"effective" Stokes radius can be calculated or determined empirically (*24*). A dextran polymer of 10,000 daltons would have an effective Stokes radius of 2.4 nm, whereas the radius of an 18,000-dalton dextran polymer would be approximately 3.1 nm. On this basis, the molecular size exclusion limit (Stokes radius) of a plasmodesma in control tobacco plants would be approximately 0.73 nm, whereas that of plasmodesmata in plants that express the 30-kD MP would be greater than 2.4 nm but less than 3.1 nm. Since Gibbs (*12*) has suggested that the molecular dimensions of TMV RNA may be approximately 10 nm, additional information is needed before we can account for the role of the MP in mediating systemic spread of TMV.

REFERENCES AND NOTES

1. B. E. S. Gunning, in *Intercellular Communication in Plants: Studies on Plasmodesmata*, B. E. S. Gunning and A. W. Robards, Eds. (Springer-Verlag, Berlin, 1976), pp. 1–13; ——— and A. W. Robards, in *Transport and Transfer Processes in Plants*, I. F. Wardlaw and J. B. Passioura, Eds. (Academic Press, New York, 1976), pp. 15–41.
2. M. G. Erwee and P. B. Goodwin, *Planta* **158**, 320 (1983); *Protoplasma* **122**, 162 (1984); ———, A. J. E. van Bel, *Plant Cell Environ.* **8**, 173 (1985); M. G. Erwee and P. B. Goodwin, *Planta* **163**, 9 (1985); D. G. Fisher, *Plant Cell Environ.* **11**, 639 (1988); K. J. Oparka and D. A. M. Prior, *Planta* **176**, 533 (1988).
3. W. W. Stewart, *Nature* **292**, 17 (1981).
4. W. R. Loewenstein and B. Rose, *Ann. NY Acad. Sci.* **307**, 285 (1978).
5. M. A. Madore, J. W. Oross, W. J. Lucas, *Plant Physiol.* **82**, 432 (1986).
6. E. B. Tucker, *Protoplasma* **113**, 193 (1982).
7. B. A. Palevitz and P. K. Helper, *Planta* **164**, 473 (1985).
8. J. Flagg-Newton, I. Simpson, W. R. Loewenstein, *Science* **205**, 404 (1979).
9. B. R. Terry and A. W. Robards, *Planta* **171**, 145 (1987).
10. K. Esau, Ed., *The Phloem: Handbuch der Pflanzenanatomie* (Gebrueder Borntraeger, Berlin, 1969).
11. E. W. Kitajima and J. A. Lauritis, *Virology* **37**, 681 (1969).
12. A. Gibbs, in *Intercellular Communication in Plants: Studies on Plasmodesmata*, B. E. S. Gunning and A. W. Robards, Eds. (Springer-Verlag, Berlin, 1976), pp. 149–164.
13. A. V. Allison and T. A. Shalla, *Phytopathology* **64**, 784 (1974); M. Weintraub, H. W. J. Ragetli, E. Leung, *J. Ultrastruct. Res.* **56**, 351 (1976).
14. C. M. Deom, M. J. Oliver, R. N. Beachy, *Science* **237**, 389 (1987).
15. T. Meshi *et al.*, *EMBO J.* 6, 2557 (1987).
16. M. Nishiguchi, F. Motoyoshi, N. Oshima, *J. Gen. Virol.* **39**, 53 (1978); *ibid.* **46**, 497 (1980).
17. I. Simpson, *Anal. Biochem.* **89**, 304 (1978).
18. U. Pick, *Arch. Biochem. Biophys.* **212**, 186 (1981).
19. Transgenic tobacco plant line 277 (expressing the 30-kD protein) and line 306 (harboring the intermediate plasmid only) were used for all experiments. Seeds were germinated and plants were grown for 3 weeks in an insect-free greenhouse and then transferred to a growth chamber for 1 to 2 weeks before the measurements. Temperature regime in the growth chamber was 27°/18°C (day/night) with 16 hours of light.
20. S. Wolf and W. J. Lucas, unpublished results.
21. E. B. Tucker and R. M. Spanswick, *Protoplasma* **128**, 167 (1985).
22. J. G. Atabekov and Y. L. Dorokhov, *Adv. Virus Res.* **29**, 313 (1984).
23. W. J. Lucas and T. C. Pesacreta, unpublished results.
24. T. C. Laurent and J. Killander, *J. Chromatogr.* **14**, 317 (1964); R. H. Pearce and B. J. Grimmer, *ibid.* **151**, 548 (1978); P. G. Squire, *ibid.* **210**, 433 (1981); E. Poitevin and P. Wahl, *Biophys. Chem.* **31**, 247 (1988).

25. Supported by grants from the National Science Foundation to R.N.B. (DMB 87017012) and W.J.L. (DMB 8703624).

12 June 1989; accepted 5 September 1989

The Gene for Enhancer Binding Proteins E12/E47 Lies at the t(1;19) Breakpoint in Acute Leukemias

JULIA D. MELLENTIN, CORNELIS MURRE, TIMOTHY A. DONLON, PATRICK S. MCCAW, STEPHEN D. SMITH, ANDREW J. CARROLL, MARCY E. MCDONALD, DAVID BALTIMORE, MICHAEL L. CLEARY*

The gene (*E2A*) that codes for proteins with the properties of immunoglobulin enhancer binding factors E12/E47 was mapped to chromosome region 19p13.2–p13.3, a site associated with nonrandom translocations in acute lymphoblastic leukemias. The majority of t(1;19)(q23;p13)–carrying leukemias and cell lines studied contained rearrangements of *E2A* as determined by DNA blot analyses. The rearrangements altered the *E2A* transcriptional unit, resulting in the synthesis of a transcript larger than the normal-sized *E2A* mRNAs in one of the cell lines with this translocation. These observations indicate that the gene for a transcription factor is located at the breakpoint of a consistently recurring chromosomal translocation in many acute leukemias and suggest a direct role for alteration of such factors in the pathogenesis of some malignancies.

CHROMOSOMAL TRANSLOCATIONS are implicated as important events in the pathogenesis of tumors of the hematopoietic lineage. The majority of acute lymphoblastic leukemias (ALLs) carry such karyotypic abnormalities, although more than 15 different consistently recurring rearrangements have been described (*1*). One of the most frequently reported cytogenetic changes in ALL is the t(1;19)(q23;p13.3) chromosomal translocation, first reported in 1984 and subsequently observed in up to 6% of pediatric acute leukemias and in approximately 30% of leukemias with a pre-B cell phenotype (*2*). The association of this translocation with a particular leukemia phenotype has prompted investigations into the identity of genes that may map to the breakpoint loci on chromosomes 1 and 19 (*3*). Despite suggestive chromosomal localizations, neither the insulin receptor gene at 19p13.2–p13.3 nor the proto-oncogene c-*ski* at 1q22–24 has been shown to map to the breakpoint in the t(1;19) translocation. We now report that the gene for a transcription factor that appears to regulate immunoglobulin kappa light chain gene expression is located at the breakpoint on chromosome 19 and, furthermore, we demonstrate that this gene is structurally altered by most t(1;19) chromosomal translocations.

Various trans-acting factors and sequence motifs required for tissue-specific expression of the immunoglobulin genes have been described (*4*). One class of such sequences is the "E-box motif," found in both the heavy chain and kappa light chain enhancers (*5–7*); trans-acting factors that interact with the E-box sites both in vivo and in vitro have been identified (*5, 6*). In the enhancer region of the kappa light chain gene, E-box elements are required for optimal transcription and have been shown to bind distinct proteins in vitro (*6, 7*). Two cDNAs (*E12/E47*) that encode proteins that bind to the kappa E-box site kE2 have been isolated by an oligonucleotide screening procedure (*8*) and have been found to be derived from one gene, which has been called *E2A* (*9*). The cDNAs code for nearly identical proteins, which contain a region that appears to represent a helix-loop-helix DNA binding motif and a dimerization domain. This region is similar to regions in several proteins that participate in the control of differentiation and prolif-

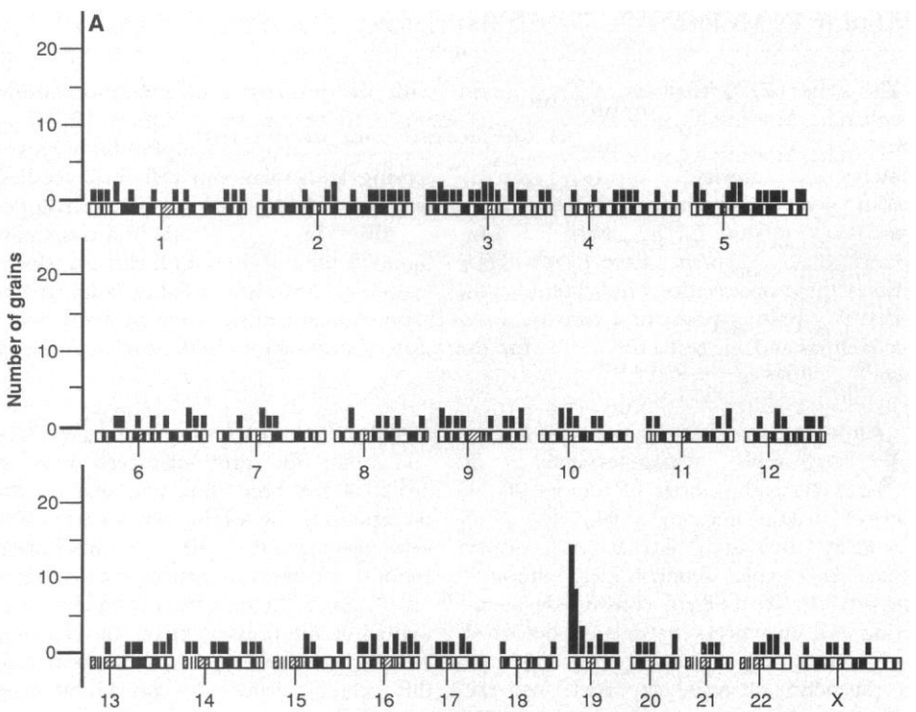
J. D. Mellentin, T. A. Donlon, M. L. Cleary, Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94025.
C. Murre, P. S. McCaw, D. Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
S. D. Smith, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94025.
A. J. Carroll, Laboratory of Medical Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, and Pediatric Oncology Group, St. Louis, MO 63108.
M. E. McDonald, Neurogenetics Laboratory, Massachusetts General Hospital, Boston, MA 02114, and Department of Genetics, Harvard Medical School, Boston, MA 02115.

*To whom correspondence should be addressed.

eration in various cellular lineages (*8, 10, 11*). Because several members of this group, the Myc proteins and Lyl-1, are associated with genetic rearrangements and abnormalities of growth control in various neoplasms (*11, 12*), our studies were undertaken to ascertain the potential role of *E2A* in similar processes.

To determine the chromosomal localization of *E2A*, we carried out in situ hybridization to human chromosomes (*13*) with a ^{3}H-labeled subclone (pE47M) containing a 847-bp insert fragment that codes for 283 COOH-terminal amino acids of the *E2A*-encoded protein E47, including the helix-loop-helix DNA binding motif and dimerization domain (*8*). Of 34 grains observed on chromosome 19, 25 (or 74%) were located on bands 19p13.2–p13.3, with a peak on the distal subband 19p13.3 (Fig. 1A). In addition, out of 206 chromosomal grains observed in 100 metaphase cells, 25 (or 12.1%) were found over 19p13.2–p13.3, and no other sites were labeled above background.

The chromosomal assignment of the gene detected by pE47M was confirmed by DNA blot analysis of rodent-human somatic cell hybrids carrying fragments of human chromosome 19 with well-characterized breakpoints (*14*) (Fig. 1B). Analysis of Eco RI–digested DNA showed two bands of 23 and 11 kb homologous to pE47M in human genomic DNA (Fig. 1B, lane 1). The 23-kb band was present in somatic cell hybrids containing DNA from human chromosome 19q13.2-19pter (Fig. 1B, lanes 2 and 3). This band was not observed in DNA from hybrid cells carrying human chromosome 19p13.2-19qter and 19q (Fig. 1B, lanes 4 and 5). These results are consistent with the in situ hybridization data, which localized pE47M-homologous sequences to 19p13.2–p13.3.



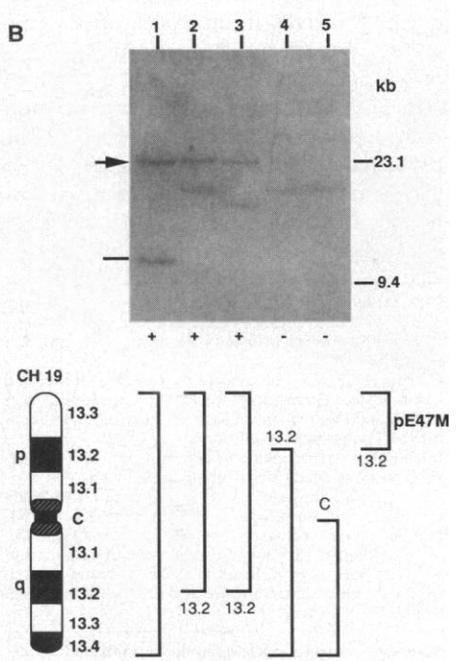


Fig. 1. Localization of *E2A*. (**A**) Histogram showing the distribution of silver grains over the human chromosome complement. Twenty-three percent of the metaphases contained label over at least one chromosome 19 at bands p13.2–p13.3. Conditions and methods used for in situ chromosomal hybridizations were as described (*13*). (**B**) Hybridization of pE47M probe to DNA from human-rodent hybrid cell lines. Cell lines were derived as described (*14*). The human chromosome 19 (CH 19) content of each cell line is indicated by brackets below the autoradiogram (C stands for centromere). Eco RI–digested DNA (10 μg) was applied to all lanes, and DNA blots were hybridized with pE47M as described (*22*). The pE47M-homologous band in the autoradiogram that localizes to chromosome 19 is denoted by an arrow, and its presence (+) or absence (−) is indicated below each lane. An additional cross-hybridizing band not present on chromosome 19 and observed only in lane 1 is denoted by a dash. Faint bands at approximately 16 and 18 kb represent cross-hybridizing hamster and mouse bands, respectively. Right-hand bracket indicates smallest region of chromosome 19 containing detectable pE47M-hybridizing DNA. Lane 1, total human DNA; lanes 2 to 5, somatic cell hybrids CF100-1, G35F3, G24A9, and CF104-22, respectively (*14*).

The 11-kb Eco RI band observed in total human DNA (Fig. 1B, lane 1) does not map to chromosome 19 because it was not seen in the somatic cell hybrids (Fig. 1B, lanes 2 to 5). Thus the presence of an additional pE47M-homologous human sequence that maps to a site other than chromosome 19 is indicated. This secondary site may not have been observed in the chromosomal in situ hybridization experiments because of a higher stringency of hybridization (*13*). Additional studies indicate that the 23-kb Eco RI band contains *E2A* and that the 11-kb Eco RI band contains a cross-hybridizing pseudogene (*9, 15*). *E2A* appears to be highly conserved in rodents because pE47M cross-hybridized with a single band of approximately 16 or 18 kb present in the somatic cell hybrid DNAs (Fig. 1B, lanes 2 to 5) but not in human DNA.

To determine whether *E2A* mapped to the breakpoint on chromosome 19 in t(1;19)(q23;p13) chromosomal translocations, we performed blot analysis on DNA obtained from nine leukemia specimens and two cell lines that carry t(1;19) cytogenetic abnormalities (*16–18*). Using pE47M as a probe, we observed DNA rearrangements in all but one of these DNAs after digestion with either Eco RI, Bam HI, or Hind III (Fig. 2 and Table 1). Although germline DNA was not available from the individuals from which the leukemia specimens came, our inability to detect non-germline bands in an equal number of acute leukemia DNAs lacking cytogenetic alterations of chromosome 19 (*19*) strongly suggests that the observed rearrangements were not inherited polymorphisms, but rather resulted from translocation breakpoints within or near *E2A* in each of the leukemias.

In most cases, only a single non-germline pE47M-homologous fragment was observed; however, DNA prepared from the leukemic cells of patient 3 and the cell line SUP-B27 had two rearranged fragments.

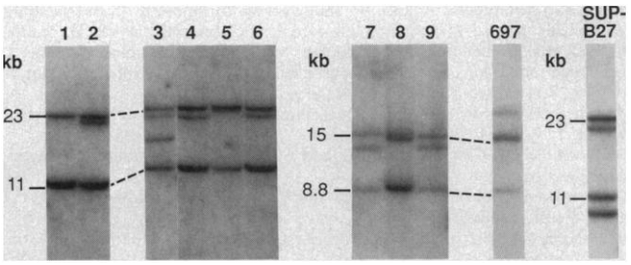


Fig. 2. Hybridization analysis of various cell line and leukemia DNAs. The DNAs were subjected to DNA blot analysis with the *E2A* cDNA subclone pE47M as a hybridization probe. DNAs from specimens 7 to 9 and cell line 697 were analyzed after Bam HI digestion; all other DNAs were digested with Eco RI. Dashes indicate germline bands of 15 and 8.8 kb for Bam HI and 23 and 11 kb for Eco RI. Fragments observed in non-germline positions represent rearrangements of pE47M-homologous DNA. Designations above the lanes refer to patients and cell lines (*16–18*) as shown in Table 1.

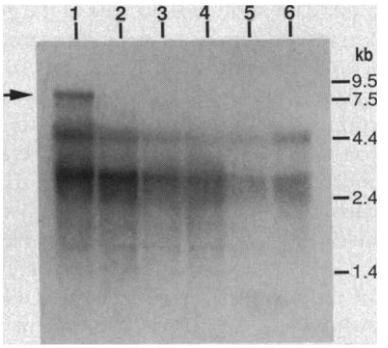


Fig. 3. RNA blot analysis of *E2A* mRNA in various lymphoid cell lines. Polyadenylated mRNAs were size-fractionated in a formaldehyde-agarose gel, transferred to a nylon membrane, and hybridized with the pE47M cDNA probe (*22*). An 8.3-kb mRNA present in cell line SUP-B27 is indicated with an arrow. Lane 1, t(1;19)–carrying cell line SUP-B27; lanes 2 to 4, B cell lineage lymphoma lines FL18, SU-DUL-5, and SU-DHL-4, respectively; lanes 5 and 6, T cell lineage ALL lines SUP-T7 and SUP-T8, respectively. Derivation and characterization of cell lines have been described (*17, 18, 23*). Size standards consisted of a ladder of phage λ–homologous marker RNAs (Bethesda Research Laboratories).

These two cases were the only ones that carried balanced t(1;19) translocations (Table 1). All other analyzed samples carried the more common form of t(1;19), whereby the derivative chromosome 1 had been lost by the leukemia cells and only the derivative chromosome 19 was retained (*1, 2*). Thus, there was complete correlation between the DNA blot data and the karyotype data, indicating that both products of the translocation were detected as rearranged DNA fragments for the two cases in which they were present. The fact that pE47M hybridized to both derivative chromosome 1 and 19 translocation products in cases with a balanced t(1;19) indicates that the breakpoint occurred within the portion of *E2A* detected by this probe, thereby resulting in a structural interruption of the gene. Furthermore, because pE47M consisted of a cDNA fragment coding for 283 COOH-terminal amino acids and no untranslated portions of the E47 mRNA (*8*), the data indicate that the predicted protein must also be structurally altered following some, if not all, t(1;19) translocations.

To further investigate this possibility, we examined the transcriptional activity and products of *E2A* in several different cell lines. Blot analysis of RNAs hybridized with pE47M showed two major *E2A* transcripts of 2.9 and 4.4 kb in all lymphoid cell lines examined (Fig. 3). However, in the t(1;19)–carrying cell line SUP-B27, an additional RNA of approximately 8.3 kb was also observed. The presence of an abnormal-sized RNA in SUP-B27 is consistent with the DNA blot data indicating that the *E2A* transcriptional unit is disrupted by the translocation. The fact that this 8.3-kb RNA was significantly larger than the normal-sized *E2A* mRNAs observed in other lymphoid cell lines suggests that it may cross the t(1;19) breakpoint to produce a fusion transcript. Additional studies in which ribonuclease (RNase) protection assay was used showed that two other t(1;19)–carrying cell lines have *E2A* mRNA abnormalities identical to those observed in SUP-B27, providing further confirmation for a structural alteration of *E2A* by this translocation (*20*).

Our data demonstrating t(1;19) breakpoints within *E2A* and an associated novel RNA transcript imply that an alteration of the *E2A*-encoded proteins (E12/E47) may be pathogenically important in leukemias carrying a t(1;19). Although these proteins appear to be expressed in a wide variety of tissues (*21*), t(1;19) may alter some of their properties to result in aberrant transcriptional control of a specific set of proliferation-associated genes. Cytogenetic studies of t(1;19)–carrying leukemias indicate that the critical genetic rearrangement is the translocation of material from chromosome 1 to chromosome 19 because the t(1;19) is most frequently unbalanced, with loss of the $1q^-$ chromosome. In cells with only a der(19) chromosome, DNA blot analyses with a subfragment of pE47M coding for the DNA binding domain showed that this portion of the gene is not present on the single rearranged bands observed in Fig. 2 (*19*). Thus, the $19p^+$ chromosome may no longer retain the exons encoding the DNA binding domains of the *E2A*-encoded proteins, suggesting that the role of these proteins in the pathogenesis of the t(1;19) may be independent of their sequence-specific DNA binding properties and is related more to other properties of *E2A* or its products.

Our results establish the identity of the gene localized to the t(1;19) breakpoint site on chromosome 19. The fact that 1 out of 11 cases could not be shown to have a DNA rearrangement most likely indicates that not all t(1;19) breakpoints occur within the portion of *E2A* detected by pE47M, which contains only a portion of the complete cDNA (*8, 21*). Alternatively, there may be another gene at 19p13.3 that is important in a subset of these leukemias, although this seems less likely because of the characteristically uniform morphologic and phenotypic features of t(1;19)–carrying cells. In addi-

Table 1. Partial karyotypes and DNA blot results on various cell lines and acute leukemia specimens. DNA samples 1 to 9 were prepared from leukemia cells obtained from children with ALL whose karyotypic evaluation showed a balanced or unbalanced t(1;19) chromosomal translocation. Cell lines 697 and SUP-B27 were established from t(1;19)–carrying ALLs that phenotypically typed as pre-B cells (*16, 17*). Abbreviations: G, germline; ND, not determined; 1R, one rearranged band; and 2R, two rearranged bands.

Patient or cell line	Partial karyotype	DNA blot analysis		
		Eco RI	Bam HI	Hind III
1	−19, +der(19) t(1;19)(q23;p13)	G	G	G
2	−19, +der(19) t(1;19)(q23;p13)	1R	ND	ND
3	t(1;19)(q23;p13)	2R	ND	ND
4	−19, +der(19) t(1;19)(q23;p13)	1R	1R	1R
5	−19, +der(19) t(1;19)(q23;p13)	1R	1R	1R
6	−19, +der(19) t(1;19)(q23;p13)	1R	1R	1R
7	−19, +der(19) t(1;19)(q23;p13)	G	1R	1R
8	−19, +der(19) t(1;19)(q23;p13)	G	1R	G
9	−19, +der(19) t(1;19)(q23;p13)	G	1R	1R
697	−19, +der(19) t(1;19)(q23;p13)	G	1R	G
SUP-B27	t(1;19)(q23;p13)	2R	1R	2R

tion, our data imply a direct role of the gene for a transcription factor in human cancer-associated genetic rearrangements. Although it is suspected that Myc may also function as a transcription factor, direct demonstration of this has not been presented. Finally, given our data suggesting that a potential fusion transcript may be formed as a result of t(1;19), it should be possible to design DNA probe–based methodologies to detect this abnormality in leukemia DNAs. Knowledge of the genes affected by the t(1;19) should also help establish whether submicroscopic lesions of *E2A* or a gene on chromosome 1 are associated with various leukemias that lack the t(1;19) chromosomal translocation.

Note added in proof: Recent review of the karyotype for leukemia cells from patient 1 showed that although this leukemia carries a chromosome 19 abnormality, it is not a t(1;19) or its unbalanced variant (*24*). Therefore, ten out of ten t(1;19) breakpoints in this study were detected by using the pE47M probe, suggesting that all t(1;19) breakpoints occur near or within *E2A*.

REFERENCES AND NOTES

1. F. Mitelman, *Catalog of Chromosome Aberrations in Cancer* (Liss, New York, 1988); S. Heim and F. Mitelman, *Cancer Cytogenetics* (Liss, New York, 1987).
2. A. J. Carroll *et al.*, *Blood* **63**, 721 (1984); P. M. Michael, M. D. Levin, O. M. Garson, *Cancer Genet. Cytogenet.* **12**, 333 (1984); D. L. Williams *et al.*, *Cell* **36**, 101 (1984); P. M. Michael, O. M. Garson, D. F. Callen, *Cancer Genet. Cytogenet.* **17**, 79 (1985).
3. T. L. Yang-Feng, U. Francke, A. Ullrich, *Science* **228**, 728 (1985); R. S. K. Chaaganti *et al.*, *Cytogenet. Cell Genet.* **43**, 181 (1986).
4. A. K. Hatzopoulos, U. Schlokat, P. Gruss, in *Eukaryotic RNA Synthesis and Processing*, B. D. Hames and D. M. Glover, Eds. (Horizons in Molecular Biology Series, IRL Press, Oxford, 1988), pp. 43–96.
5. G. M. Church, A. Ephrussi, W. Gilbert, S. Tonegawa, *Nature* **313**, 798 (1985); A. Ephrussi, G. M. Church, S. Tonegawa, W. Gilbert, *Science* **227**, 134 (1985); J. Weinberger, D. Baltimore, P. A. Sharp, *Nature* **322**, 846 (1986).
6. R. Sen and D. Baltimore, *Cell* **46**, 705 (1986).
7. M. Lenardo, J. W. Pierce, D. Baltimore, *Science* **236**, 1573 (1987).
8. C. Murre, P. S. McCaw, D. Baltimore, *Cell* **56**, 777 (1989).
9. X. H. Sun and D. Baltimore, unpublished observations.
10. R. L. Davis, H. Weintraub, A. B. Lassar, *Cell* **51**, 987 (1987); M. C. Alonso and C. V. Cabrera, *EMBO J.* **7**, 2585 (1988); M. Caudy *et al.*, *Cell* **55**, 1061 (1988); E. Legouy *et al.*, *EMBO J.* **6**, 3359 (1987); L. W. Stanton, M. Schwab, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1772 (1986); B. Thisse, C. Stoetzel, C. Gorostiza-Thisse, F. Perrin-Schmitt, *EMBO J.* **7**, 2175 (1988); R. Villares and C. V. Cabrera, *Cell* **50**, 415 (1987); W. E. Wright, D. A. Sassoon, V. K. Lin, *ibid.* **56**, 607 (1989).
11. J. D. Mellentin, S. D. Smith, M. L. Cleary, *Cell* **58**, 77 (1989).
12. F. W. Alt *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 931 (1986).
13. For in situ hybridizations, pE47M was labeled to a specific activity of 7.9×10^8 cpm/μg by hexamer priming [A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1984)]. Hybridization to chromosome preparations from a chromosomally normal female was carried out at 44°C at a final probe concentration of 10 pg/μl [M. E. Harper and G. F. Saunders, *Chromosoma* **83**, 431 (1981)]. The preparations were exposed for 5 days, and then developed and analyzed [T. A. Donlon, M. Litt, S. R. Newcom, R. E. Magenis, *Am. J. Hum. Genet.* **35**, 1097 (1984)]. Additional studies, in which less stringent hybridization conditions were used, showed a primary hybridization peak at chromosome bands 19p13.2–p13.3 and a possible secondary site on chromosome 9p (T. A. Donlon, unpublished data).
14. J. D. Brook, D. J. Shaw, L. Meredith, G. A. Brun, P. S. Harper, *Hum. Genet.* **68**, 282 (1984); A. J. Lusis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3929 (1986).
15. J. D. Mellentin, J. Nourse, M. L. Cleary, unpublished observations.
16. H. W. Findley *et al.*, *Blood* **60**, 1305 (1982); R. J. Green, H. W. Findley, Jr., A. T. L. Chen, A. H. Ragab, *Cancer Genet. Cytogenet.* **7**, 257 (1982). The cell line 697 was originally described as having a t(7;19)(q11;q13) chromosomal translocation, but subsequent review showed that it carries an unbalanced t(1;19)(q13;p13) [P. E. Barker, A. J. Carroll, M. D. Cooper, *ibid.* **25**, 379 (1987); A. J. Carroll, unpublished observations].
17. Cell line SUP-B27 was established from an individual with pre-B cell ALL under conditions similar to those previously described (*18*). Karyotype studies showed that the cell line was representative of the patient's leukemia because both carried a reciprocal t(1;19) and trisomy chromosome 8 as the only detectable cytogenetic abnormalities (S. D. Smith, T. A. Donlon, M. L. Cleary, unpublished data).
18. S. D. Smith *et al.*, *Blood* **71**, 395 (1988); S. D. Smith *et al.*, *ibid.*, in press.
19. J. D. Mellentin and M. L. Cleary, unpublished observations.
20. N. Galili and M. L. Cleary, unpublished observations.
21. C. Murre and D. Baltimore, unpublished observations.
22. M. L. Cleary *et al.*, *Cell* **47**, 19 (1986).
23. A. Epstein *et al.*, *Cancer* **42**, 2379 (1978); S. H. Doi *et al.*, *Blood* **70**, 1619 (1987).
24. A. G. Carroll, unpublished observations.

25. We thank the late S. A. Latt for initial indications that *E2A* might map to chromosome 19, P. Verzola for photography, G. Bruns and T. Mohandas for somatic cell hybrid DNAs, and W. Crist and the Pediatric Oncology Group, St. Louis, MO, for providing leukemia cells carrying the t(1;19). Supported by grants from NIH (CA42971, CA30969, CA42106, CA33603, CA31566, and GM39458) and a grant from the Lucille P. Markey Charitable Trust. J.D.M. was supported in part by PHS grant NRSA 5T32 CA09302. C.M. is supported by a grant from the Leukemia Society of America. D.B. is supported by a grant from the American Cancer Society. M.L.C. is a scholar of the Lucille P. Markey Charitable Trust.

13 June 1989; accepted 28 August 1989

A Role for a 70-Kilodaton Heat Shock Protein in Lysosomal Degradation of Intracellular Proteins

HUI-LING CHIANG,* STANLEY R. TERLECKY, CHARLES P. PLANT, J. FRED DICE†

A 73-kilodalton (kD) intracellular protein was found to bind to peptide regions that target intracellular proteins for lysosomal degradation in response to serum withdrawal. This protein cross-reacted with a monoclonal antibody raised to a member of the 70-kD heat shock protein (hsp70) family, and sequences of two internal peptides of the 73-kD protein confirm that it is a member of this family. In response to serum withdrawal, the intracellular concentration of the 73-kD protein increased severalfold. In the presence of adenosine 5′-triphosphate (ATP) and $MgCl_2$, the 73-kD protein enhanced protein degradation in two different cell-free assays for lysosomal proteolysis.

STUDIES OF THE DEGRADATION OF proteins microinjected into cultured cells have shown that ribonuclease A (RNase A) is degraded within lysosomes and that lysosomal degradation of RNase A is increased when cells are deprived of serum growth factors. The increased degradation during serum withdrawal results from an increased rate of protein transfer to lysosomes (*1, 2*).

Amino acid residues 7 to 11 (KFERQ) (*3*) of RNase A constitute the essential region for the enhanced degradation (*4*), and similar, but not identical, peptide sequences exist in fibroblast proteins that are preferentially degraded when cells are deprived of serum (*5*). A detailed description of the probable peptide motif has been presented (*6*).

To determine whether cytosolic proteins participate in the lysosomal proteolysis of microinjected RNase A seen in response to serum withdrawal, we looked for proteins that could specifically recognize KFERQ. We found a 73-kD protein in the cytosol of serum-deprived fibroblasts that bound to RNase S-peptide (residues 1 to 20 of RNase A) linked to Sepharose (but not to underivatized Sepharose) and could be eluted with the peptide KFERQ (Fig. 1), RNase S-peptide, or RNase A, but not by several unrelated peptides or proteins (7). We refer to this protein as the 73-kD peptide recognition protein (prp73). A smaller protein (30

Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.

*Present address: Department of Biochemistry, University of California, Berkeley, CA 94720.
†To whom correspondence should be addressed.