Thus, 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 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 chromosome 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.

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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.

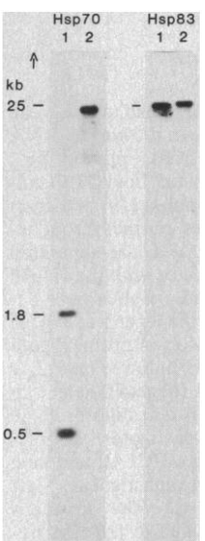

Fig. 1. (A) Identification of heat shock gene homologous sequences in the nuclear DNA of *T. brucei* and *L. major*. Nuclear DNA of *T. brucei* stock 427 (lanes 1) (*24*) and *L. major* strain WR 300 (lanes 2) (*19*) was prepared (*25*). Nuclear DNA was digested with Hind III, size-separated in a 0.7 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. Posthybridizational washes were done with 3× standard saline citrate (SSC) at 65°C.

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 insect to anaerobic respiration 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 noninfective 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 (nontemperature-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.

When nuclear DNA's of *T. brucei* and *L. major* were probed with the drosophila Hsp70 (*13*) and Hsp83 (*14*) heat shock genes under relaxed hybridization conditions, sequences related to heat shock genes were detected (Fig. 1). The same restriction enzyme fragments detected with drosophila Hsp70 and Hsp83 probes are also recognized by yeast Hsp70 (*15*) and Hsp83 (*16*) probes. The hybridizations were therefore not due to fortuitous homology. Heat shock sequences related to the class of small genes, Hsp24 (*1, 2*), could not be detected.

In *T. brucei* bloodstream–derived trypanosomes (trypomastigotes), both an Hsp70- and an Hsp83-related gene are expressed. To detect Hsp transcripts we prepared RNA directly from trypanosome-infected rat blood to minimize degradation of transcripts that may occur during the normally lengthy, low-temperature trypanosome purification. With the drosophila Hsp70 and Hsp83 genes a 2300-nucleotide (nt) and a 2600-nt transcript, respectively, were detected in Northern blots with total RNA (Fig. 2A). RNA prepared from uninfected rat blood showed no hybridization. These messenger RNA (mRNA) levels for trypomastigotes were considerably higher than those seen in the low-temperature (27°C), culture-form procyclic trypanosomes for both the Hsp70 and Hsp83 transcripts, showing that a heat shock response exists (Fig. 2A). The difference was measured by scanning the autoradiograms and comparing the hybridization signals to that of tubulin, which does not hybridize to rat RNA under these conditions. Relative to tubulin, the amount of Hsp mRNA found in procyclic trypanosomes was 25 times less than in the bloodstream form. However, we found a twofold difference in the tubulin hybridization signal of trypomastigotes and procyclic trypanosomes, reflecting a true decrease of tubulin mRNA levels in the procyclic trypanosomes. Furthermore, since the trypomastigote RNA was prepared from whole infected rat blood (ratio of erythrocytes to trypanosomes, 3 to 1), it was contaminated with rat RNA. Thus the 25-fold Hsp mRNA increase is a lower limit and the true increase is likely to be at least 100-fold.

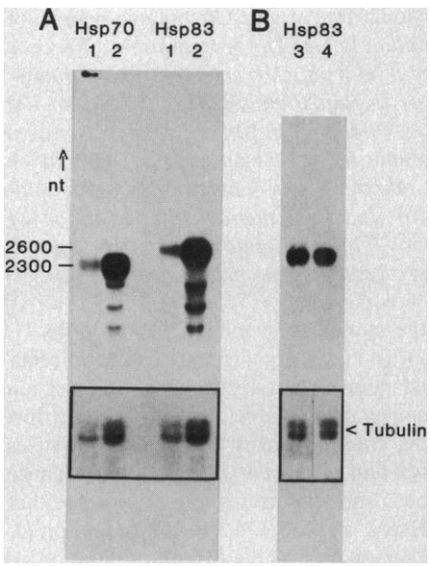

Fig. 2. (A) Quantitation of heat shock gene transcripts in procyclic trypanosomes (*26*) and trypomastigotes. RNA was prepared from infected rat blood (variant 118a) and culture trypanosomes (*26, 27*). The RNA was treated with deoxyribonuclease I, separated in a 1.5 percent agarose gel, and blotted to nitrocellulose filters (*28*). RNA (20 μg) was added to each lane and hybridized with the drosophila Hsp70 and Hsp83 gene probes (lanes 1, RNA from procyclic culture-form trypanosomes; lanes 2, trypomastigote RNA); posthybridizational washes were done with 3× SSC (standard saline citrate) at 65°C. The three hybridizing bands in the low molecular weight range are an artifact that results from the abundance of ribosomal RNA's in this region of the gel. The same filters were hybridized with a *T. brucei* α-β-tubulin gene probe (*29*). The tubulin hybridization signals are indicated in the insets. (B) Comparison of heat shock gene transcripts in trypomastigote cultures at 22°C (lane 3) and 34°C (lane 4). *Trypanosoma brucei* stock 427 (variant 1.208) was adapted to culture with a feeder layer of rat embryo fibroblast in IMDM medium supplemented with 20 percent rat serum at 34°C (*23*). After growth in culture for 10 days the trypanosomes were propagated in BALB/c mice. These trypanosomes were reintroduced to culture and divided approximately every 12 hours at 34°C. RNA was prepared from cultures grown at 34°C and cultures that were transferred from 34° to 22°C and grown for 3 days. The RNA's were separated by size and hybridized with the drosophila Hsp83 probe (10 μg of RNA per lane). The control hybridization performed on the same filter with a *T. brucei* α-β-tubulin gene probe is indicated in the inset.

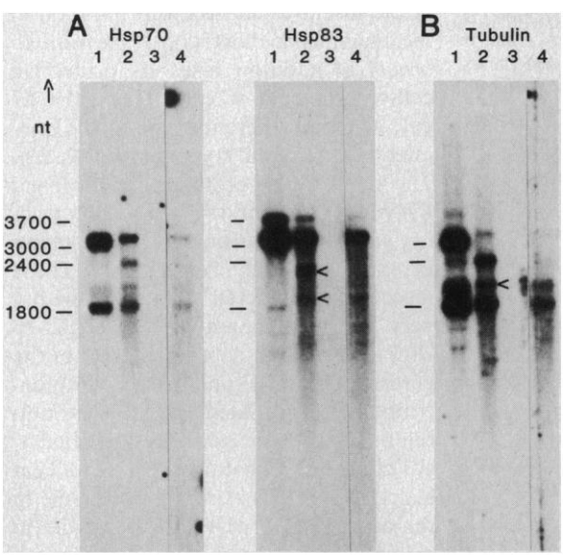

Fig. 3. (A) Heat shock gene transcripts in *L. major*. RNA prepared from cultured promastigotes (25° and 37°C culture conditions) (*19*) and from amastigotes purified from lesions (*30*) was size-separated and hybridized with the Hsp70 and Hsp83 probes as described in the legend to Fig. 1. Lane 1, 20 μg of promastigote RNA, 25°C culture; lane 2, 1 μg of RNA (oligo dT–selected) from lesion amastigotes; lane 3, lesion amastigotes (A^- fraction, 10 μg of RNA); and lane 4, cultured amastigotes (5 μg of RNA). (B) The Northern filter indicated in (A) was hybridized with a *T. brucei* α-β-tubulin gene probe (hybridization at 65°C and 3× SSC).

Leishmania major has a life cycle in an insect vector, the sand fly, and an intracellular stage in the macrophages of mammalian hosts (*17*). In *L. major*, mRNA levels of the Hsp70- and Hsp83-related genes show both qualitative and quantitative differences between promastigotes (insect stage) and amastigotes (mammalian stage) (Fig. 3A). With an Hsp70 gene probe we detected four transcripts in lesion-derived amastigotes and two in promastigotes. The hybridization signals of the 1900- and 3100-nt RNA molecules in promastigotes and the 1900- and 3200-nt molecules in lesion amastigotes were different, showing the quantitative and qualitative differences in these mRNA's. In addition, 2200- and 2400-nt transcripts were found in the amastigotes. Differences in the transcription pattern between promastigotes and lesion-derived amastigotes for the Hsp83-related heat shock genes were less obvious. The two most abundant of the four transcripts comigrated with the promastigote Hsp RNA's. The two additional minor transcripts were detected only in the lesion amastigotes; however, these minor transcripts may have been specific degradation products.

If the differentiation of the parasite, from the insect to the bloodstream form, is controlled by the temperature shift between the hosts, then it should be possible to induce differentiation by growing the low-temperature insect stage at elevated temperatures. We performed this experiment on *L. major* culture promastigotes, comparing the morphology and RNA levels of promastigotes cultured at 25°C with those of promastigotes cultured at 37°C for 5 days

and with those of lesion-derived amastigotes. The promastigotes cultured at 25°C were long slender cells (Fig. 4A), while the amastigotes from lesions (Fig. 4B) and the promastigotes from 37°C cultures (Fig. 4C) were small round cells [as was also recently indicated for *Leishmania mexicana* (*18*)]. The high-temperature promastigotes remained fully viable and infective to BALB/c mice.

On the basis of three additional criteria, we conclude that the temperature shift produces a transformation into an amastigote. First, when serum from infected mice was used to detect parasite antigens in a dot enzyme-linked immunosorbent assay (*19*), the high-temperature promastigotes showed a tenfold increase in antigenicity over the low-temperature promastigotes. Second, the heat shock–specific transcription patterns were compared in promastigotes, lesion amastigotes, and high-temperature promastigotes. The hybridization patterns with the Hsp70 and Hsp83 heat shock genes for the lesion amastigotes and the high-temperature promastigotes were similar (lanes 2 and 4 in Fig. 3). Third, a characteristic pattern of α- and β-tubulin gene expression results from differentiation of promastigotes to amastigotes in *Leishmania enriettii* (*20*). We examined *L. major* promastigotes, lesion amastigotes, and high-temperature promastigotes to see whether a temperature shift induced a characteristic pattern of tubulin transcripts. After heat shock, the mRNA's hybridizing with the tubulin gene probe were identical in lesion and high-temperature promastigotes except for the level of expression of the 2400-nt transcript, which was less abundant in the high-temperature promastigote (Fig. 3B). The loss of the 3000-nt transcript together with the smear of hybridization at 2000 nt were the most obvious results of the temperature shift. The transcription pattern of the high-temperature promastigote was therefore intermediate between promastigotes and amastigotes, and other factors may be needed to obtain complete transformation. From these results we conclude that the temperature-treated promastigote differentiates into an amastigote that we call a "culture-form" amastigote.

As has been shown in *Drosophila* (*21, 22*), the induction of heat shock genes results not only from heat stress but also from metabolic stimuli. In *T. brucei*, heat shock gene expression is retained after a shift from 34° to 22°C when trypanosomes are grown under conditions where they remain infective to laboratory animals and thus do not dedifferentiate to the procyclic insect stage. This was demonstrated by adapting trypomastigotes to culture with a feeder layer of rat embryo fibroblasts (*23*). These culture-form trypomastigotes (division time, 12 hours at 34°C) were grown at 22° or 34°C for 3 days. Heat shock gene transcription patterns and infectivity to mice were then tested. Figure 2B shows that the amount of Hsp83 heat shock gene transcripts is identical at both temperatures. Similar results were obtained for Hsp70. The levels of expression of Hsp mRNA's in the cultures at both temperatures were elevated compared with those in the procyclic trypanosomes. Since both cultures retained infectivity to mice (inoculation with 10^5 trypanosomes led to a parasitemia in 3 days), the temperature shift did not result in dedifferentiation to procyclic stages. This indicates that a heat shock response can be mimicked by altered growth conditions. The high level of expression of several of the *L. major* heat shock gene transcripts in promastigotes may also reflect this phenomenon.

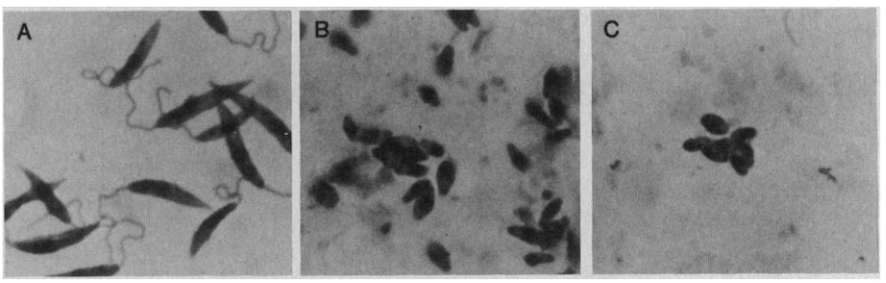

Fig. 4. Light microscopy of cultured promastigotes, lesion amastigotes, and culture-form amastigotes (×1000). (A) *Leishmania major* promastigotes (*19*) cultured 5 days in RPMI-1640 medium with 10 percent fetal bovine serum at 25°C. (B) Lesion amastigotes (*30*) from BALB/c ByJ mice 2 months after infection. (C) Culture-form amastigotes cultured for 5 days in RPMI-1640 with 10 percent fetal bovine serum at 37°C.

Our results show that a heat shock response exists in parasitic protozoa that naturally shuttle between a poikilothermic (22° to 28°C) (*11, 12*) insect vector and a homeothermic (37°C) mammalian host. While a heat shock response in other eukaryotes (37° to 42°C) serves to shut down overall protein synthesis, the response in these parasitic protozoa (25°C to 37°C) does not seem to interfere with cell proliferation. Trypanosomes and leishmania divide normally at the higher temperature and therefore are not in the quiescent state of heat-shocked cells. The in vitro temperature-shift experiments with *L. major* support the hypothesis that differentiation results from a temperature shift which affects the heat shock genes. The role of temperature in the control of parasite development is also illustrated by the failure of parasites to survive when their insect vectors are maintained outside the narrow limits of 22° to 28°C (*11, 12*). However, we do not know at what level regulation of heat shock mRNA occurs. The elevated levels of heat shock transcripts in *T. brucei* and the newly detected transcripts in *L. major* may arise from enhanced transcription of their heat shock genes at the higher temperature, as is the case for heat shock genes in other organisms. Since our experiments show that the differentially expressed tubulin genes may be under the control of heat shock genes, the temperature shift could, by enhancing Hsp transcription, result in adaptation to life in the mammalian host tissues.

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Mitochondrial DNA Size Variation Within Individual Crickets

Abstract. *The mitochondrial DNA's of two closely related cricket species (genus* Gryllus*) share a size polymorphism as evidenced by analysis of restriction fragment patterns. Moreover, 12 of 100 field-collected crickets are heteroplasmic, that is these individuals have more than one size class of mitochondrial DNA. No heteroplasmy for restriction site variation is observed. Intraindividual variation in cricket mitochondrial DNA provides a useful marker for studying the transmission genetics of mitochondrial DNA. Available data on patterns of variation in mothers and offspring suggest that random segregation of mitochondrial DNA variants does not occur rapidly in cricket germ-cell lineages.*

Studies of mitochondrial DNA (mtDNA) in animals have revealed high levels of intraspecific variation and substantial differentiation between closely related species (*1–3*). It has been suggested that mtDNA evolves at a rate significantly faster than that of single-copy nuclear DNA (*3, 4*). Despite the rapid accumulation of differences between lineages, there is generally little or no variation in populations of mtDNA molecules within individuals. Virtually all individuals of animal species examined thus far (mostly vertebrates and *Drosophila*) are homoplasmic; that is, they carry only one type of mtDNA (*2*). Heteroplasmy has been observed or inferred in only a very few species, and in most cases has been analyzed only in a single individual or maternal lineage (*2, 5, 6*).

Homoplasmy is, in part, a consequence of the maternal inheritance of mtDNA. However, the relation between degrees of variation within individuals and degrees of differentiation between individuals also depends on (i) rates of mutation (which generate diversity) and (ii) rates of fixation and loss within germ-cell lineages (which reduce diversity within but not between such lineages) (*5, 7, 8*). A high mutation rate coupled with rapid fixation or loss can lead to the rapid evolution of mtDNA and the absence of individual heteroplasmy (*5, 7, 8*). Under these conditions, the heteroplasmic state (which must intervene if maternal lineages are to become differentiated) exists only briefly.

Here we report the existence of heteroplasmy for mtDNA size variants in natural populations of field crickets. We show that two closely related cricket species share a mtDNA size polymorphism and that in both species there are a substantial number of heteroplasmic individuals. We suggest that the addition or deletion (or both) events responsible for the size variation occur with high frequency and that the size polymorphism has arisen independently in the two lineages. Direct observations of patterns of mtDNA variation in a heteroplasmic female and her offspring indicate that the heteroplasmic state is not transient but is transmitted from mother to offspring. Segregation of mtDNA variants in cricket germ-cell lineages does not occur rapidly.

Our initial reason for examining mtDNA variation in field crickets was to identify suitable genetic markers for characterizing patterns of genetic exchange across a hybrid zone between the closely related species *Gryllus pennsylvanicus* and *Gryllus firmus* (*9*). To do this, we collected crickets from a series of populations across the hybrid zone in Connecticut (*10*). Total DNA was isolated from field-collected individuals and digested with restriction endonucleases (*11*). The resulting digests were processed on 0.7 or 1.2 percent agarose gels and transferred to nitrocellulose filters (*12*). Pure mtDNA was isolated from a laboratory isofemale line of *Gryllus assimilis* (*13*). The purified mtDNA was nick-translated (*14*) to yield a ^{32}P-labeled probe that could be hybridized with the nitrocellulose filters. The mtDNA fragment patterns were then visualized by autoradiography.

In an initial survey of fragment patterns produced by 16 restriction endonucleases, four of the enzymes (Apa I, Hinc II, Hind III, Xba I) showed variation in fragment patterns that could be attributed to gain or loss of a recognition site. Using these variable sites as markers, we determined the composite mtDNA genotypes of all field-collected individuals. Two composite genotypes, labeled AAAA and BBBB (*15*), accounted for 96 percent of our sample. These differed from each other by gain or loss of a single recognition site for each of the four enzymes. In addition, we detected two rare mtDNA genotypes, which in each case could be derived from the common ones by one further single site

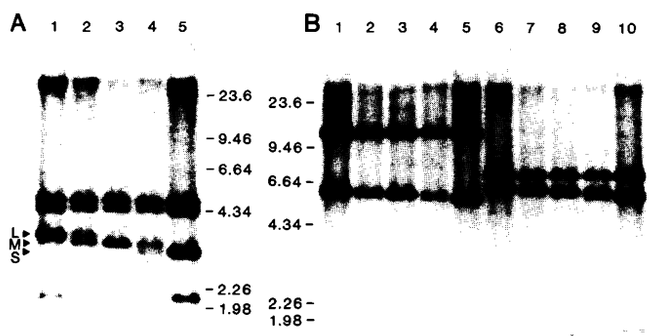

Fig. 1. (A) Eco RI digests of DNA from five individuals of *G. firmus* from Guilford, Connecticut. The fragment patterns for each individual include fragments of approximately 4.9, 4.6, 2.1, and 1.2 kb (the smallest fragment cannot be seen in this autoradiograph). In addition, there is a fifth fragment which appears to come in three sizes (labeled L, M, S). Individuals in lanes 2 and 4 appear to have two size classes of this variable sized fragment. (B) DNA from the same individuals digested with Ava I (lanes 1 to 5) and Hind III (lanes 6 to 10). In both cases gels were 0.7 percent agarose.