tion of additional genetic influences inherited from her father's (IV-17) branch of the family, might contribute to her susceptibility to bilateral breast cancer. In fact, other incidences of cancer have been reported in the pedigree of IV-17 (ref. 12). More extensive sequencing of the *p53* gene, encompassing areas beyond the four hot-spot regions, should provide further clarification.

It has been suggested that point mutations in the conserved region of the *p53* gene can cause conformational changes in the encoded nuclear phosphoprotein. Mutant p53 protein forms a complex with wild-type p53, thereby inhibiting the normal functions of the latter[17,18]. Furthermore, mutations in wild-type p53 protein can abolish the transformation-suppressing activity of the normal p53 in a *ras* complementation assay[19]. The addition of wild-type *p53* complementary DNA can suppress the growth of human glioblastoma[20] and colorectal carcinoma[21] cells. Although the precise functions of the p53 protein remain to be elucidated, several studies of the interaction between p53 protein and viral oncoproteins such as the simian virus 40 T antigen[22,23], adenovirus E1b[24], and papilloma virus E6[25], or studies of the functions of p53 involving the microinjection of anti-p53 antibody[26], indicate that p53 may be essential for the control of cell proliferation. Because affected individuals of the family we studied often have more than one type of neoplasm, a defect in a gene such as *p53*, which is expressed in a variety of cell types, might disadvantage cells of these individuals in terms of the control of normal cell proliferation. Additionally, this mutation in *p53* was found only in NSFs of affected individuals.

Comparisons between normal and tumour tissue from patients with non-hereditary colorectal[7] or lung cancer[8], have demonstrated that neither allelic loss nor *p53* mutations occur in these patients' unaffected cells. The inherited nature of the *p53* mutation that we have now identified in noncancerous cells may represent an early genetic alteration having a predisposing effect in carcinogenesis in the family we studied.

Note added in proof: While this paper was being accepted for publication, similar findings in Li-Fraumeni syndrome were reported[27]. □

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Site-independent expression of the chicken β^A-globin gene in transgenic mice

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THE level of expression of exogenous genes carried by transgenic mice typically varies from mouse to mouse and can be quite low. This behaviour is attributed to the influence of the mouse chromatin near the site of transgene integration[1,2]. This 'position effect' has been seen in transgenic mice carrying the human β-globin gene. It was however, abolished when DNase I hypersensitive sites (normally found 65 to 44 kilobases (kb) upstream) were linked to the human β-globin transgene[3–5]. Thus, the upstream DNA (previously named a dominant control or locus activation region, now denoted a locus control region) conferred the ability to express human β-globin at high levels dependent on copy number on every mouse carrying the construct. We report here an investigation of chicken β^A-globin gene expression in transgenic mice. A 4.5-kb fragment carrying the β^A-globin gene and its downstream enhancer[6–9], without any far upstream elements, is sufficient to ensure that every transgenic mouse expresses chicken globin messenger RNA at levels proportional to the transgene copy number. Thus the chicken DNA elements that allow position-independent expression can function in mice. In marked contrast to the human β cluster, these elements are no farther than 2 kb from the gene. The location of the elements within the cluster demonstrates that position independence can be mediated by DNA that does not define a gene cluster boundary.

To examine the elements contributing to tissue-specific and integration site-independent expression, we produced transgenic mice containing the chicken β^A-globin gene with (β^AE) or without ($\beta^A\Delta$E) the downstream enhancer (Fig. 1*a*). Six of 33 mice developing from embryos injected with β^AE, and 5 of 62 mice developing from $\beta^A\Delta$E-injected embryos carried the transgene. All transgenic mice carried intact β^A-globin genes, integrated in head-to-tail tandem arrays (Fig. 1*b*, and data not shown). Breeding resulted in the establishment of five β^AE lines (one founder did not breed) and six $\beta^A\Delta$E lines (one founder was not bred, and another contained three independently segregating integration stites).

Transgene expression was measured by primer extension. Correctly initiated β^A mRNA was detected in RNA isolated from blood of all six β^AE lines, but not in non-transgenic mice, nor in any of the $\beta^A\Delta$E mice (summarized in Table 1). β^A-globin RNA present at 0.02% of the mouse β^{maj}-globin RNA abundance would have been detected. Pretreatment of the mice with phenylhydrazine[10] increased the total RNA yield 8- to 12-fold (to ~1 μg μl^{-1} blood), but did not affect the relative expression of the mouse α, mouse β^{maj}, and chicken β^A genes. β^A-globin RNA expression was erythroid-specific (present at high levels in blood and spleen; Fig. 2*a*). We presume that the β^A RNA in non-erythroid tissues is due to blood contamination as the $\beta^A:\alpha:\beta^{maj}$ ratio was constant (Fig. 2*a*).

We were curious as to what developmental pattern β^A would exhibit in the mouse. In the chicken, the β^A gene is first expressed in yolk sac in mid-stage embryos and is the predominant β-globin thereafter[11]. In both transgenic mouse lines tested (Fig. 2*b*), the β^A transgene is expressed in 11.5-day embryos (when all of the embryonically derived erythroid cells are produced in

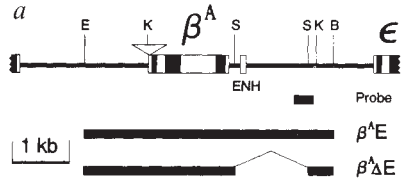
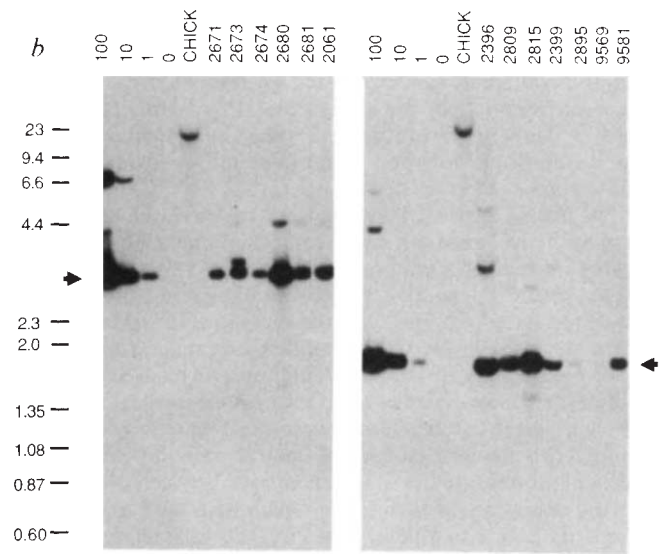


FIG. 1 Production of transgenic mice. *a*, Map of the chicken β-globin locus. The β^A and ε genes (transcribed from left to right), β^A/ε enhancer (ENH), DNA fragments (β^AE, $\beta^A\Delta$E) used for making transgenics, and hybridization probe are indicated. β^AE is a 4.45-kb fragment extending from 1.05 kb upstream of the β^A transcription start site to 0.94 kb 5′ of the ε start site. It contains a 35-base pair (bp) insert with a new *Kpn*I site in the 5′, transcribed, untranslated region of the β^A gene[26]. $\beta^A\Delta$E is the same as β^AE, except for a 1.25-kb *Sac*I deletion that begins ~90 bp 3′ of the polyadenylation site and removes the β^A/ε enhancer. The probe, I142, is a 362-bp *Bgl*II/*Kpn*I fragment. Restriction sites are: E, *Eco*RI; K, *Kpn*I; S, *Sac*I; and B, *Bam*HI. *b*, Southern blots of DNA from transgenic mice. Transgenic FVB/N mice were produced by injection of DNA[27], their tail DNA was digested with *Kpn*I, and subjected to Southern blotting. Left panel, β^AE founder animals (except for 2061 which is derived from 2702). Right panel, $\beta^A\Delta$E founder animals and two 2396-derived progeny (2809, carrying integration site A, and 2815 carrying sites A and C). Arrows show the fragments expected from intact, unrearranged transgenes. Fragments smaller than the arrows are derived from rearranged copies of the transgenes. The 4.5-kb (left panel) and 3.2-kb (right panel) bands are the products expected from partial digestion of head-to-tail tandemly integrated transgenes. Non-transgenic mouse DNA augmented with plasmids containing β^AE (left panel) or



$\beta^A\Delta$E (right panel) at the indicated copy number (per diploid genome) is in lanes marked 0, 1, 10, and 100 (bands larger than those indicated by the arrows are partially digested plasmids). Chicken genomic DNA is also shown at a loading corresponding to a copy number of four; the hybridizing band is of the appropriate size as genomic DNA does not carry the inserted *Kpn*I site. The positions of size markers, in kilobases, are at the left.

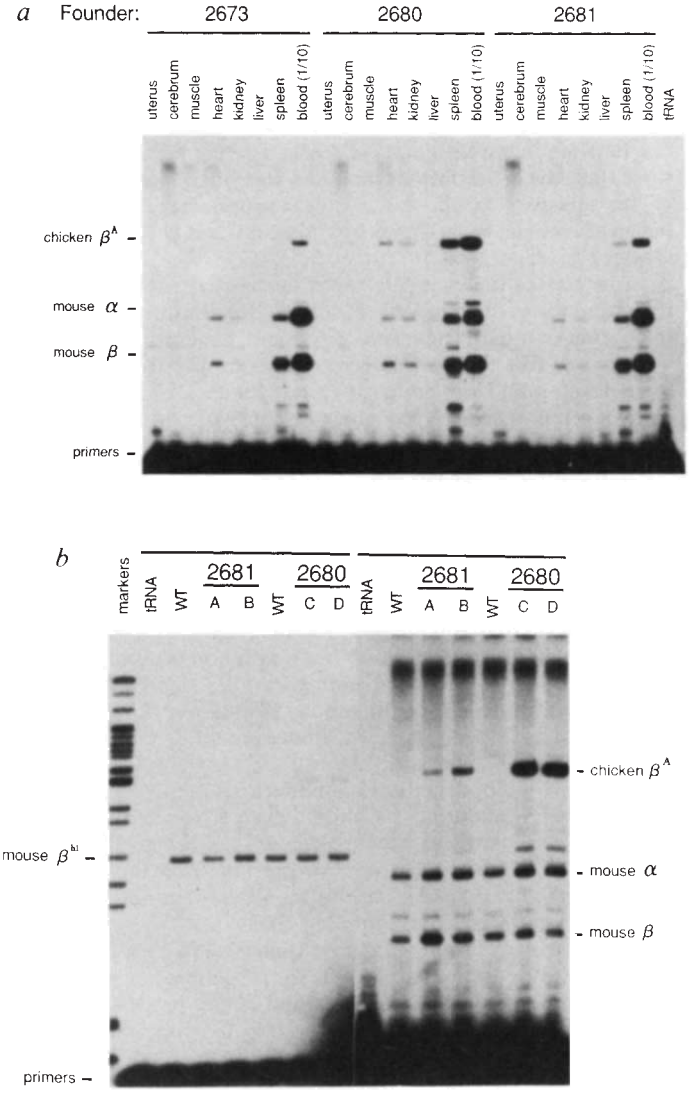


FIG. 2 Tissue and developmental specificity of transgene expression. *a*, RNA was isolated from the indicated tissues of adult female mice (derived from the indicated founders) and measured by primer extension. The chicken β^A, mouse α, and mouse β^{maj} products are so indicated. *b*, Primer extension using total RNA from 11.5-day-old embryos (including placenta and membranes; at 11.5 days all the embryonically produced blood is derived from the yolk sac[12]). The left lanes show primer extensions using the mouse embryonic β^{h1} primer with, from left to right: tRNA, a negative control template; three litter mates, one non-transgenic (WT) and two transgenic (A and B) from breeding a 2681-derived male with a non-transgenic female; and three litter mates, one non-transgenic (WT) and two transgenic (C and D) from breeding a 2680-derived male with a non-transgenic female. The right lanes use the chicken β^A, mouse α, and mouse β^{maj} primers with the same RNAs. Measurement of β^{h1} RNA allows estimation of the contribution of the embryonic erythroid tissue to the total RNA isolated.

METHODS. RNA was isolated[28], primer extension[13] performed with 10 μg (1 μg for blood) of RNA, and the products separated on denaturing 8% polyacrylamide gels. The primers and product sizes are: chicken β^A, 5′-GGTGATGAGCTGCTTCTCCTC-3, 115 nucleotides; mouse α, 5′-CAGGCAGCCT-TGATGTTGCTT-3′, 65 nucleotides; mouse β^{maj}, 5′-TGATGTCTGTTTCTGGGGT-TGTG-3′, 53 nucleotides; and mouse β^{h1}, 5′-ATAGCTGCCTTCTCCTCAGCT-3′, 87 nucleotides. the primers are specific for the indicated mRNAs, except that the β^{h1} primer also detects β^A mRNA. The chicken primer detects the other chicken β-like globins, none of which is present in mice. Assays using a single primer gave identical results to those using multiple primers.

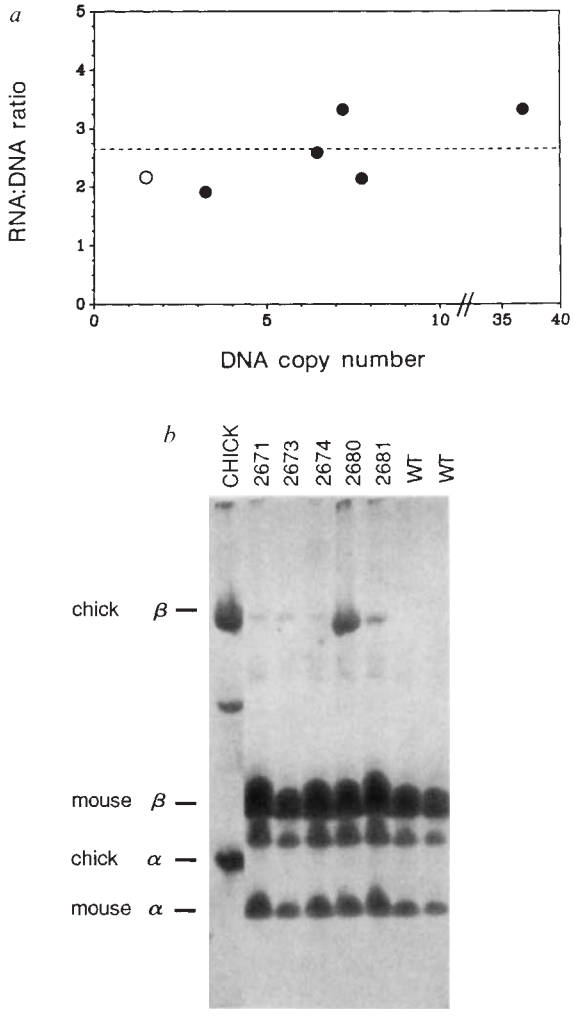


FIG. 3 Copy number dependence of RNA expression. *a*, β^A RNA abundance divided by DNA copy number plotted against DNA copy number. Solid circles represent the mean progeny data and the open circle is the datum for founder mouse 2674 (data from Table 1). The RNA:DNA ratio is the amount of transgene RNA, per transgene copy, expressed as a percentage of the amount of mouse β^{maj} RNA, per gene copy (assuming equally efficient priming and reverse transcription of the different primers and 2 β^{maj} genes per diploid genome). The β^A mRNA abundance per copy averages 2.7% that of mouse β^{maj}. *b*, Triton/acid/urea gel electrophoresis of erythrocyte lysates. A Coomassie-stained gel with lysates from five founder mice, two non-transgenic (WT) mice, and an adult chicken (CHICK) is shown. Positions of the various globins are as indicated. Lysate preparation and electrophoresis were essentially as described[29]. The FVB/N strain used here carries the 'diffuse' (*Hbb*^{d}) allele (C. Hansen, personal communication).

TABLE 1 Transgene DNA copy number and RNA abundance

Line	Founder DNA copy number	Founder RNA abundance	Progeny DNA copy number	Progeny RNA abundance
β^AE				
2671	1.7	2.1	3.2±0.5 (3)	3.1±0.2 (3)
2673	2.2	6.3	6.5±1.0 (11)	8.3±1.6 (6)
2674	1.5	1.6		
2680	30		37±7.6 (5)	61±21 (4)
2681	5.7	7.1	7.2±0.1 (2)	11.9±0.7 (4)
2702	1.8	1.4	7.7±0.5 (2)	8.3±2.1 (3)
$\beta^A\Delta$E				
2396(A+B+C)	72	<0.2		
2396(A)			28±12 (6)	<0.02 (3)
2396(B)			46±17 (3)	<0.02 (1)
2396(C)			63±7 (2)	<0.02 (2)
2399	10	<0.02	9.8±0.1 (3)	<0.02 (2)
2404	0.3	<0.02	0.9±0.1 (7)	<0.02 (3)
9569	1	<0.02		
9581	11	<0.02	24±10 (2)	<0.02 (2)

DNA copy number is presented in copies per diploid genome. β^A RNA abundance is expressed as percentage of mouse β^{maj} RNA, uncorrected for either β^A or β^{maj} gene copy number. Numbers are mean ±s.d. (number of independent observations). Independent observations are defined as different animals, except for pairs of RNA measurements on the same animals bled at different times. Founder 2396 had three separate integration sites, denoted A, B, and C.

DNA copy number was measured by slot blotting[24]. Tail DNA (3 μg) was bound to GeneScreen Plus, in duplicate, and detected by hybridization with probe l142. Linearly exposed autoradiograms were scanned and copy number was calculated from standards on the same blot by linear interpolation. Correct slot loading was confirmed by rehydridizing the blot to a glyceraldehyde 3-phosphate dehydrogenase cDNA probe[25]. The actual copy number is integral in the progeny mice without any rearrangements. RNA abundance in blood was measured by primer extension (Fig. 2). The β^A, α, and β^{maj} bands were scanned, and integrated, and β^A was normalized to the mouse globins in the same sample. From the specific activities of the primers, β^A RNA abundance as a percentage of mouse β^{maj} RNA was calculated.

the yolk sac)[12]. Thus in the mouse, β^A expression begins in the yolk sac and continues through adulthood (Fig. 2*b* and data not shown).

To determine whether locus control region (LCR) activity was present in the β^A fragment, we measured the transgene DNA copy number and RNA abundance (Table 1). Mouse α-globin mRNA served as an internal control for measurement of β^A RNA, confirming equal amounts of erythroid RNA in each sample. The constant mouse β^{maj} message levels demonstrated that transgene expression does not affect the transcription of the endogenous mouse β^{maj} gene. The copy number data suggested that the founder mice were mosaic, and this was supported by the percentage of progeny that were transgenic (25, 28, 18, 28 and 20% for the β^AE lines). Measurement of the DNA copy number and RNA abundance in progeny mice eliminated the confounding effects of mosaicism. Figure 3*a* shows a plot of β^A RNA abundance per copy number against copy number. It is striking that over a 25-fold range of copy number, the RNA:DNA ratio is constant, exhibiting less than twofold variation.

These data provide evidence that transgene expression is position-independent. Proof of position independence requires only that all lines carrying the same number of gene copies express the same amount of mRNA. That the abundance of β^A RNA per copy number is the same over a 25-fold range of copy number suggests in addition that all copies of the integrated genes are expressed equally. We can compare the coefficient of variation (CV) of the RNA:DNA ratios shown in Fig. 3*a* with that obtained for the human β-blobin gene: the CV is 23% for the chicken gene, comparable to the value of 12% for the human β-globin gene with upstream elements. In contrast, the CV is 130% without these elements (calculated respectively from ref. 5 and ref. 13, −815hβG construct).

The relatively low level of β^A RNA (2.7% that of mouse β^{maj}, per copy) is perhaps not surprising, as far upstream hypersensitive sites[14] (which include enhancers (unpublished observations, see ref. 15)) which might augment the local elements, were not included in the β^AE fragment. It is also possible that in the 250 million years since the common ancestor of chickens and mice, divergence of *cis* elements or *trans* factors may have occurred (see ref. 16 for an example), resulting in less efficient expression of the heterologous gene. Dissociation of

position independence from high level expression also occurs in the human β-globin locus[17]. Irrespective of the level of β^A RNA, it is clear that the chicken elements mediating position-independent expression can function in mice. Thus the mechanisms and mediators responsible for expression independent of integration site arose before the divergence of Mammalia and Aves and have not changed significantly since then. The β^AE transgenic mice also express β^A-globin protein in a copy-number dependent manner (Fig. 3*b*).

This strict dependence on copy number, together with the observation that every transgenic mouse expresses the chicken β^A-globin gene, is evidence that the β^AE fragment carries the information necessary for position independence. We do not know which regions within β^AE cause position independence, but the β^A/ε enhancer is nuclease-hypersensitive in chromatin and thus a likely candidate. It is interesting that both the chicken β^AE fragment and complete constructs of the human β-globin LCR contain the strongest enhancer of their respective clusters[15,18]. Both the chicken β^AE and the larger, more effective versions of the human β LCR constructions contain matrix attachment regions (putative sites of DNA attachment to the nuclear matrix or scaffold)[19,20]. The functional contribution of matrix attachment regions to LCR activity is, however, unclear.

The chicken globin element we describe is located in the midst of the gene cluster, whereas the elements of the human β-globin LCR are far upstream from the genes they regulate[5,15,21–23]. The intracluster location demonstrates that position independence is not necessarily mediated by regions at the boundary of a gene cluster or chromatin domain.

Little is known about the mechanisms bestowing position-independent gene expression. For example, there is no evidence that the elements mediating this effect share a unique, defining sequence motif or bind a distinct protein. The ability of a relatively well understood part of the chicken β-globin locus to confer position independence bodes well for our ability to dissect the details of this phenomenon. □

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