

The immunoglobulin heavy-chain B-lymphocyte enhancer efficiently stimulates transcription in non-lymphoid cells

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The mouse immunoglobulin heavy-chain (IgH) B-lymphocyte enhancer stimulates transcription from heterologous promoters 20- to 40-fold when transfected into several non-lymphoid cell lines. Stimulation in B-lymphocyte melanoma cell-lines is only about 5–10 times better. A central sequence is equally active in both cell types, whilst flanking sequences, on either side of the common enhancer sequences, specifically stimulate transcription in myeloma cells. These results suggest that there are factors in non-lymphoid cells that can interact with the IgH enhancer to stimulate transcription.

Key words: mouse immunoglobulin enhancer/heterologous promoters/relative activity

Introduction

Enhancers were first identified as components of viral protein-coding gene promoters which stimulate transcription over large distances (several thousand bp) when they are located either upstream or downstream from the RNA startsite. However, they are most active when they are directly upstream from other promoter elements (the TATA box and upstream sequences), between about 50 and 150 bp from the RNA startsite. In addition they can stimulate transcription bidirectionally, from both heterologous and substitute promoters, and they organise chromatin structure. Enhancers have been found in both viral and cellular genes, and are naturally located either upstream, far upstream, or downstream from the RNA startsite. There are several short sequence homologies between enhancers, which are important for enhancer activity, and are probably binding sites for specific trans-acting factors (for reviews and refs see Gluzman and Schenk, 1983; Khoury and Gruss, 1983; Chambon *et al.*, 1984; Yaniv, 1984; Serfling *et al.*, 1985; Wasylyk, 1986; see also Ephrussi *et al.*, 1985; Church *et al.*, 1985; Zenke *et al.*, 1986; Wildeman *et al.*, 1985).

The immunoglobulin heavy-chain (IgH) gene is expressed specifically in cells of the B-lymphocyte lineage. The IgH gene contains an enhancer in an intron several thousand bp downstream from the variable-region promoter (reviewed in Calame, 1985; Figure 1). The enhancer (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Mercola *et al.*, 1983), the other promoter elements (Mason *et al.*, 1985; Grosschedl and Baltimore, 1985) and as yet undefined intragenic sequences (Grosschedl and Baltimore, 1985) all appear to contribute to tissue-specific expression of the IgH gene. Previous studies have suggested that the immunoglobulin enhancer is inactive in non-lymphoid cells (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Mercola *et al.*, 1983). We show that a subset of the IgH enhancer sequences which are active in B cells can efficiently stimulate transcription in non-lymphoid cells. The implications of these results for regulation of the IgH gene are discussed.

Results

The IgH B-lymphocyte enhancer can efficiently stimulate transcription in non-lymphoid cells

The recombinant pTCTMI (Figure 1E), composed of the SV40 early coding region, the chicken conalbumin promoter (–102 to +62) and the *PvuII*(384)–*EcoRI*(687) fragment of the IgH enhancer (with the *PvuII* site directly upstream from the promoter, see Figure 1) was transfected into mouse 3T3TK[–] fibroblast cells by the calcium phosphate technique. A different recombinant was included in the transfection to correct for variations in the transfection efficiency (see legend to Table I and Wasylyk *et al.*, 1983 for details). Total RNA was isolated

Table I. Stimulation by the IgH enhancer of specific transcription from the conalbumin and β -globin promoters in fibroblasts and B-lymphocytes

Promoter	Recombinant	Fibroblasts LMTK [–]	B-Lymphocytes MPC11BU4
C	pTCT	1	1
O	pTCTMI	40	400
N	pTCTMI3	NM	30
A	pTCTM	7	90
L	pTCTM3	NM	25
B	pTCTMDI	40	55
U	pTCTMD	7	30
M	pTCTMOI	10	10
I	pTCTMO	4	5
N			
	pG1	1	1
	pG1MI	15	100
	pG2	0.5	0.5
	pG2MI	2	20
G	pG1M	4	90
L	pG2M	2	15
O	Δ 105	2	70
B	Δ 111	1	60
I	Δ 107	0.5	150
N	Δ 118	25	150
	Δ 122	3	100
	Δ 110	1	70
	Δ 108	1	2

Specific transcription from the β -globin and conalbumin promoters was measured by quantitative S1 nuclease mapping using an excess of 5' ³²P-labelled probes. Band intensities were measured by densitometry of appropriately exposed autoradiograms. For large differences, the RNA was diluted with non-specific RNA prior to S1 nuclease mapping. Values are corrected for transcription from a co-transfected internal control, which was pTCTB for the β -globin recombinants, or p β (244+) (de Vililers *et al.*, 1981) for conalbumin-containing recombinants. The internal control represented half (by weight) of the total specific DNA transfected. Control experiments, without the internal co-transfected standard, showed that its presence did not influence the results. Values represent fold stimulation compared with the enhancerless recombinants and are averages from at least three transfections with two independent DNA preparations. The amount of DNA used in the transfections was not saturating, because decreasing the quantity of specific DNA in the transfection decreased specific transcription. NM = not measured, but much less than 40.

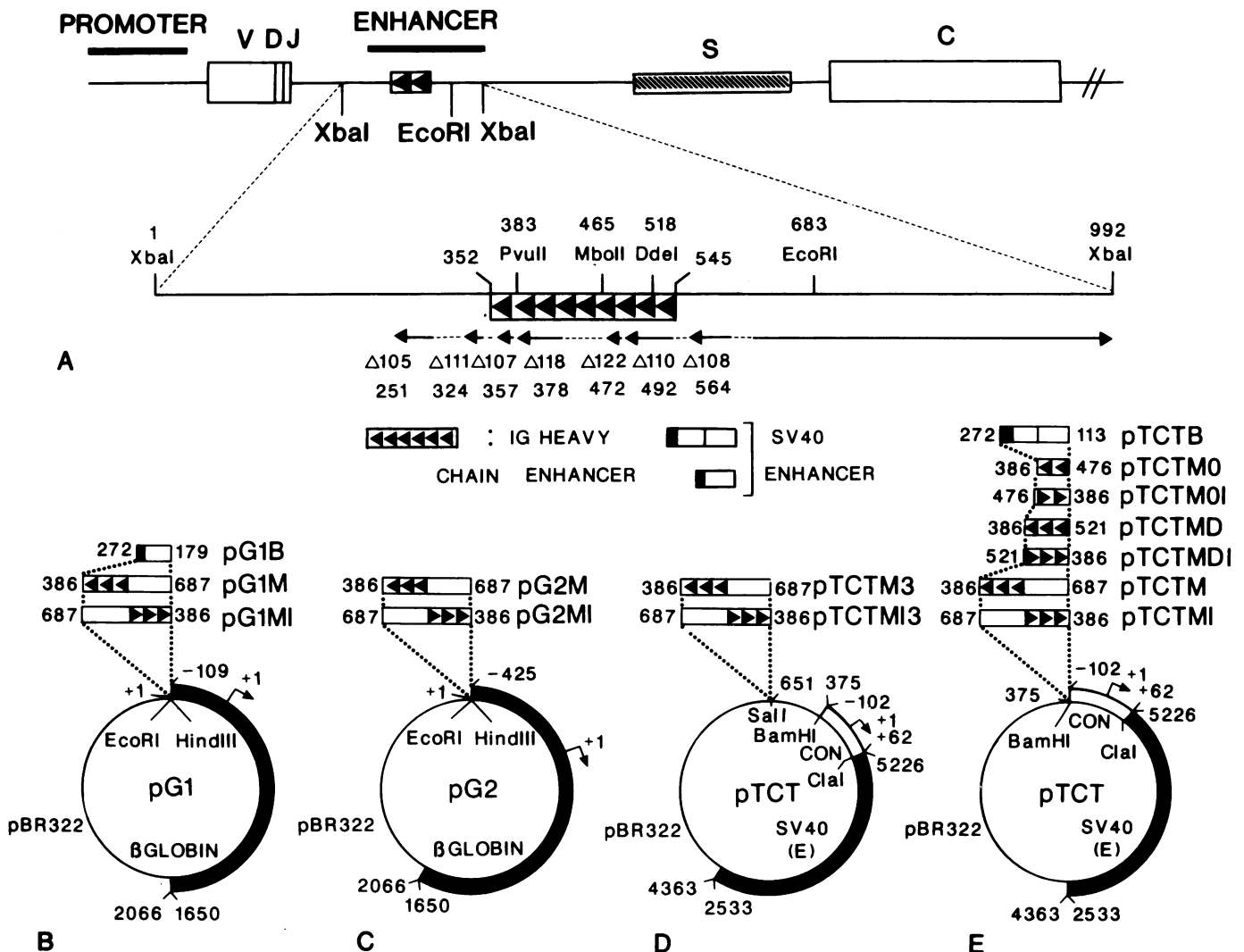


Fig. 1. Scheme of a rearranged IgH gene and structure of recombinants. (A) The IgH enhancer is located on a 992-bp *XbaI* fragment situated downstream from the IgH promoter, and the rearranged variable region (V) and the diversity (D) and joining (J) segments. The switch (S) region is involved in switching of the IgH constant (C) regions. Restriction enzyme recognition sequences on the *XbaI* fragment are numbered according to Ephrussi *et al.* (1985). The boundaries of the B-lymphocyte specific IgH enhancer (boxed arrowheads) are the limits of the genomic footprint of Ephrussi *et al.* (1985). The sequences present in mutants Δ105, Δ111, Δ107, Δ118, Δ122, Δ110 and Δ108 are indicated. (B) pG1 contains rabbit β-globin coding sequences from -109 to +1650 relative to the startsite (+1) (derived from a deletion mutant of Dierks *et al.*, 1983), pBR322 sequences, from 2066 to 4363, and the M13mp12 polylinker from the *EcoRI* to *HindIII* restriction sites. Either the SV40 (open box) or the IgH (box with arrowheads) enhancers were inserted in the polylinker upstream from the β-globin promoter. (C) The pG2 recombinants resemble pG1 series, except that β-globin sequences extend to -425 (Dierks *et al.*, 1983). (D) pTCT contains sequences from the chicken conalbumin gene (-102 to +62, CON), the SV40 early region [5226 to 2533, SV40 (E)] and pBR322 (4362 to 375) (see Moreau *et al.*, 1981). In pTCTM3 and pTCTMI3, the IgH enhancer is present in the unique *SalI* site of pBR322. (E) In pTCTM, pTCTMI, pTCTMD, pTCTMDI, pTCTMO and pTCTMOI different fragments (boxed arrowheads) of the IgH enhancer are introduced in the *BamHI* site at -102 of the conalbumin promoter. pTCTB contains the SV40 enhancer (open boxes) in the same position.

40–46 h later and analysed for specific transcription from the conalbumin promoter by quantitative S1 nuclease mapping using a 5' ³²P-labelled conalbumin probe (see Materials and methods). The IgH enhancer stimulated specific transcription from the conalbumin promoter 40-fold (compare pTCTMI, lane 4', with the enhancerless recombinant, pTCT, in lanes 1' and 2', Figure 2A and Table I). The strong SV40 enhancer, in the equivalent recombinant pTCTB, was only about 10 times more efficient than the IgH enhancer (compare lanes 3 and 4, Figure 2A). This surprisingly efficient stimulation of transcription by the IgH enhancer in non-lymphoid cells prompted us to transfect pTCTMI into a different mouse fibroblast cell line.

pTCTMI was transfected into LMTK⁻ cells with DEAE-dextran and chloroquine (see Materials and methods) and after 40–46 h total RNA was analysed by quantitative S1 nuclease

mapping. Transfections with pTCTMI clearly gave much more specific RNA than the enhancerless recombinant pTCT (Figure 4A, lanes 1 and 7). We also isolated stably transformed LMTK⁺ mouse fibroblast clones by co-transfecting pTCTMI or pTCT with the herpes thymidine-kinase gene into LMTK⁻ cells, and selecting HAT resistant clones (see Materials and methods). DNA blot analysis of four independent pTCTMI clones showed that they contained between one and ten intact copies of the hybrid gene (Figure 2C, lanes 23'–26'), while two pTCT clones contained over ten intact copies of the enhancerless gene (Figure 2C, lanes 27' and 28'). Quantitative S1 nuclease mapping of total cell RNA showed that the pTCTMI clones contained at least 5–10 times more specific transcripts than the pTCT clones (compare lanes 23–26 with 27–28, Figure 2B). Similar results were obtained in over 30 different clones analysed (not shown),

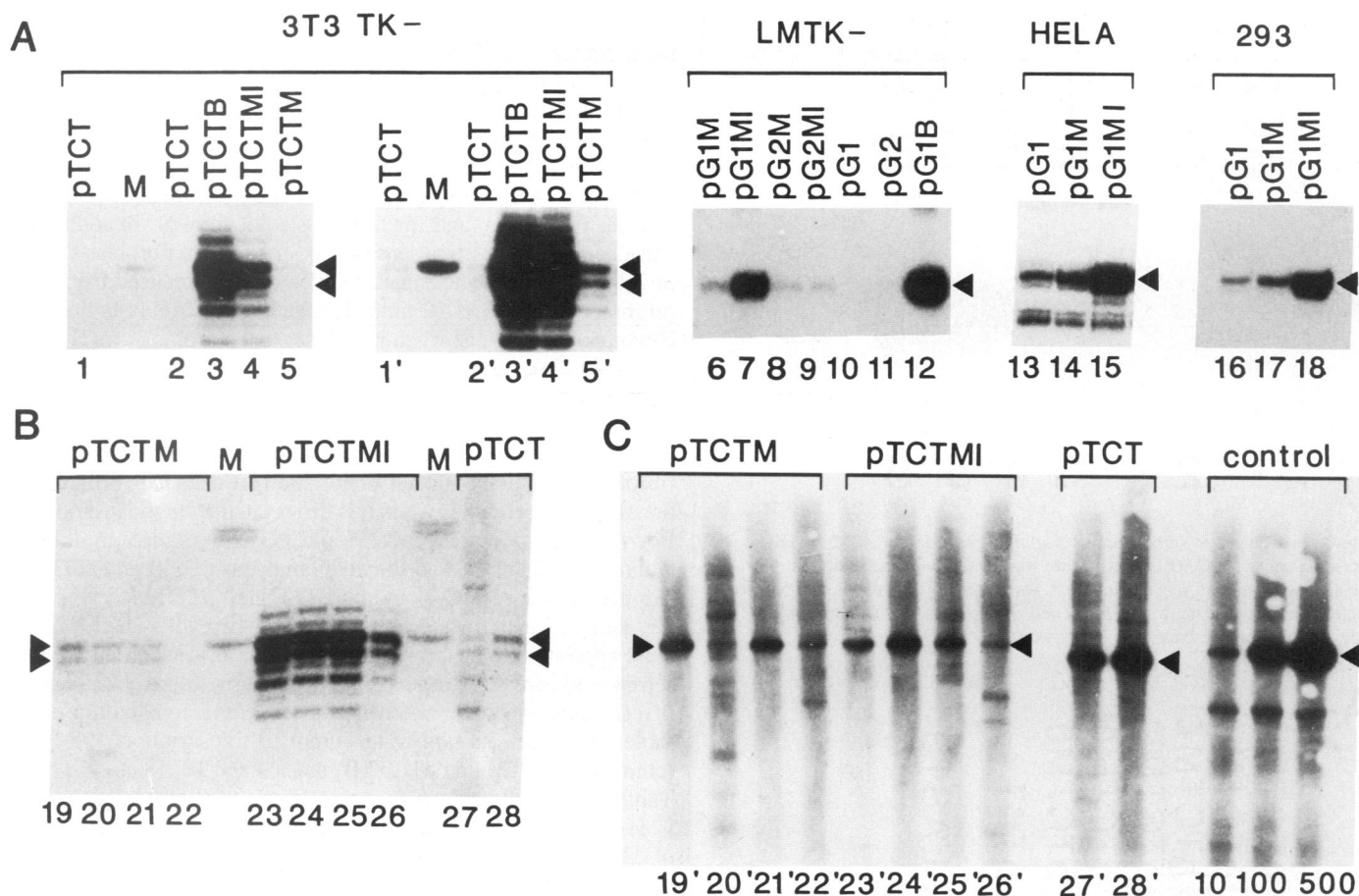


Fig. 2. Activity of the IgH enhancer in non-lymphoid cells. The indicated recombinants were either (A) transfected into mouse 3T3 TK⁻ or LMTK⁻ cells, human HeLa cells or 293 cells by either the calcium phosphate (3T3 TK⁻, HeLa and 293 cells) or the DEAE dextran and chloroquine (LMTK⁻ cells) techniques, or (B) co-transformed into LMTK⁻ cells with the HSV TK gene. Total RNA (15 μ g) from transfected cells (A) or individual clones (B) was analysed by quantitative S1 nuclease mapping using either the 5' ³²P-labelled +136 to -86 β -globin or +62 to -102 conalbumin coding strand probes. Lanes 19-22 and 23-26 represent results from different clones. Specific bands are indicated by arrowheads (Wasylyk *et al.*, 1983), M = 5' ³²P-labelled *Msp*I digest of pBR322. Lanes 1-5 and 1'-5' are different expositions of the same autoradiogram. In (C) 10 μ g of DNA from the corresponding LMTK⁻ clones in (B) was digested with *Xmn*I (which cuts twice in the pBR322 sequences of pTCT, pTCTM and pTCTMI, at 2031 and 3963), electrophoresed on 1% agarose, transferred to nitrocellulose and hybridized with nick-translated SV40 viral DNA. The expected 5100 bp band is labelled with an arrow. The control lanes contained 10, 100 and 500 pg of *Xmn*I-digested pTCT.

demonstrating that the IgH enhancer also stimulates transcription in fibroblasts when it is integrated into the cellular genome.

We also studied the effect of the IgH enhancer on transcription of the rabbit β -globin gene. pG1MI (Figure 1B), which contains the *Pvu*II-*Eco*RI IgH enhancer sequences directly upstream from the β -globin promoter (at position -109), was transfected into LMTK⁻ cells with DEAE dextran and chloroquine. pG1MI gave 25 times more specific globin transcripts than the equivalent enhancerless recombinant pG1 (Figure 2A, lanes 7 and 10). In contrast pG1B, containing the SV40 enhancer, gave 100 times more specific transcripts than pG1 (Figure 2A, lanes 10 and 12). pG1MI and pG1 were also transfected into two human cell lines by the calcium phosphate technique. The IgH enhancer stimulated specific globin transcription ~ 10-fold in HeLa cells (Figure 2A, lanes 13 and 15), and 20-fold in 293 cells (Figure 2A, lanes 16 and 18), showing that the IgH enhancer can stimulate transcription in non-lymphoid cells of both human and mouse origin.

To compare the efficiency of the IgH enhancer in B-lymphocytes and fibroblasts, pG1MI and pTCTMI were transfected into MPC11BU4 myeloma cells. The IgH enhancer stimulated specific transcription from the rabbit β -globin promoter about 100-fold (compare pG1MI and pG1 in lanes 5 and 2, Figure 3),

and from the conalbumin promoter about 400-fold (compare pTCTMI and pTCT in lanes 8 and 10, Figure 4B). Similar results were obtained in another B-lymphocyte cell line (X63Ag8, results not shown). In contrast, in similar constructions, the SV40 enhancer was about half as active as the IgH enhancer in MPC11BU4 cells (not shown, see also Banerji *et al.*, 1983), ruling out the possibility that the higher level of expression in myeloma cells is due to a general enhancer stimulation as opposed to a specific IgH enhancer effect. These results demonstrate that the IgH enhancer is about 5-10 times more efficient in stimulating specific transcription in B-lymphocytes than in fibroblasts.

Effect of distance and inversion on IgH enhancer activity in fibroblasts and B-lymphocytes

In both fibroblasts and myeloma cells the IgH enhancer is less efficient in stimulating transcription when it is moved away from the promoter. The IgH enhancer, when inserted 425 bp upstream from the rabbit β -globin startsite in pG2MI (Figure 1C), stimulated transcription in LMTK⁻ fibroblasts about 4-fold (compare pG2MI and pG2, lanes 9 and 11, Figure 2A and Table I), and about 40-fold in MPC11BU4 myeloma cells (compare

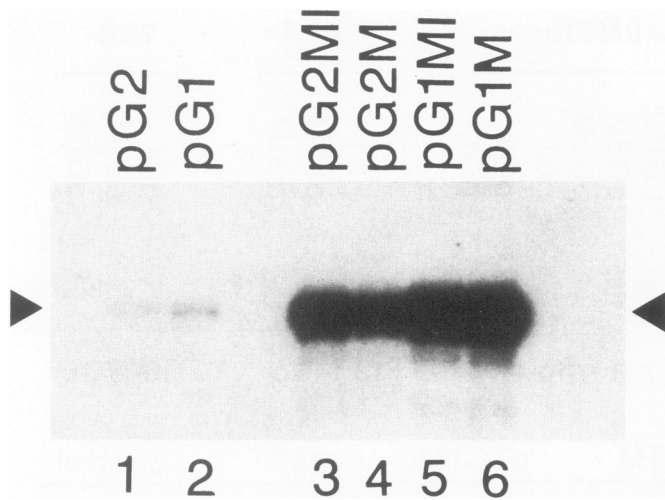


Fig. 3. IgH enhancer stimulation of β -globin transcription in MPC11BU4 myeloma cells. MPC11BU4 cells were transfected with the indicated recombinants, and 15 μ g of total RNA was analyzed by quantitative S1 nuclease mapping using a 5' 32 P-labelled β -globin probe. The arrowhead indicates the specific band (see Wasylyk *et al.*, 1983).

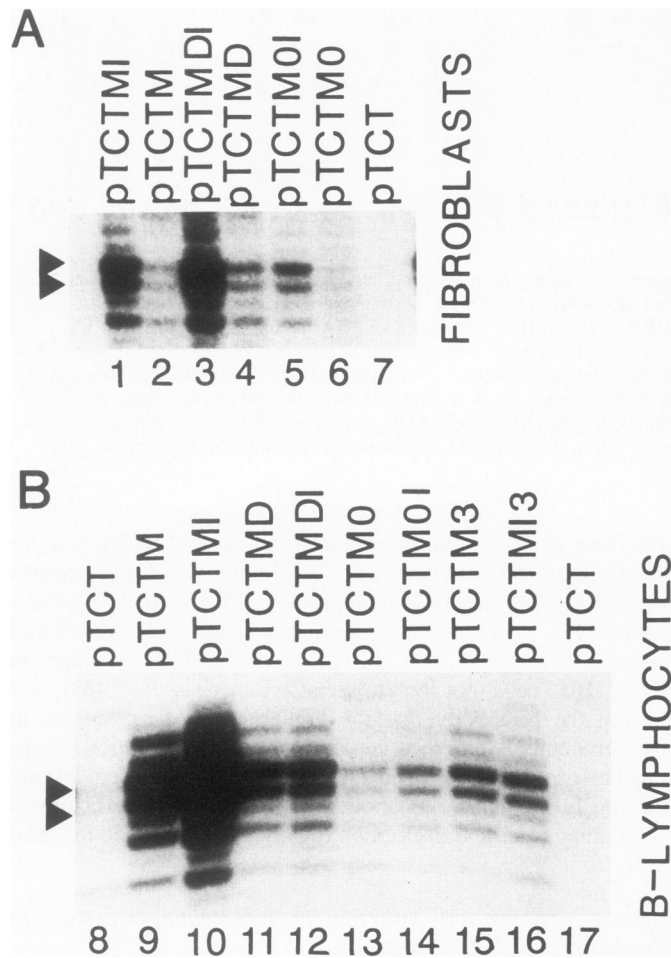


Fig. 4. Effect of enhancer deletion, inversion and distance on stimulation of specific conalbumin transcription in fibroblasts and B-lymphocytes. Conalbumin promoter-containing recombinants were transfected into LMTK⁻ fibroblasts (DEAE dextran and chloroquine) or MPC11BU4 myeloma cells (calcium phosphate) and 15 μ g of RNA was analysed by quantitative S1 nuclease mapping using a 5' 32 P-labelled +62 to -102 conalbumin probe. Specific bands are labelled with arrowheads.

pG2MI and pG2, lanes 3 and 1, Figure 3, and Table I). In contrast, stimulation is 25- and 100-fold in fibroblast and myeloma cells, respectively, when the enhancer is closer to the promoter (see PG1MI above). Similarly, dissociating the IgH enhancer from the conalbumin promoter (pTCTMI3, Figure 1D) decreased its efficiency in both fibroblasts and myeloma cells (for MPC11BU4 cells compare pTCTMI and pTCTMI3 in lanes 10 and 16, Figure 4B, and for fibroblasts not shown). In addition, enhancer activity is more sensitive to 'distance' in fibroblasts than in B-lymphocytes. The enhancer, when it is dissociated from the promoter (pG2MI) is 10 times less active in fibroblasts than in B-lymphocytes, whilst when it is closer to the promoter (pG1MI), it is only about four times less active (see Table I).

For each of the recombinants described above, an equivalent recombinant, containing the IgH enhancer fragment in the opposite orientation, was constructed and transfected into both fibroblast and myeloma cell-lines. The IgH enhancer is bidirectional in both cell types when it is dissociated from the promoter. For fibroblasts compare pG2M and pG2MI (Figure 2A, lanes 8 and 9 and Table I), and for myeloma cells pG2M and pG2MI (Figure 3, lanes 3 and 4) and pTCTM3 and pTCTM13 (Figure 4B, lanes 15 and 16, see also Table I). However, when it is in close apposition to the promoter, the enhancer is apparently more sensitive to inversion, especially in fibroblasts. In myeloma cells, inverting the enhancer when it is close to the conalbumin promoter decreases its ability to stimulate transcription ~5-fold (compare pTCTM and pTCTMI, lanes 9 and 10, Figure 4B and Table I). In contrast, transcription from the β -globin promoter is less sensitive to enhancer inversion (compare pG1MI and pG1M, lanes 5 and 6, Figure 3 and Table I). In fibroblasts, inverting the enhancer decreases its ability to stimulate transcription 5- to 10-fold (Figure 2A and B, compare pTCTM and pTCTMI, in lanes 4 and 5, also 19-22 and 23-26, and pG1M and pG1MI in lanes 6 and 7, 14 and 15, and 17 and 18, and Table I). The sensitivity to inversion may result from the asymmetric disposition of the enhancer sequences in the DNA fragment used, so that inversion leads to a change in the distance of the enhancer from the promoter (see below and Discussion).

The 3' boundary of the IgH enhancer is different in B-lymphocytes and fibroblasts

To delimit the 3' boundary of the IgH enhancer two mutants were constructed which carry deletions of the enhancer sequences in pTCTMI between 521 and 687 (pTCTMDI, Figure 1E) and 476 and 687 (pTCTMOI). The 521-687 deletion had no effect on enhancer activity in fibroblasts (compare pTCTMI and pTCTMDI, lanes 1 and 3, Figure 4 and Table I), but led to an 8-fold decrease in enhancer activity in B-lymphocytes (compare pTCTMI and pTCTMDI in Figure 4B, lanes 10 and 12, and Table I). The more extensive 476-687 deletion resulted in a 4-fold decrease in enhancer activity in fibroblasts (compare pTCTMI and pTCTMOI, lanes 1 and 5, Figure 4A and Table I) and a 40-fold decrease in enhancer activity in myeloma cells (compare pTCTMI and pTCTMOI, lanes 10 and 14, Figure 4B). These results are supported by 3' deletion mutants similar to the 5' deletion mutants described below, in which the large (*Xba*I) enhancer fragment in the sense orientation was systematically deleted. Mutants extending to nucleotide 602 or 497 both stimulated transcription to the same extent in fibroblast (~5-fold) but led to a decrease in enhancer activity in myeloma cells (results not shown). It is striking that the 384-476 enhancer fragment (in pTCTMOI) has similar stimulatory activity in fibroblasts and myelomas, and that the larger 384-521 enhancer fragment (in

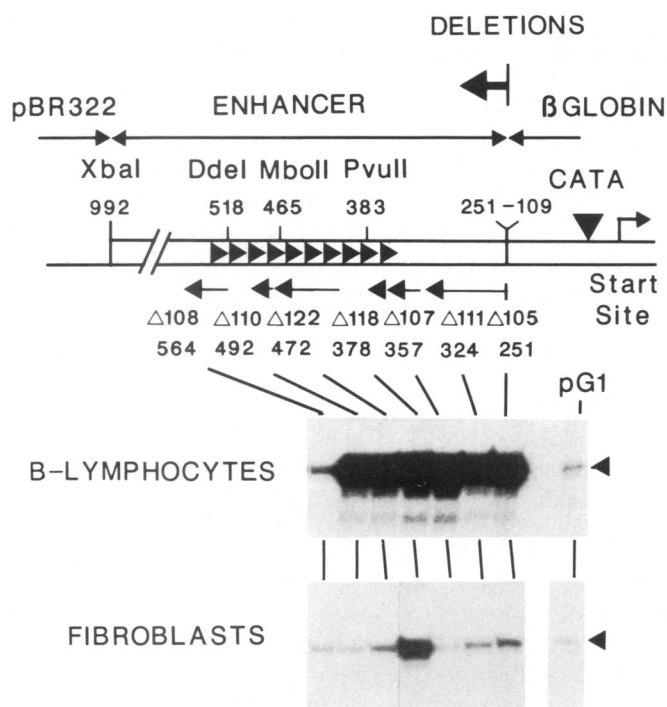


Fig. 5. Effect of IgH enhancer deletions on specific β -globin transcription in B-lymphocytes and fibroblasts. pG1 type recombinants containing IgH sequences from 992 to the endpoint of deletion (from 251 to 564 in $\Delta 105$ to $\Delta 108$, the arrows indicate the extent of deletion) were transfected into LMTK⁻ fibroblasts, or MPC11BU4 myeloma cells, and 15 μ g RNA was analysed for specific β -globin transcription by quantitative S1 nuclease mapping. The arrowheads indicate bands corresponding to specific β -globin transcripts. CATA = β -globin TATA-box like sequence.

pTCTMDI) is about 75% as active in fibroblasts as in B-lymphocytes. These results show that the 3' boundary of the IgH enhancer is different in fibroblasts and B-lymphocytes.

The 5' boundary of the IgH enhancer is different in B-lymphocytes and fibroblasts

To test whether the 5' boundary of the IgH enhancer sequences active in fibroblasts extends beyond nucleotide 387, a larger DNA fragment from the IgH gene intron, extending from nucleotide 251 to 992 (Figure 1), was cloned directly upstream from the β -globin promoter in pG1 to give $\Delta 105$ (Figure 5). The β -globin promoter was chosen because transcription, in the absence of an enhancer, was more easily detected than from the conalbumin promoter. The IgH enhancer, in $\Delta 105$, stimulated transcription 2-fold in fibroblasts and 70-fold in myelomas (Figure 5). Deletions were made in the IgH sequences from nucleotide 251, thereby bringing the enhancer sequences closer to the β -globin promoter. This increases the sensitivity of the assay, because the enhancer is most active when it is adjacent to the promoter (see above). Transcription in fibroblasts from $\Delta 111$ and $\Delta 107$, which retain IgH sequences from 992 to 324 and 357, respectively, was less than from $\Delta 105$ (Figure 5 and Table I). In contrast, in B-lymphocytes, these recombinants were transcribed 60-fold ($\Delta 111$) and 145-fold ($\Delta 107$) better than the enhancerless recombinant pG1 (Figure 5). A further deletion of 21 bp ($\Delta 118$), results in a large 30-fold increase in transcription in fibroblasts (compare $\Delta 107$ and $\Delta 118$, Figure 5 and Table I), whilst in myelomas no change in transcription stimulation is observed. Further deletions which remove enhancer sequences ($\Delta 122$, $\Delta 110$ and $\Delta 108$) lead to a decrease in activity in both fibroblasts and B-lymphocytes. However, the enhancer sequences are shorter in fibroblasts than

in B-lymphocytes. In $\Delta 110$, the IgH sequences have no effect on transcription from the β -globin promoter in fibroblasts, but stimulate transcription 70-fold in B-lymphocytes (Figure 5 and Table I). These results show that the 5' boundary of the IgH enhancer is different in both cell types (see in particular mutant $\Delta 107$). In addition they support our conclusions (see above) that the 3' boundary is different too (see mutant $\Delta 110$).

Discussion

The IgH enhancer contains elements which stimulate transcription in non-lymphoid cells

We have shown that the IgH enhancer can efficiently stimulate transcription both in transient assays and when integrated into the genome of mouse LMTK⁻ cells. Enhancer activity is observed in several non-lymphoid cell lines, and with two heterologous promoter elements. This finding complements previous transfection studies, in which it was concluded that the IgH enhancer is inoperative in fibroblasts (Banerji *et al.*, 1983; Gillies *et al.*, 1983). In the latter studies a larger DNA fragment was used for transfection, and the enhancer was at distant locations from the promoter, which precluded detection of enhancer activity in these cells (see below). The IgH enhancer sequences which are active in fibroblasts are situated between nucleotides 378 and 492 (Figure 1, see $\Delta 118$, $\Delta 110$, pTCTMDI and pTCTMOI, Table I), whilst flanking sequences, on both sides, are uniquely active in B-lymphocytes. The 'fibroblast' enhancer sequences contain homologies to other enhancers, in particular three GTGG(T/A)(T/A)(T/A)G core homologies between 433–440, 458–465 and 471–478 (see Banerji *et al.*, 1983; Gillies *et al.*, 1983; Church *et al.*, 1985). The $\Delta 122$ to $\Delta 110$ deletion, which eliminates enhancer activity in fibroblasts, removes a core sequence (nucleotides 471–478). Interestingly, the IgH enhancer sequence 521–687, which does not function in fibroblasts, contains a cd element (nucleotides 539–548, Falkner and Zachau, 1984; Parslow *et al.*, 1984), which confers cell-type specificity to the α light chain and IgH promoters (Bergman *et al.*, 1984; Falkner *et al.*, 1984; Foster *et al.*, 1985; Grosschedl and Baltimore, 1985; Mason *et al.*, 1985). Further systematic mutagenesis (McKnight and Kingsbury, 1982; Zenke *et al.*, 1986), footprinting and purification of factors from *in vitro* extracts (Parker and Topol, 1984; Wildeman *et al.*, 1985), will be necessary to identify elements required for IgH enhancer activity in lymphoid and non-lymphoid cells.

The IgH enhancer is 5–10 times less active in fibroblasts than in myeloma cells. There are several possibilities to account for this difference. The sequence elements which are important for IgH enhancer activity and the factors interacting with the enhancer may be completely different in B-lymphocytes and fibroblasts. This appears unlikely at present, because the 384–476 fragment of the IgH enhancer (pTCTMOI, Table I) is equally active in both cell types. The IgH enhancer, like the polyoma and SV40 enhancers (Herbomel *et al.*, 1984; Zenke *et al.*, 1986; Wildeman *et al.*, 1985; Herr and Gluzman, 1985) may have a modular structure and might interact with several different positive factors which synergistically generate an efficient enhancer. Enhancer sequences which are active in both cell types might interact with common factors, whilst sequences which are active only in myeloma cells might interact with B-lymphocyte specific factors which would confer tissue specificity to the enhancer. The common factors may be similar to those which interact with the SV40 enhancer, since the SV40 and IgH enhancers compete for transacting factors in B-lymphocytes, both *in vivo* (Mercola *et al.*,

1985; our unpublished observations) and *in vitro* (Schöler and Gruss, 1985) and in HeLa cells *in vitro* (Sassone-Corsi *et al.*, 1985; Schöler and Gruss, 1985). In addition the IgH enhancer may be negatively regulated, and perhaps in our recombinants we have partially deleted negative elements, which inhibit the enhancer, preventing its activity in non-lymphoid cells. Several observations suggest that there are negative elements in non-lymphoid cells. We occasionally observed that IgH enhancer activity in non-lymphoid cells was weaker than usual, when the overall transfection efficiency was low, suggesting that it was necessary to titrate our negative factors to obtain maximum enhancer stimulation. In addition, in titration experiments, in which the specific DNA concentration was systematically varied, stimulation by the IgH enhancer sequences decreased, relative to promoter alone recombinants, as the specific DNA concentration was decreased (unpublished results). We have also identified sequences flanking the IgH enhancer which inhibit β -globin transcription in fibroblasts, but not in myelomas (in preparation). Various other observations suggest negative regulation of the IgH enhancer in non-lymphoid cells (see for example Zeuthen *et al.*, 1976; Junker, 1982; Schöler and Gruss, 1985).

Several studies have shown that the adenovirus EIA gene products inhibit enhancer activity (Borrelli *et al.*, 1984; Velcich and Ziff, 1985), and as a consequence, the SV40 enhancer is inactive in 293 cells (Borrelli *et al.*, 1984), which constitutively expresses EIA. The IgH enhancer is even more active in 293 cells than in HeLa cells (see Results) suggesting that the EIA products do not inhibit the IgH enhancer in these cells. In contrast, in myeloma cells, the EIA proteins inhibit the IgH enhancer (Hen *et al.*, 1986). Perhaps inhibition in myeloma cells is mediated through sequences and factors which are unique to myeloma cells.

IgH enhancer activity is sensitive to 'distance' and is bidirectional

The IgH enhancer is less efficient in stimulating transcription when it is dissociated from the activated promoter elements, in both fibroblasts and B-lymphocytes. The weaker IgH enhancer in fibroblasts is more sensitive to this 'distance' effect than the strong IgH enhancer in B-lymphocytes (compare pG1MI and pG2MI in Table I). However, the IgH enhancer in fibroblasts still stimulates transcription 4-fold when it is 425 bp upstream from the RNA startsite (Table I). Other enhancers, including the SV40 72 bp repeat, are also less efficient when dissociated from promoter elements (Wasylyk *et al.*, 1984; Zenke *et al.*, 1986), and the weaker MLV enhancer is more sensitive to the 'distance' effect than the SV40 enhancer (Augereau and Wasylyk, 1984). In contrast, upstream promoter elements appear to be unable to stimulate transcription when moved away from other promoter elements (reviewed in Wasylyk, 1986). For example, the SV40 21-bp repeat does not stimulate transcription when it is inserted 425 bp upstream from the β -globin start-site, in a recombinant derived from pG2 (P.Jalinot and C.Kédinger, unpublished results). This suggests that the IgH sequences act as a weak enhancer in fibroblasts, rather than as an upstream element.

In the rearranged IgH gene the enhancer is several thousand bp downstream from the IgH promoter. We have found that the IgH enhancer in B-lymphocytes is less efficient in stimulating transcription when it is dissociated from a heterologous promoter element, which raises the question of which other elements in the IgH gene can compensate for the distant location of the IgH enhancer to allow efficient transcription from the IgH promoter. One possible explanation stems from the observation that the β -globin promoter is less sensitive to the 'distance' effect than the weaker conalbumin promoter, both with the IgH (see Results)

and SV40 enhancers. We have shown that moving the SV40 enhancer from directly upstream to 2 kb upstream from the β -globin promoter decreases transcription by about 50% (B.Wasylyk and C.Wasylyk, in preparation). In contrast, with the conalbumin promoter, moving the SV40 enhancer to an equivalent distant location decreases transcription to 3% (Wasylyk *et al.*, 1983). A distance effect is observed when the β -globin promoter is 'weakened' by deleting one or both of its upstream elements (B.Wasylyk and C.Wasylyk, in preparation). Thus, if the IgH promoter is stronger in B-lymphocytes than the β -globin promoter, then it might be insensitive to the distance of the IgH enhancer. Interestingly, the IgH promoter contains an efficient B-lymphocyte specific upstream element (Mason *et al.*, 1985; Grosschedl and Baltimore, 1985) suggesting that it may be an important element for regulation of IgH gene expression. Having the enhancer at distance rather than in close apposition creates the possibility of control of the IgH gene expression through modulation of the activity of the upstream promoter element.

Inverting both the 386–687 and 386–521 IgH enhancer fragments increases enhancer activity more drastically in fibroblasts than in B-lymphocytes (see pTCTM, pTCTMI, pTCTMD and pTCTMDI in Table I). In B-lymphocytes, inverting the large fragment when it is directly upstream from the conalbumin promoter increases enhancer activity (see pTCTM and pTCTMI, Table I). In contrast, inverting this fragment when it is upstream from the β -globin promoter (pGIM and pGIMI, Table I), or the smaller fragment when it is upstream from the conalbumin promoter (pTCTMD and pTCTMDI, Table I) does not affect enhancer activity. These results are most easily interpreted as follows. The enhancer is bidirectional; however, the active sequences are asymmetrically distributed in the large fragment, so that inverting this fragment changes the distance of the enhancer from the promoter. In myeloma cells, this only affects the conalbumin promoter, because it is more sensitive to the 'distance' effect (see above). With the smaller fragment, inversion does not change the distance as much, so that enhancer activity is approximately equal in both orientations. Similar considerations can account for the results in fibroblasts, taking into account that the enhancer is 5–10 times weaker in these cells, and that the enhancer sequences are even more localised to one side of the 386–687 DNA fragment. Inverting the 386–687 IgH enhancer increases activity with both the conalbumin and the β -globin promoters (compare pGIM, pGIMI, pTCTM and pTCTMI, Table I). Similarly, inverting the smaller 386–687 fragment significantly increases enhancer activity (compare pTCTMD and pTCTMDI, Table I). In contrast, inverting the smallest 386–476 fragment has a much smaller effect on enhancer activity, which is similar to the difference observed with this fragment in B-lymphocytes (compare pTCTMO and pTCTMOI, Table I). Since this fragment contains only active sequences, inverting the enhancer does not change its distance from the promoter.

Physiological significance of IgH enhancer activity in non-lymphoid cells

The physiological significance of the IgH enhancer activity in fibroblasts is not clear at present. Immunoglobulin genes in non-lymphoid tissues are heavily methylated and are in an inactive chromatin structure (for review see Calame, 1985), which would preclude the interaction of cellular factors with the IgH enhancer. This could account for the results of Ephrussi *et al.* (1985) and Church *et al.* (1985) who detected, by genomic footprinting, specific interaction of the IgH enhancer with factors only in cells

of the B-lineage. In transient transfections and in co-transformed cell clones, the DNA is both in an open chromatin conformation, and is not methylated by eukaryotic methylases, which would facilitate the interaction with cellular factors, allowing the enhancer to function.

The IgH enhancer activity we have detected in fibroblasts is relatively weak, and efficient stimulation of transcription is observed only from proximal promoter elements. Several observations suggest that this activity could be relevant to IgH gene transcription, at least in pre-B cells and in some T cells. In pre-B cells, and in T cells, 'sterile' transcripts have been detected, at least some of which initiate close to the IgH enhancer (Kemp *et al.*, 1980; Alt *et al.*, 1982; Zuniga *et al.*, 1982; Schwaber *et al.*, 1983; Nelson *et al.*, 1983). In addition, the IgH enhancer region is in an 'open' DNase I hypersensitive chromatin conformation in some T lymphocytes (Storb *et al.*, 1981) which would allow interaction with enhancer factors. It is possible that very early in the development of B cells, or in other lymphoid cells, the enhancer is open to interaction with 'ubiquitous' enhancer factors, which stimulate proximal 'sterile' transcription. Our results would help to reconcile the observation that the IgH enhancer does not act detectably at a distance in T-cell lines (Mason *et al.*, 1985) and yet can act as a promoter element for sterile μ transcripts in the same cells (Lennon and Perry, 1985). Later in B-cell development specific IgH enhancer factors are synthesized, which increase the strength of the enhancer, allowing it to stimulate transcription from the rearranged IgH promoter. However, to obtain efficient transcription from the IgH gene, the IgH promoter may also need to be activated, since the efficiency of stimulation of transcription by the enhancer of distant promoter elements will depend upon the strength of these elements (see above). Hence IgH promoter activation may be an important step in B-cell development. Experiments are in progress to test these possibilities.

Materials and methods

Transfections, RNA extraction and analysis

Cells were transfected using either calcium phosphate (Corsaro and Pearson, 1981) as described previously (Wasylyk *et al.*, 1983) or DEAE dextran and chloroquine (Banerji *et al.*, 1983) with the following modifications: 7 μ g of DNA was used per 90 mm plate and the length of incubation with chloroquine was varied (generally 2–4 h) to avoid excessive cell death. Total cell RNA was extracted with acid-phenol (Stafford and Queen, 1983) and analysed by quantitative S1 nuclease mapping using excess 5' 32 P-labelled conalbumin and β -globin probes as described previously (Wasylyk *et al.*, 1983). LMTK⁻ cell clones were prepared as described previously (Wasylyk and Chambon, 1983).

Recombinants

Standard DNA recombinant techniques were used (Maniatis *et al.*, 1982). For the pTCT series (Figure 1E) the DNA polymerase I (Klenow) repaired PvuII(384)–EcoRI(687) (pTCTM, pTCTMI), PvuII(384)–DdeI(518) (pTCTMD, pTCTMDI) or PvuII(384)–MboII(465) fragments of the IgH enhancer (see Figure 1) were cloned with BamHI linkers into the BamHI site of pTCT (see Wasylyk *et al.*, 1983). pTCTM3 and pTCTMI3 were constructed by inserting the DNA polymerase I-repaired PvuII(384)–EcoRI(687) fragment with XhoI linkers into the SalI site of pTCT (Figure 1D). pG1 contains rabbit β -globin sequences from +1650 to –109 (from a deletion mutant of Dierks *et al.*, 1983), and the M13mp12 polylinker with the HindIII site closest to the β -globin promoter. pG2 is similar, except that the β -globin sequences extend to –425. pG1M and pG1MI were created by blunt-end ligating the DNA polymerase I repaired PvuII(384)–EcoRI(685) fragment into the DNA polymerase I-repaired HindIII site of pG1. The DNA polymerase I repaired SV40 enhancer BamHI(179), artificial site at the boundary of the enhancer created by Zenke *et al.*, 1986)–PvuII(272) fragment was cloned with XhoI linkers into the XhoI site of pG1, to give pG1B. For pG2M and pG2MI the DNA polymerase I repaired PvuII(386)–EcoRI(683) fragment was inserted with XhoI linkers into the XhoI site of pG2. Δ 105, Δ 111, Δ 107, Δ 118, Δ 122, Δ 110 and Δ 108 were constructed by Bal31 deletion from the XbaI site (nucleotide 1, Figure 1) site. Xho linkers

were inserted at the deletion end-point, and the XhoI–XbaI (nucleotide 992) fragment was introduced between the XhoI and XbaI sites of pG1. The deletion end-point was determined by dideoxy-nucleotide sequencing.

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C.Wasylyk and B.Wasylyk

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