

humans, semisynthetic vaccines composed of capsular polysaccharides of pathogenic bacteria covalently bound to carrier proteins have been manufactured and proved effective in man and monkeys (21).

The immunological screening of *P. falciparum* sporozoites from different areas of the world showed that all isolates contained representations of the epitope (NANP)₃ (22). Moreover, the (NANP)₃ sequence is repeated in each CS molecule, 37 times in the CS protein from an isolate of *P. falciparum* from Brazil (7) and at least 23 times in the CS protein from an isolate from Thailand (8). Because this sequence is so abundantly represented on the surface of the *P. falciparum* sporozoite, this stage of the parasite should be particularly susceptible to attack by antibodies to (NANP)₃. Synthetic or genetically engineered (NANP)₃-containing antigens are therefore logical candidates for the development of *P. falciparum* malaria vaccines.

FIDEL ZAVALA

Department of Medical and Molecular Parasitology, New York University Medical Center, New York 10021

JAMES P. TAM

Rockefeller University, New York 10021

MICHAEL R. HOLLINGDALE

Biomedical Research Institute, Rockville, Maryland 20852

ALLAN H. COCHRANE

Department of Medical and Molecular Parasitology, New York University

ISABELLA QUAKYI

Immunology Unit, Nogushi Memorial Institute for Medical Research, University of Ghana, Legon

RUTH S. NUSSENZWEIG

Department of Medical and Molecular Parasitology, New York University Medical Center

VICTOR NUSSENZWEIG

Department of Pathology, New York University Medical Center

stitution per gram of resin) was placed into the reaction vessel of a modified Beckman 990 synthesizer that performed the synthesis according to a computer program that optimized all coupling steps. The protected peptide-resin was deprotected batchwise (0.5 g) by a mixture of HF and anisole (9:1 by volume, 10 ml) for 60 minutes at 0°C. The cleavage yield was 91 percent based on the back hydrolysis of the resulting resin by 6N HCl. Analytical high-performance liquid chromatography (HPLC) on a reversed-phase C-18 column (4 by 300 mm, Waters Associates) using the usual aqueous CF₃CO₂H and CH₃CN gradient system [W. C. Mahoney and M. A. Hermodson, *J. Biol. Chem.* 255, 11199 (1980)] showed that the purity of the crude peptides was greater than 85 percent. Preparative scale purification of the dodecamer was carried out in a low-pressure liquid chromatography system on a 2.5 by 30 cm column. The overall yield based on the first proline attached to the resin was 61.4 percent. The purified dodecamer peptide gave a single symmetrical peak in reversed-phase analytical HPLC and on amino acid analysis gave Asp:Ala:Pro, 2.02:1:0.99 (theoretical value, 2:1:1).

11. E. H. Nardin et al., *Science* 206, 597 (1979).
12. P. Tapchaisri et al., *Am. J. Trop. Med. Hyg.* 32, 1203 (1983).
13. D. Clyde, H. Most, V. McCarthy, J. Vanderberg, *Am. J. Med. Sci.* 266, 169 (1973).
14. Questions could be raised about the nature of the immunogen that stimulates the production of the antibodies to (NANP)₃, in view of the finding of I. A. Hope et al. [*Nature (London)* 308, 191 (1984)] that a monoclonal antibody (5.1) to *P. falciparum* blood forms also recognizes the sporozoite surface membrane. This unique 5.1 antibody reacts with a 25,000-dalton protein in blood-stage parasites, present in some but not all isolates of *P. falciparum* [J. S. McBride et al., *Science* 217, 254 (1982)]. Although 5.1 also binds to the synthetic peptide (NANP)₃, none of our monoclonal antibodies to *P. falciparum* sporozoites [which, as shown here, are specific for (NANP)₃] react with the blood-stage antigen by IFA (F. Zavala, unpublished observations). Therefore (NANP)₃ is not present in blood forms and the epitope recognized by 5.1 is structurally different. Consistent with this idea are earlier observations indicating that sera from sporozoite-vaccinated volunteers contained a

high titer of antibodies to sporozoites but not to blood forms [See (12) and E. H. Nardin et al., *Bull. WHO* 57, 211 (1979)], and that mice immunized with *P. falciparum* blood forms have high levels of antibodies to blood forms that do not react with sporozoites (10). Also, in sera from humans in The Gambia, there was no correlation between the IFA titers of antibodies to sporozoites and levels of antibodies against blood-stage parasites (10). The amino acid sequence of the blood-stage antigen has been elucidated [I. A. Hope et al., *Nucleic Acids Res.* 13, 369 (1985)]. Probably the cross-reactive epitope resides in a region of the polypeptide chain containing a single NANP sequence flanked on either side by similar but not identical sequences. In view of the striking immunogenicity of the repeat region of all CS proteins, and the finding that the human sera do not react with (NANP)₃, it seems unlikely that the antibodies to (NANP)₃ detected in human sera are the result of an immune response to the weakly immunogenic and cross-reactive blood-stage epitope.

15. W. Trager and J. B. Jensen, *Science* 193, 673 (1976); T. Ifediba and J. P. Vanderberg, *Nature (London)* 249, 364 (1981).
16. M. R. Hollingdale et al., *J. Immunol.* 132, 909 (1984).
17. D. H. Schlesinger et al., *Biochemistry* 23, 5665 (1984).
18. J. Gysin et al., *J. Exp. Med.* 160, 935 (1984).
19. T. F. Creighton, *Proteins* (Freeman, New York, 1983), pp. 199-264.
20. E. A. Emini, B. A. Jameson, E. Wimmer, *Nature (London)* 304, 699 (1983).
21. M. L. Lepow, J. S. Samuelson, L. K. Gordon, *J. Pediatr.* 106, 185 (1985); R. Schneerson et al., *Infect. Immun.* 45, 582 (1984).
22. F. Zavala, A. Masuda, R. S. Nussenzweig, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 43 (Abstr.), 1808 (1984).
23. Supported by grants from the Agency for International Development (453-A00-5012 and 453-C-00-3051), the National Institutes of Health, UNOP/World Bank/WHO Special Programme for Research and Training in Tropical Medicine, and the MacArthur Foundation. We thank B. Greenwood for serum samples from The Gambia, R. Melton for technical assistance, and R. Rose for manuscript preparation.

9 April 1985; accepted 8 May 1985

Involvement of the *bcl-2* Gene in Human Follicular Lymphoma

Abstract. Recombinant DNA probes were cloned for the areas flanking the breakpoint on chromosome 18 in cells from a patient with acute lymphocytic leukemia of the B-cell type; cells of this line carry the t(14;18) chromosomal translocation. Two of the probes detected DNA rearrangements in approximately 60 percent of the cases of follicular lymphoma screened. In follicular lymphoma, most of the breakpoints in band q21 of chromosome 18 were clustered within a short stretch of DNA, approximately 2.1 kilobases in length. Chromosome 18-specific DNA probes for the areas flanking the breakpoints also detected RNA transcripts 6 kilobases in length in various cell types. The gene coding for these transcripts (the *bcl-2* gene) seems to be interrupted in most cases of follicular lymphomas carrying the t(14;18) chromosomal translocation.

Follicular lymphoma is one of the most common human B-cell neoplasms; in most patients the lymphoma cells carry a translocation between chromosomes 14 and 18 (1,2). By taking advantage of an established cell line, 380, derived from a patient with acute lymphocytic leukemia of the pre-B-cell type (3), we cloned the DNA region joining chromosomes 14 and 18 on the 14q⁺ chromosome of line 380 cells (4). Using chromosome 18-specific DNA probes flanking the chromosome breakpoint of line 380 cells, we also showed DNA rearrangements of the homologous DNA segments in follicular lymphoma cells with the

t(14;18) chromosome translocation (4). In the present study we have "walked" on the region of chromosome 18 that is involved in chromosome rearrangements in B-cell neoplasms to map the breakpoints in follicular lymphoma and to identify the putative *bcl-2* gene, which may have a role in the pathogenesis of follicular lymphoma and other B-cell neoplasms carrying the t(14;18) chromosome translocation.

By starting with DNA probes that mapped close to the t(14;18)-associated breakpoint on chromosome 18 in line 380 leukemic cells, we were able to "walk" on chromosome 18 in both directions and

References and Notes

1. I. A. McGregor, *Trans. R. Soc. Trop. Med. Hyg.* 58, 80 (1964).
2. R. S. Nussenzweig and V. Nussenzweig, *Phil. Trans. R. Soc. Lond. B* 307, 117 (1984).
3. F. Zavala et al., *J. Exp. Med.* 157, 1947 (1983).
4. J. Ellis et al., *Nature (London)* 302, 536 (1983).
5. G. N. Godson et al., *ibid.* 306, 29 (1983).
6. V. Enea et al., *Proc. Natl. Acad. Sci. U.S.A.* 81, 7520 (1984).
7. J. B. Dame et al., *Science* 225, 593 (1984).
8. V. Enea et al., *ibid.* p. 628.
9. M. R. Hollingdale et al., *J. Immunol.* 128, 1929 (1982).
10. Synthetic peptides H-(Asn-Ala-Asn-Pro)₂; H-(Asn-Ala-Asn-Pro)₃; H-(Asn-Ala-Asn-Pro)₄ were synthesized by the stepwise solid-phase method of B. Merrifield [*J. Am. Chem. Soc.* 85, 2149 (1963)]. The attachment of the COOH-terminal amino acid residue, tertbutyloxycarbonyl (BOC)-Pro, was onto a hydroxymethyl phenylacetoxymethyl (PAM) resin (copolyethylene and 1 percent divinylbenzene) support [A. R. Mitchell et al., *J. Am. Chem. Soc.* 98, 7357 (1976)] to prevent loss of peptide chains during the synthesis. BOC-Pro-OCH₂-Pam-resin (0.4 mmol sub-

...but not to
Nathan et al.,
...have high
...do not
...in sera from
...no correlation
...to sporadic
...blood-stage
...quence of the
...elated [1, A
...369 (1985)]
...resides in a
...containing a
...either side by
...In view of
...repeat region
...ing that the
...Pr, it seems
...NP, detect
...an immune
...c and cross-

...193, 673
...rg. Nature
...132, 909
...23, 5665
...s (1984)
...New York,
...Wimmer,
...Gordon,
...son et al.,
...weig, Fed.
...str.), 1808
...for Inter-
...nd 453-C
...Health,
...gramme
...edicine,
...thank B.
...he Gam-
...and R.

ma

g the
cyclic
omal
ly 60
most
hort
cific
ts 6
(the
ing

(4).
d'
is
nts
lk-
to
ch
of
li

determine the structure of the normal chromosome (Fig. 1A). DNA's were extracted from 17 randomly selected follicular lymphomas and screened for DNA rearrangements by Southern blot hybridization with chromosome 18-specific probes a, b, c, and d (Fig. 1A). Probe c detected rearranged DNA fragments in line 380 DNA (Fig. 1B) and also in DNA from line LN128 follicular lymphoma

cells with the t(14;18) chromosome translocation (4). This probe detected a rearranged DNA fragment in only one (FL1018) of the additional 16 cases of follicular lymphoma tested (Fig. 1B). The rearranged DNA fragment was not due to polymorphisms in restriction fragment length because hybridization of FL1018 follicular lymphoma DNA from different preparations from the same pa-

tient revealed unrearranged bands of various intensities; these variations were caused by the variable numbers of normal cells contaminating the neoplastic specimen.

Probe b detected rearrangements in 9 of 17 follicular lymphoma DNA's digested with Bam HI (Fig. 1C) and Sst I (Fig. 1D). Two or three (FL993) rearranged fragments and one unrearranged frag-

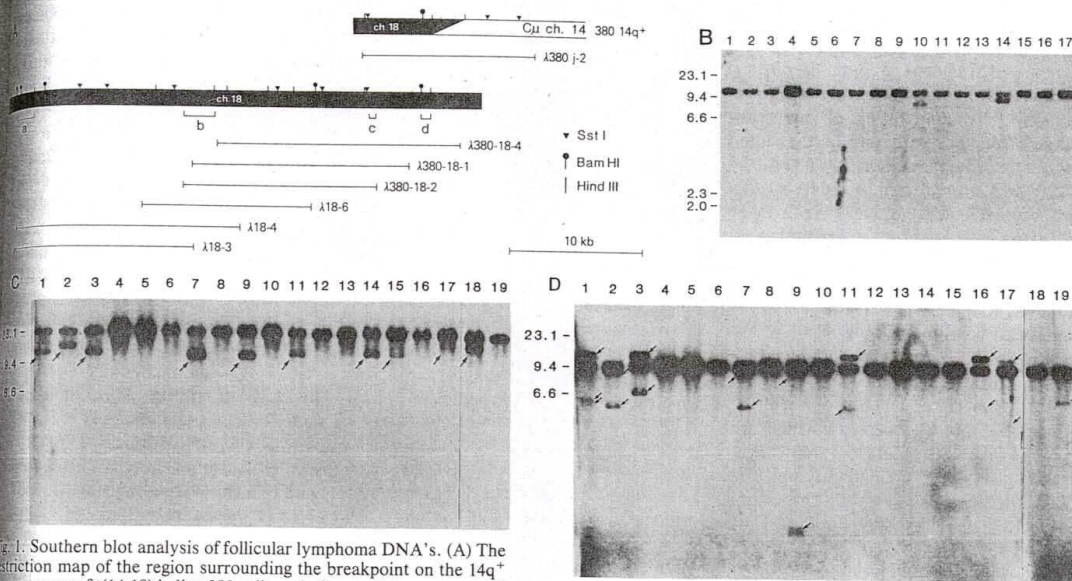


Fig. 1. Southern blot analysis of follicular lymphoma DNA's. (A) The restriction map of the region surrounding the breakpoint on the 14q+ chromosome of t(14;18) in line 380 cells and of normal chromosome 18 are shown. The restriction map of the 14q+ chromosome has been shown previously (4). The structure of normal chromosome 18 was deduced by analyzing the overlapping recombinant clones isolated from a phage library of line 380 DNA screened with the chromosome 18-specific probes. Each horizontal line indicates DNA inserts of representative recombinant clones. (B) Southern blot hybridization with DNA probe c. Sst I-digested follicular lymphoma DNA's were separated on a 0.7 percent agarose gel, transferred to a nitrocellulose filter, and hybridized with probe c. The size of fragments is given in kilobases. (Lane 1) FL993; (lane 2) FL1003; (lane 3) FL1032; (lane 4) FL1162; (lane 5) FL1213; (lane 6) FL1251; (lane 7) FL1341; (lane 8) FL1412; (lane 9) FL989; (lane 10) FL1018; (lane 11) FL1144; (lane 12) FL1175; (lane 13) FL1226; (lane 14) line 380; (lane 15) FL966; (lane 16) FL1245; (lane 17) FL1063. (C) The filter of Bam HI-digested DNA's hybridized with probe b. (Lane 1) FL993; (lane 2) FL1003; (lane 3) FL1032; (lane 4) FL1162; (lane 5) FL1164 (different specimen from the same lymph node as FL1162); (lane 6) FL1213; (lane 7) FL1251; (lane 8) FL1341; (lane 9) FL1412; (lane 10) FL989; (lane 11) FL1144; (lane 12) FL1175; (lane 13) FL1226; (lane 14) FL966; (lane 15) FL1245; (lane 16) FL1018; (lane 17) FL1063; (lane 18) FL1281 (sequential biopsy from FL1063); (lane 19) FL1352. (D) The filter of Sst I-digested DNA's hybridized with probe b. (Lane 1) FL993; (lane 2) FL1003; (lane 3) FL1032; (lane 4) FL1162; (lane 5) FL1164; (lane 6) FL1213; (lane 7) FL1251; (lane 8) FL1341; (lane 9) FL1412; (lane 10) FL989; (lane 11) FL1144; (lane 12) FL1175; (lane 13) FL1226; (lane 14) FL1352; (lane 15) FL1484 (peripheral blood leukocyte DNA from the same source as FL1352); (lane 16) FL966; (lane 17) FL1245; (lane 18) FL1063; (lane 19) FL1281. Rearranged DNA fragments are shown by arrows.

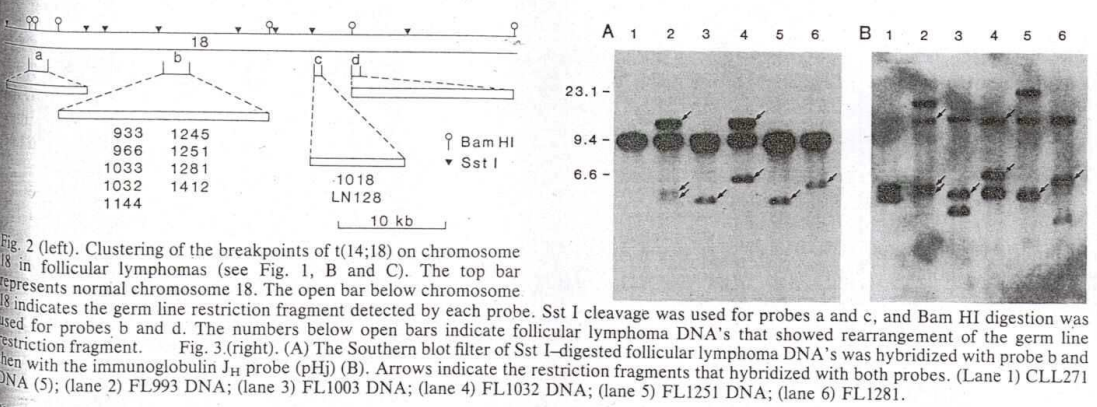


Fig. 2 (left). Clustering of the breakpoints of t(14;18) on chromosome 18 in follicular lymphomas (see Fig. 1, B and C). The top bar represents normal chromosome 18. The open bar below chromosome 18 indicates the germ line restriction fragment detected by each probe. Sst I cleavage was used for probes a and c, and Bam HI digestion was used for probes b and d. The numbers below open bars indicate follicular lymphoma DNA's that showed rearrangement of the germ line restriction fragment. Fig. 3 (right). (A) The Southern blot filter of Sst I-digested follicular lymphoma DNA's was hybridized with probe b and then with the immunoglobulin JH probe (pHj). (B) Arrows indicate the restriction fragments that hybridized with both probes. (Lane 1) CLL271 DNA (S); (lane 2) FL993 DNA; (lane 3) FL1003 DNA; (lane 4) FL1032 DNA; (lane 5) FL1251 DNA; (lane 6) FL1281.

ment (FL1281) were observed after Sst I digestion. This suggested that the breakpoint in these eight cases occurred within probe b, which was 2.1 kb (kilobases) in length (Fig. 2). The two rearranged fragments may represent probe b-hybridizing sequences that were split by the chromosome breakpoints. Thus one of the two fragments may represent the segment of chromosome 18 that is translocated to the 14q⁺ chromosome, and the other may represent the segment of chromosome 18 that remains on the 18q⁻ chromosome. The presence of only one rearranged fragment in Bam HI digests of follicular lymphoma DNA's can be explained by migration of one rearranged fragment with one of the other two hybridizing fragments. Hybridization of 20 normal human DNA's with probe b revealed no DNA rearrangement, which confirms that the rearranged fragments in follicular lymphoma DNA's reflect chromosome translocation and not restriction site polymorphism.

No rearrangements were observed after hybridization of follicular lymphoma DNA's with either DNA probe a or d. Thus DNA probes b and c, which are only 13 kb apart, detected rearrangements in 11 of 18 follicular lymphoma DNA's (62 percent) (Fig. 2). Only sample LN128 was karyotyped before DNA analysis (5) (Fig. 2). Thus, these probes can be used to detect a t(14;18) translocation in B-cell neoplasms. Our data also indicate that, in most follicular lymphomas showing rearrangements, the chromosome breakpoints are clustered within a very short segment of DNA (2.1 kb in size).

We have previously shown that the breakpoint on chromosome 14 in the t(14;18) chromosome translocation of line 380 cells is close to the J_H region of the immunoglobulin heavy-chain locus (4). By comparing the detailed restriction maps of the recombinant clone representing normal chromosome 18 sequences (λ 380-18-4; Fig. 1A) and of the clone representing the breakpoint (λ 380j-2; Fig. 1A), we conclude that the breakpoint on chromosome 14 occurs within the J_H region.

To determine whether the chromosome 18 sequences involved in follicular lymphomas with the t(14;18) chromosome translocation are also translocated to the J_H region of the heavy-chain locus, we hybridized Sst I-digested DNA's with probe b and with a J_H probe (pHj) (4, 5). In five cases tested, some of the rearranged fragments that hybridized with probe b also hybridized with the pHj probe, suggesting that the break-

points on chromosome 14 map to the J_H region (Fig. 3, A and B). We have also cloned the joining region between chromosome 14 and 18 in four (FL966, FL1003, FL1032, and FL1144) of the nine cases described in Fig. 1C and have detected both J_H and probe b sequences within the same genomic clones.

Thus the breakpoints on chromosome 14 in the cases we analyzed are also clustered and involved the J_H region of the heavy-chain locus. Clustering of breakpoints (within a region of 0.9 kb) has been observed on chromosome 11 in cells having the t(11;14) translocation from a patient with chronic lymphocytic leukemia (CLL) of the B-cell type and a patient with diffuse B-cell lymphoma (5, 6). In the case of the t(11;14) chromosome translocation in CLL of the B-cell type, the translocation seems to be sequence-specific and to involve the enzyme that takes part in VDJ joining (6). DNA sequence analysis of the breakpoints in the t(14;18) translocation may provide new findings concerning the mechanisms of this translocation.

We have proposed that a gene, for which we suggested the name *bcl-2*, has

a role in the t(14;18) translocation in follicular lymphomas (4) and in other B-cell neoplasms such as acute B-cell leukemia, from which the 380 cell line was established, and that this gene is important in the pathogenesis of these diseases (3-6).

To determine whether a cellular gene (the putative *bcl-2* gene) is involved in the t(14;18) chromosome translocation, we used probe b to detect RNA transcripts in polyadenylated [poly(A)⁺] RNA isolated from line 380 leukemic cells with the t(14;18) translocation. We detected a 6-kb RNA transcript that hybridized to the nick-translated b probe (Fig. 4, A and C). The smaller RNA band hybridizing with the probe might represent cross-hybridization to contaminating 28S ribosomal RNA. The levels of *bcl-2* transcripts in line 380 cells (pre-B cells) were higher than those in line 697 cells, which are also derived from a pre-B-cell leukemia (7) but carry a t(1;19) chromosome translocation (Fig. 4A). Hybridization of the same filter with a ³²P-labeled human phosphoglycerokinase (PGK) complementary DNA probe (pHPGK-7e) showed approximately the same levels of transcripts (Fig. 4B). Northern blot hybridization of RNA's from an Epstein-Barr virus-transformed lymphoblastoid cell line (GM1500), a chronic myelogenous leukemic cell line (Bv173), an erythroleukemia cell line (K562), and a T-cell lymphoma cell line (Jurkat) indicated the presence of the *bcl-2* transcripts, although in lower amounts than in line 380 leukemic cells carrying the t(14;18) chromosome translocation (Fig. 4C). This suggests that transcription of the *bcl-2* gene of line 380 cells is deregulated by its translocation to the heavy-chain locus. We have shown a similar phenomenon in the case of Burkitt lymphoma, where the *c-myc* oncogene is deregulated by its close proximity to one of the three immunoglobulin loci [see (8)]. Because the breakpoint on chromosome 14 in line 380 cells maps within the J_H region, the immunoglobulin enhancer element between the J_H and the S_H region (9) remains on chromosome 14q⁺, close to the breakpoint. Thus, this enhancer may have a role in *bcl-2* gene activation in line 380 cells.

Because most of the chromosome breakpoints on chromosome 18 in follicular lymphomas showing DNA rearrangement occur within probe b, and because this probe contains an exon (or exons) transcribed into the 6-kb *bcl-2* RNA transcripts, many of the breakpoints in follicular lymphoma must involve or be very close to the *bcl-2* gene.

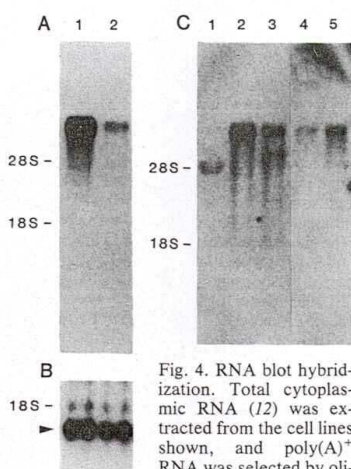


Fig. 4. RNA blot hybridization. Total cytoplasmic RNA (12) was extracted from the cell lines shown, and poly(A)⁺ RNA was selected by oligo(dT)-cellulose column chromatography. About 5 μ g of poly(A)⁺ RNA from each cell line was glyoxylated, separated on a 1 percent agarose gel, and transferred to nitrocellulose filters (13). Each filter was hybridized with nick-translated probe in 50 percent formamide-4 \times SSC (standard saline citrate) at 37°C and washed finally in 0.5 \times SSC at 55°C. (A) (Lane 1) line 380 RNA; (lane 2) line 697 RNA. The filter was hybridized with probe b. (B) The same filter as in (A) was rehybridized with a human phosphoglycerokinase complementary DNA probe, pHPGK-7e (14). The transcript is shown by the arrow. (C) (Lane 1) line K562 RNA; (lane 2) line 380 RNA; (lane 3) line GM1500 RNA; (lane 4) Jurkat cell RNA; (lane 5) line Bv173 RNA. The filter was hybridized with probe b.

location in B-cell leukemia. In at least some cases, the *bcl-2* gene may be split by the translocation, in a manner similar to the splitting of the *c-myc* gene in some Burkitt lymphomas carrying the t(8;14) translocation (10). DNA probes immediately 5' and 3' to probe b on the normal chromosome 18 were also hybridized to the 6-kb *bcl-2* transcripts, which indicates that the *bcl-2* transcription unit must be disrupted by the chromosome translocation in most follicular lymphomas with the t(14;18) translocation.

Many cellular oncogenes are conserved among species during evolution (11). To determine whether the *bcl-2* gene is similarly conserved, we hybridized probe b to cellular DNA's from human, mouse, and Chinese hamster cells. All three mammalian DNA's hybridized to probe b under stringent conditions. Thus we conclude that at least part of the *bcl-2* gene is conserved among mammalian species.

Our results represent a novel approach for the identification of genes that have a role in the pathogenesis of human cancer. Because the immunoglobulin heavy-chain locus on chromosome 14 (12) is the frequent target of the rearrangements in B-cell neoplasia, we cloned the chromosomal breakpoints involved in the t(11;14) (5, 6) and t(14;18) (4) chromosome translocations in B-cell neoplasms. Since most of the chromosomal breakpoints in cases of follicular lymphomas directly involve the transcription unit of the *bcl-2* gene, it seems likely that in most follicular lymphomas the oncogene may be structurally altered, as in the case of the *c-myc* gene in some Burkitt lymphomas. Thus it is possible to take advantage of specific chromosomal alterations in certain human neoplasms to isolate and characterize the genes that take part in the neoplastic process. A logical extension of this approach is to use these DNA probes to detect specific chromosomal alterations in human tumors. The classification and the diagnosis of human B-cell malignancies should be greatly aided by knowledge of the genomic rearrangements and of the genes involved in their pathogenesis.

YOSHIOHIDE TSUJIMOTO

Wistar Institute,
Philadelphia, Pennsylvania 19104

JEFFREY COSSMAN
ELAINE JAFFE

Hematology Section, Laboratory
of Pathology, National Cancer
Institute, Bethesda, Maryland 20205

CARLO M. CROCE

Wistar Institute and Department of
Human Genetics, University of
Pennsylvania School of Medicine

21 JUNE 1985

References and Notes

1. S. Fukuhara *et al.*, *Cancer Res.* **30**, 3119 (1979).
2. J. J. Yunis *et al.*, *N. Engl. J. Med.* **307**, 1231 (1982); J. J. Yunis *et al.*, *Cancer Genet. Cytogenet.* **13**, 17 (1984).
3. L. Pegoraro *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7166 (1984).
4. Y. Tsujimoto *et al.*, *Science* **226**, 1097 (1984).
5. Y. Tsujimoto *et al.*, *ibid.* **224**, 1403 (1984).
6. Y. Tsujimoto *et al.*, *Nature (London)*, in press.
7. D. L. Williams *et al.*, *Cell* **36**, 101 (1984).
8. C. M. Croce and P. C. Nowell, *Blood* **65**, 1 (1985).
9. S. D. Gilles *et al.*, *Cell* **33**, 717 (1983).
10. R. Dalla-Favera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982); R. Taub *et al.*, *ibid.*, p. 7442; R. Dalla-Favera *et al.*, *Science* **219**, 963 (1983).
11. D. Sheiness and J. M. Bishop, *J. Virol.* **39**, 514 (1979); D. Sheiness *et al.*, *Virology* **105**, 415 (1980).
12. C. M. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3416 (1979).
13. P. Thomas, *ibid.* **77**, 5201 (1980).
14. A. M. Michelson *et al.*, *ibid.* **80**, 472 (1983).
15. Supported in part by National Cancer Institute grants CA16685 and CA36521 and by a grant from the American Cancer Society and March of Dimes (C.M.C.).

20 March 1985; accepted 29 April 1985

Heat Shock Genes: Regulatory Role for Differentiation in Parasitic Protozoa

Abstract. The parasitic protozoa *Trypanosoma brucei* and *Leishmania major* are transmitted by insect vectors to their mammalian hosts. The temperature difference between the hosts (25° and 37°C) may induce a heat shock response in the parasite. Transcripts of heat shock genes (homologous to Hsp70 and Hsp83) were 25 to 100 times more abundant in *Trypanosoma brucei* bloodstream forms (trypomastigotes) than in insect (procyclic) stages. In *Leishmania major* the patterns of heat shock gene expression in promastigotes (insect-adapted) and amastigotes (mammal-adapted) were different. A temperature shift in vitro induced differentiation of *Leishmania major* from promastigotes to amastigotes. Therefore, heat shock genes may be responsible for differentiation of these vector-borne parasites.

Heat shock genes are activated when a cell responds to stress, such as an increase in temperature (37° to 42°C) or exposure to inhibitors of oxidative phosphorylation (1). The heat shock response involves the immediate activation of several heat shock genes, resulting in extensive synthesis of heat shock proteins (Hsp's), a rapid decrease in transcription of most other genes, and a cessation in

the synthesis of most other proteins (1, 2). In many organisms heat shock-related genes are expressed during certain stages of cell development: in mice during embryogenesis (3), in erythropoiesis (4), and in yeast at sporulation (5). These heat shock gene responses indicate that Hsp's, which have a nuclear location (6, 7), may be involved in differentiation.

Many parasitic protozoa have biphasic life cycles that involve an insect vector and a mammalian host. Adaptation of the protozoan to either of its hosts involves differentiation with extensive morphological alterations, often including a sexual life cycle in the insect vector and a switch from oxidative phosphorylation in the mammalian host (8). *Trypanosoma brucei* in addition loses its protective cell-surface coat when entering the fly gut, where it differentiates into the non-infective procyclic trypanosome (9, 10). The kinetoplastid protozoa *T. brucei* and *Leishmania major* (= *Leishmania tropica major*) are transmitted by the tsetse fly and the sand fly, respectively. These insects are restricted to habitats with a very narrow temperature range (22° to 28°C) (11, 12). In nature, transfer of the parasite from its poikilothermic (non-temperature-regulated) insect vector to the homeothermic (temperature-regulated) mammalian host might trigger a heat shock response that could play a role in the adaptation of the parasite to life in mammalian host tissues.



Fig. 1. (A) Identification of heat shock gene homologous sequences in the nuclear DNA of *T. brucei* and *L. major*. Nuclear DNA of *T. brucei* stock 427 (lanes 1) (24) and *L. major* strain WR 300 (lanes 2) (19) was prepared (25). Nuclear DNA was digested with Hind III, size-separated in a 0.7 percent agarose gel, transferred to nitrocellulose filters, and hybridized with the *drosophila* Hsp70 [1.0-kb Bam HI-Sal I fragment; clone 229.1 (13)] and Hsp83 [2.9-kb Hae II fragment derived from clone 244 (14)] gene probes. Posthybridization washes were done with 3× standard saline citrate (SSC) at 65°C.