The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice

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Transgenic mice bearing the cellular myc *oncogene coupled to the immunoglobulin* μ *or* κ *enhancer frequently develop a fatal lymphoma within a few months of birth. Since the tumours represent both immature and mature B lymphocytes, constitutive c-*myc *expression appears to be highly leukaemogenic at several stages of B-cell maturation. These* myc *mice should aid study of lymphoma development, B-cell ontogeny and immunoglobulin regulation.*

THE arguments that a small number of cellular genes, the proto-oncogenes, are crucial to the development of neoplasia are persuasive[1,2]. Nevertheless, the evidence that natural malignancies are caused by somatically altered proto-oncogenes has remained largely circumstantial[3]. Transgenic mice (reviewed in ref. 4) provide the means to test directly the efficacy of cellular oncogenes *in vivo*. Since a gene injected into a fertilized ovum typically integrates into a host chromosome within a few cell divisions, all tissues of the transgenic mouse usually acquire the gene, but the regulatory elements of the gene may direct tissue-specific expression[4]. Introduced immunoglobulin genes, for example, are expressed only in the lymphoid cells of transgenic mice[5-8].

Recently, both a viral and a cellular oncogene have been shown to elicit characteristic tumours in transgenic mice. The type of tumour induced by the large-T antigen gene of simian virus 40 (SV40) depends on the element controlling its expression; with the SV40 enhancers, papillomas of the choroid plexus develop[9,10], while the insulin regulatory region leads to pancreatic tumours[11], and the metallothionein-I control region yields still other neoplasms[12]. The cellular *myc* oncogene linked to the regulatory region within the long terminal repeat (LTR) of the mouse mammary tumour virus promotes mammary carcinomas[13]. These results suggest that tissue-specific regulators can target the action of oncogenes to particular cell types.

We wished to establish whether the c-*myc* gene can be introduced into the mouse genome in a form that promotes lymphoid malignancy. Altered regulation of c-*myc* expression has been strongly implicated in lymphoid neoplasia. In retrovirus-induced avian B lymphomas[14,15] and some rodent T lymphomas[16,17], proviral insertion near c-*myc* brings it under the control of the promoter or enhancer within the retroviral LTR. In most human Burkitt's lymphomas and murine plasmacytomas, the *myc* gene has been activated by translocation to the immunoglobulin heavy (*IgH*)-chain locus (reviewed in refs 18-20). The way in which the *IgH* locus activates c-*myc* expression is poorly understood, but the lymphoid-specific *H* locus enhancer (E_μ)[21-24] may well be the activating element in those translocations that couple it to c-*myc*.

By constructing transgenic mice bearing different forms of the c-*myc* gene, we have established that subjugation of this proto-oncogene to immunoglobulin enhancers converts it into a potent leukaemogenic agent for B lymphoid cells. Our results also support the notion that expression of the normal *myc* gene is subject to feedback regulation[18,25] and bear upon the issue of whether an activated c-*myc* gene alone is sufficient to cause tumours. Since the predisposition is heritable, the novel lines of *myc* mice described here should prove valuable in unravelling early events in lymphoma development, in defining early stages of lymphoid ontogeny, and in clarifying the mechanisms that regulate immunoglobulin gene expression.

Enhancers make c-*myc* tumorigenic

The seven constructs we have introduced into mice include an intact murine c-*myc* gene (top), a truncated form bearing the coding region (exons 2 and 3) and five versions with c-*myc* coupled to other regulatory regions (Fig. 1*a*). In the E_μ-*myc* construct, which is equivalent to the rearranged c-*myc* allele in the plasmacytoma ABPC17 (ref. 26), the *H* locus enhancer lies upstream of exon 1, while in the LTR-*myc* construct the enhancer region of a murine retroviral LTR is positioned similarly[16]. In E_κ-SV-*myc*, the lymphoid-specific immunoglobulin κ enhancer[6,27,28] is coupled through the SV40 promoter to *myc* exons 2 and 3. SV-*myc* and MT-*myc* are analogous constructs containing the enhancer plus promoter of SV40 or the murine metallothionein-I gene, respectively. In four of the constructs the *myc* 3′-untranslated region was marked (Fig. 1*b*) with an irrelevant sequence (0.6 kilobases (kb) of ΦX174 phage DNA) to distinguish its transcripts from those of the endogenous *myc* gene.

To make transgenic *myc* mice, (C57BL × SJL)F_2 eggs were injected with a construct, implanted in pseudopregnant females, and transgenic pups identified by hybridization to tail DNA[29]. Many of these primary transgenic animals developed tumours, and the incidence is expressed in Fig. 1*a* as a fraction of the mice bearing each construct.

Significantly, both the μ and κ enhancer constructs elicited tumours and all involved lymphoid tissue. The E_μ-*myc* gene was remarkably potent, 13 of 15 primary transgenic animals developing lymphomas (see below). These mice died, or became terminally ill (and hence were killed) between 6 and 15 weeks of age (median 11 weeks). With E_κ-SV-*myc*, 6 of 17 transgenic animals died with lymphomas between 11 and 44 weeks of age (median 23 weeks). The efficacy and specificity of these constructs are almost certainly due to the immunoglobulin enhancers, because no tumours have arisen during 10 months of observation in the mice carrying either the tagged normal *myc* gene or the truncated form typical of most murine plasmacytomas[19,20]. Since both immunoglobulin constructs were effective in multiple primary transgenic animals, each representing an independent insertion event, these enhancers must function in diverse chromosomal environments, as can intact μ and κ genes[5-8].

The non-immunoglobulin constructs gave few tumours. The LTR enhancer-*myc* construct, which is derived from a T lymphoma[16], gave rise to only one tumour, a thymic lymphoma. With the SV-*myc* construct, which is equivalent to one that transforms very effectively *in vitro*[30], 3 of 21 mice developed tumours: a lymphosarcoma, a renal carcinoma and a fibrosar-

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Fig. 1 *a*, The c-*myc* constructs injected into fertilized ova and tumour incidence in resulting transgenic mice. The solid bar denotes c-*myc* sequences with exons raised, untranslated regions striped, the ΦX174 marker DNA (see *b*) as a triangle, and vector sequences as a thin line. The linear fragments shown were isolated from plasmid constructs (described below) before injection. The E_μ-*myc* construct contains 2.3 kb of immunoglobulin DNA (open bar) spanning the H-chain enhancer (diamond), inserted 361 base pairs (bp) 5′ to c-*myc* exon 1, as in plasmacytoma ABPC17 (see *b* and ref. 26) and extends 7.2 kb 3′ to c-*myc*. The LTR-*myc* construct has the enhancer-containing 210 bp of the Soule murine leukaemia virus LTR (cross-hatched) inserted ~1.3 kb 5′ to c-*myc*, as in T lymphoma ST4 (ref. 16). The bottom three constructs involve fusion to the *Xba*I site directly 5′ to *myc* exon 2 (ref. 45). E_κ-SV-*myc* has an ~900-bp fragment of κ locus DNA (open bar) containing the κ enhancer (diamond) linked to the SV40 promoter (181-bp *Sph*I/*Stu*I fragment); SV-*myc* has the complete SV40 promoter/enhancer region (347-bp *Kpn*I/*Stu*I fragment); and MT-*myc* bears metallothionein-I sequences (stippled), from −750 to +6 (ref. 52), including its heavy metal-regulated promoter. *b*, Construction of c-*myc* plasmids. To mark the ABPC17 c-*myc* gene (ref. 26) within its 3′-untranslated region, the *Eco*RI fragment spanning c-*myc* was subcloned, and its *Eco*RV/*Kpn*I fragment exchanged for one bearing the ΦX174 marker. The latter was taken from pST4BamΦX, constructed by ligating a ΦX174 *Hae*III/*Xho*I fragment (nucleotides 4,984–162) via *Xho*I linkers to a *Xho*I-restricted *Bam*HI subclone isolated from the T lymphoma ST4 (ref. 16). Finally, the ΦX-marked *Eco*RI fragment was inserted at the *Eco*RI site of the ABPC17 *Sac*I/*Eco*RI subclone to give pA17E_μ*myc*ΦX. For injection, this plasmid was linearized with *Sal*I, which cuts within the pUC12 vector. The c-*myc* (ex 2+3) fragment was excised from pA17E_μ*myc*ΦX with *Bgl*II and *Kpn*I. The marked normal c-*myc* construct (*a*, top) was prepared analogously by replacing the 5′ segment of pST4BamΦX (up to the unique *Eco*RV site in exon 2) with an 11-kb *Eco*RI/*Eco*RV fragment spanning the 5′ end of the unrearranged c-*myc* gene; the 11.2-kb *Kpn*I fragment was then excised for injection. The LTR-*myc* plasmid was constructed by inserting the ΦX marker into an ~14-kb rearranged c-*myc* fragment from ST4 (ref. 16) by *Eco*RV/*Kpn*I exchange with pST4BamΦX as above, and the isolated *Eco*RI fragment injected. The E_κ-SV-*myc* plasmid was constructed by replacing the SV40 enhancer (*Sph*I/*Kpn*I) with a 900-bp fragment (nucleotides 3,390–4,290 in ref. 53) containing the κ enhancer. For MT-*myc*, the *Xba*I/*Bam*HI fragment spanning *myc* exons 2 and 3 was inserted into a MT vector containing a *Xba*I linker at +6 and a 3′ *Bam*HI site; it was linearized at *Bam*HI for injection. Restriction endonuclease sites are: K, *Kpn*I; Bg, *Bgl*II; S, *Sac*I; R, *Eco*RI; Sl, *Sal*I; B, *Bam*HI; X, *Xho*I; V, *Eco*RV.

coma. This diversity echoes the wide range of cells in which the SV40 enhancer functions. The absence of tumours of the choroid plexus with this construct may mean that the *myc* gene product is less effective than the large-T antigen[9,10] in transforming brain epithelial cells. We were surprised that no tumours ensued with the MT-*myc* construct, because that control region has induced expression of other genes in transgenic mice[12,31,32], but the vector sequences present in this construct (Fig. 1*a*) may have had a suppressive effect[33].

Aggressive lymphoma

The striking efficacy of the E_μ-*myc* construct led us to concentrate on mice bearing it. To establish whether the predisposition to lymphoma development was heritable, we analysed progeny from primary E_μ-*myc* mice that survived long enough to allow breeding. In eight independent lineages, lymphomas have developed with high fidelity. Indeed, in three generations of descendants, 59 of 63 mice (94%) have died by 4 months of age. The pathology is very similar in all lineages.

The hallmark of the disease in E_μ-*myc* mice is massive enlargement of the lymph nodes. The onset, most readily assessed by palpation of the inguinal lymph nodes, usually occurs between 3 and 18 weeks of age. A few weeks later, most mice develop 'water wings', prominent lumps on both shoulders and the neck (Fig. 2*a*), reflecting swelling of the brachial and cervical lymph nodes, and most other nodes are grossly enlarged (Fig. 2*b*). The spleen usually shows modest (about twofold) expansion and the thymus is markedly enlarged in some mice. The enlarged lymphoid organs are replete with lymphoblasts and the blood contains large numbers of similar cells. Variable invasion of other tissues is often observed. The typical pathological diagnosis is therefore multicentric lymphosarcoma (disseminated lymphoma) with associated leukaemia. Occasionally, mice have developed a thymoma without notable involvement of peripheral lymphoid organs and some have succumbed to a bowel obstruction caused by intestinal involution (intussusception) over focal growths of lymphoblasts in the wall. The typical pathology is somewhat like that induced by Abelson murine leukaemia virus[34].

Transplantation into syngeneic mice established that the enlarged lymphoid organs of E_μ-*myc* mice represented frank tumours rather than massive proliferations of 'normal' cells. Rapidly growing tumours arose in the (C57×SJL)F_1 hybrid recipients a few weeks after injection of 10^5–10^6 cells from each of 10 E_μ-*myc* mice, including all those in Table 1. Indeed, 10^2 cells from one tumour tested at lower cell numbers (4Met in Table 1) initiated tumours within 2–3 weeks. The disease in E_μ-*myc* mice is patently highly malignant.

Tumours from single B-lymphoid clones

We wished to identify the cell lineage(s) from which the tumours were derived. The pathology was highly suggestive of a lymphoid disease but did not rule out myeloid involvement. Transcriptional studies of the μ constant-region (C_μ) locus[35–37] and gene transfer studies[7,24] suggest that E_μ can operate in many T lymphocytes and certain other haematopoietic cells as well as B cells, but all the tumours so far analysed in detail are B lymphoid in origin. All cell lines derived from them are non-adherent and have an absolute requirement for 2-mercaptoethanol, a strong indication of lymphoid rather than myeloid origin. As expected for B cells, all tumours and cell lines displayed rearrangement and expression of the *IgH* and/or κ loci, as illustrated in Figs 3 and 4 and summarized in Table 1 for the tumours of five mice. Arguing against a T-cell origin, no tumour so far studied has displayed the Thy-1 surface antigen, nor exhibited rearrangement or transcription of the T-cell antigen receptor β locus (data not shown). Moreover, since the κ locus (unlike the *H* locus) appears to be transcriptionally silent in T lymphocytes (refs 6, 38 and our unpublished results), the presence of κ-locus transcripts in all the E_μ-*myc* tumour cell lines (see below) strongly favours a B-lymphoid origin.

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Table 1 Representative tumours arising in E_{μ}-*myc* transgenic mice

Pathology	Cell line	Ig genes‡		Ig RNA¶		sIg #		Cell type
		H	κ	H	κ	1°	2°	
Mouse 1 (285-5-6)♂								
Multicentric lymphoma/leukaemia with very large pancreatic lymph node	1 Pan*	R1, R2	G	C_{μ}++	κ^0+	0	0	Pre-B clone *A*
	1 Bra*	R3	G	C_{μ}++	κ^0+	0	0	Pre-B clone *B*
Mouse 2 (285-5-12)♀								
Multicentric lymphoma/leukaemia with thymoma	2 Thy†	R1, R2	G	C_{μ}++	κ^0+	0	0	Pre-B clone *A*
	2 Bra*	R3, G§	G	C_{μ}++	κ^0+	ND	0	Pre-B clone *B*
	2AxL	R3, G§	G	C_{μ}++	κ^0+	0	0	Pre-B clone *B*
	2 Mes*	R3, G§	ND	C_{μ}++	κ^{0+}	ND	<5	Pre-B clone *B*
Mouse 3 (285-5-9)♀								
Multicentric lymphoma/leukaemia with intussusception of intestine	3 Mes	R1, R2	R1	μ++	κ++	5-10	>90	Pre-B → B clone *A*
	3 Bra	R1, R2	G	μ++	κ^0+	5-10	10**	Pre-B → B clone *A*
Mouse 4 (292-5-5)♂								
Multicentric lymphoma/leukaemia with enlarged thymus	4 Thy†	R1, R2	R1, R2	(μ, C_{μ})+++	κ+++	80-90	>90	B clone *A*
	4 Mes†	R3, R4	R3, R4	(μ, C_{μ})+++	κ+++	80-90	>90	B clone *B*
	4 Bra†	R3, R4	ND	(μ, C_{μ})+++	κ+++	80-90	>90	B clone *B*
	4 Met†	R3, R4	ND	(μ, C_{μ})+++	κ+++	80-90	>90	B clone *B*
Mouse 7 (292-1-1-11)♂								
Multicentric lymphoma/leukaemia with thymoma and lymphoblast infiltration of perinodal fat	7 Mes*	R1, R2	R‖	C_{μ}++	κ++	0	0	Pre-B clone *A*
	7 BM	R1, R2	R‖	C_{μ}++	κ++	ND	ND	Pre-B clone *A*
	7 ThyD*	R1, R2	R‖	C_{μ}++	κ++	0	ND	Pre-B clone *A*
	7 ThyV*	R1, R2	R‖	C_{μ}++	ND	ND	ND	Pre-B clone *A*
	7 Spl*	R1, R2	R‖	C_{μ}++	ND	ND	ND	Pre-B clone *A*

Mice specified here were first- or second-generation descendants of three primary transgenic mice (285-5, 292-5 and 292-1) bred with normal (C57BL × SJL)F_1 hybrids. Sick mice killed by exsanguination under anaesthesia were dissected for assessment of gross pathology. Tissue sections were stained with haematoxylin and eosin and blood smears stained with Giemsa for histopathological assessment. Cell lines are designated by their origin as tumours in the pancreatic (Pan), brachial (Bra), axillary (AxL), mesenteric (Mes) lymph nodes; diffuse mediastinal tissue (Met), thymus (Thy), spleen (Spl) or bone marrow (BM). ThyD and ThyV derived from discrete masses situated dorsally and ventrally in the position of normal thymus. ND, not determined.

* Primary tumour and cell line shown to have equivalent J_H rearrangements.

† Primary tumour oligoclonal but dominated by the single clone present in cell line.

‡ Rearrangement of J_H alleles was determined by Southern blot analysis of *Eco*RI digests using ^{32}P-labelled probe *A* (Fig. 3), while J_{κ} analysis used *Bam*HI digests and probe *C* (Fig. 4, top). G indicates a fragment the same size as that in germline (liver) DNA from C57BL or SJL mice, while R1-R4 indicate rearranged fragments of different sizes.

§ Other rearranged J_H fragments were present in minor yield. We are investigating whether the fragment in germline position is truly unrearranged.

‖ Four rearranged bands were detected and their relationship to each other is under investigation.

¶ μ and κ denote authentic μ and κ mRNA, while C_{μ} denotes all other C_{μ}-bearing RNA species (see refs 35, 37, 39) and κ^0 denotes transcripts of the unrearranged κ locus (see Fig. 4). +++ indicates a level comparable to that in a conventional B lymphoma (WEHI-231), and ++ and + about one-third and one-tenth that level.

\# % Cells with surface immunoglobulin (sIg) in either the primary tumour (1°) or the cell line derived from it (2°).

** Growth used for RNA and DNA analysis was 10% sIg$^+$, but the line subsequently progressed to >90% sIg$^+$.

Although E_{μ}-driven *myc* expression might be expected to trigger proliferation of the entire B-cell compartment, the simplicity of the *IgH* locus rearrangement patterns (Fig. 3) provided strong evidence that most tumours are monoclonal. Most contain two fragments bearing a heavy-chain joining region (J_H) in equimolar yield but lack the 6.5-kb 'germline' fragment found in liver (G), precisely as expected for a clonal lymphoid cell line that has undergone diversity-joining region (DJ) or variable-diversity-joining region (VDJ) recombination at both *IgH* alleles. The apparent single rearranged fragment in tumours such as 1BraC probably reflects two co-migrating fragments. While a few primary tumours (for example, 2AxL, Table 1) contained four to six rearranged J_H fragments, indicative of oligoclonality, one or two bands always strongly predominated.

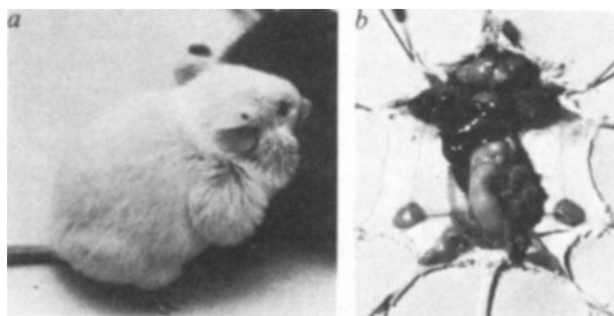

Fig. 2 *a*, Fourth-generation E_{μ}-*myc* mouse, 11 weeks old, with swellings due to grossly enlarged lymph nodes. *b*, Interior of same mouse, displaying gross bilateral enlargement of (from the top) cervical, axillary, brachial, mesenteric and inguinal lymph nodes.

The J_H rearrangement pattern also confirmed that the cultured line (C) represented either the sole or very dominant clone within the primary tumour (T) mass (compare C and T for several tumours in Fig. 3).

The tumour masses at different sites in a *myc* mouse are frequently derived from the same clone. In mouse 7, for example, tumours from five different sites displayed identical J_H rearrangements (Fig. 3, Table 1) and even the liver had been invaded by this clone (Fig. 3). Some mice, however, appeared to bear more than one independent tumour (Table 1). For example, the pancreatic and brachial lymph node tumours in mouse 1 (1Pan and 1Bra) had different J_H rearrangements, while the thymic tumours in mice 2 and 4 differed from the lymph node tumours (Fig. 3). These differences may reflect independent transformation events, or maturation of a single primary clone by further rearrangement, such as recombination of *V* with *DJ* segments.

B-lymphocyte transformation

The pre-B cell, the earliest defined stage in B-cell ontogeny, displays rearrangement at the heavy-chain locus (*DJ* or *VDJ*) but lacks surface immunoglobulin (sIg), usually because the κ or λ light chain loci are unrearranged. Fusion of a V_{κ} gene with J_{κ} (or, more rarely, V_{λ} with J_{λ}) permits κ or λ expression and progression to the mature (sIg$^+$) B cell. Although c-*myc* has been implicated only in tumours of mature B cells and plasma cells, the E_{μ}-*myc* tumours include some with pre-B characteristics, others that represent mature B cells and one that progressed from the pre-B to the B-cell stage (Table 1). Hence, *myc*-induced tumorigenesis is not stage-specific.

Tumours of pre-B type developed in mice 1, 2 and 7 (Table 1). No sIg was present, and μ_m messenger RNA, which encodes the membrane form of μ-chain, was not detected by blot

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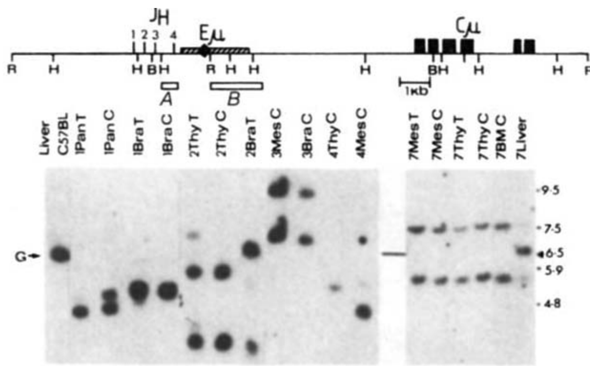

Fig. 3 Rearrangement at the immunoglobulin heavy-chain locus in tumours arising in E_μ-*myc* mice. The diagram indicates the structure of the J_H-C_μ locus before rearrangement (germline configuration), with exons indicated as solid boxes, and the enhancer (E_μ) as a diamond. Hatched area is the region inserted 5′ to c-*myc* in the E_μ-*myc* construct, and *Eco*RI (R), *Bam*HI (B) and *Hin*dIII sites are shown. The Southern blot shows J_H rearrangements revealed in *Eco*RI-cut DNA by hybridization with ^{32}P-labelled probe *A*, a 450-bp *Hin*dIII/*Nae*I fragment, subcloned to eliminate sequences within the E_μ-*myc* insert. An additional fragment detected by probe *A* in 4Thy (and weakly in 4Mes) is too small (2.8 kb) to be shown by this autoradiograph. An arrow marks the position of the unrearranged J_H-bearing fragment (G) and sizes of marker fragments are given in kilobase pairs (kb). Probe *B*, a 1.5-kb *Eco*RI/*Hha*I fragment, is the S_μ probe used in Fig. 5.

hybridization with a C_μ probe (Table 1). As will be documented elsewhere, the C_μ-bearing RNAs in these lines are those expected[35-41] from either partially rearranged (DJ_H) or unrearranged heavy-chain locus alleles. The tumour in mouse 7 is of an unusual pre-B type, having a κ rearrangement and κ mRNA without μ_m mRNA (Table 1).

Although the cell lines from mice 1 and 2 lack κ rearrangement (Table 1), we found not only the known 8-kb transcript of the germline C_κ locus[42], which initiates upstream of J_κ (Fig. 4, top), and a 0.8-kb species[43], but also an ~1.1-kb species, the size of κ mRNA (Fig. 4*a*). Unlike κ mRNA, however, this species bears sequences upstream of J_κ, since it hybridizes with probe *A*+*B* (Fig. 4*b*) and probe *A* alone (not shown). We therefore suggest that processing of the 8-kb RNA generates this previously unrecognized[42] κ RNA species (Fig. 4, top).

An intriguing tumour that progressed from a pre-B to a B cell developed in mouse 3. The J_H rearrangement patterns assign its mesenteric and brachial lymph node tumours (3Mes and 3Bra) to the same clone (Fig. 3), but only 3Mes had a J_κ rearrangement (Table 1). However, after only a few weeks in culture, over 90% of 3Mes cells became sIg$^+$, as later occurred for 3Bra. We conclude that this leukaemic pre-B clone generates mature B-cell progeny, as can a few lymphomas induced by Abelson murine leukaemia virus[44]. Thus a *myc*-induced tumour need not be 'frozen' at one stage of differentiation.

Expression of *myc* gene

Abundant c-*myc* mRNA was detected in all the E_μ-*myc* cell lines examined, as illustrated for six lines in Fig. 5*a*. The levels in all the lines, which included tumours from three separate E_μ-*myc* lineages, were comparable and fell within the upper range of that we have observed in plasmacytomas[45,46]. Due to the 0.6-kb ΦX174 insert within the *myc* 3′ untranslated region (Fig. 1), all the lines contained an ~3-kb *myc* mRNA, which also hybridized to the appropriate ΦX174 probe (not shown), whereas a normal, unrearranged *myc* gene yields ~2.3-kb transcripts, as shown for plasmacytoma ABPC103 and B lymphoma WEHI-231 in Fig. 5*a*. Significantly, no normal c-*myc* mRNA was detectable in any E_μ-*myc* cell line, even after lengthy autoradiographic exposures (for example, Fig. 5*b*). Moreover, treatment of four of the lines with the B-cell mitogen lipopolysaccharide (LPS) at 20 μg ml^{-1} for 24 or 48 h did not induce normal

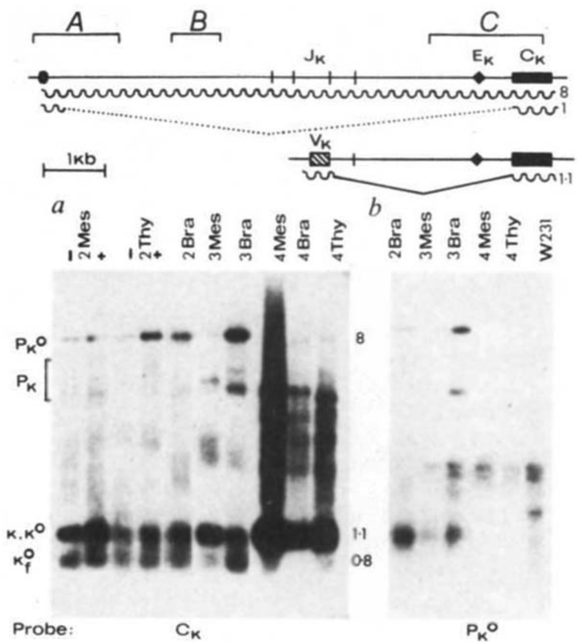

Fig. 4 Transcripts of the κ locus in lymphoma cell lines from E_μ-*myc* mice. The diagram shows that transcripts from the germline C_κ locus (top) include a previously described[42] 8-kb unprocessed (nuclear) RNA initiated at a promoter (filled circle) 3.2 kb upstream from $J_\kappa 1$ and a proposed spliced ~1.1-kb product (κ°) derived from it (see text), while an assembled $V_\kappa J_\kappa C_\kappa$ gene yields 1.1-kb κ mRNA. *a*, C_κ-bearing transcripts in the tumour lines. + and − denote cultures grown for 24-48 h in the presence (+) or absence (−) of 20 μg ml^{-1} bacterial LPS. The C_κ probe was probe *C* (top), a subcloned 1.5-kb *Hin*dIII/*Bgl*II fragment. κ_f° denotes a 0.8-kb C_κ-bearing transcript proposed to be derived from a germline allele (see text), while $P_\kappa{}^\circ$ denotes the unprocessed precursor of authentic κ mRNA. *b*, Transcripts revealed with upstream probes (a mixture of probes *A*+*B* above), showing that an ~1.1-kb poly(A)$^+$ RNA bears upstream sequences. Probe *A* alone also labelled the 1.1- and 8-kb species. Probe *A* is a 1.4-kb *Hin*dIII/*Xba*I fragment and probe *B* a 0.7-kb *Hin*dIII fragment (see Fig. 1 in ref. 42), prepared from a 12.7-kb *Bam*HI clone kindly provided by M. Weigert. These probes are free of C_κ sequences because they did not reveal a 1.1-kb species in WEHI-231 (W231), which contains abundant κ mRNA, nor in several plasmacytoma lines in which both alleles are rearranged (not shown).

Methods. Tumour lines were established in Dulbecco's modified Eagle's medium supplemented with 50 μM 2-mercaptoethanol, 100 μM asparagine and 10% fetal calf serum. Within a few weeks of initiation into culture, the cells were grown in roller bottles and collected while in exponential growth phase (1-2 × 10^6 cells ml^{-1}). Poly(A)$^+$ total cellular RNA was isolated from ~10^9 cells by a method[54] involving digestion with proteinase K and oligo-dT cellulose chromatography. RNA (2 μg) heated for 5 min at 65 °C in buffered 2 M formaldehyde/50% formamide was fractionated electrophoretically on a 1.2% agarose/2 M formaldehyde gel and blotted on to nitrocellulose[55]. Hybridization was carried out overnight in 50% formamide/5 × SSC (SSC is 0.15 M NaCl, 15 mM sodium citrate, *p*H 7.0) at 42 °C, and washing in 2 × SSC at 65 °C.

c-*myc* transcripts (or alter E_μ-*myc* expression), although shorter times might be needed to reveal the c-*myc* stimulation observed in normal B cells[47]. At least three of the cell lines were clearly responsive to LPS, since their growth rate increased and the levels of some immunogloblin RNAs increased slightly (Fig. 4 and our unpublished results). The absence of normal c-*myc* transcripts favours a feedback control model for *myc* expression (see below).

Two classes of *myc* RNA of similar size are apparently made from the E_μ-*myc* insert (Fig. 5, top). One class bears *myc* exon 1 sequences (Fig. 5*c*) and appears to initiate at the normal *myc* promoters P_1 and P_2, judging by S_1 nuclease protection results (W.A., unpublished results). The second class, apparently of

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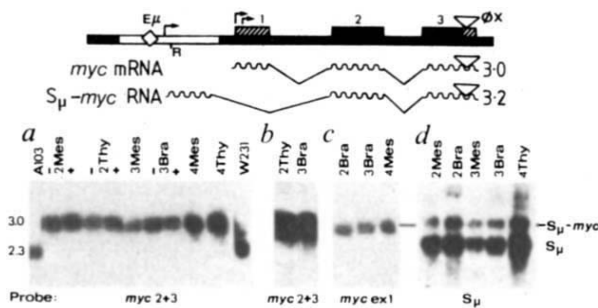

Fig. 5 Transcripts of the *myc* gene in lymphoma cell lines from E_μ-*myc* mice. Proposed transcripts from the E_μ-*myc* insert are shown at the top (see text). *a*, *myc* transcripts in transgenic lines showing absence of the normal ~2.3-kb *myc* mRNA present in ABPC103 (A103) and WEHI-231, whether the cells were treated with LPS (+) or untreated (−). *b*, Longer exposure of two lanes from *a* (3 days rather than 8 h) *c*, Transcripts bearing exon 1 sequences. *d*, Transcripts bearing S_μ sequences, indicating the proposed S_μ-(*myc* exons 2+3) hybrid RNA (S_μ-*myc*). The *myc* 2+3 probe was a 1.7-kb *Xho*I fragement from a murine *myc* complementary DNA clone kindly provided by K. Marcu; *myc* ex 1, a *Bam*HI/*Xho*I fragment from exon 1 (see map in ref. 45); S_μ, a unique-sequence 1.5-kb *Eco*RI/*Hha*I fragment indicated as bar *B* in Fig. 3 (top).

comparable abundance, may have a 5′ segment equivalent to that on certain 'S_μ' RNAs, which bear sequences extending ~0.7 kb downstream from E_μ, spliced to C_μ (ref. 39). A μ switch region (S_μ)-*myc* hybrid transcript would explain the novel ~3.0-kb RNA species that hybridizes in all the tumour lines to an S_μ probe (Fig. 5*d*) but not to a C_μ probe (data not shown). Splicing of the ~0.7-kb S_μ sequence to *myc* exon 2 (where the first known splice acceptor occurs), would yield a *myc* RNA marginally larger than the species bearing the normal 0.55-kb *myc* exon 1 (3.2 kb compared with 3.0 kb) and indeed the S_μ-bearing species is slightly larger than that detected by a *myc* exon 1 probe on the same filter. The S_μ-*myc* RNA is unlikely to encode an altered *myc* polypeptide, because all the ATG sequences within the relevant segment of S_μ are followed shortly by stop codons.

Discussion

The novel lymphoid neoplasia described here demonstrates dramatically that subjugation of c-*myc* expression to the immunoglobulin μ or κ enhancer converts this proto-oncogene into a potent leukaemogenic agent *in vivo*. Presumably the malignancy is a consequence of high constitutive *myc* expression within the lymphoid compartment, forced by these enhancers, which are activated by lymphoid factors[21–23,27,28]. In primary transgenic animals, the E_μ-*myc* and E_κ-SV-*myc* constructs induced an equivalent pathology, but the E_μ-*myc* gene gave a higher tumour incidence and somewhat shorter latent period. This might reflect the greater enhancer activity of E_μ over E_κ with certain promoters[28], or a larger pool of susceptible cells, since E_μ is probably activated earlier in lymphoid ontogeny than E_κ.

The predisposition to neoplasia in the transgenic lines of E_μ-*myc* mice is nearly absolute: over four generations, more than 100 mice involving eight lineages have succumbed within a few months of birth. The typical disease is an aggressive multifocal lymphoma/leukaemia involving lymphoid organs, including the bone marrow, and often invading other tissues. To date, all tumours studied in detail are of the B-lymphoid lineage, but the indications that the E_μ enhancer is also active in other haematopoietic cells[7,35,36,38] leave open the possibility that malignancies of T lymphocytes and myeloid cells arise at a lower frequency. The induction of tumours representing pre-B as well as B cells indicates that *myc*-promoted tumorigenicity is not restricted to one stage.

Our observation that the normal endogenous *myc* genes of the E_μ-*myc* tumour lines are silent (Fig. 5) is highly reminiscent of findings regarding *myc* translocations[18–20]: in almost all murine plasmacytomas and Burkitt's lymphomas, only the translocated *myc* allele is active[45,46,48]. One model to account for such results proposes that the normal *myc* gene is subject to negative feedback control by an excess of the *myc* polypeptide or products induced by it[18,25]. Translocation to the *IgH* locus, or the influence of the E_μ enhancer, frees that c-*myc* gene from this restraint, and the ensuing constitutive *myc* expression silences the unaltered allele(s). An alternative model suggests that c-*myc* would not be expressed in a normal cell at the stage of B-cell maturation equivalent to the tumour[19,49]. A corollary of this model is that c-*myc* expression should be tumorigenic only at those stages where it is normally silent. Judging from Burkitt's lymphomas and murine plasmacytomas, the susceptible stage(s) would be relatively late in B-cell ontogeny, either sIg^+ B cells or plasma cells. Our evidence that c-*myc* can participate in the transformation of pre-B cells as well as mature B cells argues against this model but is consistent with feedback control.

It has been possible to argue that the major effect of most *myc* translocations is to dissociate the coding region from a negative regulatory element within *myc* exon 1 or its 5′-flanking region, the immunoglobulin locus being merely a passive bystander caught up because of its propensity for rearrangement (see refs 18–20). We found, however, that a *myc* construct lacking the entire 5′ portion but bearing all the cryptic promoters within intron 1 (*myc* ex 2+3 in Fig. 1) was completely ineffectual *in vivo*, as was the intact *myc* gene, in marked contrast to the E_μ and E_κ constructs. These results bolster the case that the immunoglobulin loci have a positive regulatory input. The puzzle that only a minority of *myc* translocations leave *myc* and E_μ on the same chromosome may be partially resolved by evidence that *IgH* genes remain active *in vivo* even when deletions remove E_μ (ref. 50). If E_μ is needed to initiate but not maintain an active chromatin configuration within the C_H locus, the active C_H locus may suffice for *myc* activation.

A critical issue in oncology is whether a single oncogene can shift a normal cell to the fully malignant state. The potency *in vivo* of the acute retroviruses, such as those bearing v-*myc*, seems to argue that one oncogene suffices, but *in vitro* studies suggest that transformation of normal cells usually requires particular combinations of active oncogenes, such as a *myc* plus a *ras* gene[30]. The nearly invariant leukaemogenesis of E_μ-*myc* mice might be taken as evidence that an active *myc* gene is sufficient for lymphoid neoplasia, but several observations make us think that other events (presumably genetic) influence tumour development. First, the latent period before any overt signs arise is variable and can be as long as 5 months, whereas we would expect E_μ-driven *myc* expression to commence concomitantly with B-cell ontogeny, well before birth. Second, there appears to be a true pre-neoplastic state in these mice, since no malignant cells, as assessed by transplantation, are detectable within the lymphoid organs of young transgenic mice free of overt disease (A.W.H., unpublished results). Finally, we are struck by the fact that many of the tumours are monoclonal (Table 1). Since the E_μ-*myc* gene should be active within the entire B-lymphoid compartment, clonality implies that one lymphocyte acquired a decided growth advantage, probably by some still undefined genetic alteration. Conceivably the background of the mice (C57BL×SJL)F_2 might also have some role, since aged SJL mice are subject to a neoplastic disorder of the lympho-reticular system[51].

If dysregulated c-*myc* expression is insufficient for malignancy, why does it elicit high-frequency tumorigenesis? *myc* expression is normally associated with dividing cells and drops precipitously as the cell enters the resting (G_0) state[18–20]. Thus, constitutive *myc* expression above a certain threshold may promote cell division at the expense of differentiation. The probability that a given cell will proliferate (self-renew) rather than generate differentiated progeny may be set by the level of c-*myc*, or a balance between *myc* and differentiation-promoting elements. This model predicts that excess *myc* expression in a given

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lineage will increase the proportion of cells that are less mature and therefore have greater proliferative capacity. Any accidental event that further increases the proliferation/differentiation ratio in a member of this large vulnerable population then creates the malignant clone.

It already seems clear that the diverse set of B-lymphoid cell lines generated by E_μ-*myc* mice will aid studies on lymphoid ontogeny and the mechanisms that regulate immunoglobulin gene rearrangement and expression. Even more significant, the very high predisposition of these mice to neoplasia provides new opportunities for exploring the pre-neoplastic state. It will be intriguing to discover in what ways the haematopoietic populations have been perturbed by this cellular oncogene freed from its normal restraints.

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Role of acetylcholine receptor subunits in gating of the channel

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The Torpedo *and calf acetylcholine receptors and hybrids composed of subunits from the two species have been produced in* Xenopus *oocytes by the use of the cloned complementary DNAs. Single-channel current measurements indicate that these receptors form channels of similar conductance but with different gating behaviour.*

THE nicotinic acetylcholine receptor (AChR) is a transmembrane protein constituting a ligand-gated ionic channel. In the absence of acetylcholine(ACh), the channel is in the closed state. When ACh is bound to the receptor, the channel opens for a few milliseconds, giving rise to an elementary current pulse[1], the size and duration of which can be observed directly by the patch-clamp technique[2]. Biochemical evidence has shown that the AChR from *Torpedo* electroplax consists of four kinds of homologous subunits assembled in a molar stoichiometry of α_2, β, γ, δ and that the α-subunit carries the ACh-binding site[3-5]. The primary structures of all four subunits of the *Torpedo* electroplax[6-11] and mammalian muscle AChRs[12-17], the γ- and δ-subunits of the avian AChR[18] and the newly found ε-subunit of the calf muscle AChR[19] have been elucidated by cloning and sequencing complementary DNAs or genomic DNAs encoding these polypeptides. The cloned cDNAs encoding the α-, β-, γ- and δ-subunits of the *Torpedo* AChR can be expressed to produce the functional receptor in *Xenopus* oocytes[20,21] and analysis of the functional properties of AChR mutants possessing α-subunits altered by site-directed mutagenesis of the cDNA has enabled functional regions of the subunit molecule to be localized[21].

One possible approach to studying the functional roles of the individual subunits in ion transport and gating of the AChR channel would be to construct hybrid AChR molecules using subunits from different species and to compare their channel properties with those of the parental AChRs. It has been shown recently that the hybrid AChRs composed of the *Torpedo* α-,

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