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5. In our previous studies of hair cell loss after acoustic trauma in young chicks, we reported a gradual increase in the amount of hair cell loss over a period from 10 to 30 days after trauma with no further increase in cell loss after 30 days. In these animals a 125-dB pure tone [5 dB stronger than that used in (4)] was used [E. W. Rubel and B. M. Ryals, *Acta Oto-laryngol.* **93**, 31 (1982)].
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 7. Fourteen 3- to 6-month-old quail were exposed to a 1500-Hz pure tone at 115 dB for 12 hours. After noise exposure they were returned to their cages and allowed to survive for 10, 30, or 60 days. One ear from each animal was analyzed for hair cell number: 10-day survival, $n = 6$; 30-day survival, $n = 5$; 60-day survival, $n = 3$.
 8. Groups of three or four sections (3 μ m thick) were collected at each 100- μ m interval throughout the cochlea, mounted in serial order, and stained with Toluidine Blue. Quantitative analysis of the number of hair cells was performed by viewing each section under a 40 \times planapochromatic oil immersion objective (numerical aperture, 1.0) at a total magnification of $\times 500$. The criteria for the presence of a hair cell were presence of cuticular plate, stereocilia, and cell body.
 9. No statistical difference was found in hair cell size (short hair cells, 11.9 μ m; tall hair cells, 6.06 μ m) between the noise-exposed and control birds [short hair cells, $F(3,52) = 2.30$, $P > 0.05$; and tall hair cells, $F(3,52) = 1.98$, $P > 0.05$]. An additional analysis of cell density by means of scanning electron microscopy ($n = 2$) showed no obvious change in cell density outside the damaged area. Finally, statistical comparisons were made on total number of hair cells. The average total number of hair cells (± 1 SEM) in each group was as follows: control, 275 ± 6.2 ; 10 day, 188 ± 8.5 ; 30 day, 236 ± 18.9 ; and 60 day, 255 ± 8.1 . An F test for simple randomized design showed a significant difference between control and experimental groups: $F(3,16) = 11.73$, $P < 0.001$. Individual comparisons with the t test for differences among several means [J. L. Bruning and B. L. Kintz, *Computational Handbook of Statistics* (Scott, Foresman, Glenview, IL, 1968)] revealed least significant differences at the 0.01 level and at the 0.05 level: between normal controls and the 10-day survival group ($P < 0.01$); between 10- and 60-day survival group ($P < 0.01$); between normal controls and the 30-day survival group ($P < 0.05$); and between the 10- and 30-day survival group ($P < 0.05$). Thus recovery of cells could not be accounted for by migration of undamaged cells into the damaged area.
 10. Ten 4-month-old adult quail were used. Five birds were exposed to the 1500-Hz, 115-dB acoustic trauma, and five birds were used as age-matched controls. Control and experimental birds received two intramuscular injections of [3 H]thymidine daily for 10 days after noise exposure; controls were paired with experimental animals for injections. Each injection consisted of 380 μ Ci of [3 H]thymidine [84 Ci per millimole of [3 H]thymidine in 0.38 ml of sterile distilled water (0.001 Ci/ml) (NEN). This amount corresponds to approximately 2.5 μ Ci per gram of body weight, a dose similar to that used by other investigators studying adult birds (G. D. Burd and F. Nottebohm, *J. Comp. Neurol.* **240**, 143 (1985)].
 11. Cochleae were embedded and sectioned serially as in (8). Sections (3 μ m thick) were mounted on acid-cleaned slides in basal to apical order and prepared for autoradiography by using Kodak MTB-2 emulsion and routine methods. After exposure for 4 to 7

weeks, the autoradiographs were developed in D19 and stained through the emulsion with Toluidine Blue.

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A Point Mutation in the *c-myc* Locus of a Burkitt Lymphoma Abolishes Binding of a Nuclear Protein

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A 20-base pair region in the first intron of the human *c-myc* gene was identified as the binding site of a nuclear protein. This binding site is mutated in five out of seven Burkitt lymphomas sequenced to date. To investigate the protein-recognition region in greater detail, the abnormal *c-myc* allele from a Burkitt lymphoma line (PA682) that carries a t(8;22) chromosomal translocation was used. A point mutation in the binding region of the PA682 *c-myc* DNA abolished binding of this nuclear protein. This protein may be an important factor for control of *c-myc* expression, and mutations in its recognition sequence may be associated with *c-myc* activation in many cases of Burkitt lymphoma.

IN BURKITT LYMPHOMA (BL), THE *c-myc* allele present on the normal chromosome 8 is transcriptionally silent, while the *c-myc* locus located on the translocated chromosome is activated (1). The *c-myc* transcriptional activation has been suggested to result from juxtaposition of the *c-myc* with an immunoglobulin gene regulatory sequence (2), from truncation within the gene (3), or from mutation in the exon I and/or its 5' flanking sequence (4, 5). However, the relative contributions of the above mechanisms to *c-myc* expression in BL remain controversial. Cell line PA682 is an Epstein-Barr virus (EBV)-positive BL cell line derived from a patient with acquired immunodeficiency syndrome (AIDS) (6), which contains a t(8;22) chromosomal translocation. In this cell line the *c-myc* gene has not been rearranged and the translocation occurred at least 16 kb downstream of the *c-myc* gene (7). The *c-myc* gene in PA682 is activated, as demonstrated by ribonuclease (RNase) protection experiments in which transcription of the *c-myc* gene was shown to occur predominantly from the abnormal allele (8). We isolated from PA682 cells two different *c-myc* gene loci corresponding to the normal and abnormal alleles. The two alleles could be distin-

guished by an approximately 40-nucleotide deletion at the 5' end of the first intron as determined by restriction enzyme mapping (7). Clone λ -PA22, representing the abnormal allele, was subcloned, and the nucleotide sequence for the first exon, part of the first intron, as well as 80 bp of the 5' flanking region, was determined (Fig. 1). Comparison of the sequences from the abnormal *c-myc* allele of the λ -PA22 clone with the normal *c-myc* gene (9) revealed mutations (base substitutions as well as single base deletions) scattered throughout the 3' region of the first exon and the 5' region of the first intron (Fig. 1). In addition, intron I contained a 38-bp deletion of a thymidine-rich sequence 9 bp downstream of the exon I splice-donor site.

Since the elongation of the *c-myc* mRNA, initiated at the P1 and P2 promoters located at the 5' end of exon I, has been shown to be blocked in the vicinity of the exon I-intron I boundary (10, 11), we examined the read-through efficiency of *c-myc* transcripts in PA682 cells. If the elongation of the *c-myc* mRNA transcription is attenuated or terminated because of the thymidine runs (T₄ T₃ T₇) present in the first intron, then deletion of the poly T segments should abrogate the block to *c-myc* RNA elongation. We performed nuclear run-off experiments on PA682 cells and used human cord blood (CB) cells immortalized with the strain of EBV derived from PA682 cells as a control. We examined transcription of the first and second exons using single-stranded probes for each DNA strand (Fig. 2B). Both the

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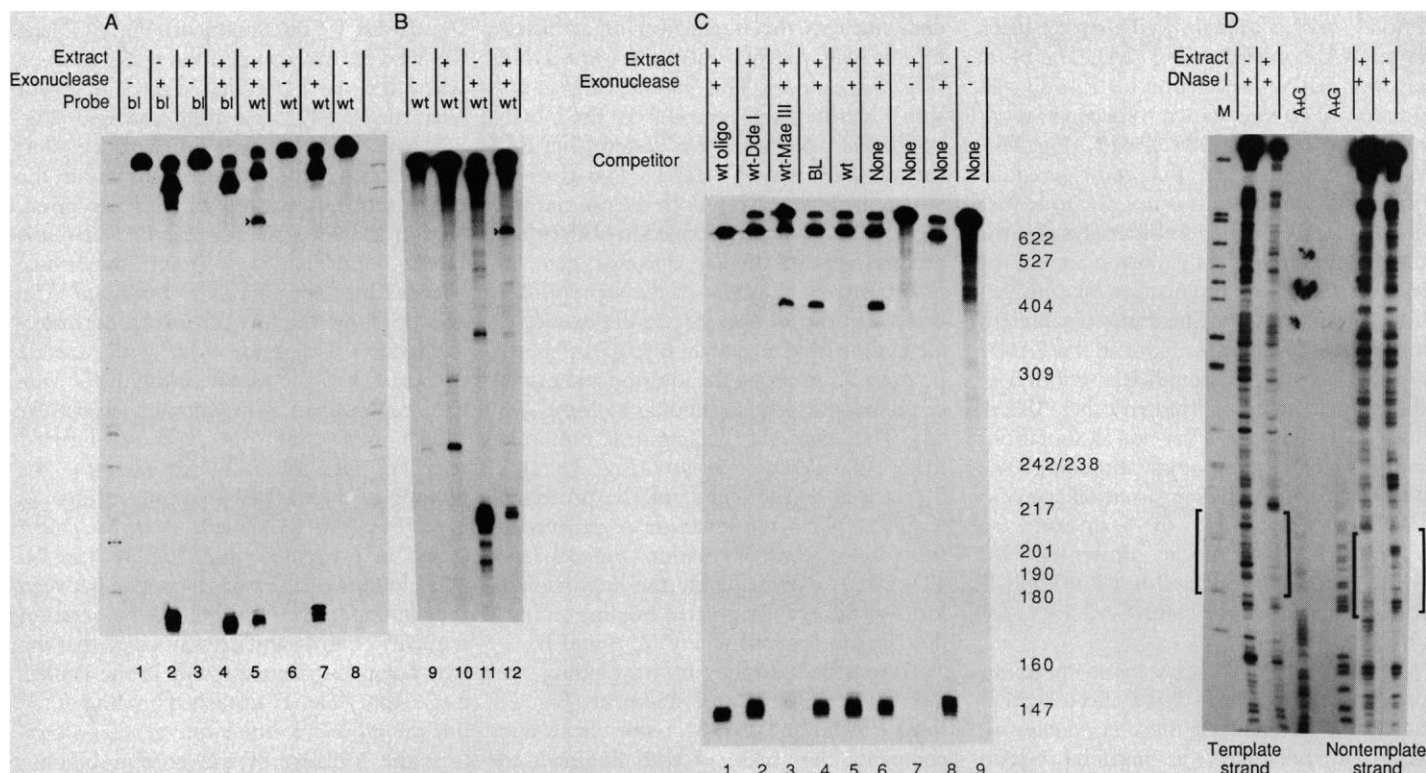


Fig. 3. Binding of a nuclear factor to the first intron of the *c-myc* gene. **(A)** Exonuclease protection assay demonstrating binding of an HL60 nuclear factor to the wild-type (wt) *c-myc* DNA (arrowhead in lane 5 points to the protected fragment) but not to the corresponding DNA from Burkitt lymphoma (bl) PA682 cells (lane 4). An identical protected fragment was obtained when extracts from either differentiated HL60 cell or HeLa cell nuclei were used. The Pvu II–Sst I fragment from normal and abnormal *c-myc* were both cloned into Sma I/Sst I site of the pUC18 vector. The Pvu II–Pvu II restriction fragments, containing the *c-myc* as well as the pUC18 flanking sequences, were 3' end-labeled with 32 P, immobilized to immunobeads by a *lac* repressor– β -galactosidase protein, incubated with 50 μ g of HL60 nuclear extract (30) in the presence of 5 μ g of poly[d(I–C)] and treated with T7 gene 6 exonuclease as described previously (13, 14). A 485-bp DNA fragment was protected from exonuclease hydrolysis. Accounting for 190 bp of pUC sequence, the 5' boundary of the segment protected occurs 295 bp upstream of the Sst I site. **(B)** The Pvu II–Sst I fragment from the wt *c-myc* DNA was cloned into pUC19, which allowed exonuclease hydrolysis to proceed from the opposite orientation. The protected fragment

(arrowhead, lane 12) is 430 bp in length. Subtracting 216 bp of pUC19 sequence from the 430-bp protected segment, we determined that the 3' boundary of the binding site occurs 214 bp downstream of the Pvu II site. **(C)** Competition of protein binding. The probe used was the Pvu II–Sst I fragment of the wt *c-myc* gene cloned in pUC19 and labeled as described in (A). Cold competitors used: lanes 4 and 5, Taq I fragment (Fig. 1) purified from BL and wt *c-myc*, respectively; lanes 2 and 3, Taq I fragment from wt *c-myc* DNA digested with Dde I and Mae III, respectively; lane 1, double-stranded oligonucleotide prepared from sequence spanning the Mae III site (shown in the small box of Fig. 1). For each competition 100 ng of cold DNA was used. **(D)** DNase I footprint analysis of the protein binding site (31). The protected region of 20 bp (shown in brackets) is demonstrated in the presence of HL60 nuclear extract. The Taq I DNA from wt *c-myc* gene, cloned in pUC18 vector (5' \rightarrow 3'), was digested with Hind III and labeled with polynucleotide kinase or DNA polymerase large fragment after Bst NI digestion, thus labeling the coding or noncoding strands, respectively. The fragment was bound to immunobeads, incubated with or without HL60 nuclear extract, and DNase I treated as described (13, 14).

Mae III recognition sequence (shown in a small box on Fig. 1). In an exonuclease protection assay the synthetic double-stranded DNA competed stoichiometrically with normal sequence for protein binding to the *c-myc* locus (Fig. 3C, lane 1). Deoxyribonuclease (DNase) I footprinting analysis demonstrated binding to the same segment of the normal *c-myc* allele defined by exonuclease mapping (Fig. 3D).

We then prepared three synthetic duplex oligonucleotides that contained either one or both point mutations (G \rightarrow A and A \rightarrow T) present in the PA682 *c-myc* sequence (Fig. 4). The mutated and the wild-type (wt) oligonucleotides were used as probes or competitor DNA in gel retardation assays (14) (Fig. 4). When a wt oligonucleotide probe was used with HeLa cell nuclear extract, two closely spaced bands were observed (Fig. 4, lane 1). When wt

oligonucleotide was then used as a competitor, the probe-protein complex was no longer seen (Fig. 4, lane 3). In contrast, the doubly mutated oligonucleotide did not compete binding of the nuclear protein to the synthetic wt probe (Fig. 4, lane 4). Similarly, the oligonucleotide with only the G \rightarrow A point mutation, did not compete binding to the wt oligomer probe (Fig. 4, lane 6), while incomplete competition was observed with the oligonucleotide constructed with the A \rightarrow T point mutation (Fig. 4, lane 5). This suggested that the G \rightarrow A mutation in the palindromic sequence (GTAAC) of the binding site was a critical change that abrogated binding of the nuclear protein.

We have also used the doubly mutated oligonucleotide as a probe in a gel retardation assay (Fig. 4, lanes 7 to 12) where we detected a trace amount of binding (Fig. 4,

lane 7). In contrast, in the exonuclease protection assay, no binding was observed to the 427-bp Pvu II–Sst I fragment derived from the PA682 *c-myc* gene (Fig. 3A). The background binding observed when the mutated oligonucleotide was used in the gel retardation assay may have been due to the higher sensitivity of the assay or to conformational differences between the 20-bp duplex oligonucleotide and the 427-bp DNA fragment. The competition with wt and mutated oligomers and with doubly mutated oligonucleotide as a probe (Fig. 4, lanes 9 to 12) confirmed the specificity of the binding. Comparison of nuclear extracts from undifferentiated HL60 cells and retinoic acid- or dimethyl sulfoxide-treated HL60 cells revealed alterations in the mobilities of the DNA-protein complexes formed, suggesting regulation of the protein binding to this sequence (7).

PA682 and CB cells showed a strong block in mRNA elongation (Fig. 2A). The block of *c-myc* mRNA elongation for PA682 cells was similar to that shown to occur in retinoic acid-induced HL60 cells (10) (Fig. 2A). We therefore conclude that *c-myc* deregulation in BL PA682 cells is not due to release of the mRNA elongation block and that the thymidine runs in the first intron are not the sole signal for the attenuation or termination of transcription. The mutations present in the exon I-intron I region of the PA682 do not allow read-through transcription of the *c-myc* gene, in contrast to other BL cell lines (12), indicating that not all mutations in this region abolish attenuation. All *c-myc* transcription detectable in a run-off assay for PA682 was sensitive to α -amanitin (2 μ g/ml) (7). In addition, antisense *c-myc* transcription was detected in exon I and II for BL PA682 and the control cell lines used (10, 11) (Fig. 2A).

To analyze if the other somatic mutations present in PA682 may have affected other functional elements of the *c-myc* gene, we asked whether there was interference with the binding of nuclear proteins to the *c-myc* exon I-intron I region. By means of an exonuclease protection procedure that allows the screening of large DNA fragments for site-specific protein binding (13-15), we

demonstrated the interaction of a nuclear protein with normal intron I *c-myc* DNA (Fig. 3A, lane 5). This interaction was not seen when the same region from the mutated PA682 *c-myc* allele was tested (Fig. 3A, lane 4). We used the 427-bp Pvu II-Sst I fragment (Fig. 1) from both the normal and the PA682 *c-myc* allele cloned in both orientations next to the *lac* operator gene of *Escherichia coli*. Binding of the *lac* repressor protein to the *lac* operator before exonuclease hydrolysis served as an internal reference point in determining the location and extent of the nuclear protein binding to *c-myc* (14, 15). The length of the fragment protected from exonuclease hydrolysis (Fig. 3A, lane 5), as well as the length of the protected fragment when the exonuclease proceeded from the opposite orientation (Fig. 3B, lane 12), allowed us to define the approximate locus of the cellular protein binding site (as described in legends to Fig. 3, A and B).

To test the specificity of this binding, we purified a 183-bp Taq I fragment (Fig. 1) from normal and PA682 *c-myc* alleles and compared the ability of each fragment to compete in an exonuclease protection assay with binding to the Pvu II-Sst I fragment from the normal *c-myc* locus. The 183-bp purified Taq I DNA from the normal *c-myc* locus fully competed binding to the labeled

Pvu II-Sst I *c-myc* fragment (Fig. 3C, lane 5), whereas no competition was observed when the corresponding Taq I fragment from the PA682 *c-myc* allele was used (Fig. 3C, lane 4). Two restriction enzyme sites, Mae III and Dde I, were present within the DNA sequence where binding occurred. Within the Mae III site the PA682 *c-myc* DNA contained two point mutations, whereas the Dde I site was unaltered. The 183-bp Taq I fragment from the normal *c-myc* locus was digested either with Mae III or Dde I, and the digested fragments were used as competitors in binding assays. As shown in Fig. 3C, the Mae III-digested fragment lost its ability to compete the binding of the nuclear protein to normal *c-myc* DNA (Fig. 3C, lane 3), whereas Dde I digestion showed no effect (Fig. 3C, lane 2). These results established an overlap between the binding site and the Mae III recognition sequence (Fig. 1) and demonstrated that the two point mutations present in the PA682 *c-myc* clone (Fig. 1) abolished binding of the nuclear protein to the *c-myc* gene. To confirm the location of the protein binding sequence, we constructed a synthetic 20-bp duplex oligonucleotide encompassing the

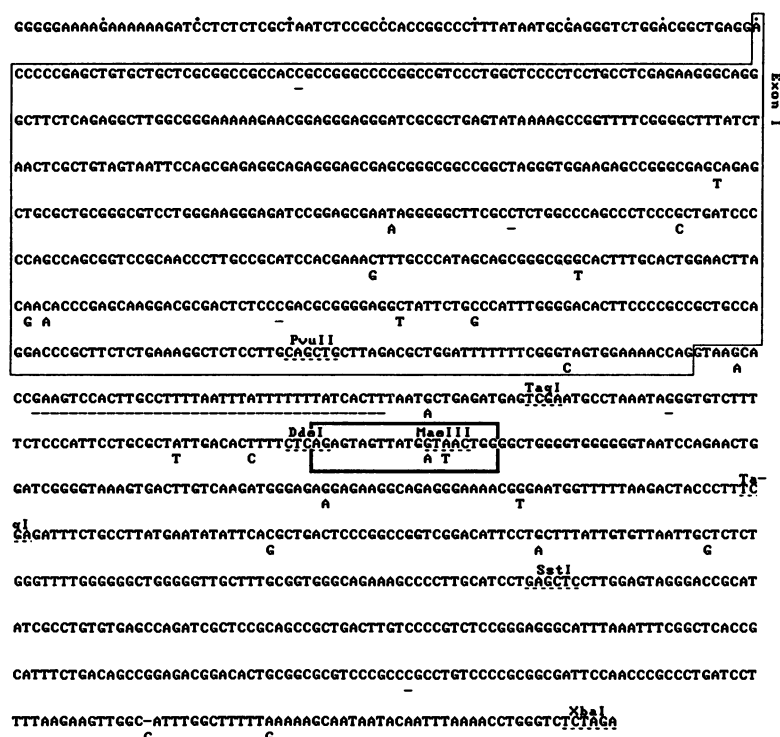


Fig. 1. Comparison of Burkitt lymphoma, PA682, and normal *c-myc* sequences (9). Bal 31-derived DNA fragments from the PA682 *c-myc* gene were cloned into M13 vectors, and the nucleotide sequence of both strands was determined (26). The top line shows the sequence from exon I and intron I of the normal *c-myc* locus while the letters below the line display differences in the PA682 sequence. The dashed lines indicate base-pair deletions in the PA682 cell lines. The first exon is enclosed in a large box. The protein binding sequence is shown in the small box. Restriction enzyme sites used in the analysis of the protein binding site are indicated.

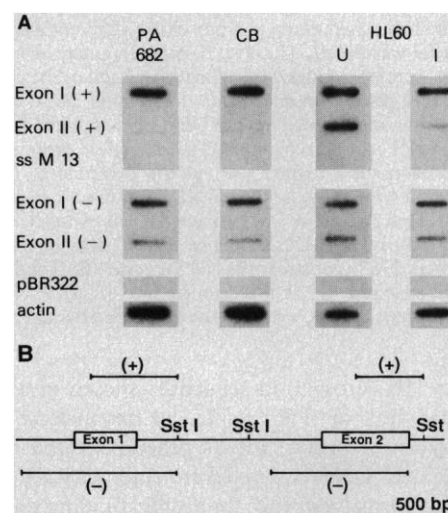


Fig. 2. (A) The *c-myc* nuclear run-off transcription from Burkitt lymphoma PA682 and CB cells. HL60 cells untreated (U) and induced (I) for 2.5 days in 1 μ M retinoic acid were used as controls (10). *C-myc* probes marked (+) detect sense transcripts and (-) detect antisense transcripts. M13 phage DNA alone, actin in pBR322 (27), and pBR322 alone were used as controls. (B) Map of the *c-myc* exon I and exon II. PA682 DNA probes shown were cloned in M13 vectors. Fragments marked (+) detect sense transcripts. Exon I (+) is 720 bp, exon II (+) is 600 bp. DNA fragments marked (-) detect antisense transcripts. Exon I (-) is 1.35 kb and exon II (-) is 1.2 kb. The exon I (+) and exon II (+) probes contain 22 and 18.6% thymidine, respectively. Nuclei were prepared from PA682, CB, and HL60 cells as described previously (28). The run-off transcription was performed as described (28, 29).

Fig. 4. DNA gel retardation assay (14) confirms binding of a nuclear protein to a wild-type oligonucleotide spanning the DNA binding sequence. Duplex oligonucleotides were synthesized from sequence encompassed in the small box shown in Fig. 1. The wild-type oligonucleotide (wt or w) represents normal *c-myc* sequence (9, 32), whereas bl represents sequence from PA682 containing two distinct point mutations within the binding site. Duplex oligomer, 0.4 ng ³²P end-labeled, was incubated either with (+) or without (–) HeLa nuclear extracts (25 µg) (30) in presence of 5 µg of poly[d(I-C)]. Competitor duplex oligonucleotides (50 ng) were included in the incubations as shown; bl 1 + 2 (same as bl), oligomer carrying both G → A and A → T point mutations; bl-1 and bl-2 oligomers constructed with G → A or A → T mutations, respectively. The free probe was run off the gel to better resolve the retarded doublet. The band that migrates 40% down the gel is reproducibly seen in all the experiments and is not competed by the mutant or wild-type *c-myc* intron I sequence.

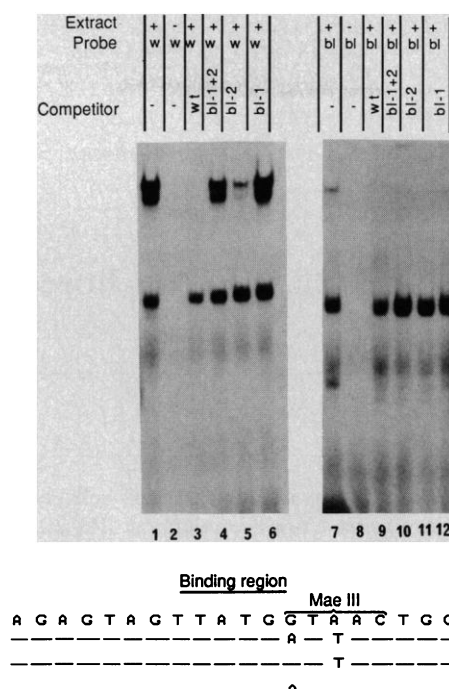


Fig. 5. Nucleotide sequence comparison of the wild-type DNA binding sequence from the first intron of the *c-myc* gene with the analogous region from seven independently derived Burkitt lymphoma cell lines. Pertinent regions from *c-myc* clones from BL37 and LY47 were sequenced with synthetic oligonucleotide primers.

Cell line	Binding region	Translocation	Ref.
Normal	A G A G T A G T T A T G G T A A C T G G	(None)	(9,32)
BL37	----- A - T -----	(8;22)	(19)
Daudi	----- T -----	(8;14)	(5)
PA682	----- T -----	(8;22)	this report
BL2	----- T -----	(8;22)	(16)
KK124	----- T -----	(8;22)	(17)
Raji	--- A --- C C T --- G ---	(8;14)	(18)
LY47	--- A --- G T C ---	(8;22)	(20)

To determine whether point mutations in this 20-bp region of intron I were present in other cases of BL, we reviewed all previously published *c-myc* intron I sequences. We found four BL cell lines [Daudi (5), BL2 (16), KK124 (17), and Raji (18)] for which intron I sequence containing the binding site was available. All cell lines, with the exception of Daudi, contained point mutations located precisely within the binding site we have identified (Fig. 5). Two BL cell lines, BL2 (16) and KK124 (17), each contained only two point mutations in a 300-bp region spanning the 5' end of the first intron, and in each cell line one of the point mutations was located within the protein binding sequence. In addition, we have sequenced the binding region from two other BL clones, BL37 (19) and LY47 (20), and found mutations in the binding sequence of the LY47 *c-myc* clone, but not in the BL37 *c-myc* DNA (Fig. 5). Therefore, out of seven BL *c-myc* clones whose first intron sequences we were able to analyze, five contain mutations precisely within the binding site we have identified. This suggests that specific mutations in the cognate sequence may be a frequent feature associated with *c-*

myc activation in these BL cell lines.

How protein binding in the midst of intron I may influence *c-myc* expression remains unknown. It was recently proposed that the 5' half of the first intron contains sequences that compete for one or more putative negative regulatory factors (21). Thus, the nuclear protein we identified may act as a negative element in the regulation of *c-myc* expression. This protein may also interact with the RNA synthetic apparatus at the initiation site of transcription and alter *c-myc* expression in an orientation- and position-independent manner similar to silencers (22) and enhancers (23). Alternatively, this protein could act synergistically or antagonistically with *c-myc*-specific cis and trans elements (15, 21) in which case the intron factor activity would be apparent only in the context of additional *c-myc* cis-acting elements. The magnitude of the mRNA elongation block present in BL PA682 cells (Fig. 2A) indicates that the intron I factor probably plays no significant role in the attenuation or termination signal. Antisense transcription which, in general, could provide an extremely sensitive means of regulating gene expression, particularly for threshold all-or-

none events (24), has been observed for *c-myc* as shown in Fig. 2A. The *c-myc* exon I antisense transcription may require cis and trans elements present in intron I. In addition, DNA sequences have been shown recently to influence posttranscriptional events, and such a role of the protein and binding site described here cannot be excluded (25). The purification and characterization of this nuclear protein may allow us to better understand its role in the regulatory control of the *c-myc* gene.

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Restricted Lateral Diffusion of PH-20, a PI-Anchored Sperm Membrane Protein

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The rate of lateral diffusion of integral membrane proteins is constrained in cells, but the constraining factors for most membrane proteins have not been defined. PH-20, a sperm surface protein involved in sperm-egg adhesion, was shown to be anchored in the plasma membrane by attachment to the lipid phosphatidylinositol and to have a diffusion rate that is highly restricted on testicular sperm, being more than a thousand times slower than lipid diffusion. These results support the hypothesis that lateral mobility of a membrane protein can be regulated exclusively by interactions of its ectodomain.

THE LATERAL DIFFUSION COEFFICIENTS (D) of most integral membrane proteins are much smaller than would be predicted on the basis of hydrodynamic size alone. Interactions beyond those mediated by lipid bilayer viscosity must be at work. The first indication of the possible nature of these interactions came out of studies on erythrocyte membranes; this work demonstrated the constraints on mobility imposed by interactions of cytoplasmic portions of the proteins with underlying cytoskeletal structures (1). Studies that made use of artificial lipopolysaccharides and genetically engineered plasma membrane proteins with shortened cytoplasmic domains indicated that noncytoplasmic interactions might be responsible for restricting the lateral diffusion of membrane molecules (2-4). The discovery that some proteins are anchored to the outer leaflet of the lipid bilayer by phosphatidylinositol (PI) rather than by a membrane-spanning stretch of hydrophobic amino acids suggested the possibility of measuring the mobility of proteins that are completely free from direct interaction with the cytoskeleton. Measurements on Thy-1, alkaline phosphatase, and decay accelerating factor, all PI-anchored, show that these proteins are freely diffusing at rates comparable to those of lipid probes (5-7). We show that the guinea pig sperm

protein PH-20 is anchored in the plasma membrane by PI but that its rate of diffusion is highly restricted on testicular sperm. Because PH-20 is localized to surface domains at some stages of sperm differentiation, these findings also show that direct interaction with the cytoskeleton is not required for membrane protein localization.

The PH-20 membrane protein is involved in sperm adhesion to the glycoprotein coat of the egg, the zona pellucida, during the initial steps of fertilization (8). A potential connection has been pointed out between the function of certain proteins involved in cell adhesion and PI-anchoring in the membrane (9, 10). To determine whether PH-20 is anchored in the bilayer by PI, we treated cells treated with a PI-specific phospholipase C (PI-PLC), which has been shown to cleave PI-anchored plasma membrane proteins from other cells (9-13). Sperm were surface-iodinated and the release of PH-20 into the medium after treatment with PI-PLC was detected by immunoprecipitation (14, 15). Sperm at three progressive stages of differentiation were used: (i) testicular sperm, (ii) cauda epididymal sperm before sperm exocytosis (the acrosome reaction), and (iii) cauda epididymal sperm after the acrosome reaction. PH-20 was immunoprecipitated from the supernatants of sperm at all three stages after treatment of the cells with PI-PLC; little or no PH-20 protein was detected in the supernatants of untreated controls (Fig. 1).

A comparison of ^{125}I counts found in immunoprecipitable PH-20 released by PI-PLC into the medium with the total PH-20 extracted by NP-40 from untreated testicu-

lar sperm indicates that ~60% of the protein is released by 1 hour of exposure to PI-PLC. Incomplete release from intact cells might result from inaccessibility of some of the protein molecules to the enzyme, or it could indicate a population of PH-20 that is either not PI-anchored or has a PI anchor that is insensitive to this particular PI-PLC. Other PI-anchored proteins have also

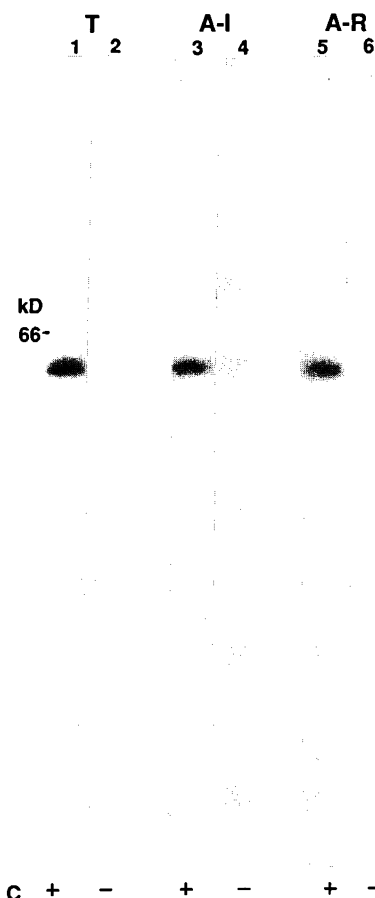


Fig. 1. Immunoprecipitation of PH-20 released from the surface of guinea pig sperm after exposure to PI-PLC. (Lanes 1 and 2) Immunoprecipitates of supernatants from 3×10^6 testicular (T) sperm per lane. (Lanes 3 and 4) Supernatants from 2×10^7 acrosome-intact (A-I) epididymal sperm per lane. (Lanes 5 and 6) Immunoprecipitates of supernatants from 2×10^7 acrosome-reacted (A-R) epididymal sperm per lane. Lanes 1, 3, and 5 are from sperm exposed to PI-PLC; lanes 2, 4, and 6 are from control sperm incubated without PI-PLC. Male Hartley guinea pigs (>700 g) were killed by CO_2 asphyxiation, and live sperm at 1×10^7 to 7×10^7 per milliliter were surface-iodinated with 1 to 4 mCi of Na^{125}I with Iodogen (15). The labeled cells were washed with Mg^{2+} -Hepes buffer and evenly distributed to each of two flasks containing either buffer or buffer plus *Bacillus thuringiensis* PI-PLC (13) for 1 hour at 37°C . PH-20 was immunoprecipitated from the separated supernatants with either a rabbit polyclonal antiserum to PH-20 plus protein A-Sepharose beads or with a monoclonal antibody (PH-22) bound to Sepharose 4B beads. After boiling and removal of the beads, the samples were run on 10% SDS-polyacrylamide gel electrophoresis (24, 25).

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