high expression of angiotensinogen in testis and salivary gland, while the BALB/cBy progenitor has low expression in both organs. To determine the genotype of angiotensinogen in the CXB recombinant inbred set, we used an *Eco*RI restriction fragment length polymorphism (RFLP) identified by the mouse angiotensinogen exon 2 cDNA probe (Figure 7). In the C57BL/6By progenitor (designated 'B' by Bailey 1981) there is a 4.8 kb band, whereas in the BALB/cBy progenitor (abbreviated as 'C' by Bailey, 1981) there is a 3.9 kb band. Therefore, the angiotensinogen genotype of the CXB recombinant inbred strains D to K is B C B C B B B (Figure 7).

We next determined the expression phenotype of angiotensinogen in salivary gland and testis of the CXB recombinant inbred mice by Northern analysis (Figure 8). The strain distribution pattern of angiotensinogen mRNA in both organs is B C B C B B B. This is identical to the angiotensinogen genotype (Figure 7). This concordance between genotype and phenotype suggests that mutant *cis*-acting elements in the BALB/c angiotensinogen gene determine the expression phenotypes observed in both testis and salivary gland.

To characterize further this genetic lesion within the angiotensinogen locus, we searched for an RFLP co-

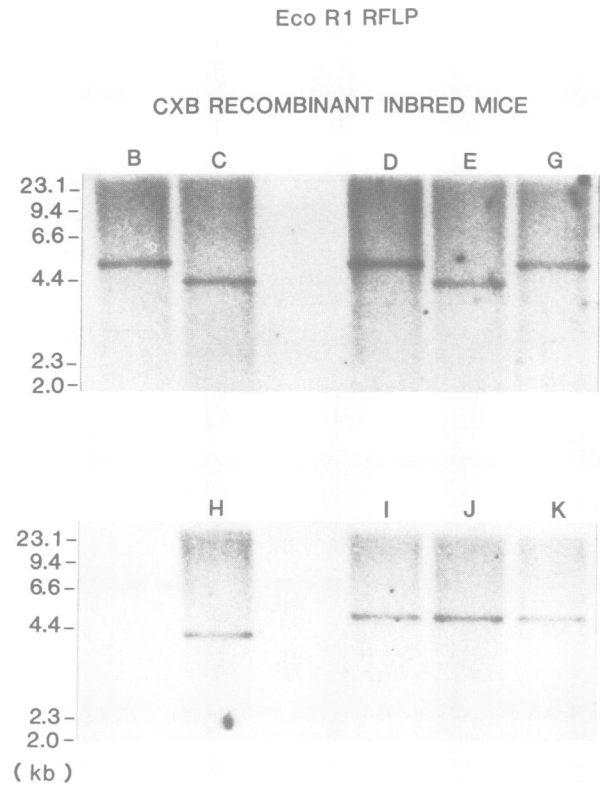

Fig. 7. Genotype of angiotensinogen in CXB recombinant inbred mice. A Southern blot of *Eco*RI-digested genomic DNA from the parental strains C57BL/6By (**B**) and BALB/cBy (**C**), and the CXB recombinant inbred stains **D** to **K** was probed with the mouse angiotensinogen exon 2 cDNA. Size markers are λ DNA digested with *Hin*dIII.

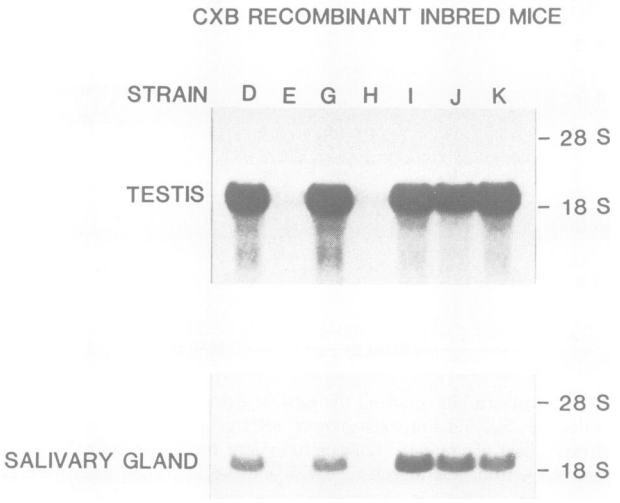

Fig. 8. Northern blot showing angiotensinogen expression phenotypes in the testis and salivary gland of CXB recombinant inbred strains **D**–**K**. Ribosomal RNA size-markers are shown. The strain distribution patterns of expression are identical in both organs.

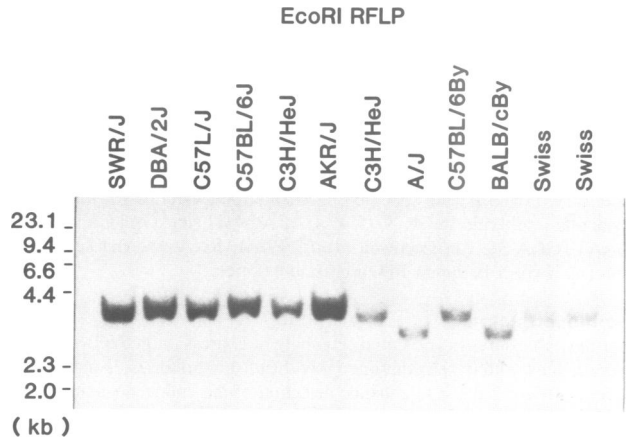

Fig. 9. Genomic Southern blot showing segregation of an *Eco*RI restriction fragment length polymorphism (RFLP) for angiotensinogen into different mouse strains. The lower mol. wt band (designated the Ang^l allele) shows concordance with low angiotensinogen expression in the salivary gland and testis of BALB/c and A/J mice. Conversely, the higher mol. wt band (designated the Ang^h allele) is present in strains having high levels of angiotensinogen expression. The difference in intensity of the bands is due to variation in amounts of DNA loaded, as judged by ethidium bromide staining (data not shown). Size markers are λ DNA digested with *Hin*dIII.

segregating with the expression phenotypes in various inbred mouse strains. A *Bam*HI RFLP recognized by the angiotensinogen exon 2 probe (Clouston and Richards, 1989) does not correlate with the expression patterns (Figure 6). However, the *Eco*RI RFLP used to genotype the CXB recombinant inbred mice (Figure 7) also segregates with the angiotensinogen expression phenotypes observed in the salivary gland and testis of inbred mouse strains (Figures 6 and 9). The alleles recognized by this RFLP have been designated Ang^l and Ang^h (Figure 9). The Ang^l allele is associated with low expression in strains BALB/c and A/J, whereas the Ang^h allele is associated with the high expression seen in all other strains tested.

Discussion

We have shown that the 0.75 A/2 minigene contains sufficient information to direct appropriate expression in the majority of mouse tissues which produce angiotensinogen. The deletion mutants allowed us to map the hormone and acute-phase response elements to the shorter construct. Finally, this analysis has identified and characterized strain polymorphisms affecting angiotensinogen expression in the testis and salivary gland.

Recent interest has centred on the design of transgenic constructs capable of directing copy number-dependent expression free of positional interference. For the human β-globin gene, this was achieved by the assembly of a minilocus containing far upstream DNase I-hypersensitive sites to produce a dominant expression phenotype (Grosveld *et al.*, 1987; van Assendelft *et al.*, 1989). In the case of the human CD2 gene, inclusion of 3′ flanking sequences resulted in position-independent expression in the thymus but not in the brain (Greaves *et al.*, 1989). Our angiotensinogen transgenic lines responded to a variety of stimuli at greater levels than the endogenous gene, suggesting that multiple copies of the minigene were transcriptionally active (Figure 3). However, the fact that this response was not proportional to copy number, and the observation that two out of the eight transgenic lines expressed the construct poorly suggests that these minigenes are not capable of position-independent expression. DNase I hypersensitivity analysis of the angiotensinogen gene will help to determine whether other regions are required to achieve independence of integration site effects. Another potential mechanism contributing to site of integration effects is transcriptional interference (Proudfoot, 1986), and this may be overcome by including strong transcription termination sequences upstream of the construct (Heard *et al.*, 1987).

Our genetic analysis using both transgenic and recombinant inbred mice has defined strain polymorphisms affecting angiotensinogen expression in the testis and salivary gland. This may not be unique to inbred mouse strains as the rat angiotensinogen gene is also expessed in the ovary, but not in testis or salivary gland (Ohkubo *et al.*, 1986; Campbell and Habener, 1986). The evidence supporting a *cis*-interaction as the basis of the testicular expression phenotypes in mice is strong and consistent (Figures 4−8). In the salivary gland, the over-expression of the minigene is paradoxical because the BALB/c angiotensinogen gene is expressed at low levels in this tissue. The recombinant inbred analysis suggests that the genetic lesion in the salivary gland is also in *cis*, and this conclusion is supported by the identification of an *Eco*RI RFLP co-segregating with the expression phenotypes into inbred mouse strains (Figure 9). Based on these findings, deletion of a repressor element from the minigene is the most likely explanation for the over-expression observed in the salivary gland. Using the informative *Eco*RI RFLP, it will now be possible to backcross the BALB/c angiotensinogen gene into a Swiss genetic background to produce congenic strains, which will provide definitive proof of this hypothesis. It should be possible to subsequently isolate the mutant region, as we have already cloned the BALB/c angiotensinogen gene (Clouston *et al.*, 1988).

There are several precedents for deletions and insertions affecting the tissue-specific expression of mouse genes. The *Slp* gene is closely linked to the complement C4 gene, and by virtue of an insertion 2 kb upstream of the cap-site, has acquired androgen-responsiveness (Loreni *et al.*, 1988). A retroviral insertion has recently been identified as the cause of the *hairless* mutation (Stoye *et al.*, 1988). By analysis of a revertant of the mutation, excision of this retroviral element was proven by DNA sequencing (Stoye *et al.*, 1988). The renin gene in mice shows sexually dimorphic expression in the salivary gland (Wilson *et al.*, 1978). In this case, there is a gene duplication event, with the *Ren1* gene being expressed in the kidney and the duplicated *Ren2* gene being expressed in the male salivary gland at high levels, as well as in the kidney (Piccini *et al.*, 1982; Field and Gross, 1985). DNA sequence analysis of the *Ren2* promoter shows the insertion of two repetitive elements (Nakamura *et al.*, 1988), one of which interrupts a potential cyclic AMP response element.

We conclude that the expression polymorphisms observed for the BALB/c angiotensinogen minigene and the endogenous Swiss angiotensinogen gene (Figures 5 and 6) illustrate a potentially important cause of variability in expression of other genes in transgenic mice. Such variability is often attributed to site of integration effects (Palmiter and

Brinster, 1986). Most transgenic laboratories use outbred or F2 hybrid zygotes to make transgenic mice, because mice of a genetically heterogenous background yield greater zygote numbers, and these give higher transformation rates (Brinster *et al.*, 1985). The disadvantage of this approach is that it does not control for heterogeneity of *trans*-acting factors between the parental strains of the F2 cross (Donner *et al.*, 1988; Duncan *et al.*, 1988). This heterogeneity can only be dissected by subsequent backcrossing to produce ongenic lines. In selected cases, it may be worth accepting the lower breeding and transformation efficiency offered by inbred mouse lines to produce more uniform results.

Materials and methods

Mice

Random bred Swiss, DBA/2J, SJL, A/J, C57BL/6By, BALB/cBy, BALB/cJ and CXB recombinant inbred mice were obtained from the Animal Resources Centre, Perth, Australia. C3H/HeJ, CBA, BALB/cJ and AKR/J adult male mice were purchased from the Department of Pathology, University of Melbourne, Australia.

DNA

Genomic DNA from SWR/J, DBA/2J, C57L/J, C57BL/6J, C3H/HeJ and AKR/J mice used in Figure 9 was generously provided by Dr B.A.Taylor, The Jackson Laboratory, Bar Harbor, Maine.

Constructs

Our BALB/c mouse genomic clones (Clouston *et al.*, 1988) were the starting material for the constructs (Figure 1). To make a minigene with 4 kb of 5′ flanking region (4.0 A/2), a 3.3 kb *Bam*HI−*Hin*dIII fragment containing exon 5 and part of exon 4 was subcloned from λgMA2 into pJB8 (see Figures 2 and 4 of Clouston *et al.*, 1988 for a detailed restriction map). A 0.71 kb *Eco*RI−*Bam*HI fragment from cosmid c2, containing the 5′ end of exon 2 was then fused to the exon 4 and 5-containing plasmid. This in-phase fusion of exons 2 and 4 was confirmed by DNA sequencing, and effectively deleted 720 bp from the centre of the mature mRNA. The resulting 4-kb *Eco*RI−*Hin*dIII fragment containing the fused exons 2−4 and 5 was then ligated to a 6.8 kb *Hin*dIII−*Eco*RI fragment from cosmid c2, which had 4.0 kb of 5′ flanking DNA upstream of exon 1 (Figure 1), using *Hin*dIII-cut, dephosphorylated pJB8 as a vector. The resulting construct was designated 4.0 A/2. Note that there is also a deletion of 1.6 kb in the first intron which contains a cluster of restriction sites. This deletion is well upstream of the splice acceptor sequences of this intron.

To construct a shortened version of the minigene, a 5.6 kb *Xba*I−*Hin*dIII fragment spanning the exon 2/4 fusion was isolated from the 4.0 A/2 plasmid, and ligated to a 2.1 kb *Pst*I−*Xba*I fragment containing exon 1 (see Figure 4A of Clouston *et al.*, 1988). This construct contains 0.75 kb of 5′ flanking DNA, and was designated 0.75 A/2 (Figure 1).

All vector sequences were removed for microinjection, which was performed essentially as described by Hogan *et al.* (1986), except that a compressed air system (I.Lyons, in preparation) was used for microinjection. The BALB/c minigene constructs were microinjected at a concentration of 200 copies/pl into fertilized ova from random bred Swiss mice.

DNA analysis of transgenic animals

For genomic Southern identification of positive animals, tail DNA was digested with *Bam*HI and probed with a 397 bp *Stu*I−*Bam*HI fragment from exon 2 of the mouse angiotensinogen gene. This probe identifies an allelic polymorphism of the endogenous angiotensinogen gene, with 2.1 kb and 1.7 kb alleles (Clouston and Richards, 1989). The diagnostic *Bam*HI minigene fragment identified by this probe is 0.71 kb (Figure 1). Copy number of positive transformants was estimated by a molecular dynamics computing densitometer, with reference to the signal from a single allele of the endogenous gene.

For screening tail DNA of F1 and F2 animals by dot-blot analysis, we identified a unique area of the construct at the fusion of exons 2 and 4. A 24mer synthetic oligodeoxyribonucleotide containing 12 base pairs on either side of the exon 2/4 junction (5′-dCAGATTGTAGGATCCAAG-GTAGAA-3′) discriminated single-copy transformants from negative animals when used under high-stringency conditions (hybridization at 42°C in 30% formamide and washing at 55°C in 1 × SSC).

RNA analysis

Total RNA was isolated by homogenization of tissue in 5 M guanidinium thiocyanate, with subsequent centrifugation through a 5.7 M CsCl cushion (Chirgwin *et al.*, 1979). Northern blots were hybridized with a 30mer synthetic mouse angiotensinogen oligodeoxyribonucleotide (5′-dTGCTCT-TGTTGTGGTAAAGGAGATGGAAGG-3′ at 42°C in the 30% formamide buffer described by Ullrich *et al.* (1984) and washed to a stringency of 0.5 × SSC at 42°C. Control probes were a β-tubulin cDNA (Valenzuela *et al.*, 1981) and a gene-specific oligodeoxyribonucleotide for the mouse glandular kallikrein mGK-3 (5′-dTCACACAGTAGAGATCATCTGT-GAATTGGA-3′; van Leeuwen *et al.*, 1986). RNA size markers were purchased from Bethesda Research Laboratories.

Hormone treatment

For the tissue surveys and initial screening of expression in the liver, mice were treated with dexamethasone (David Bull Laboratories; 7 mg/kg i.p. twice daily for 2 days) to enhance detection of the endogenous and mini-angiotensinogen mRNAs. For studies of acute induction of gene expression, a single dose of either dexamethasone (7 mg/kg; Campbell *et al.*, 1984) or lipopolysaccharide (Sigma; 1 mg/kg; Kageyama *et al.*, 1985) was given by i.p. injection 6 h prior to RNA isolation. For induction by oestrogen, animals were given 0.3 mg/kg of s.c. ethinyl oestradiol (Wyeth Laboratories; dissolved in 95% peanut oil and 5% benzoic acid) in a single daily dose for 2 days prior to tissue extraction (Darby, 1986).

Acknowledgements

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