more examples will be needed to base conclusions about the folding mechanism.

These data are encouraging with regard to predicting secondary structure from amino acid sequence because they suggest that helix formation in the isolated S-peptide is localized to the same, or nearly the same, residues as in native RNase A. It is interesting that present schemes for predicting secondary structure are more successful in finding the locations of helices than in defining their endpoints[17]. Experiments of the type described here, with peptides of specified amino acid sequence, may provide clues about the nature of helix termination signals.

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Note added in proof: A recent ^{1}H-NMR study[25] (published after submission of this article) of helix formation by S-peptide (1–19) agrees with our conclusion that the helix is limited to certain residues including the helical residues of RNase A.

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Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus

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A tissue-specific transcriptional enhancer element that is associated with the human immunoglobulin heavy-chain locus is defined. In a non-Hodgkin's lymphoma that contains a translocated c-myc *gene this enhancer is retained on the 14q$^+$ chromosome and occurs within sequences shown to activate previously cryptic promoters of the* c-myc *gene.*

A FUNCTIONAL immunoglobulin gene is generated somatically during the differentiation of B lymphocytes by a set of developmentally regulated gene rearrangements[1]. The major function of these rearrangements is to generate a diverse set of complete immunoglobulin genes from a limited number of inherited gene segments.

Another important role of these rearrangements has recently been identified at least for the mouse immunoglobulin heavy-chain genes[2–4]. In an individual B lymphocyte and its progenies, only one of a few hundred copies of the variable (V) gene segments is expressed. This activation of a specific V gene segment results from its direct rearrangement into the vicinity of a transcriptional enhancer element that is located upstream of the constant (C) region gene segments, between the joining (J) segments and the switch (S) region. Interestingly, the immunoglobulin gene-associated enhancer functions in a tissue-specific manner, suggesting a more general involvement of cellular enhancer elements in tissue-specific expression of eukaryotic genes during cell differentiation.

Another biological process in which gene rearrangements and enhancer elements may play a critical, albeit adventitious, role is tumorigenesis. A model supporting this notion comes from the studies on the chicken leukaemogenesis induced by the non-acute avian leukosis virus (ALV). By integrating close to the cellular oncogene, c-*myc*, the ALV provirus via its transcriptional promoter or enhancer element can lead to increased levels of c-*myc* transcription[5,6]. It has recently been shown that the c-*myc* gene is translocated into an immunoglobulin locus in certain lymphoid neoplasms of both mice and men[7–12]. Thus, murine c-*myc* gene is recombined into the immunoglobulin heavy-chain locus in BALB/c plasmacytomas characterized by t(12:15) translocations[7,9–11]. Likewise, in a majority of the many Burkitt's lymphomas or non-Hodgkin's lymphomas characterized by t(8:14) (q24; q32) translocations, the c-*myc* gene is recombined into the immunoglobulin heavy-chain locus[8,11,12]. It has generally been considered that in these and other neoplasms where nonrandom chromosomal translocations have been observed, c-*onc* genes are activated by the translocations[13–15].

To understand the relationship between the translocation and activation of a c-*myc* gene we have been analysing the structure and expression of normal and translocated human c-*myc* genes. The results presented here demonstrate that the human immunoglobulin heavy-chain locus carries a transcriptional enhancer element analogous to its mouse counterpart, and that this enhancer element may be playing a direct part in the activation of the translocated c-*myc* gene in some lymphoid neoplasms.

c-*myc* translocation in Manca cells

The non-Hodgkin's lymphoma, Manca[16], shows a chromosome translocation (t(8:14)(q24; q32)) that is characteristic of many

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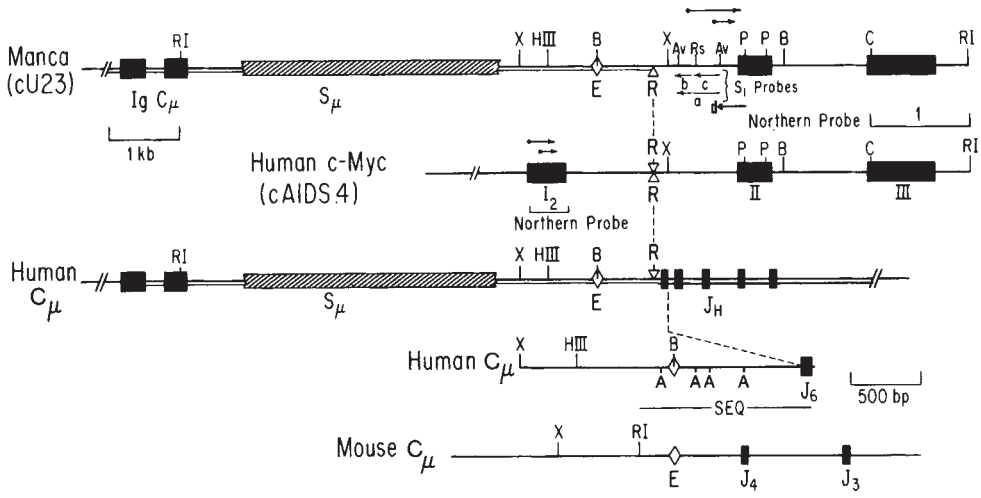

Fig. 1 A comparison of the human c-*myc* gene and the C_μ region of the human immunoglobulin heavy-chain locus to the C_μ–c-*myc* joining region of the 14:8 translocated chromosome from Manca cells. Exons are shown as solid boxes; chromosome 14 sequences as a double line; and chromosome 8 sequences as a single line. R indicates the site of recombination between the two chromosomes. E is a transcriptional enhancer element, which was previously identified for the mouse immunoglobulin heavy-chain locus[2–4] and the identification of the human equivalent is reported here. S_μ, C_μ switch sequences; J_μ, joining segments. The J_μ segments are defined on the basis of data presented in ref. 40, and our own sequencing[41]. The horizontal arrows indicate the initiation sites and directions of transcription. Restriction sites: RI, *Eco*RI; X, *Xba*I; H, *Hin*dIII; Av, *Ava*I; Rs, *Rsa*I; P, *Pst*I; B, *Bgl*II; C, *Cla*I; A, *Alu*I (the complete map is only shown for the first three of these). 1 and 2 delineate the extent of the probes used in the Northern blot experiments. The cloning of the recombination region of the translocated chromosome into cosmid pTCF[19] will be described elsewhere (K.W. *et al.*, manuscript in preparation). The unrearranged c-*myc* gene was also cloned into a cosmid. Colonies were screened with the Northern probe 1. The definition of the c-*myc* gene will be described[22].

Burkitt's lymphomas[14,15]. *In situ* hybridization of a v-*myc* probe[17] to Manca chromosomes indicates that the human c-*myc* locus is on that portion of chromosome 8 [12,18] that is translocated (data not shown).

From a cosmid library[19] of Manca DNA a clone, cU23 was isolated that hybridized both to v-*myc* and to pH18-C1-10, a plasmid containing the immunoglobulin heavy-chain C_μ gene and sequences immediately 5′ to it[20]. Southern blot analysis[21] of Manca DNA indicated that the linkage of c-*myc* and immunoglobulin heavy-chain gene sequences detected in cU23 had occurred in the human cells, and not upon cloning (data not shown). A detailed map of clone cU23 in the region of c-*myc*–C_μ linkage was derived by restriction analysis and DNA sequencing (K. W. *et al.*, manuscript in preparation), and was compared with maps of human germ-line C_μ and c-*myc* genes, contained on plasmid pH18-Cl-10 and cosmid cAIDS4 respectively (Fig. 1). The latter clone was described previously[22]. It can be deduced that in Manca DNA, the human c-*myc* gene is fused head-to-head with the C_μ gene, about 6.5 kilobases (kb) 5′ to the first C_μ exon. The junction on chromosome 14 lies between S_μ and J_H6, at a point that is not normally used for the productive rearrangement of an immunoglobulin heavy-chain gene. The junction on chromosome 8 occurs between exons I and II of the c-*myc* gene. As a result, the Manca DNA cloned in cU23 contains neither exon I nor the normal transcriptional start sites[22] of the human c-*myc* gene. Instead, c-*myc* exons II and III are fused to C_μ sequences that include a stretch of DNA between S_μ and J_H that is in an analogous location to sequences in the mouse genome shown to have tissue-specific transcriptional enhancing activity[2–4] (region 'E' in Fig. 1). The translation of transcripts of the human c-*myc* gene begins within exon II, at an initiation AUG codon that is retained, and presumably used, in transcripts of the translocated c-*myc* gene in Manca cells.

Multiple transcription initiation sites

Northern blot analysis of RNA from Manca cells indicates a high level of c-*myc* transcription (Fig. 2*A*). It is clear that there are multiple transcripts hybridizing to the c-*myc* Northern probe 1 (see Fig. 1). The level of c-*myc* transcription in Manca cells is the highest that we have detected in any cell line (which include other Burkitt's lymphomas, and Epstein–Barr virus-immortalized lymphoblastoid cells) except the promyelocytic leukaemia cell line HL60 (data not shown). The relevance of this comparison is unclear, however, as all these cells are growth transformed. An alternative approach is to compare the level of transcription of the rearranged c-*myc* gene in Manca cells with that of the unrearranged allele; with a probe specific for exon I no transcription of the unrearranged c-*myc* gene can be detected in Manca cells (K.W. *et al.*, manuscript in preparation). Therefore, the high c-*myc* level of transcription in Manca cells all derives from the translocated allele.

The initiation points of c-*myc* transcripts in Manca cells were mapped by S_1 nuclease protection experiments[23,24] using the probes shown at the bottom of Fig. 2. The major initiation site (site 1; see Fig. 2) for c-*myc* transcripts occurs about 636 base pairs (bp) upstream of c-*myc* exon II, within what would ordinarily be intron I of the c-*myc* gene. The nucleotide numbering system used to denote the initiation points is that of Colby *et al.*[25], whose sequence begins within intron I of the human c-*myc* gene such that nucleotide 1,000 defines the start of exon II. Transcripts initiating at site I (nucleotide 364) form S_1-resistant hybrids with probes b and a of 66 base pairs (bp) and 359 bp respectively (Fig. 2*B*, *C*). Downstream of site I, at nucleotide 378, is a weaker initiation point, Ia (Fig. 2*B*, *C*). As has been previously noted[25], upstream of initiation sites I and Ia are sequences related to the consensus TATA box[26,27] commonly found 5′ of genes transcribed by RNA polymerase II (see Fig. 3). Initiating at around nucleotide 873 are further transcripts, which form S_1 nuclease-resistant hybrids of length 192 bp with probe d (data not shown), and 28 bp upstream of this site occurs the sequence TTTATT (Fig. 3). In addition to these initiation sites, an S_1 sensitive site (X) in hybrids between Manca RNA and either probe a or c is detected at around nucleotide 520. However, the assignment of an initiation site to this position is uncertain, an S_1-sensitive site also being apparent at this position in hybrids between probes a or c and yeast RNA on long exposures.

If transcripts initiating at these sites were to have no functional splice sites 5′ of the junction between exon II and the following intron, then the processed RNAs would range in size from 1,660 to 2,450 nucleotides (not including the poly(A) tail[28]). That this is the case is suggested by cDNA cloning, which has revealed a contiguous transcript extending upstream from exon II at least as far as position 660. Beyond this point the S_1 nuclease-sensitive sites have been confirmed as initiation (as opposed to splice) sites by primer extension analyses. For example, reverse transcripts of Manca cell RNA using the primer shown in Fig. 2*B* extend as far as nucleotide 364. Further primer extensions (for example using S_1 probe b) indicate no initiation sites upstream of those characterized here (data not shown).

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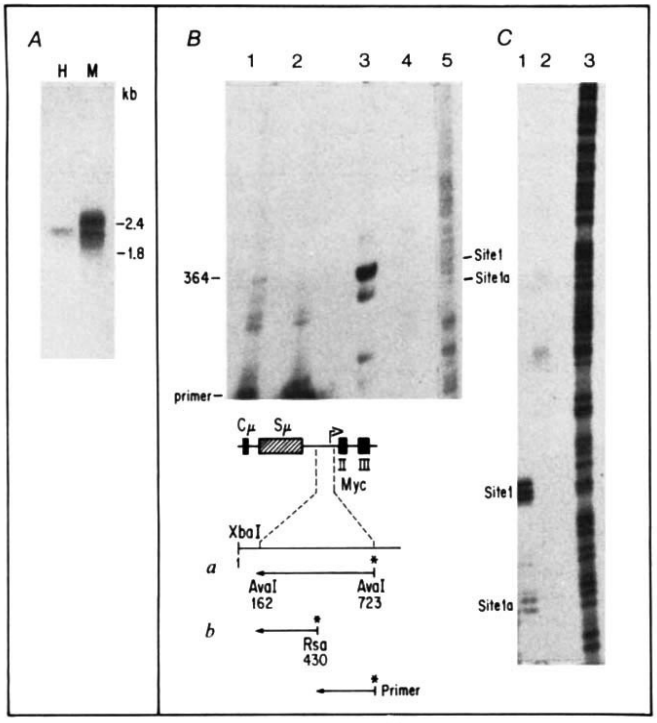

Fig. 2 ***A***, Northern blot analysis[42] using the probe 1 shown in Fig. 1 of mRNA from: lane H, HeLa cells (1 μg); lane M, Manca cells (0.3 μg). ***B***, Lanes 1 and 2 show a primer extension analysis[43], and lanes 3 and 4 an S_1 nuclease mapping analysis[23,24] of: lanes 1 and 3, Manca cell mRNA; lanes 2 and 4, yeast mRNA. The probes used are 'a' and 'primer' shown in the diagram below the photograph of the blot, and were strand separated and end labelled[41] before hybridization. The nucleotide numbering system derives from ref. 25, and starts (nucleotide 1) at the *Xba*I site in intron I of the human c-*myc* gene. Sites 1 and 1a are referred to in the text; site 1 centres on nucleotide 364, and the mark indicating this position on the right of the figure shows the correspondence between the primer extension and S_1 signals in lanes 1 and 3. Lane 5 is a pyrimidine analysis[41] of the sense strand of the probes, from which the nucleotide positions of the DNA–RNA discontinuity could be determined (see Fig. 3). ***C***, S_1 nuclease analysis using the probe 'b' shown in the diagram below *B* of mRNA from: lane 1, Manca cells; lane 2, yeast cells. Lane 3 is the same type of reaction as shown in lane 5 of *B*, but using probe b.
Methods: The S_1 nuclease reactions contained either 100 ng Manca cell mRNA plus 20 μg yeast RNA, or only the yeast RNA. For the S_1 analysis 5×10^4 c.p.m. of probe was used per reaction, and for the primer extension analysis 2×10^4 c.p.m. of probe was used per reaction. The probes were annealed to the RNA at 53 °C for 4 h (except in the case of probe b, which was annealed at 30 °C for 12–16 h). S_1 reactions were performed at 15 °C for 3 h, and primer extension reactions at 41 °C for 45 min. A 6% acrylamide gel was used for *B*, and run for 7 h, and an 8% gel run for 3 h for *C*.

Transcriptional enhancer element

We have previously shown that sequences derived from the J_H–C_μ region of the mouse immunoglobulin heavy-chain locus contain a DNA element that enhances the transcription of the associated gene in a manner largely independent of both position and orientation[2]. The translocation of the c-*myc* gene in Manca cell DNA just 300 bp downstream of the human J_H cluster (Fig. 1) suggested that the high level of c-*myc* transcription in Manca cells (Fig. 2*A*) might be due to a human enhancer element located in an analogous region to that in the mouse ('E' in Fig. 1).

To test this hypothesis, we first attempted to identify the putative human enhancer element. For this purpose, the 2.2 kilobase (kb) *Xba*I fragment (see Fig. 1) containing most of the sequence between the J_H cluster and S_μ, plus about 330 bp derived from the 5′ flanking region of the translocated, truncated c-*myc* gene, was inserted into the *Eco*RI site of plasmid pSER[2]. This plasmid was derived from pSV2.gpt[29] (which contains the mycophenolic acid-resistance conferring gene *Ecogpt*) by deletion from the latter of the SV40 enhancer sequences. As a result, pSER transforms cells to mycophenolic acid resistance (gpt^+ phenotype) at a much lower frequency than does pSV2.gpt (see Table 1). The original level of transformation can be restored by the insertion into the *Eco*RI site of pSER of the mouse immunoglobulin heavy-chain locus enhancer element[2]. As shown in Table 1, the insertion in either orientation of the human *Xba*I fragment into the *Eco*RI site of pSER (2.3 kb upstream of the *Ecogpt* gene) restored the gpt^+ transformation efficiency to a level even exceeding (by about eightfold) that of pSV2 gpt. The transformation efficiency of pSV2 gpt was itself increased 5–10-fold by insertion of the *Xba*I into its *Eco*RI site. Other fragments of mouse and human immunoglobulin heavy-chain loci DNA had no significant effects on transformation frequencies when inserted into pSER of pSV2.gpt. Also, like its murine counterpart the effect of the *Xba*I fragment was tissue-specific: with rodent fibroblasts the transformation efficiencies of pSERXa and pSERXb were over 100 times lower that of pSV2gpt. In a further experiment the 2.2 kb *Xba*I fragment was dissected by cleavage with *Alu*I, and some of the resulting fragments also tested in the pSER assay. Most, if not all the enhancing activity could be attributed to the 279 bp fragment, *Alu*.a (delineated by the two *Alu*I sites flanking the *Bgl*I site shown in the expanded map of the human C_μ gene in Fig. 1).

These transformation experiments strongly suggest that the 2.2 kb human *Xba*I fragment contains a tissue-specific transcriptional enhancer element. To demonstrate this point more directly we examined the effect of this DNA fragment on the transcription of a mouse immunoglobulin γ2b gene. The plasmid pSV.γ2bΔ$X_{2/4}$ contains a functionally rearranged mouse γ2b gene from which most of the sequences (including the enhancer element) between *V–D–J* and *C* exons have been deleted[2]. The plasmid also contains the selectable *Ecogpt* gene. J558L cells transformed to mycophenolic acid resistance by pSV.γ2bΔ$X_{2/4}$ express almost no γ2b heavy-chain RNA[2]. A high level of γ2b expression can be restored by re-insertion (either upstream or downstream of the *V–D–J* promoter) into plasmid pSV · γ2bΔ$X_{2/4}$ of the mouse enhancer[2], and similarly by insertion of the human *Xba*I fragment (Fig. 4). In this experiment, pools of cells transformed to a gpt^+ phenotype by pSV · γ2bΔ$X_{2/4}$ and its derivatives were assayed for γ2b gene expression by S_1 nuclease protection. The mouse enhancer was equally effective whether inserted upstream or downstream of the γ2b *V–D–J* promoter. By contrast the level of the γ2b gene transcription attained by the human enhancer was somewhat variable depending on its position, the highest level of expression being obtained when the enhancer was inserted upstream of the promoter in the opposite orientation to the direction of transcription (Fig. 4). Interestingly, the relative orientations of enhancer and promoter in this case are equivalent to those of the enhancer and the altered c-*myc* promoters in Manca cell DNA (see Fig. 1).

The nucleotide sequence of a portion of the J_H–C_μ region of the human germ-line clone pH18-CL-10 was determined and compared with that of the corresponding region of mouse DNA (Fig. 5), which contains the enhancer core sequences[30] which are common to enhancers so far defined. Core-like sequences are present in the human DNA: there is a 67% matching between the 140 bp of mouse DNA that contains most, if not all, of the murine enhancing activity and the corresponding human sequence. The similarity between the mouse and human sequences throughout the region shown in Fig. 5 is sufficiently strong to allow a unique alignment, with several insertions and deletions.

Activation of c-*myc* transcription

To begin to investigate whether the high level of c-*myc* transcription in Manca cells was due to proximity to the immunoglobulin

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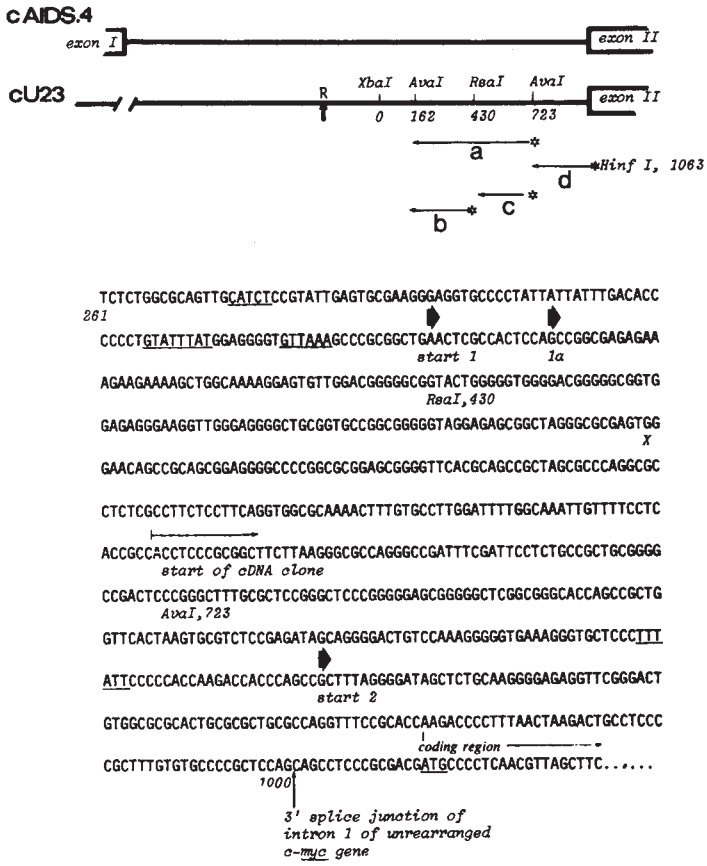

Fig. 3 Shows the nucleotide sequence from intron I of the germ-line c-*myc* gene that contains the initiation sites of the transcripts of the translocated c-*myc* gene. The recombination site of the translocated Manca chromosome is 336 nucleotides 5′ of the start of the sequence shown. Exon II of the germ-line c-*myc* gene starts at nucleotide 1,000. The transcription initiation sites (1, 1a and 2), as deduced by S_1 and primer extension analyses, are marked by the thick arrows. The probes used in these analyses (a–d, a and b being those used in Fig. 2) are shown near the top of the figure. The asterisks mark the ^{32}P-labelled ends of the probes. Underlined sequences are similar to those commonly found 5′ of RNA polymerase II-transcribed genes[27]. The initiator ATG codon of the protein encoded by c-*myc* is also underlined. X is an S_1-sensitive site, and R the recombination site. The sequence data derive from ref. 25 plus our own data in the regions of designated promoters.

Table 1 Transformation efficiency of pSV2.gpt and derivative plasmids

Plasmid	Frequency of transformation to gpt$^+$ phenotype	
	J558L cells*	rat-2 cells†
pSV2.gpt	1×10^{-4}	5×10^{-3}
pSV2.gpt.Xa‡	6×10^{-4}	ND
pSER	5×10^{-6}	ND
pSER.Xa‡	8×10^{-4}	1×10^{-5}
pSER.Xb‡	8×10^{-4}	1×10^{-5}
pSER.Ra§	8×10^{-6}	ND
pSER.Alu.a‖	2×10^{-4}	ND
pSER.Alu.z‖	5×10^{-6}	ND

Escherichia coli cells containing the above-mentioned plasmids were converted to spheroplasts[39], and fused[2] to recipient cells (10^{10} spheroplasts per 2×10^6 cells), after which the cells were plated at 5×10^3 (J558L) cells per well or 1×10^5 (rat-2) cells per culture dish. The selective medium, which contained xanthine (250 μg ml^{-1}), hypoxanthine (15 μg ml^{-1}) and mycophenolic acid (6.5 μg ml^{-1}), was added 48 h after fusion, and colonies were counted after 14 days. The experiments were routinely repeated three times. ND, not done.

* A mouse plasmacytoma cell line.

† A rodent fibroblast cell line.

‡ Xa/Xb plasmids contain an insertion in either orientation (a or b) of the 2.2 kb *Xba*I fragment from cU23.

§ pSER.Ra contains an insertion of a 4 kb *Eco*RI fragment from the mouse immunoglobulin heavy-chain C_α gene.

‖ Alu.a/Alu.z plasmids contain an insertion of either of two *Alu*I fragments (a and z) derived from the 2.2 kb *Xba*I fragment.

heavy-chain locus enhancer, the transcription of the translocated c-*myc* gene was studied in a murine myeloma cell line, J558L. The myeloma cells were transformed by spheroplast fusion with either of two plasmids, pSV.26 or pSV2.Δ4. Plasmid pSV2.26 contains the 11.7 kb *Eco*RI fragment from cU23 (containing the translocated c-*myc* gene plus about 8 kb of 5′ flanking sequences which include the immunoglobulin heavy-chain locus enhancer, see Fig. 1) the translocated c-*myc* gene cloned into the *Eco*RI site of pSV2gpt. Plasmid pSV2Δ4 contains a 9.5 kb *Eco*RI fragment (derived from the 11.7 kb *Eco*RI fragment of cU23 by deletion of the central 2.2 kb *Xba*I fragment) cloned into the *Eco*RI site of pSV2.gpt. Plasmid pSV2Δ4 thus lacks the sequences identified above as having transcriptional enhancing activity.

After transforming with these plasmids, gpt$^+$ transformants of J558L cells were isolated and assessed for the presence of exogenous DNA. Clones 26.1–26.9 were nine cloned isolates of cells transformed by pSV2.26; Δ4.2 is a cell line developed after transformation with pSV2.Δ4; Δ4.1 is a pool of several colonies transformed by pSV2.Δ4. Most cells transformed by pSV2.26 have acquired only single or a few copies of the exogenous DNA, and in most of these cases the integration has occurred within the 11.7 kb *Eco*RI fragment, so that *Eco*RI fragments smaller than 11.7 kb are detected with the c-*myc* probe[31]. Analysis with enzymes that cut within this fragment, however, indicate that in at least four out of nine cases (clones 26.1, 26.2, 26.4 and 26.7) contiguous DNA sequences that extend from the enhancer through the transcriptional initiation sites mapped above, and into the truncated c-*myc* gene, are intact (Fig. 6).

By contrast, the cells transformed in identical conditions by pSV2.Δ4, contain multiple, tandem copies of the exogenous DNA, and in each case the c-*myc* transcription unit and upstream sequences are intact. This difference in copy number of transforming DNA in cells selected for gpt$^+$ phenotype may be due to a positive effect (from over 7 kb away) of the enhancer on *Ecogpt* transcription: in the absence of the enhancer, high levels of *Ecogpt* transcription may only be obtained from an increased copy number. Consistent with this, the transformation frequency of J558L cells by pSV2Δ4 is 5–10-fold lower than the frequency of transformation by pSV2.26 (data not shown). The difference in copy number may also result from a deleterious effect of excess c-*myc* gene product on J558 cells, which already contain an actively transcribed translocated mouse c-*myc* gene[32]. If such an effect exists, and if the immunoglobulin heavy-chain locus enhancer does activate Manca cell c-*myc* transcription, then there may be a selective advantage to cells transformed with pSV2.26 that retain only one or even no intact copies of the Manca cell c-*myc* gene.

Both total and poly(A)$^+$ RNA were isolated from groups of transformants, and were analysed by Northern transfer and hybridization to probe 1 (see Fig. 1). Although transformants receiving pSV2.Δ4 contained multiple intact copies of the transfected c-*myc* gene, none of them showed evidence for much c-*myc* transcription over and above the level of the mouse c-*myc* transcription endogenous to J558L cells (data not shown). By contrast several lines of cells transformed by pSV2.26 showed strongly hybridizing c-*myc* transcripts (data not shown).

Because of the variability in retention of c-*myc* DNA sequences, and the cross-reactivity of the Northern probe with the mouse c-*myc* transcripts in J558L cells, a quantitative com-

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parison of human translocated c-*myc* gene transcription was better made by S_1 nuclease mapping. The S_1 probe a (Fig. 3), which detects the major initiation site, was protected to a similar quantitative degree by Manca cell RNA and by RNA of several lines transformed by pSV2.26 (for example, lines 26.1, 26.4 and 26.7, see Fig. 7). That RNA in these cell lines is qualitatively the same as in Manca cells was further indicated by use of S_1 probe b. Use of either probe shows almost no protection by RNA of cell lines Δ4.1 or Δ4.2 (see Fig. 7) even though these cells contain multiple intact copies of the transforming DNA (Fig. 6). It cannot be argued that in either of these cases the transforming DNA has become sequestered in a transcriptionally inactive area, since continual selection for *Ecogpt* gene activity is made. In addition, several isolates of both total and poly(A)$^+$ RNA from these cells gave the same result, and these RNAs were positive in an S_1 nuclease protection assay of *Ecogpt* transcripts (data not shown). It appears then, that the activity of the major transcriptional initiation site for translocated c-*myc* gene transcription is severely reduced by deletion of sequences 360 bp upstream of it. Deletions at this distance upstream of a

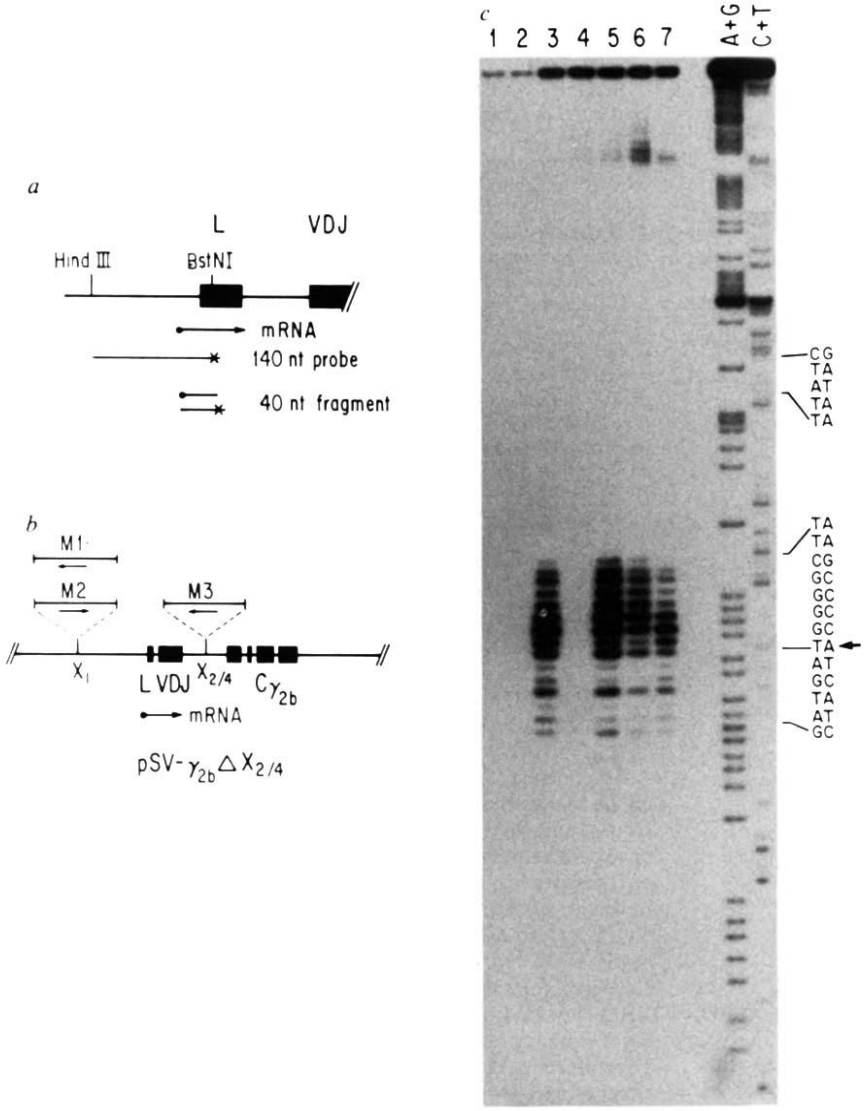

Fig. 4 *a*, Strategy of the S_1 nuclease mapping experiment shown in *c*. The probe is an end-labelled *Hin*dIII–*Bst*NI fragment, which was strand separated and annealed to total cell mRNA before S_1 digestion and electrophoretic resolution, which were carried out as described previously[23,24]. The 40 bp fragment protected by correctly initiated γ26 mRNA is as predicted by primer extension analysis[44]. The solid blocks indicate the leader (*L*) exon and 5′ end of the *V–D–J* exons. ***b*** Shows the derivatives of pSV-γ2bΔ$X_{2/4}$ used for transfection. The 2.2 kb *Xba*I fragment into the X_1 (M1 and M2) or $X_{2/4}$ (M3) sites[2] in the indicated orientations. ***c*** Shows the S_1 nuclease resistant hybrids formed between the probe shown in *a* and: lane 1, 20 μg tRNA; lane 2, 20 μg RNA from J558L cells; lanes 3–7, 20 μg RNA from J558L cells transfected with: lane 3, pSV-γ2bVC[2]; lane 4, pSV-γ2bΔ$X_{2/4}$; lane 5, pSV-γ2bΔ$X_{2/4}$ M1; lane 6, pSV-γ2bΔ$X_{2/4}$ M2; lane 7, pSV-γ2bΔ$X_{2/4}$ M3. In the two right-hand lanes are co-electrophoresed sequencing reactions[34] of the probe, which allow the identification of the transcription initiation site (arrow) and the GATAA box at position −30.

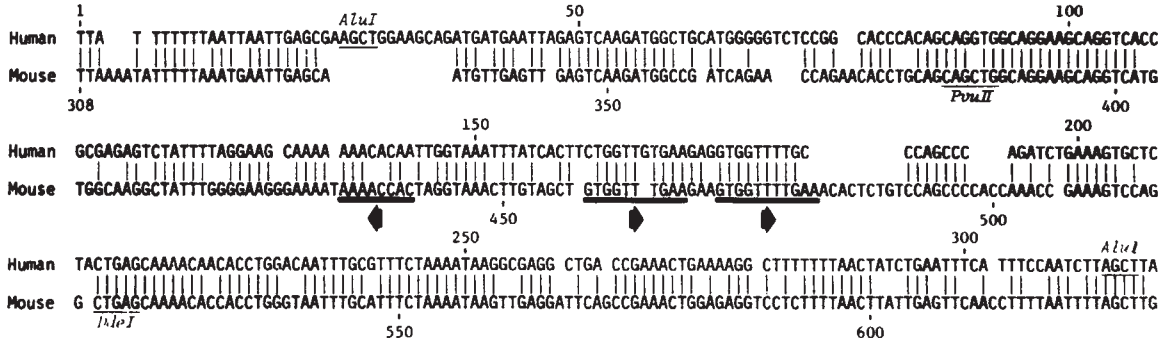

Fig. 5 Sequences of the murine[2] and human enhancer elements, which were compared using the SEQ computer program[45].

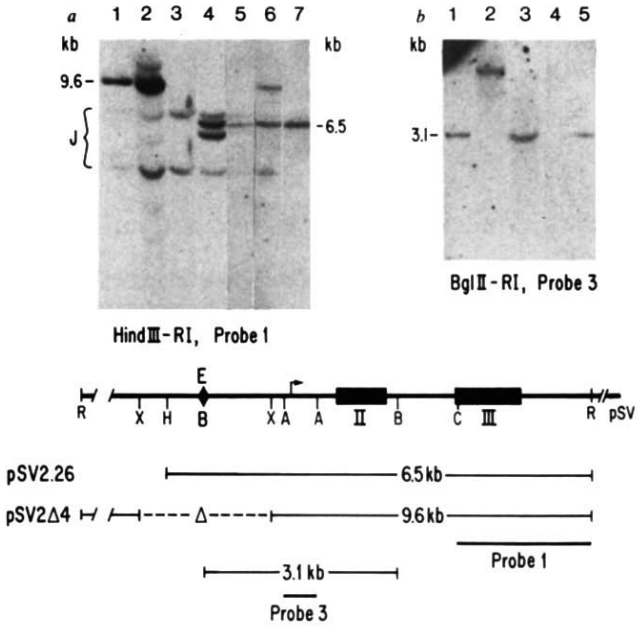

Fig. 6 Southern blot analyses of c-*myc* containing sequences in J558L cells, and in their derivatives transformed with either pSV2.26 or pSV2.Δ4. ***a*** Shows a blot obtained using probe 1 (as indicated in the diagram below) and *Hin*dIII plus *Eco*RI digested DNA from: lane 1, Δ4.2 cells; lane 2, Δ4.1 cells; lane 3, J558L cells; lane 4, 26.7 cells; lane 5, 26.2 cells; lane 6, 26.1 cells; lane 7, 5 pg of pSV2.26 cleaved with *Hin*dIII plus *Eco*RI, co-electrophoresed as a marker, ***b*** Shows a blot obtained using probe 3 and *Bgl*II plus *Eco*RI cleaved DNA from: lane 1, 26.1 cells; lane 2, 26.5 cells; lane 3, 26.4 cells; lane 4, J558L cells; lane 5, as in lane 7 of *a*, but cut with *Bgl*II. The diagram below the blots shows the 11.7 kb *Eco*RI fragment contained in pSV2.26 and also the position of the 6.5 kb *Hin*dIII–*Eco*RI fragment. The deletion (Δ) in pSV2Δ4 removes the *Hin*dIII site, as a result of which a 9.6 kb *Eco*RI fragment is the *Hin*dIII plus *Eco*RI digestion product that hybridizes to the c-*myc* probe. As a further result of this deletion the 3.1 kb *Bgl*II fragment detected with probe 3 (indicated in *b*) is only detected in pSV2.26 transformants. Bands labelled J in *a* are due to the cross-reactivity of probe 1 with normal and rearranged mouse c-*myc* genes in J558L cells[11,32]. Restriction sites marked in the lower diagram: R, *Eco*RI; X, *Xba*I; H, *Hin*dIII; B, *Bgl*II; A, *Ava*I; C, *Cla*I.

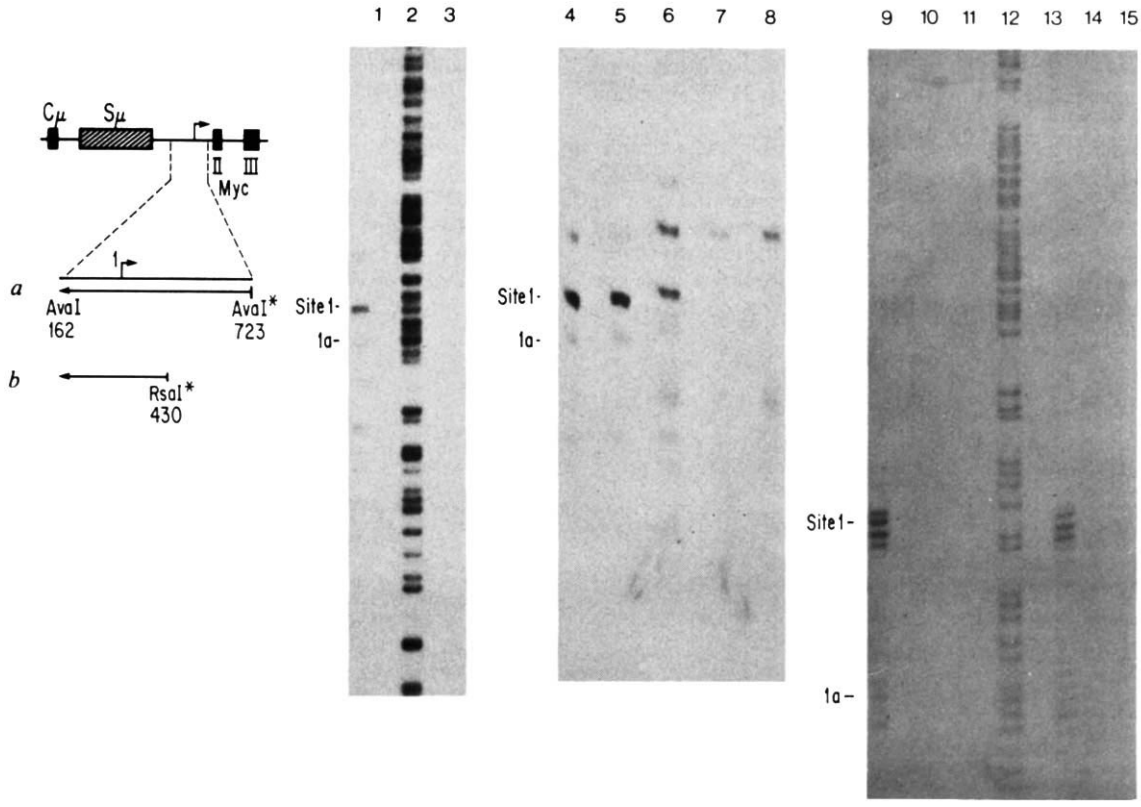

Fig. 7 S_1 nuclease analysis of mRNA from Manca cells, J558L cells, and transformed derivatives of J558L cells. Lanes 1–8 were reactions involving probe a, and lanes 9–15 involved probe b. Lane 2 is a pyrimidine sequencing reaction of probe a, and lane 12 is a similar reaction with probe b. The other lanes are the S_1 reactions, with mRNA from: lane 1, 26.7 cells; lane 3, J558L cells; lane 4, 26.1 cells; lane 5, 26.4 cells; lane 6, Manca cells; lane 7, Δ4.1 cells; lane 8, yeast cells; lane 9, Manca cells; lane 10, 26.3 cells (which retained no human c-*myc* sequences); lane 11, Δ4.1 cells; lane 13, 26.1 cells; lane 14, Δ4.2 cells; lane 15, J558L cells. The end-labelled, strand-separated[41] probes were melted at 85 °C, then annealed to the RNAs at 51 °C for probe a, and at 30 °C for 12 h for probe b. S_1 digestions were carried out at 15 °C for 2 h. Electrophoresis was carried out as described previously, for lanes 1–8 on a 6% gel run for 7 h, for lanes 9–15 on an 8% gel run for 3 h. All reactions contained equal amounts of sample RNA made up to 20 μg with yeast RNA. S_1 nuclease-sensitive sites specific for the presence of lymphoid RNA are indicated.

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promoter are characteristic of enhancer mutations[33]. As the enhancing activity of the deleted fragment has been demonstrated and largely localized to one fragment (the *Alu* 'a' fragment), it appears likely that it is the removal of the immunoglobulin heavy-chain locus enhancer that has drastically reduced transcription of the translocated c-*myc* gene.

Multiple mechanisms of c-*myc* activation

We show here that Manca cells contain a translocated c-*myc* gene that is transcribed from previously cryptic start sites that ordinarily form part of intron I of the c-*myc* gene. This transcription is reproduced with fidelity after introduction of the gene into mouse myeloma cells. Use of this system has allowed us to demonstrate that transcription from at least the major cryptic promoter is dramatically dependent on the presence of sequences over 350 bp upstream of the RNA initiation site. These sequences include an element that we demonstrate to be a human immunoglobulin gene-associated enhancer element, and it may be this element itself that is responsible for activating translocated c-*myc* transcription in Manca cells. The remaining possibility, that it was the removal of other sequences (including about 300 bp of DNA that are also ordinarily part of c-*myc* intron I) that drastically reduced transcription from initiation site I is presently being investigated. The unrearranged c-*myc* gene is still present in Manca cells, but our preliminary results from use of Northern probes (probe 2 in Fig. 1), S_1 mapping and cDNA cloning indicate that this allele is barely, if at all, expressed. Such a situation has already been reported for a mouse plasmacytoma and may be true for other Burkitt's lymphomas[11,32,34]. Hence, transcriptional activation of the c-*myc* gene appears to be an important result of translocation and in the case described here, it is possible that such activation is largely contributed to by the immunoglobulin heavy-chain locus enhancer.

The retention of the immunoglobulin associated enhancer element adjacent to the translocated c-*myc* gene is not a common feature of c-*myc* translocations so far documented in either mouse[7,10,11,35] or man[12,36]. In these instances, the translocated gene is still transcribed[7,10,11,37], whilst again the untranslocated gene may be silent[11,32,34]. In these cases, transcription of the translocated c-*myc* gene may be maintained by a cellular enhancer-type element yet to be identified, and which may be a considerable distance from the translocated c-*myc* gene.

Alternatively, translocated c-*myc* genes may not be directly dependent upon immunoglobulin sequences for their transcription, but rather their transcription may be activated by the accumulation of mutations in regulatory regions. For example, it has been suggested[34] that the c-*myc* gene product itself may regulate its own transcription, and that, in lymphomas harbouring translocated genes, the translocated but not the normal c-*myc* gene, escapes this repression. At this stage, the hypothesis of positive activation, and that of escaping repression, are not mutually exclusive.

The detachment of c-*myc* exon I sequences from c-*myc* exons II and III is a consequence for all BALB/c and for several human c-*myc* translocations thus far documented[7,10–12,38]. We have previously suggested[22,31] that this event, in itself, leads to increased translation of c-*myc* RNA in cells harbouring such translocations.

In summary, there are probably multiple steps in the activation of the human c-*myc* gene in non-Hodgkin's lymphomas. From this article it is clear that previously cryptic promoters of the c-*myc* gene are activated upon translocation, and that this activation is dependent upon upstream sequences. In Manca cells, the critical element in these upstream sequences may be the human immunoglobulin heavy-chain locus enhancer element. As both the translocated c-*myc* gene from a mouse plasmacytoma and the translocated human c-*myc* gene from Manca cells have detectable biological activity (ref. 46 and our unpublished data) it is apparent that the activation of the c-*myc* gene contributes to either initiation or maintenance of oncogenesis. It is possible that the misuse of a developmentally regulated, cellular enhancer element will be found, in many cases, to be responsible for activating oncogenes.

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Note added in proof: The integrity of all RNA preparations has been confirmed by succcessful S_1 analysis of gpt transcription.

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