MOLECULAR AND CELLULAR BIOLOGY, Apr. 1984, p. 591–598
0270-7306/84/040591-08$02.00/0

Vol. 4, No. 4

Repetitive *Dictyostelium* Heat-Shock Promotor Functions in *Saccharomyces cerevisiae*

JOE CAPPELLO, CHARLES ZUKER, AND HARVEY F. LODISH*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 26 September 1983/Accepted 6 January 1984

The *Dictyostelium* genome contains 40 copies of a 4.7-kilobase repetitive and apparently transposable DNA sequence (DIRS-1) and about 250 smaller elements that appear to be deletions or rearrangements of DIRS-1. Transcripts of these sequences are induced during differentiation and also by heat shock treatment of growing cells. We showed that one such cloned element, pB41.6 (2.5 kilobases) contains a nucleotide sequence identical to the *Drosophila* consensus heat shock promotor. To test whether this sequence might indeed control the expression of DIRS-1-related RNAs, we have cloned this genomic segment into yeast cells. In yeast cells, 41.6 directs synthesis of a 1.7-kilobase RNA that is induced at least 10-fold by heat shock. Transcription initiates at about 124 bases 3′ of the putative promotor sequence and terminates within the 41.6 insert. A 381-base-pair subclone that contains the putative promotor sequence is sufficient to induce the heat shock response of 41.6 in yeast cells.

We have been studying the structure and expression of a family of repetitive DNA elements in the genome of *Dictyostelium discoideum*. There are approximately 250 members of this family dispersed throughout the genome (18). About 40 of these exist as a single discrete unit of about 4.7 kilobases (kb) in length, an element called DIRS-1 (*Dictyostelium* intermediate repeat sequence 1). The remaining members of this family are very heterogeneous in size and appear to represent deletion products or rearrangements (or both) of the intact DIRS-1 element. The genomic locations of DIRS-1 differ in several genetic strains of *Dictyostelium* (2). Thus, we have proposed that the 4.7-kb DIRS-1 element sequence is a transposable element.

DIRS-1-related sequences transcribe a heterogeneous population of RNAs that is expressed in response to various cellular stresses (17). DIRS-1 RNAs are almost undetectable in normal growing cells. Their abundance increases very rapidly after the cells are plated for development. Vegetative cells express DIRS-1 RNAs if submitted to heat shock or if grown to very high cell densities (Zuker and Lodish, manuscript in preparation). Individual DIRS-1 RNAs that are induced very early in development (from 0.5 to 2.5 h after plating) are also specifically induced by heat shock. Under both conditions, the increased abundance of DIRS-1 RNAs is due, in part, to a four- to fivefold induction in the rate of transcription. The maximum level of DIRS-1 RNA is achieved by any single stress; it is not enhanced by the application of an additional stress condition (i.e., development and heat shock together). Thus, the expression of DIRS-1 RNAs is induced and regulated in response to stress, examples of which are heat shock, high cell density, and the manipulations which precede development (17).

The nucleotide sequence of a fragment of DIRS-1, clone pB41.6, has revealed the presence of a putative heat shock promotor. A 14-base sequence was identified which bears a striking homology to the consensus heat shock promotor sequence of *Drosophila* sp. (12, 17). The presence of a functional heat shock promotor in DIRS-1-related sequences could explain the pattern of expression of these RNAs. Unfortunately, because transcripts of DIRS-1 sequences are very heterogeneous in size, probably reflecting the heterogeneity of the genomic templates from which they are derived, the study of RNA transcribed from any specific DIRS-1 copy is impossible in *Dictyostelium* sp. For this reason, we assayed the transcription of 41.6 DNA in a heterologous system, *Saccharomyces cerevisiae*.

We incorporated the 41.6 fragment into a yeast plasmid transformation vector and introduced it into yeast cells. We show here that 41.6 DNA indeed produces a 1.7-kb RNA whose expression is induced at least 10-fold by heat shock. Transcription initiates 124 base pairs (bps) downstream from the putative heat shock promotor sequence. Additionally, heat shock induction of the 41.6 transcript is correlated to the presence of a 381-bp *Eco*RI-*Hin*dIII fragment which contains the heat shock promotor sequence.

MATERIALS AND METHODS

Growth and heat shock treatment of yeast cells. *S. cerevisiae* BWG2-9a-1 (α *his4-519 ade ura3-52 gal4*) was grown on a 2% agar plate or in suspension culture in a medium (YPD) consisting of (grams per liter): yeast extract, 10; peptone, 20; and glucose, 20. URA^+ transformed cells were grown in minimal media containing 0.7% nitrogen base (without amino acids), 40 μg of adenine per ml, 40 μg of histidine per ml, and 2% glucose. Minimal media for growth of untransformed 9a-1 was supplemented with 40 μg of uracil per ml.

Heat shock was routinely performed on 50-ml cultures at an optical density at 600 nm of 0.5 to 0.7. Cultures were shaken in a 50°C water bath until the internal temperature of the culture became 37°C (usually 2 to 2.5 min). The cultures were then immediately transferred to a 37°C shaking incubator for 1 h.

Construction of plasmids. The use of restriction enzymes, DNA ligase, and transformation of *Escherichia coli* HB101 were as described previously (11).

The shuttle vector used in these experiments was β72 (a gift from G. Fink). This plasmid contains selectable markers for both *E. coli* (Amp^r and Tet^r) and *S. cerevisiae* (*URA3*). It also contains origins of replication for both organisms (from

* Corresponding author.

pBR322 and 2μ circle, respectively). 41.6 was ligated into β72 via the unique *Eco*RI and *Hin*dIII restriction endonuclease sites of this vector. The 2.5-kb *Eco*RI fragment of 41.6 was purified from pB41.6 (18) by electroelution from a 1% agarose gel. Briefly, the DNA band was excised from the gel and sealed in a dialysis bag containing 50 mM Tris-borate (pH 8.0), 1 mM EDTA, and 5 μg of bovine serum albumin per ml. The bag was placed in an electrophoresis chamber containing the same buffer without bovine serum albumin. Electrophoresis was conducted for 1 to 2 h at 150 mA. The DNA fragment was recovered by ethanol precipitation and ligated with *Eco*RI-digested β72. Selection for ampicillin-resistant *E. coli* transformants identified 18 β72:41.6 recombinants which were all of the same orientation (β72:41.6-1; see Fig. 1). The next ligation reaction of the above products was subsequently transformed into *E. coli* and selected on tetracycline-containing plates. This procedure yielded nine recombinants, five of the β72:41.6-1 orientation and four of the reverse, β72:41.6-2 orientation. Upon assaying the putative β72:41.6-2 recombinant plasmids in yeast cells (as described below), we found them all to be nonresponsive to heat shock, in comparison with β72:41.6-1. We suspected that some rearrangements occurred during growth in *E. coli*, and thus we constructed β72:41.6-2R by transforming an identical ligation reaction directly into yeast cells. Screening and identification of β72:41.6-2R is described below.

The construction of β72:41.6-3 and β72:41.6-4 was accomplished by ligation of the 2.5-kb *Eco*RI 41.6 fragment, digested with *Hin*dIII, with β72 cleaved with both *Eco*RI and *Hin*dIII. The plasmids were isolated from *E. coli* and analyzed by restriction enzyme analysis. β72:41.6-5 is a reconstruction of the full-length 2.5-kb *Eco*RI 41.6 insert by insertion of a *Sau*A-*Hin*dIII fragment of β72:41.6-1, which contains the 381-bp *Eco*RI-*Hin*dIII fragment of 41.6, into β72:41.6-4 after cleavage of the latter with *Hin*dIII and *Bam*HI. This plasmid was isolated directly from yeast cells and characterized by restriction digests.

Transformation of yeast cells and RNA isolation. Yeast cells were transformed with either 0.5 to 1.5 μg of partially purified plasmid DNA isolated from *E. coli* or with a ligation reaction containing 1 μg of restriction enzyme-digested vector DNA and 0.5 to 1.0 μg of purified insert fragment. Transformation was by the lithium acetate-polyethylene glycol procedure described previously by Ito et al. (6). Transformed cells were selected for growth on minimal plates in the absence of uracil.

URA^+ transformants were grown at 30°C in 100 ml of liquid minimal cultures to an optical density at 600 nm of 0.4 to 0.7. The culture was then split equally; half was allowed to grow for one additional hour at 30°C, and the other half was heat shocked at 37°C for 1 h as described above. Total cellular RNA was purified from each culture by the glass bead disruption method (1) with the following modifications. Cultures (50 ml) were washed several times in cold cycloheximide solution (0.05 mg/ml) before disruption. Glass bead disruption was performed in 250 μl of 50 mM Tris-hydrochloride (pH 6.8)–2 mM EDTA–0.05 mg of cycloheximide per ml. Total RNA (~100 to 600 μg) from each culture was dissolved in 0.1 to 0.2 ml of sterile deionized water, and the concentration was determined spectrophotometrically by absorption at 260 nm. The relative concentration of each solution was also verified by the intensity of ethidium bromide-stained rRNA bands after electrophoresis in denaturing agarose gels.

RNA blot hybridizations. Total RNA (15 μg per lane) was size fractionated on a 1.5% denaturing agarose-formaldehyde gel as described previously by Chung et al. (2). The RNA was transferred to a Gene Screen filter (New England Nuclear Corp.) according to the specifications of the manufacturer, and the filters were hybridized with 1×10^6 to 5×10^6 cpm of nick-translated [^{32}P]DNA probes per ml (●). Hybridizations were carried out in 1.5 M NaCl–0.1 M sodium citrate–0.04% polyvinylpyrrolidone–0.04% bovine serum albumin–0.04% Ficoll–20 mM sodium phosphate (pH

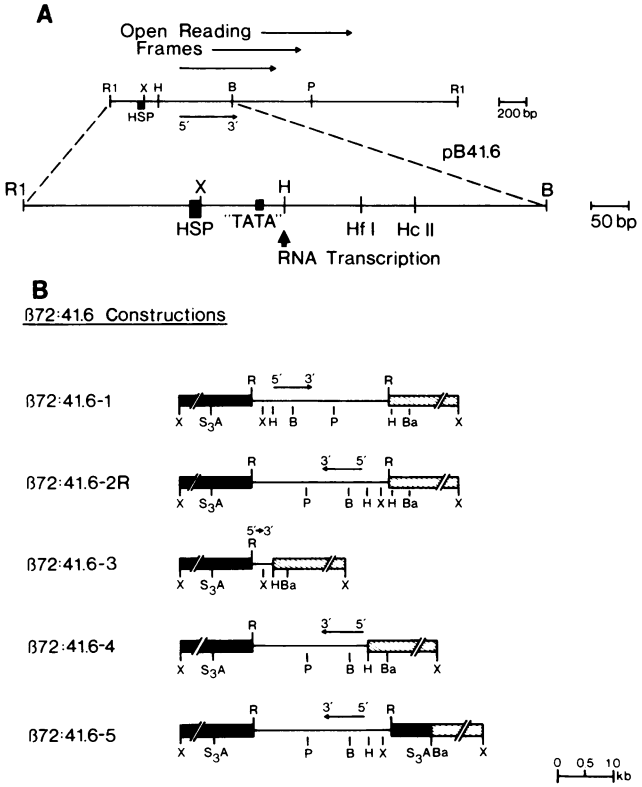

FIG. 1. Structures of the 41.6 repetitive DNA element and the 41.6-β72 transformants studied. (A) The restriction map of 41.6 and the positions of the three longest open reading frames are depicted by arrows above the map (17). The arrow below the map shows the direction of transcription of 41.6. The locations of the putative heat shock promotor (HSP), a TATA box, and the initiation of transcription are also indicated. The positions of the sites for the restriction endonucleases *Hin*fI (Hf I) and *Hin*cII (Hc II) are shown with reference to the S1 mapping experiment shown in Fig. 4. (B) The 2.5-kb *Eco*RI fragment of 41.6 was cloned into the yeast plasmid vector β72 either directly or after cleavage with the restriction endonuclease *Hin*dIII. The structure and orientation of the 41.6 sequence (single line) in each of these clones is diagrammed. β72:41.6-1 and β72:41.6-2R contain the entire 2.5-kb *Eco*RI fragment cloned into the *Eco*RI site of β72 in either of the two possible orientations. β72:41.6-3 and β72:41.6-4 contain the 381-bp and the 2.2-kbp *Eco*RI-*Hin*dIII fragments of 41.6, respectively. β72:41.6-5 is an insertion of a *Hin*dIII-*Sau*3A fragment from β72:41.6-1 into the *Hin*dIII and *Bam*HI sites of β72:41.6-4, thus reforming the full-length 2.5-kb *Eco*RI fragment in this clone. More detailed descriptions of these constructions are contained in the text. The structure and integrity of each of the recombinant molecules was determined by restriction mapping and Southern blotting as described in the text. The shaded and hatched boxes represent the β72 vector sequences on either side of the insertion region. The arrow above each construction indicates the orientation of the insert as established in (A). Abbreviations: R and R1: *Eco*RI; X: *Xba*I; H: *Hin*dIII; B: *Bgl*II; P: *Pvu*II; Ba: *Bam*HI; S_3A: *Sau*3A.

6.8)–50 μg of denatured salmon sperm DNA per ml–150 μg of polyadenylic acid per ml. After incubation in this mixture at 63°C for 72 h, the filter was washed for several hours in four changes of 15 mM NaCl–1.5 mM sodium citrate at 50°C. The filter was autoradiographed for 1 to 2 h at −80°C with an intensifying screen.

S1 nuclease mapping. Si nuclease mapping was performed with asymmetrically end-labeled double-stranded DNA fragments exactly as described previously by Weaver and Weissmann (15). A typical reaction mixture contained 0.5 pmol of end-labeled fragment (0.2×10^6 to 1.0×10^6 cpm) hybridized with 20 μg of total yeast RNA at 37°C for 18 to 24 h in 70% formamide–0.4 N NaCl–0.04 M PIPES [piperazine-*N*,*N*′-bis (2-ethanesulfonic acid)] (pH 6.4)–0.001 M EDTA. The hybridization reaction (volume, 20 μl) was diluted by adding 180 μl of cold S1 nuclease buffer (0.25 M NaCl, 0.03 M sodium acetate [pH 4.6], 0.001 M $ZnSO_4$, 20 μg of denatured salmon sperm DNA per ml) and digested with 200 U of S1 nuclease (Bethesda Research Laboratories). The S1-protected nucleic acids were collected by ethanol precipitation and electrophoresed on a denaturing 8% polyacrylamide–7 M urea sequencing gel. The gel was autoradiographed for 48 h at −70°C.

RESULTS

The plasmid β72 was used as the transformation vector for the introduction of the *Dictyostelium* 41.6 sequence into yeast cells. β72 contains the yeast *URA3* gene which allows its selection in *ura3* host cells. It also contains a fragment of the yeast 2μ circle DNA that functions as an origin of replication. The remainder of the vector is essentially pBR322 DNA that facilitates plasmid growth and selection in *E. coli*. Therefore, plasmids constructed in β72 can propagate in either *E. coli* or *S. cerevisiae*.

41.6 is the 2.5-kb *Eco*RI *Dictyostelium* insert from clone pB41.6 (Fig. 1A). This fragment was cloned into the single

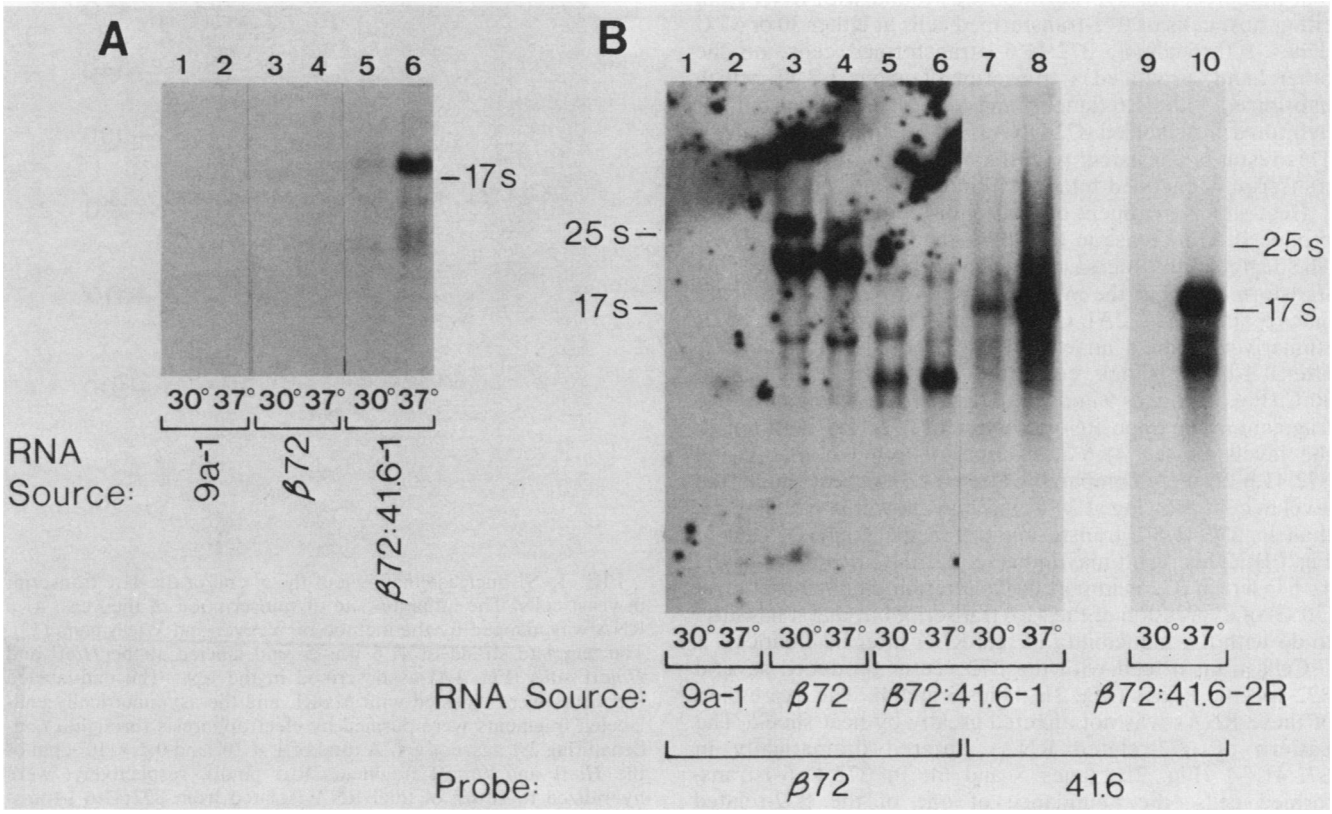

FIG. 2. RNA complementary to 41.6 in yeast cells transformed with 41.6-β72 recombinants. *S. cerevisiae* BWG2-9a-1 (*his4-519 ade ura3-52 gal4*) was transformed with 0.5 to 3 μg of partially purified plasmid DNA from β72, β72:41.6-1, or β72:41.6-2R recombinants. $Ura3^+$ transformants were isolated and were tested for the presence of the appropriate plasmid by Southern blots (data not shown). Total RNA was purified from the yeast transformants before and after heat shock (1 h at 37°C). The RNAs (15 μg per lane) were size fractionated on a denaturing 1.5% agarose-formaldehyde gel and transferred to Gene Screen filters as described in the text. After hybridization for 72 h at 64°C with 1.0×10^6 to 5.0×10^6 cpm of ^{32}P-labeled 41.6 insert or β72 DNA per ml, the blots were washed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 50°C for several hours. The filters were autoradiographed for 2 h at −80°C with an intensifying screen. (A) Lanes 1 and 2 contain total RNA from untransformed host cells, 9a-1, before (30°C) and after heat shock (37°C); lanes 3 and 4 contain RNA from β72-transformed cells; and lanes 5 and 6 contain RNA from β72:41.6-1-transformed cells. The blot was hybridized with nick-translated purified 2.5-kb *Eco*RI 41.6 fragment. 17S refers to the migration of the yeast rRNAs. (B) An RNA blot similar to the one shown in (A) was hybridized with nick-translated β72 vector DNA to distinguish the vector-derived transcripts from the 41.6-specific transcript. Total RNA (15 μg) from the following cells was applied to the following lanes. Lanes 1 and 2, 9a-1 host cells; lanes 3 and 4, β72-transformed cells; lanes 5 through 8, β72:41.6-1-transformed cells; and lanes 9 and 10, β72:41.6-2R-transformed cells. RNAs in lanes 1, 3, 5, 7, and 9 were obtained from cells grown at 30°C, and lanes 2, 4, 6, 8, and 10 contain RNA from the same cells heat shocked for 1 h at 37°C. Lanes 1 to 6 were probed with β72 vector DNA. After removal of the hybridized probe from lanes 5 and 6, the blot was reprobed with nick-translated 41.6 DNA (lanes 7 and 8). Lanes 9 and 10 were also hybridized with nick-translated 41.6. 25S and 17S refer to the migration of the yeast rRNAs.

*Eco*RI site of β72. The two possible orientations, designated β72:41.6-1 and β72:41.6-2R, are shown in Fig. 1B. 41.6 contains a single *Hin*dIII site at position 381 which was used in subcloning the two *Eco*RI-*Hin*dIII fragments of 41.6 into β72. The resulting clones, β72:41.6-3 and β72:41.6-4, contain the 381-bp and the 2.2-kb *Eco*RI-*Hin*dIII fragments of 41.6, respectively. The final plasmid construction, β72:41.6-5, is a reconstruction of the full-length 41.6 *Eco*RI fragment in β72:41.6-4 by insertion of a *Hin*dIII-*Sau*3A fragment of β72:41.6-1 into the *Hin*dIII-*Bam*HI-digested β72:41.6-4.

The expression of 41.6 in yeast cells was conducted by selecting clones of cells transformed with β72:41.6-1 that were URA positive. These were tested for the presence of 41.6-specific transcription products. Total cellular RNA was purified from host 9a-1 cells, β72-transformed cells, and β72:41.6-transformed cells, both after growth at normal temperature (30°C) and after heat shock treatment for 1 h at 37°C. The RNA was size fractionated on a denaturing 1.5% agarose-formaldehyde gel, transferred to Gene Screen filters, and hybridized with 41.6 [^{32}P]DNA. Figure 2A shows that no detectable hybridization was found with RNA from either host cells or β72-transformed cells at either 30 or 37°C (lanes 1 through 4). β72:41.6-1-transformed cells, on the other hand, produced a transcript of about 1.7 kb which hybridized with 41.6 (lanes 5 and 6). This transcript did not hybridize with labeled β72 DNA (Fig. 2B, lanes 5 through 8). This result is consistent with the notion that the 1.7-kb 41.6 transcript is encoded totally within the 41.6 sequence.

Heat shock treatment of yeast cells containing β72:41.6-1 produced an increase in the abundance of the 1.7-kb RNA. The degree of this increase varied among different β72:41.6-1 transformants, but the average was 10- to 12-fold (compare lanes 5 and 6, Fig. 2A). Cells transformed with β72:41.6-2R similarly contained an elevated level of this 1.7-kb RNA after a 1-h heat shock, compared with control cells kept at 30°C (Fig. 2B, lanes 9 and 10). β72:41.6-2R contains the 41.6 fragment in the opposite orientation of β72:41.6-1. Although the levels of the 41.6 transcript in both β72:41.6-1 and β72:41.6-2R were comparable after the 37°C heat shock, the level in cells growing at 30°C was much lower in β72:41.6-2R than in β72:41.6-1 transformants (compare lanes 7 and 9, Fig. 2B). This result may indicate that the orientation of the 41.6 insert in β72 is important in determining the basal level (30°C) of expression of the 41.6 transcript but that it has little to do with the inducibility of this RNA after heat shock.

Cells transformed with the β72 vector produced several β72-specific RNAs (Fig. 2B, lanes 3 and 4). The expression of these RNAs was not affected greatly by heat shock. The pattern of β72-related RNAs differed dramatically in β72:41.6-1 (Fig. 2B, lanes 5 and 6). In β72:41.6-1-transformed cells, the abundance of one of the β72-related transcripts (1.3 kb in size) was reduced by heat shock, and the other (0.9 kb in size) was induced by about threefold. Neither of these species hybridized with 41.6 (Fig. 2B, lanes 7 and 8). We do not yet understand the reason for the fluctuation in the expression of the β72-derived transcripts in β72:41.6-1 transformants, but the involvement of the 41.6 sequences in this phenomenon is under investigation (see below).

Site of transcriptional initiation in 41.6. The initiation site of transcription of 41.6 RNA was determined by the S1 nuclease mapping procedure of Weaver and Weissmann (15). The 5′ end-labeled probes used for this analysis were generated by digestion of 41.6 with *Hin*fI and *Hin*cII. The position of the label in each of these fragments was at 484 and 543 bp, respectively, within the 41.6 insert (Fig. 1). Each

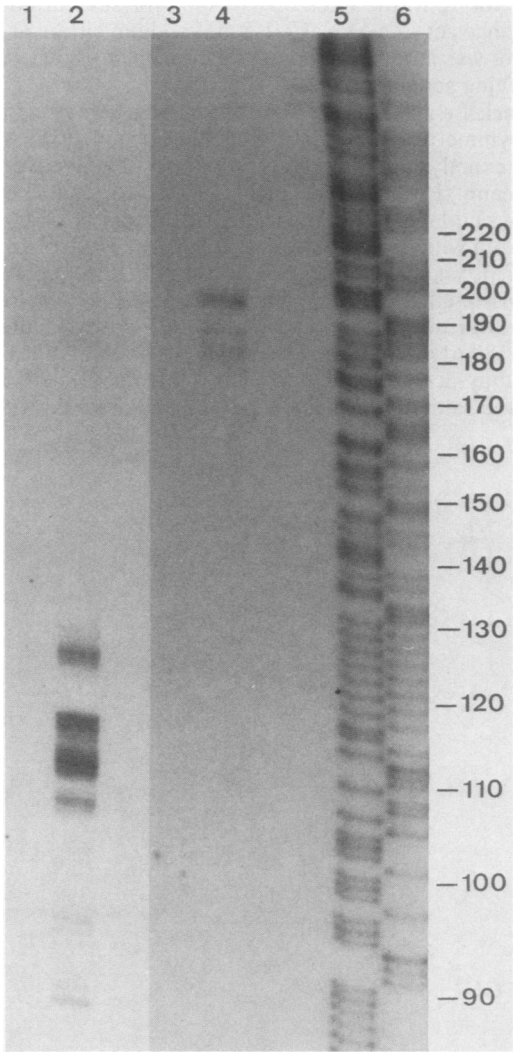

FIG. 3. S1 nuclease mapping of the 5′ end of the 41.6 transcript in yeast cells. The initiation site of transcription of the yeast 41.6 RNA was mapped by the method of Weaver and Wiessmann (15). The template strand of 41.6 was 5′ end labeled at the *Hin*fI and *Hin*cII sites (Fig. 1A) as described in the text. The end-labeled fragments were digested with *Eco*RI, and the asymmetrically end-labeled fragments were purified by electrophoresis through a non-denaturing 2% agarose gel. A total of 1×10^6 and 0.2×10^6 cpm of the *Hin*fI and *Hin*cII fragments (0.5 pmol), respectively, were hybridized to 20 μg of total RNA isolated from β72:41.6-1-transformed yeast cells heat shocked for 1 h at 37°C. The hybridization was carried out in 70% formamide–0.4 M NaCl–0.04 M PIPES (pH 6.4)–0.001 M EDTA for 18 h at 37°C. The hybridization reaction was digested with S1 nuclease as described in the text and applied to an 8% polyacrylamide–7 M urea sequencing gel. Lanes are as follows. Lane 1: S1 endonuclease protection of *Hin*fI end-labeled fragment hybridized with 20 μg of yeast tRNA. Lane 2: total RNA (20 μg) from β72:41.6-1-transformed cells after heat shock. Lane 3: S1 endonuclease protection of *Hin*cII end-labeled fragment hybridized with 20 μg of yeast tRNA. Lane 4: RNA (20 μg) from β72:41.6-1-transformed cells after heat shock. A Maxam and Gilbert (10) sequencing ladder generated by G+A and C+T chemical cleavage reactions for size markers is shown in lanes 5 and 6. Marker sizes, in nucleotides, are given by numbers at the right.

of the probes was hybridized with RNA from heat-shocked β72:41.6-1 cells. The hybridization mixture was then digested with S1 nuclease, and the S1-protected products were size fractionated on an 8% polyacrylamide-urea sequencing gel. Each DNA probe produced four major S1-protected fragments (Fig. 3). (A certain degree of band heterogeneity occurred; most likely this represents the inherent imprecision of S1 nuclease digestion.) Band sizes were determined for the most intense band of a cluster; they are 121, 113, 108, and 104 nucleotides for the *Hin*fI probe and 192, 184, 178, and 175 nucleotides for the *Hin*cII probe.

The sizes of these DNA bands are a measure of the extent of hybridization of the probe and its complementary RNA. Figure 4 marks the distance to the end of this complementarity for each of the four bands generated from both probes in the nucleotide sequence of 41.6. Both the *Hin*fI and *Hin*cII probes map the four sites of initiation to positions 362, 370, 376, and 379. This positions the sites of transcription initiation 124 to 141 bases from the putative heat shock promotor (Fig. 4).

Location of the sequences responsible for the heat shock response of 41.6. As noted above, 41.6 DNA contains a 14-base sequence which is almost identical to the heat shock promotor of *Drosophila* sp. (17). This sequence begins at nucleotide 221, within the 381-bp *Eco*RI-*Hin*dIII fragment (Fig. 1). If this sequence is indeed responsible for the heat shock inducibility of the 41.6 transcript, then the absence of this fragment from a β72:41.6 plasmid should eliminate its ability to respond to heat shock. Plasmid β72:41.6-4 contains only the 2.2-kb *Hin*dIII-*Eco*RI fragment of 41.6 and therefore lacks the 381-bp *Eco*RI-*Hin*dIII fragment which contains the presumptive promotor. Figure 5, lanes 5 and 6, shows that three or four 41.6-specific transcripts are produced in β72:41.6-4-transformed yeast cells. None of these transcripts is inducible by heat shock.

Insertion of the 381-bp *Eco*RI-*Hin*dIII fragment into the appropriate location of β72:41.6-4 should, on the other hand, reinstate normal heat shock control over 41.6 expression. β72:41.6-5 is a plasmid formed by the insertion of a *Hin*dIII-*Sau*3A fragment from β72:41.6-1 into β72:41.6-4 digested with *Hin*dIII and *Bam*HI (Fig. 1). Figure 3, lanes 7 and 8, show that cells transformed with β72:41.6-5 produce the same 1.7-kb 41.6 transcript as does β72:41.6-1 and also induce its expression after heat shock. These results are consistent with the notion that sequences required for both the heat shock induction and the correct transcriptional initiation of the 1.7-kb 41.6 transcript are located within the 381-bp *Eco*RI-*Hin*dIII fragment.

β72:41.6-3 tests whether or not the 381-bp *Eco*RI-*Hin*dIII fragment is sufficient to place the transcription of an adjacent non-41.6 sequence under heat shock control. This plasmid contains the 381-bp *Eco*RI-*Hin*dIII fragment of 41.6 inserted in β72 such that transcription, proceeding from the *Hin*dIII site of 41.6 away from the *Eco*RI site, would continue into the tetracycline resistance gene of β72. Figure 5 shows that cells transformed with β72:41.6-3 do not produce any RNAs that hybridize with 41.6 (lanes 3 and 4). However, the cells do produce a 1.3-kb RNA that hybridizes with β72 DNA and that is inducible by heat shock. Its abundance increases over 10-fold after heat shock. Thus, the 381-bp *Eco*RI-*Hin*dIII fragment of 41.6 contains all of the sequences required to induce transcription of adjacent sequences by heat shock.

DISCUSSION

We have previously shown that the cloned 2.5-kb *Dictyostelium* repetitive element, pB41.6, contains a 14-nucleotide sequence at residues 221 to 237 that greatly resembles the *Drosophila* consensus heat shock promotor (12, 17). The results presented here demonstrate that in yeast cells, 41.6 is a functional transcription unit whose expression is induced by heat shock.

41.6 contains all of the necessary sequences for initiation, termination, and regulation of its own transcription. When introduced into yeast cells, 41.6 produces a 1.7-kb transcript. The abundance of this transcript increases over 10-fold upon heat shock treatment. This transcript initiates and terminates within 41.6 sequences. Four distinct but closely

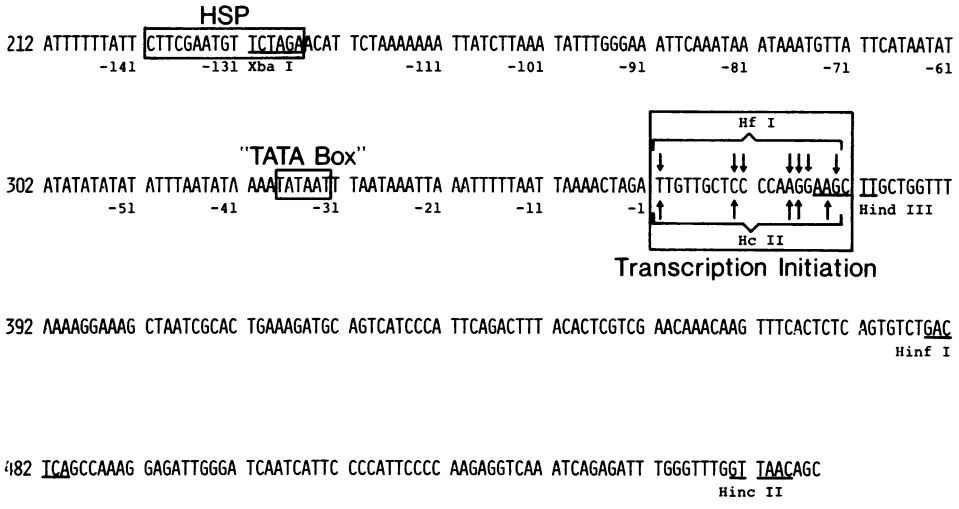

FIG. 4. Nucleotide sequence of the transcriptional control region of 41.6. The diagram shows the nucleotide sequence of the region surrounding the site of transcriptional initiation as determined in Fig. 3. The positions of the S1 nuclease-protected fragments are indicated by arrows within the box enclosing nucleotides 362 to 381; the arrows above the sequence show the positions of the *Hin*fI-protected fragments and those below the *Hin*cII-protected fragments. The locations of the putative TATA box and the consensus heat shock promotor sequence (HSP) are also indicated. The numbers below the sequence denote upstream distance in relation to the 5'-most nucleotide that initiates transcription. Numbers at the left represent distance from the *Eco*RI site at the 5' end of 41.6.

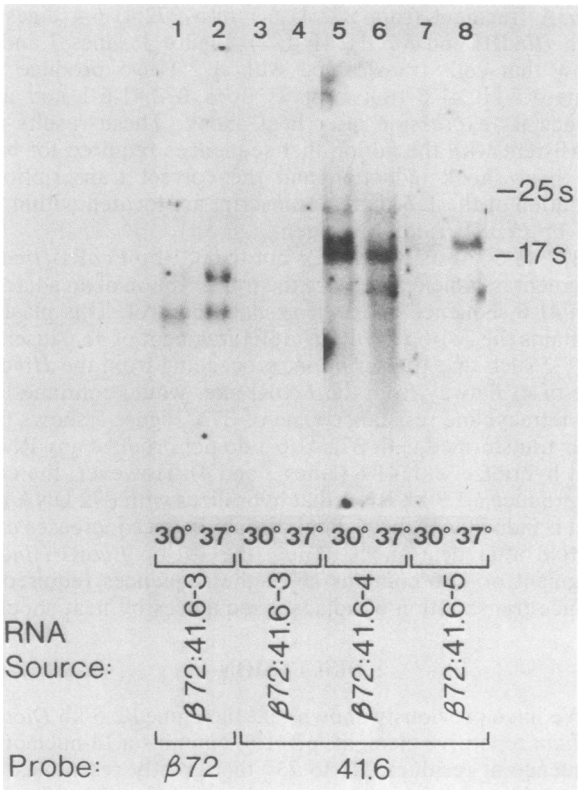

FIG. 5. RNAs present in cells transformed with derivatives of β72:41.6. Total RNA was isolated from cells transformed with β72:41.6-3, β72:41.6-4, and β72:41.6-5 before (30°C) and after heat shock (37°C). Total RNA (15 μg per lane) was size fractionated on a 1.5% denaturing agarose-formaldehyde gel and transferred to Gene Screen filters. After hybridization for 72 h at 64°C with 10^6 cpm of ^{32}P-labeled 41.6 insert or β72 DNA per ml, the blots were washed in 0.1× SSC at 50°C. Exposure was for 2 h at −80°C with an intensifying screen. Lanes are as follows. Lanes 1 through 4 contain RNA from β72:41.6-3-transformed cells; lanes 5 and 6 contain RNA from β72:41.6-4-transformed cells; and lanes 7 and 8 contain RNA from β72:41.6-5-transformed cells. Lanes 1, 3, 5, and 7 contain RNA purified from cells grown at normal temperature (30°C), and lanes 2, 4, 6, and 8 contain RNA from cells heat shocked for 1 h at 37°C. Lanes 1 and 2 were hybridized with β72 DNA, and lanes 3 through 8 were hybridized with 41.6 DNA. 25S and 17S refer to the migration of the yeast rRNAs.

spaced sites of transcription initiation are mapped to positions 362 through 380 of 41.6 (Fig. 4) or about 124 bases downstream of the putative heat shock promotor. We stated previously that transcriptional initiation of 41.6 or 41.6-like RNAs in *Dictyostelium* sp. occurs at about position 320 of 41.6 (17). This difference of 40 bases between the initiation sites in *Dictyostelium* sp. and yeasts might reflect an inherent difference in the preferred nucleotide sequences surrounding sites of transcription initiation of this promotor in the two organisms. Yeast cells also initiate the transcription of a *Dictyostelium* discoidin gene at a novel position, in this case 100 bases upstream of the normal site (7). The direction of transcription of 41.6 in yeast cells is such that the RNA could transcribe proteins encoded by the three long open reading frames contained in the 41.6 sequence (17).

Sequences containing the heat shock promotor (the 381-bp *Eco*RI-*Hin*dIII fragment) are responsible for the heat shock a plasmid that lacks this fragment is not heat shock inducible. Moreover, the presence of this sequence, alone, is sufficient to render transcription of an adjacent vector sequence subject to heat shock control. This analysis demonstrates that the DNA fragment which contains the consensus heat shock promotor (located at bases 221 to 237) is indeed responsible for the heat induction of 41.6 in yeast cells.

The relationship between 41.6 and the transposable element DIRS-1 is shown in Fig. 6. Genomic clone SB41 contains an 8.7-kb fragment of *Dictyostelium* genomic DNA containing an intact 4.7-kb DIRS-1 element. 41.6 is defined as the 2.5-kb *Eco*RI fragment found at the right of the clone, and thus contains the right inverted terminal repeat of the central intact DIRS-1 (2; C. Zuker, J. Cappello, H. F. Lodish, P. George, and S. Chung, Proc. Natl. Acad. Sci. U.S.A., in press). The sequence of 41.6 downstream of the *Hin*dIII site is very closely related to the right half of the intact DIRS-1, as determined both by restriction mapping and partial nucleotide sequencing (Fig. 6; unpublished data). We hypothesize that this peculiar genomic arrangement of a DIRS-1 element flanked by a DIRS-1-related sequence was generated by an insertion of the intact transposon within a preexisting DIRS-1-related sequence. Therefore, the heat shock promotor sequence assayed here is part of the right terminal repeat of DIRS-1, and the 41.6-specific RNA transcribed from this promotor represents sequences flanking the central DIRS-1. The induction of flanking sequence transcription is commonly linked with transposable elements and may play an interesting role in the evolutionary dispersal of regulatory sequences to new locations in the genome (3, 4).

Whether the heat shock promotor found in the right inverted terminal repeat of DIRS-1 is involved in the transcription of the transposon itself is not clear. However, we can show that the 41.6 promotor, when isolated in a plasmid, can function bidirectionally in yeast cells. Figure 2, lanes 5 and 6, and Fig. 5, lanes 1 and 2, show that β72:41.6-1 and β72:41.6-3 produce a 0.9-kb vector RNA whose size and abundance are apparently increased as a response to heat shock. This RNA contains sequences found in the vector upstream of the heat shock promotor of 41.6 and presumably is a transcript of much if not all of the ampicillin resistance gene (data not shown). This induction by heat shock of the expression of this transcript requires the presence of the 41.6 promotor in the adjacent region. No changes in the expression of this transcript during heat shock were seen in β72:41.6-2R or β72:41.6-4 (data not shown). If, in *Dictyostelium* sp., the 41.6 promotor is indeed bidirectional, it could be involved in the heat shock expression of some DIRS-1 sequences.

The *Dictyostelium* genome contains approximately 250 copies of DIRS-1-related sequences. Approximately 40 of these copies are contained within the 4.7-kb transposable element (2). The other 41.6-related sequences appear to be insertions or deletions of DIRS-1. DIRS-1 and pB41.6 hybridize with a large number of cytoplasmic polyadenylated RNAs which accumulate in a coordinated fashion in response to heat shock and development. These RNAs are derived, most likely, from the different DIRS-1-related genomic copies.

The terminal repeats of DIRS-1, shown here to contain a functional heat shock promotor, are found associated with intact as well as deleted copies of DIRS-1 (Zucker et al., in press). The demonstration that these sequences contain functional heat shock promotors explains, at least in part, the coordinate transcriptional induction of the DIRS-1 RNAs

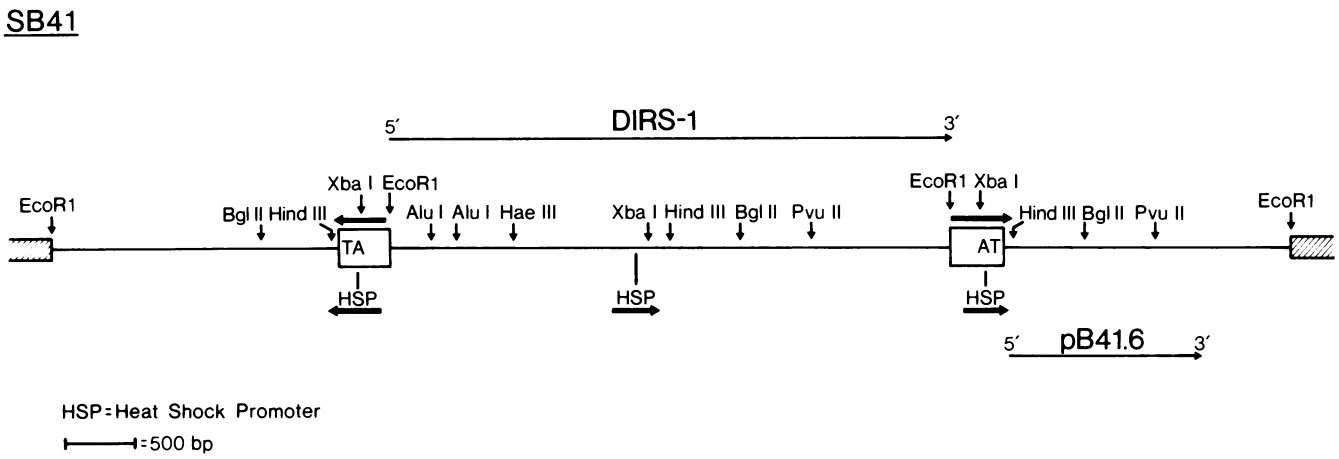

FIG. 6. Structure and transcription of DIRS-1 in genomic clone SB41. The diagram shows the physical map of genomic clone SB41. The center 4.0-kb *Eco*RI fragment corresponds to DIRS-1 and the two open boxes at each of its ends, the terminal inverted repeats. The arrows on top of the repeats indicate the direction of the repeats. The 2.5-kb *Eco*RI fragment on the right of DIRS-1 is clone pB41.6. This subclone of SB41 contains the complete right repeat of DIRS-1. The arrow below the 41.6 fragment indicates the nucleotide sequence encoding the 1.7-kb heat shock RNA transcribed in yeast cells. HSP refers to sequences that resemble heat shock promotors, and the arrows show their orientation.

during heat shock and development. The simultaneous activation of all of these presumptive promotors upon heat shock is consistent with the cytoplasmic appearance of the large number of DIRS-1-related RNAs (17). Because of the numerous DIRS-1 related sequences in the genome of *Dictyostelium* sp., it is impossible to show directly that the heat shock promotor of 41.6, here demonstrated to be functional in yeast cells, is in fact the same sequence responsible for the heat shock induction of all DIRS-1-related sequences. However, the presence of the conserved consensus promotor sequence in a large number of DIRS-1-related genomic fragments is certainly consistent with such a notion.

The heat shock response is known to be highly conserved in plants and animals, as well as in bacteria (14). Pelham (12) have shown that promotors of *Drosophila* heat shock genes function normally in mammalian cells. In fact, the heat shock induction of a non-heat shock gene only requires the insertion of the 13-base consensus heat shock promotor sequence (13). At least one of the heat shock genes of *Drosophila* spp., *hsp 83*, has been shown to be functionally regulated in yeasts (8). Our results demonstrate that a *Dictyostelium* heat shock promotor found in 41.6 is also functional in yeast cells. The putative heat shock promotor of 41.6 matches the *Drosophila* consensus heat shock promotor sequence in 14 of 15 positions (17). Given that the evolutionary distance between *Dictyostelium* and *Drosophila* is hundreds of millions of years, the apparent conservation of this promotor sequence is remarkable. Our results also suggest that conservation of the heat shock response is due, at least in part, to the conservation of the promotor DNA sequence that controls the activation of the genes, rather than the presence of different yet functionally equivalent regulatory elements. Further characterization of heat shock genes of other organisms should reveal the predicted conservation of the heat shock promotor sequence.

In *Dictyostelium* sp., DIRS-1-related RNAs are induced during normal development. It is noteworthy that the *Drosophila HSP 83*, *28*, and *26* heat shock genes are also expressed during normal ovarian development, in the absence of heat shock (16). In *Drosophila* spp., other heat shock proteins are not activated during this developmental program. Similarly, synthesis of certain *Dictyostelium* heat shock proteins is not induced at any time during normal development, unless the cells are subjected to a heat shock (9; unpublished data). Expression of DIRS-1-related RNAs may represent a bifunctional expression system, one that responds to one or more stress factors present both during heat shock and during development. It will be of interest to determine whether the heat shock promotor of 41.6 is the same sequence responsible for the induction of 41.6-related RNAs during differentiation. Recently described transformation systems for *Dictyostelium* sp. may make this possible (5).

ACKNOWLEDGMENTS

This work was supported by grant PCM 79-00839 from the National Science Foundation. J.C. is an American Cancer Society Fellow supported by fellowship no. PF-2235.

We especially thank Leonard Guarente and Beth A. Lalonde for their expert technical advice and assistance. We also thank M. Boucher for typing the manuscript.

LITERATURE CITED

1. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28**:145–154.
2. **Chung, S., C. Zuker, and H. F. Lodish.** 1983. A repetitive and apparently transposable DNA sequence in *Dictyostelium discoideum* associated with developmentally regulated mRNAs. Nucleic Acids Res. **11**:4835–4852.
3. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element TY1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. U.S.A. **80**:2432–2436.
4. **Gorman, C. M, G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promotor when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. U.S.A. **79**:6777–6781.
5. **Hirth, K. P., C. A. Edwards, and R. A. Firtel.** 1982. A DNA-

mediated transformation system for *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. U.S.A. **79**:7356–7360.
6. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
7. **Jellinghaus, U., U. Schatzle, W. Schmid, and W. Roewekamp.** 1982. Transcription of a *Dictyostelium discoidin*-I gene in yeast: alternative promoter sites used in two different eukaryotic cells. J. Mol. Biol. **159**:623–636.
8. **Lis, J., N. Costlow, J. deBanzie, D. Knipple, D. O'Connor, and L. Sinclair.** 1982. Transcription and chromatin structure of *Drosophila* heat shock genes in yeast, p. 57–62. M. Schlesinger, M. Ashburner, and A. Tissieres (ed.), Heat shock from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Loomis, W. F., and S. Wheeler.** 1980. Heat shock response of *Dictyostelium*. Dev. Biol. **79**:399–408.
10. **Maxam, A., and W. Gilbert.** 1980. Sequencing of end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
11. **Morrison, D. A.** 1979. Transformation and preservation of competent bacterial cells by freezing. Methods Enzymol. **68**:326–330.
12. **Pelham, H. R. B.** 1982. A regulatory upstream promotor element in the *Drosophila* HSP 70 heat shock gene. Cell **30**:517–538.
13. **Pelham, H. R. B., and M. Bienz.** 1982. A synthetic heat shock promotor element confers heat inducibility on the *Herpes simplex* virus thymidine kinase gene. EMBO J. **1**:1473–1477.
14. **Schlesinger, M., M. Ashburner, and A. Tissieres (ed.).** 1982. Heat shock from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Weaver, R. F., and C. Weissmann.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ terminal of 1.5S β-globin mRNA precursor and mature 10S β-globin mRNA have identical map coordinates. Nucleic Acids Res. **7**:1175–1193.
16. **Zimmerman, L. J., W. Petri and M. Meselson.** 1983. Accumulation of a specific subset of *D. melanogaster* heat shock mRNAs in normal development without heat shock. Cell **32**:1161–1170.
17. **Zuker, C., J. Cappello, R. Chisholm, and H. F. Lodish.** 1983. A repetitive *Dictyostelium* gene family that is induced during differentiation and by heat shock. Cell **34**:997–1005.
18. **Zuker, C., and H. F. Lodish.** 1981. Repetitive DNA sequences cotranscribed with developmentally regulated *Dictyostelium discoideum* mRNAs. Proc. Natl. Acad. Sci. U.S.A. **78**:5386–5390.