and 30 monoclones were obtained from clones 3.2G11 and 3.2F7, respectively, by the limiting dilution method (*12*). All of these monoclones belonged to the immunoglobulin G3 isotypes, as determined by the Ouchterlony method with antibodies to the mouse isotype.

Monoclonal antibodies were harvested from culture supernatants when cell titers reached 5×10^6 to 7×10^6 cells per milliliter and were used to detect the AY agent in diseased lettuce. As shown in Table 1, the AY agent could be detected when such monoclonal antibodies were diluted up to 1600 times and vascular preparations were diluted 20 times with coating buffer (0.05*M* carbonate buffer, *p*H 9.6). Reciprocally, serial dilutions of lettuce vascular preparation were also used to test the detecting ability of the antibodies. The AY agent could be easily detected when the antigen was diluted 4,860 to 14,580 times (Table 2). Using the same antibodies we found that they also reacted specifically to AY antigen prepared from infected periwinkle plants (*Catharanthus roseus* L.) or inoculative *M. fascifrons*, but not to preparations from healthy periwinkles or noninoculative insects. On the other hand, the AY monoclonal antibodies did not react with vascular preparations of corn infected with maize bushy stunt and from six other MLO-infected periwinkle plants: ash yellows, loofah witches'-broom, paulownia witches'-broom, sweet potato witches'-broom, peanut rosette, or elm yellows (elm phloem necrosis) (Table 3).

Monoclonal antibody–producing hybridoma cells were used to induce ascites by injecting 10^6 cells into pristane-primed BALB/c mice. The ascitic fluids harvested resulted in a 100 to 1000 times higher antibody titer (160,000 to 1,600,000) than those of the culture supernatants (1600).

Until the MLO's that are associated with AY diseases can be cultured, their identification will depend on techniques that are not based on pure antigens. Antibodies developed by our hybridoma technique are reliable for AY diagnosis and for comparison with similar yellows disease agents, providing that antigens can be obtained from them. The technique may be the only means for preparing specific antibodies against these agents because of their apparent noncultivability and their inevitable contamination by plant or insect antigens when they are purified by the techniques of virology. Because of their highly discriminatory capacity, such monoclonal antibodies can now act as a useful tool in yellows disease diagnosis, forecasting, and epidemiology, and they may also be used in taxonomic differentiation among the various MLO's associated with plant yellows diseases.

Note added in proof: Since this report was submitted, five more hybridoma clones have been obtained by using the partially purified MLO preparations from diseased lettuce as immunogen.

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References and Notes

1. L. O. Kunkle, *Am. J. Bot.* **13**, 646 (1926).
2. ———, *Adv. Virus Res.* **3**, 251 (1955).
3. Y. Doi, M. Teranaka, K. Yora, H. Asuyama, *Ann. Phytopathol. Soc. Jpn.* **33**, 259 (1967).
4. T. Ishiie, Y. Doi, K. Yora, H. Asuyama, *ibid.*, p. 267.
5. R. F. Whitcomb and L. M. Black, in *Plant and Insect Mycoplasma Techniques*, M. J. Daniels and P. G. Markham, Eds. (Wiley, New York, 1982), pp. 40–81.
6. R. C. Sinha and N. Benhamou, *Phytopathology* **73**, 1199 (1983).
7. M. F. Clark, D. J. Barbara, D. L. Davies, *Ann. Appl. Biol.* **103**, 251 (1983).
8. R. C. Sinha and L. N. Chiykowski, *Virology* **33**, 702 (1967).
9. Splenic cells (10^8) were mixed with myeloma cells (5×10^7) and pelleted by centrifugation at 200*g* for 10 minutes. The pellet was gently loosened and resuspended by adding 1.5 ml of 40 percent (weight to volume) polyethylene glycol (PEG) 4000 (Sigma) in serum-free RPMI 1640 medium dropwise over 45 seconds. The PEG suspension was first diluted by dropwise addition of 5 ml of serum-free RPMI 1640 medium over 10 minutes; then an additional 10 ml of medium was added over another 10 minutes. The cell mixture was centrifuged and resuspended in 80 ml of RPMI 1640 complete medium (*10*). After 3 hours of incubation in a CO_2 incubator the cell suspension was diluted by the same volume of medium supplemented with 2× concentrated HAT medium (*10*) and plated in 96-well plates.
10. RPMI 1640 complete medium is RPMI 1640 medium supplemented with 15 percent fetal bovine serum. HAT medium is complete medium supplemented with $10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine.
11. C. P. Lin and T. A. Chen, *Phytopathology* **74**, 798 (1984).
12. E. L. Halk, H. T. Hsu, J. Aebig, J. Franke, *ibid.*, p. 367.
13. We thank J. D. Lei for helpful discussions in developing the screening methods, Y. P. Jiang for supplying inoculative insects, and J. M. Wells for generously sharing essential equipment. This is New Jersey Agricultural Experiment Station publication D-11160-1-84, supported by state funds and the Hatch Act.

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Coexpression of Translocated and Normal c-*myc* Oncogenes in Hybrids Between Daudi and Lymphoblastoid Cells

Abstract. *Mechanisms that affect the transcription of the* c-myc *oncogene take part in the development of B-cell neoplasias such as Burkitt's lymphoma. Daudi Burkitt lymphoma cells, which express only the translocated* c-myc *oncogene, were hybridized with human lymphoblastoid cells, which express the normal* c-myc *gene; the hybrids were phenotypically lymphoblastoid and expressed both the translocated and the normal* c-myc *gene. This result contrasts with the findings that the decapitated* c-myc *gene, translocated to an immunoglobulin switch* μ *or* α *region, is transcriptionally silent in lymphoblastoid hybrids. Thus, there may be at least two distinct enhancer-like elements capable of deregulating* c-myc *transcription in lymphomas and leukemias with t(8;14) chromosome translocations. In addition, since the Daudi × lymphoblastoid hybrids express both the translocated and the normal* c-myc *gene, the* c-myc *gene product does not autoregulate* c-myc *transcription.*

Approximately 75 percent of Burkitt lymphomas carry t(8;14) chromosome translocations; the remaining 25 percent carry either t(8;22) or t(2;8) chromosome translocations (*1*). In Burkitt lymphomas with a t(8;14) translocation, the c-*myc* gene, which may remain intact or lose a portion of its 5′ end, translocates to various sites within the heavy-chain locus on chromosome 14 (*2*). In the variant t(8;22) and t(2;8) translocations, the c-*myc* oncogene remains on chromosome 8, and either the λ or the κ locus translocates to a region distal (3′) to the c-*myc* gene (*3, 4*). These chromosome translocations result in transcriptional deregulation of the c-*myc* oncogene, causing elevated constitutive expression (*3, 5*). Because the breakpoint near the c-*myc* locus—whether 5′ or 3′ of the gene or close to, within, or distant from it—and the breakpoints within the immunoglobulin loci are heterogeneous, it is difficult to propose a single model for c-*myc* deregulation. An additional complication is that the exact stage of B-cell differentiation represented by Burkitt lymphoma cells is not known and may differ among different tumor lines. In experiments to determine the mechanisms taking part in regulation of c-*myc* expression during B-cell differentiation, we have used somatic cell hybridization methods to construct cells of various phenotypes within the B-cell lineage that carry both translocated and normal c-*myc* genes within the same cell; that is, these hybrids have a Burkitt genotype with a less differentiated lymphoblastoid or more differentiated plasma cell phenotype (*3, 5, 6*).

Table 1. Phenotype of somatic cell hybrids between Daudi Burkitt lymphoma cells and GM1500-6TG-OUB lymphoblastoid cells. Secreted and cytosol immunoglobulins (Ig's) were labeled with [^{3}H]leucine (100 μCi/ml) and immunoprecipitated by means of antiserum to human Ig and subsequent addition of a 10 percent suspension of fixed *Staphylococcus aureus* antibodies (50 μl) as described (*13, 20*). Daudi cells secrete membrane-bound IgM; GM1500-6TG-OUB cells secrete IgG. Expression of the antigen recognized by B532 antibodies was studied by indirect immunofluorescence as described (*12*).

Cells	Immunoglobulin chains				Secretion	Antigen expression
	μ	γ	κ	κ1*		
Daudi	+	−	−	+	−	−
GM1500-6TG-OUB	−	+	+	−	+	+
AA1	+	+	+	+	+	+
AC4	+	+	+	+	+	+
BB3	+	+	+	+	+	+
AB4	+	+	+	+	+	+

*An aberrant κ polypeptide expressed by Daudi cells.

For example, we have hybridized human lymphoblastoid cells with two different Burkitt lymphoma cells, ST486 and CA46, carrying t(8;14) translocations in which the decapitated c-*myc* oncogene is translocated to the immunoglobulin switch (S) μ and α regions, respectively, of the heavy-chain locus on the 14q$^+$ chromosome (*6, 7*). Thus, the heavy-chain enhancer normally located between the heavy-chain joining (J_H) region and the S_μ region is on the 8q$^-$ chromosome and not on the 14q$^+$ in these cell lines (*6, 7*). The hybrids expressed the lymphoblastoid phenotype and the immunoglobulin chains of both parental cells and transcribed only the normal and not the translocated c-*myc* oncogene (*6*). Because the translocated c-*myc* oncogene was expressed in the parental lymphoma cells and in their hybrids with plasmacytoma cells, we concluded that the translocated c-*myc* oncogene was under the control of an unidentified *cis*-acting enhancer-like element active in Burkitt lymphoma and plasma cells but not in lymphoblastoid cells (*6*). This result does not support the hypothesis (*8, 9*) that loss of, or mutations in, the c-*myc* 5′ exon and the concomitant lack of repressor binding is the cause of differential regulation of the translocated and normal c-*myc* genes. If loss of its 5′ end resulted in activation of translocated c-*myc* in Burkitt lymphomas CA46 and ST486, then these decapitated, translocated c-*myc* genes should also be (but are not) expressed in the lymphoblastoid-type somatic cell hybrids (*6*). This argument is strengthened by the observation that the translocated, decapitated c-*myc* gene of ST486 and CA46 cells is not expressed in hybrids with mouse 3T3 fibroblasts (*10*) and that the translocated intact c-*myc* oncogene of P3HR-1 Burkitt lymphoma cells, in which mutations in the first *myc* exon have occurred, is also repressed in hybrids with mouse fibroblasts (*5*). Thus, c-*myc* genes with mutated or missing 5′ ends can still be turned off or, alternatively, not positively activated when placed within a lymphoblastoid or fibroblast context.

In the present study we investigated the expression of c-*myc* transcripts in hybrids between Daudi Burkitt lymphoma cells—where the breakpoint on chromosome 14 is 5′ to the J_H segment and to the heavy-chain enhancer between J_H and S_μ—and the same human lymphoblastoid cells (GM1500-6TG-OUB) that we used to produce the hybrids with ST486 and CA46 cells (*6*). Daudi Burkitt lymphoma and GM1500-6TG-OUB cells were fused at a ratio of 1:1 in the presence of polyethylene glycol 1000, and the hybrids were selected in HAT medium (*11*) containing $10^{-5}M$ ouabain (*6*). The hybrids expressed and secreted the immunoglobulin chains of both parental cells and reacted with a monoclonal antibody specific for lymphoblastoid cells (*12*) (Table 1). In addition, the hybrids were morphologically similar to the lymphoblastoid parental cells and aggregated to form large clumps, whereas parental Daudi cells grew as single cells.

To determine whether the hybrids retained the 14q$^+$ chromosome carrying the translocated c-*myc* oncogene, we examined the hybrids for the 18.0-kilobase (kb) Bam HI J_H-C_μ (immunoglobulin constant μ region) fragment derived from the 14q$^+$ chromosome (*13*). All four independent hybrids examined (AA1, AC4, AB4, and BB3) retained the 18.0-kb band. They also retained the 14.0-kb band from the normal chromosome 14, which represents the productively rearranged μ gene of Daudi cells (*13*). After digestion with Hind III, the parental J_H bands in all four hybrid clones were collected (Fig. 1).

Cleavage of Daudi DNA with Bam HI indicated that Daudi cells carry a translocated but unrearranged c-*myc* oncogene (Fig. 2) (*2*). However, analysis of the 5′ exon of the translocated c-*myc* gene from Daudi cells showed mutations in this exon (*9*). By using a probe specific for the *myc* first exon in an S_1 nuclease protection experiment (*4, 5*), we could distinguish the normal *myc* transcripts of GM1500-6TG-OUB cells as 515- and 350-nucleotide S_1 nuclease-resistant DNA products and the *myc* transcripts of Daudi cells as 290- and 195-nucleotide products on a DNA sequencing gel (Fig. 3). Daudi cells expressed only the trans-

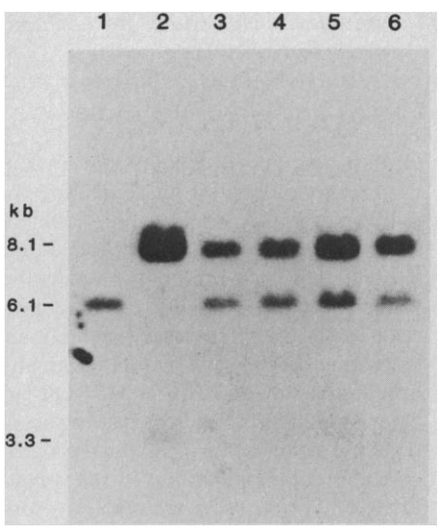

Fig. 1. Southern blot analysis of parental and hybrid cell DNA's after digestion with Hind III and hybridization with a human J_H probe (*13*). The DNA's from the parental GM1500-6TG-OUB cells (lane 1) and Daudi cells (lane 2) and from the hybrids AA1 (lane 3), AC4 (lane 4), AB4 (lane 5), and BB3 (lane 6) are shown.

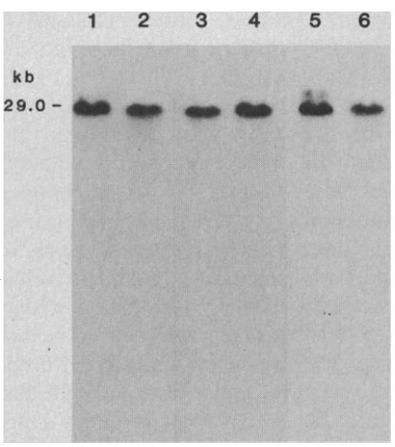

Fig. 2. Southern blot analysis of parental and hybrid cell DNA's after digestion with Bam HI and hybridization with a human c-*myc* complementary DNA probe (Ryc 7.4) (*5*). The DNA's from the parental GM1500-6TG-OUB cells (lane 1) and Daudi cells (lane 2) and from the hybrids AA1 (lane 3), AC4 (lane 4), AB4 (lane 5), and BB3 (lane 6) are shown.

located c-*myc* gene and not the normal c-*myc* gene on chromosome 8 (Fig. 3, lane 2). In contrast, GM1500-6TG-OUB expressed normal c-*myc* transcripts initiated from the two normal *myc* transcription initiation sites (lane 7). All four hybrid clones expressed both the translocated c-*myc* oncogene on chromosome $14q^+$ and the normal c-*myc* gene on chromosome 8 (lanes 3 to 6). At present it is not possible to determine whether the normal c-*myc* oncogene on the normal chromosome 8 contributed by Daudi cells is reexpressed in the hybrids. We have shown, however, that somatic cell hybrids between mouse fibroblasts and Burkitt lymphoma cells do not transcribe the silent Burkitt *myc* allele on the normal chromosome 8 but do transcribe the normal mouse *myc* gene (*10*). These results suggest that the silent c-*myc* allele of Burkitt lymphoma cells is irreversibly inactivated and cannot be reactivated in hybrids expressing the normal c-*myc* gene of the non-Burkitt parent. Thus it seems likely that the normal c-*myc* transcripts in the Daudi × GM1500 hybrids derive from the GM1500 c-*myc* genes.

The results shown in Fig. 3 contrast with our finding that a c-*myc* oncogene translocated to either an S_μ or S_α region is not transcribed in hybrids with lymphoblastoid cells (*6*) and indicate that the *cis*-acting genetic elements deregulating c-*myc* transcription in Daudi cells can activate gene transcription not only in Burkitt lymphoma and plasmacytoma cells (*3*, *5*) but also in lymphoblastoid cells. Because in ST486 the c-*myc* oncogene is translocated to the S_μ region and is not transcribed in ST486 × GM1500-6TG-OUB hybrids, we can also conclude that the *cis*-acting enhancer responsible for the translocated c-*myc* transcription in Daudi cells must be located 5′ of the S_μ region. Thus, it seems possible that in Daudi cells the heavy-chain enhancer between the J_H and S_μ regions (*14*) is responsible for the enhancement of translocated c-*myc* transcription. This enhancer is active in human lymphoblastoid cells because the separated first exon of the c-*myc* oncogene that remains on the $8q^-$ chromosome of ST486 cells and to which the enhancer is translocated is expressed at elevated levels in ST486 × GM1500-6TG-OUB hybrids (*6*).

Because the c-*myc* oncogene of Daudi cells appears to be unrearranged (Fig. 2) (the upstream Bam HI site is 14 kb 5′ of the oncogene), and because the breakpoint on chromosome 14 appears to include the region carrying heavy-chain variable region (V_H) genes (*13*), the distance between translocated c-*myc* and this enhancer must be large (>30 kb), which suggests that the immunoglobulin heavy-chain enhancer may extend its influence over distances longer than were previously supposed.

A model has been proposed in which the c-*myc* product autoregulates c-*myc* transcription (*9*). This model was based on the observation that Raji Burkitt lymphoma cells produce an altered c-*myc* product coded for by the translocated *myc* gene and express not only the translocated *myc* but also the normal *myc* gene (*9*). As a possible interpretation of these results, the mutated c-*myc* product in Raji cells was suggested to be incapable of repressing normal c-*myc* transcription (*9*). A corollary of this suggestion is that, in other Burkitt lymphomas (in which the normal *myc* gene is not expressed), the c-*myc* gene product takes part directly or indirectly in the regulation of c-*myc* transcription.

We have shown that Daudi cells express only the translocated and not the normal c-*myc* gene (Fig. 3, lane 2). Because the translocated c-*myc* gene of Daudi cells codes for a normal c-*myc* product (*9*), this result is consistent with the interpretation of Rabbitts *et al.* (*9*). However, if the translocated c-*myc* oncogene of Daudi cells were able to repress the expression of the normal c-*myc* gene, we would not observe normal c-*myc* transcripts in the Daudi × GM1500-6TG-OUB hybrids. Because we did observe normal c-*myc* transcripts in all hybrids, we conclude that the c-*myc* gene product does not autoregulate c-*myc* transcription. This interpretation is strengthened by the observation that the levels of the normal c-*myc* transcripts in the hybrids were similar in all four hybrid clones examined, even though the levels of the translocated c-*myc* transcripts varied from clone to clone (Fig. 3). In addition, the level of c-*myc* transcripts in hybrid AA1 is essentially the same as in the Daudi parent, indicating that comparable levels of translocated *myc* expression occur in B cells in which the normal c-*myc* gene is totally suppressed (Fig. 3, lane 1) and in B cells in which the normal c-*myc* gene is expressed. The possibility that subtle alterations in the *myc* protein structure could influence autoregulation is excluded by the finding that the coding exons of the translocated c-*myc* oncogene of Daudi cells are normal (*9*).

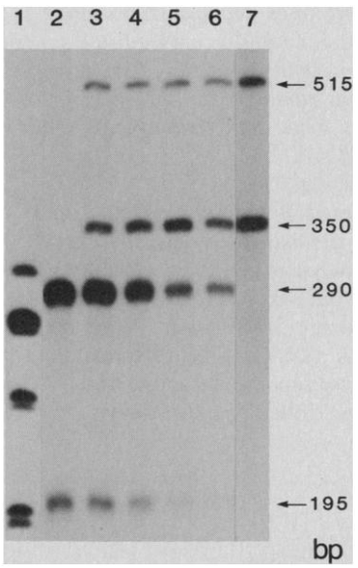

Fig. 3. S_1 mapping analysis of c-*myc* messenger RNA's synthesized in Daudi Burkitt lymphoma and somatic cell hybrids. The RNA was analyzed by a modification of the S_1 nuclease mapping procedure (*14*) with the use of a uniformly labeled DNA probe (*16*). The Xma I–Pvu II DNA fragment (610 base pairs) of the c-*myc* gene containing a portion of the 5′ flanking sequences and the first exon was cloned in M13, and the clone was uniformly labeled with ^{32}P and used as an S_1 probe. Cytoplasmic RNA's (20 μg) prepared from various cells by the cesium chloride method (*17*) were used for each assay. As described earlier (*18*), the ^{32}P-labeled DNA probe was denatured by heating, hybridized in 80 percent formamide to cytoplasmic RNA's at 57.5°C for 10 hours, digested with 80 units of nuclease S_1, and analyzed by electrophoresis on a 7*M* urea–4 percent polyacrylamide gel (*19*). (Lane 1) ϕX174 Hae III digests labeled at the 5′ ends with ^{32}P; (lane 2) Daudi cells; (lane 3) hybrid AA1; (lane 4) hybrid AB4; (lane 5) hybrid AC4; (lane 6) hybrid BB5; (lane 7) GM100-6TG-OUB cells.

Our results indicate that different genetic elements play a role in the *cis* activation of the translocated c-*myc* oncogene in B-cell neoplastic diseases with t(8;14) (q24;q32) chromosome translocations. On one side, 5′ of the C_μ region, are genetic elements (possibly the enhancer located between the J_H and S_μ regions) capable of activating c-*myc* transcription in pre–B cells (*15*), lymphoblastoid cells, Burkitt cells, and plasma cells. On the other side, 3′ of the C_μ region, are elements that can activate c-*myc* transcription only in the more differentiated B cells (*6*). Thus B-cell neoplasms carrying t(8;14) chromosome translocations may represent a heterogeneous group of diseases in which related mechanisms result in the same biologic consequence: a transcriptional deregulation of the c-*myc* oncogene involved in the reciprocal chromosomal translocation that leads to its constitutive expression in the B cells.

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References and Notes

1. G. Manolov and Y. Manolova, *Nature (London)* **237**, 33 (1972); L. Zech, V. Haglund, N. Nilsson, G. Klein, *Int. J. Cancer* **17**, 47 (1976); H. Van den Berghe *et al.*, *Cancer Genet. Cytogenet.* **1**, 9 (1979); A. Bernheim, R. Berger, G. Lenoir, *ibid.* **3**, 307 (1981).
2. R. Dalla Favera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982).
3. C. M. Croce *et al.*, *ibid.* **80**, 6922 (1983); J. Erikson *et al.*, *ibid.*, p. 7581.
4. B. S. Emanuel *et al.*, *ibid.* **81**, 2444 (1984).
5. K. Nishikura *et al.*, *ibid.* **80**, 4822 (1983).
6. C. M. Croce, J. Erikson, A. ar-Rushdi, D. Aden, K. Nishikura, *ibid.* **81**, 3170 (1984).
7. A. ar-Rushdi *et al.*, *Science* **222**, 390 (1983); E. P. Gelmann, M. C. Psallidopoulos, T. S. Papas, R. Dalla Favera, *Nature (London)* **306**, 799 (1983); L. Showe *et al.*, *Mol. Cell. Biol.*, in press.
8. P. Leder *et al.*, *Science* **222**, 765 (1983).
9. T. H. Rabbitts, A. Foster, P. Hamlyn, R. Baer, *Nature (London)* **309**, 592 (1984).
10. K. Nishikura *et al.*, *Science* **224**, 399 (1984).
11. J. Littlefield, *ibid.* **145**, 709 (1964). HAT medium contains hypoxanthine, aminopterin, and thymidine.
12. D. Frisman, S. Slovin, I. Royston, S. Baird, *Blood* **62**, 1224 (1983).
13. J. Erikson, J. Finan, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5611 (1982).
14. R. A. Sharp, A. J. Berk, S. M. Berget, *Methods Enzymol.* **65**, 750 (1980).
15. L. Pegoraro *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7166 (1984).
16. T. S. Ley, N. P. Anagnou, G. Pepe, A. W. Neinhuis, *ibid.* **79**, 4775 (1982).
17. A. ar-Rushdi, K. B. Tan, C. M. Croce, *Somatic Cell Genet.* **8**, 151 (1982).
18. K. Nishikura and G. A. Vuocolo, *EMBO J.* **3**, 689 (1984).
19. T. Maniatis, A. Jeffery, A. van Sande, *Biochemistry* **14**, 3787 (1975).
20. J. Érikson and C. M. Croce, *Eur. J. Immunol.* **12**, 697 (1982).

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A Second Nuclear Protein Is Encoded by Epstein-Barr Virus in Latent Infection

Abstract. *A region of the Epstein-Barr virus (EBV) genome that is important in inducing cell proliferation includes a single long open reading frame. Part of this open reading frame has been fused to the* lacZ *gene and expressed in* Escherichia coli. *Antisera to the fusion protein identify a protein in the nuclei of latently infected growth-transformed lymphocytes and in Burkitt tumor cells grown in vitro. This nuclear protein is encoded by a different virus gene than that which encodes the previously described EBV nuclear antigen, EBNA.*

Epstein-Barr virus (EBV) infection of human B lymphocytes results in cellular proliferation (*1*). The virus causes infectious mononucleosis and is believed to be an etiologic factor in two prevalent human tumors, Burkitt lymphoma and nasopharyngeal carcinoma (*2*). EBV does not productively replicate in most infected B lymphocytes or tumor cells but persists in a latent state (*3*). The latent virus genome is usually episomal (*4*) but may integrate into the cell genome (*5*). At least three messenger RNA's (mRNA's) are transcribed from widely separated regions of the 170-kilobase (kb) EBV genome in latently infected cells (*6–8*). One of these RNA's, encoded from the IR1-U2 region (Fig. 1A), appears to be important in initiation of growth transformation since a virus isolate (P3HR-1) with a deletion in the U2 domain cannot initiate growth transformation (*9, 10*). Recombinants between P3HR-1 and cloned U2 DNA can be obtained that are capable of transforming cells (*11*). Furthermore, the U2 domain transfected into lymphocytes induces DNA synthesis (*12*). We now demonstrate that the U2 domain of EBV encodes a recently identified nuclear antigen, EBNA2, that is characteristic of latently infected, growth-transformed cells (*13*).

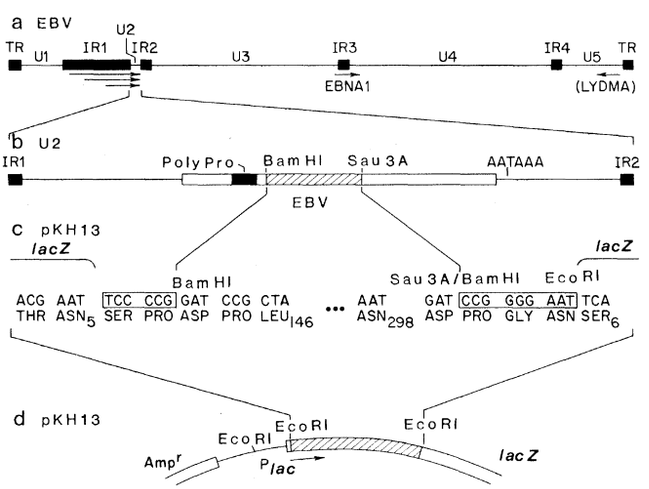

Fig. 1. EBV genome and pKH13 construction. (a) The EBV genome showing the location of major internal repeats (IR), terminal repeats (TR), and single-copy (U) domains (*3*). Transcripts and proteins characteristic of latently infected cells are indicated by arrows. Two other polyadenylated RNA's and two small nonpolyadenylated RNA's have also been detected from U1. Parentheses indicate tentative mapping of the LYDMA function to the membrane protein encoded by the U5 RNA (*8, 14*). (b) The U2 domain (*15*). The major open reading frame (open boxes) contains a polyproline-encoding triplet repeat (solid box). The location of the 465-bp Bam HI–Sau 3A fragment expressed in bacteria is shown (hatched box). (c) DNA sequence of the boundaries between the pKH13 insert and vector and the predicted amino acid sequence. Amino acids are numbered for their position in β-galactosidase (*25*) or in the EBV open reading frame (*15*). The boxed regions are characteristic of the polylinker in the pSKS104 vector (*17*). (d) Diagram of pKH13 showing the relationship of the gene fusion to the *lac* promoter.

A membrane protein and two nuclear proteins have been detected in cells latently infected with EBV (*8, 13–15*). The membrane protein is encoded by the 3′ end of the EBV genome (*8, 14*) (Fig. 1). This region probably is the source of the new cell membrane antigen, LYDMA, which is recognized by T cells from previously infected individuals. Its amino acid sequence is known from RNA mapping and DNA sequencing (*8*). One nuclear protein, EBNA, has been mapped to an EBV open reading frame which contains a polymorphic triplet repeat element (*6*). Mapping of this protein was facilitated by correlating the DNA repeat reiteration polymorphism among different EBV isolates with a polymorphism in protein size (*6*). Transfection with an EBV DNA fragment containing the open reading frame resulted in induction of a nuclear antigen detected by polyvalent, human sera (*16*). Antibody to a protein expressed in *Escherichia coli* from part of the EBNA or putative LYDMA gene identified these proteins in latently infected cells, thus confirming the earlier results (*13, 14*). A second, antigenically unrelated nuclear protein, EBNA2, was identified by EBV-immune human sera in all cells of most latently infected B lymphocyte cultures (*13*). Recent evidence has suggested that EBNA2 is encoded by the U2 domain of EBV DNA, a region which contains a polymorphic 1.5-kb open reading frame transcribed in latently infected, growth-transformed cells (*7, 15*) (Fig. 1). Five EBV isolates with similar U2 domains were shown to induce similar EBNA2 proteins; a virus with a shorter U2 open reading frame induced a smaller EBNA2 protein and a virus in which U2 was deleted lacked EBNA2 (*15*).

To prove that the U2 open reading frame encodes EBNA2, we inserted a 465-base-pair (bp) U2 Bam HI–Sau 3A fragment into the Bam HI site of the *lacZ* bacterial expression vector