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Dictyostelium transposable element DIRS-1 has 350-base-pair inverted terminal repeats that contain a heat shock promoter

(transposon/developmental regulation/DNA sequence)

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ABSTRACT **DIRS-1 is a 4.7-kilobase-pair repetitive and apparently transposable *Dictyostelium* genetic element that is transcribed during differentiation or after heat shock. The terminal regions of DIRS-1 are inverted repeats of 330 base pairs. The repeats are highly conserved both within a given element as well as between different members of the family (<10% divergence). At the distal end of all left repeats is a 32-nucleotide sequence composed almost entirely of A and T residues. In addition to this 32-base A+T sequence, the distal region of all right repeats is extended by a 28-base-pair A+T-rich sequence that is identical in all copies. The sequences flanking each DIRS-1 sequence are completely dissimilar, and there appears to be no duplication of the genomic DNA sequence at the presumed point of DIRS-1 insertion. The terminal repeats can also be found interspersed in the genome independently of the complete element. In addition, the terminal repeats carry a 15-nucleotide sequence that greatly resembles the *Drosophila* consensus heat shock promoter and may be involved in the transcriptional induction of the DIRS-1 sequences.**

DIRS-1 (*Dictyostelium* intermediate repeat sequence 1) is a 4.7-kilobase-pair (kbp) *Dictyostelium* genomic fragment that has many of the properties of a transposable element (1). There are ≈40 copies of the complete DIRS-1 element in the genome, inserted at locations having no apparent homology with each other. The genomic locations of DIRS-1 vary in different *Dictyostelium* genetic stocks, while the 4.7-kbp element itself is highly conserved. The *Dictyostelium* genome also contains about 200 sequences that appear to be fragments of DIRS-1.

A common feature of most eukaryotic and prokaryotic transposable elements is the presence of terminally repeated sequences—either direct (*copia*, Ty-1, Tn*9*) or inverted (most bacterial transposon elements) (2–4). We report here that the terminal regions of the *Dictyostelium* DIRS-1 element are inverted repeats of 330 base pairs (bp); all of the repeats on the right side of the transposon are extended by an additional 28 bp rich in A and T residues. We also show that the repeats are found interspersed in the genome, independent of the complete element. We discuss the possible origins of such genomic fragments.

DIRS-1 hybridizes to a large number of differently sized cytoplasmic polyadenylylated RNAs that accumulate in a coordinated fashion during development (5). These RNAs appear to be mRNAs as they are polyadenylylated and are specifically associated with polysomes (unpublished data). Most of the RNAs carrying sequences complementary to DIRS-1 are induced in response to heat shock or to other "stresses" which precede the initiation of development (5). Moreover, one of the many genomic fragments related to DIRS-1 (clone pB41-6) contains a 15-base sequence that resembles a *Drosophila* heat shock promoter (5) and directs the synthesis of a heat-inducible RNA when introduced into yeast (6). We demonstrate here that the heat shock promoter responsible for the thermal induction of pB41-6 is contained within the terminal repeats of DIRS-1.

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MATERIALS AND METHODS

Restriction Mapping. Restriction maps of genomic clones were generated as described (1). Restriction enzymes were obtained from Bethesda Research Laboratories and New England BioLabs.

DNA Blot Hybridization. Labeling of DNA (nick-translation), gel fractionation of RNA and DNA, and blotting onto nitrocellulose filters were performed as described (7, 8). Prehybridization, hybridization, and washing of filters were conducted exactly as described (5). Filters were hybridized with hybridization solution containing 1–2 × 10^7 cpm of denatured labeled DNA. Complete digestion of the genomic DNA by restriction enzymes was confirmed by probing one of the lanes of the gel with a cloned single-copy sequence (not shown).

Isolation of Genomic Clones. The *Dictyostelium* *Eco*RI genomic library in λ gt-λWEs has been described (8). The Charon 28 library was constructed by ligating partially digested *Sau*3A genomic framents to the *Bam*HI site of the vector (9) and will be described in detail elsewhere (unpublished data). Genomic clones carrying sequences complementary to DIRS-1 were isolated by the plaque hybridization assay of Benton and Davis (10) using labeled pB41-6 DNA as probe (7).

DNA Sequence Analysis. DNA sequence analysis was carried out according to the chain termination procedure of Sanger *et al.* (11). M13mp8 and M13mp9 were used as sequencing vectors and JM103 as the host strain. The M13 recombinants were generated as described by Messing *et al.* (12). Gel electrophoresis was as described by Sanger and Coulson (13). Chemical sequence analysis was carried out exactly as described by Maxam and Gilbert (14).

RESULTS

DIRS-1 Contains 330-bp-Long Terminal Inverted Repeats. DIRS-1 is defined as a 4-kbp *Eco*RI fragment that is repeated ≈40 times in the *Dictyostelium* genome (1). The *Eco*RI sites in all cloned DIRS-1 elements are flanked by an *Xba* I restriction site 250 bp distant (Fig. 1). To test the notion that the terminal *Eco*RI–*Xba* I fragments of DIRS-1 represent inverted terminally repeated sequences, we have determined the nucleotide sequence at both ends of several cloned copies of DIRS-1. Fig. 1 shows the restriction maps and location of the terminal sequences in the DIRS-1 elements.

The different clones containing DIRS-1 were selected by virtue of their hybridization to the genomic clone pB41-6, a

Abbreviations: kbp, kilobase pair(s); bp, base pair(s).

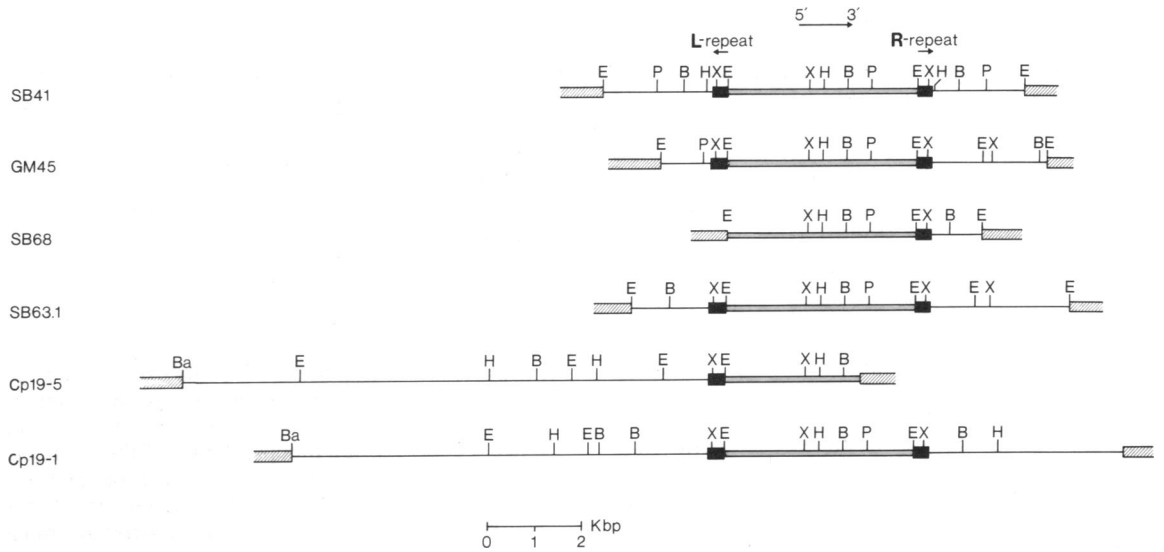

FIG. 1. Structure of genomic DIRS-1 clones. The diagram displays the source of the different repeats. L (left) and R (right) refer to the position of the repeats within DIRS-1, those farthest from and closest to the internal *Pvu* II restriction site, respectively. DIRS-1 sequences have been oriented such that transcription proceeds from left to right. Open boxes represent the DIRS-1 4.0-kbp *Eco*RI internal region and dark boxes indicate the terminal repeats of DIRS-1. The arrow on top of SB41 indicates the direction of transcription in DIRS-1 (unpublished data). Hatched boxes represent λ vector sequences. The right repeat of clone SB41 is contained in subclone pB41-6. Ba, *Bam*HI; B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pvu* II; X, *Xba* I.

2.5-kbp *Eco*RI fragment that contains sequences complementary to the right half of DIRS-1 (1, 7). This clone is an apparent deletion product of DIRS-1 and was isolated as the *Eco*RI fragment that flanks the right end of the intact DIRS-1 element contained in genomic clone SB41 (Fig. 1; see Fig. 6). Thus, pB41-6 contains the R-repeat of DIRS-1.

Fig. 2 shows the nucleotide sequence of four left (L) and five right (R) terminal repeats of DIRS-1. The left repeat is defined as the one farthest from the internal *Pvu* II site. To facilitate alignment, the nucleotide sequences of the inverted repeats are written 5′ to 3′, beginning at the *Eco*RI site and extending into the flanking sequences of each element. All nine repeats are highly conserved (<10% divergence) throughout the 330 nucleotides that define the lengths of the

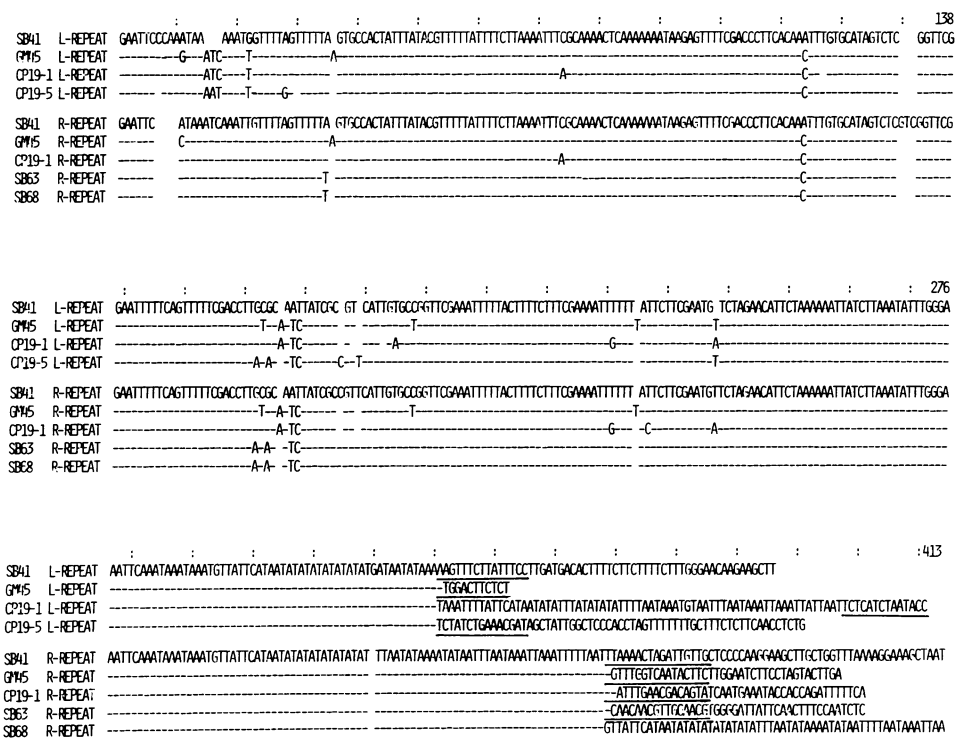

FIG. 2. Nucleotide sequence of the inverted repeats flanking DIRS-1. DNA sequences are written 5′ to 3′ beginning at the *Eco*RI restriction site (position 1–6) and extending beyond the *Xba* I site and into the flanking genomic sequence. The *Eco*RI restriction site is defined as the start of the repeat sequence because the nucleotide sequences 5′ from the *Eco*RI sites in clones SB41 and Cp19-1 show no linear homology to each other (data not shown). The sequences are aligned to maximize homology by using both repeats of SB41 for comparison. Dashed lines indicate the presence of the same nucleotide as SB41 at the same position. Blank spaces indicate deletions, and extra bases represent base insertions. Underlined nucleotides represent the presumed genomic flanking sequences.

repeats. All five right repeats are extended by a conserved 28-bp sequence; thus, the right repeats can be said to be 358 bp in length. Except for Cp19-1 L-repeat and SB68 R-repeat, which are discussed below, sequences beyond these end points are nonhomologous and are presumed to be flanking genomic sequences. The terminal repeats are A+T-rich (76% A+T, compared to 60% A+T for the internal regions of DIRS-1). The final 31 and 58 nucleotides of the left and right repeats, respectively, are almost entirely A+T.

A limited number of sequence alterations do occur within the repeats; the majority are single base changes but small insertions and deletions are also observed. Although base changes are present throughout the first 240 nucleotides of the nine repeats studied, repeat sequences beyond this point are completely invariant. When a change from an established consensus sequence, deduced from a majority of the repeats, does occur, in 50% of the cases, the same base alteration is found in both repeats flanking the same DIRS-1 element. Examples are the insertion of an A residue at position 35 in both repeats of GM45, the C to A substitution at position 74 in the repeats of Cp19-1, and the C to A substitution at position 114 in the repeats of SB41.

Although all repeats flanking DIRS-1 are highly conserved there are several consistent distinctions between the left and right repeats. First, the sequence C-C-A-A is found at position 7–10 of all left repeats and is absent in all right repeats. The G-A dinucleotide at position 320 of all four left repeats similarly is found in none of the right repeats. Importantly, left repeats are 28 bases shorter than right repeats. The precise end points of the left repeats argue against the possibility that the termini of the left repeats are generated by random deletion of A+T-rich nucleotides found in the longer right repeat.

Two of the repeats that we examined, Cp19-1 left repeat and SB68 right repeat, contain a sequence duplication of 66–71 bases of the A+T-rich termini. Fig. 3 aligns these terminal sequences with the sequences of the "consensus" left and right repeats. The additional 66 nucleotides of the right repeat of SB68 can be explained simply as a direct tandem duplication of the adjacent repeat sequence. However, the additional sequence in the left repeat of Cp-19-1 is not as simple. The additional sequence can be aligned quite well with the final 71 nucleotides of the consensus right repeat. Thus, in both cases where a deviation from the consensus termini of a left and right repeat has occurred, this deviation is in the form of a duplication or a transposition of right repeat sequence. This additional sequence is homologous to the consensus final 66–71 nucleotides of the right repeat irrespective of the sequence (left or right) to which it is adjacent. The additional sequences are inserted immediately adjacent to the consensus end point for the left and right repeats and terminate with the consensus end point of the right repeat.

Because of the consistent nature of the end points of the nine left and right repeats studied here, flanking genomic sequences adjacent to the DIRS-1 inverted terminal repeats can be delineated rather easily (see underlined residues in Fig. 2). The flanking sequences of six different elements show no relationship to each other, thus supporting the notion that the insertion of DIRS-1 into the genome is not sequence specific. In the three genomic clones where we have sequenced both repeats of the element (SB41, GB45, and Cp19-1), these flanking sequences show no evidence for duplication of any DNA sequence. This type of analysis cannot exclude the possibility that a duplication might have been lost by mutation after the insertion event. To clarify this point, a preinsertion target site sequence must be determined.

The Inverted Terminal Repeats Are Also Found Interspersed in the Genome, Independent of the Complete DIRS-1 Element. To determine whether the inverted repeats of DIRS-1 can be found independently of the complete element in the *Dictyostelium* genome, we have mapped the genomic locations of the *Eco*RI–*Xba* I terminal repeat fragment by Southern filter hybridizations. *Dictyostelium* nuclear DNA was digested with *Xba* I or with *Xba* I plus either *Bgl* II or *Pvu* II, restriction enzymes that cut DIRS-1 only once (Fig. 1). The digested DNA was size fractionated on a 1% agarose gel, transferred to nitrocellulose paper, and hybridized with a labeled 255-bp *Eco*RI–*Xba* I terminal repeat fragment isolated from clone SB68 (Fig. 4). If all terminal repeats are associated exclusively with complete, intact DIRS-1 sequences, one would expect that upon digestion with either *Xba* I, *Xba* I–*Bgl* II, or *Xba* I–*Pvu* II, the 4.7-kbp DIRS-1 element would be replaced by two smaller hybridizing bands of similar intensity. The size of the bands should correspond to that of the fragments generated by digestion of a cloned copy of DIRS-1 with the same restriction endonucleases.

Digestion of genomic DNA with *Xba* I generates three restriction fragments that hybridize to DIRS-1 (2.4, 1.9, and 1.5 kbp, respectively) (1). The two larger restriction fragments correspond to the two ends of DIRS-1. The 1.5-kbp band is repeated as many times as DIRS-1 but it is not found linked to DIRS-1. Its possible origin and its relationship with DIRS-1 are not known (1). The presence of any minor hybridizing bands would indicate either restriction site heterogeneity or the existence of isolated repeats. Indeed, the two major expected bands, derived from the 40 copies of intact DIRS-1, are present in all three digestions. Their molecular weights are those expected from the restriction map of DIRS-1. In all cases, however, a number of distinct minor bands also hybridized with purified repeat. Thus, even though the majority of the repeats are associated with intact DIRS-1 elements, a fraction are also found scattered in the genome independent of the complete element.

The minor bands are not all due to restriction site polymorphisms within DIRS-1. We have isolated two genomic clones that contain a terminal *Eco*RI–*Xba* I repeat but not a complete DIRS-1 element (data not shown). However, both

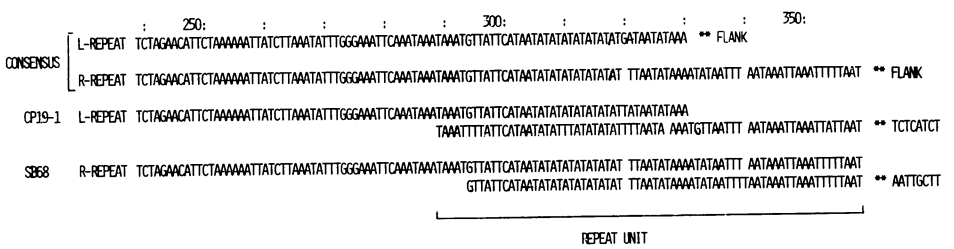

FIG. 3. DIRS-1 inverted terminal repeats with additional A+T-terminal sequences. The nucleotide sequences of Cp19-1 L and SB68 R are displayed beginning at position 239. The consensus (SB41) repeat sequences for this region of the left and right repeats are provided for comparison. The additional A+T sequences found in Cp19-1 L and SB68 R are realigned with the A+T-terminal sequences to maximize their homology. **, The presumed junction with flanking genomic sequences. The numbers above the sequence correspond to the same numbering used in Fig. 2.

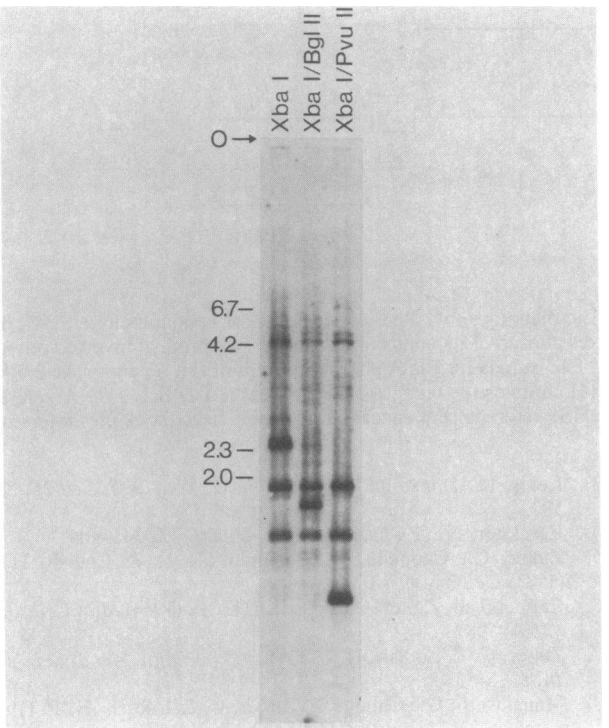

FIG. 4. Distribution of the DIRS-1 terminal repeats in the *Dictyostelium* genome. *Dictyostelium* DNA (1 μg per lane) was digested with *Xba* I (lane 1), *Xba* I and *Bgl* II (lane 2) or *Xba* I and *Pvu* II (lane 3). After fractionation on a 1% agarose gel, the gel was transferred to a nitrocellulose filter. The filter was hybridized with the nick-translated purified 225-bp pair *Eco*RI–*Xba* I terminal repeat fragment isolated from the right repeat of clone SB68. After 40 hr at 37°C, hybridization buffer was removed and the blot was washed in 15 mM sodium chloride/1.5 mM sodium citrate at 55°C for several hours. The blot was exposed for 15 hr at −80°C with an intensifying screen. O, origin. Sizes of marker bands are shown in kbp.

clones contain sequences complementary to DIRS-1 flanking the terminal repeat.

The Terminal Repeats Carry a Heat Shock Promoter. Many of the cytoplasmic RNAs complementary to DIRS-1, though absent in vegetative cells, can be induced by heat shock to levels comparable to those observed upon the initiation of development (5). Interestingly, sequence analysis of the terminal repeats has revealed that each carries a sequence almost identical to the *Drosophila* heat shock consensus promoter (Fig. 5). In addition, the promoter (position 229–244) is contained within the highly conserved domain of the repeats. Although a single nucleotide is variable within the promoter itself (position 238), the promoter sequence and the adjacent 86 or 114 nucleotides to the end of each repeat are completely invariant.

HEAT SHOCK CONSENSUS PROMOTER		208 CTG-GAATNTTCTAGA	264
SB68	(R-REPEAT)	TCTTTCGAAAATTTTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	
SB41	(L-REPEAT)	TCTTTCGAAAATTTTTTATTCTTcGAATG TCTAGAacATTCTAAAAAATTATCTT	
SB41	(R-REPEAT)	TCTTTCGAAAATTTTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	
Cp19-5	(L-REPEAT)	TCTTTCGAAAATTTTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	
SB63	(R-REPEAT)	TCTTTCGAAAATTTTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	
Cp19-1	(R-REPEAT)	TCTTTCGAAAATTGTTTACTCTTcGAATGaTCTAGAacATTCTAAAAAATTATCTT	
Cp19-1	(L-REPEAT)	TCTTTCGAAAATTGTTTATTCTTcGAATGaTCTAGAacATTCTAAAAAATTATCTT	
GM45	(R-REPEAT)	TCTTTCGAAAATTGTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	
GM45	(L-REPEAT)	TCTTTCGAAAATTGTTTATTCTTcGAATGTTCTAGAacATTCTAAAAAATTATCTT	

FIG. 5. Heat shock promoter sequence of DIRS-1. All sequences have been aligned at the *Eco*RI restriction site of the repeats (see Fig. 2). The origin of the different repeats is diagramed in Fig. 1. The *Drosophila* heat shock consensus promoter is from Pelham (15). The divergent arrows below the sequence of the putative heat shock promoter of SB68 indicate the palindromic nature of the terminal repeat promoters. The right repeat of SB41 is the promoter sequence of pB41-6 (6).

DISCUSSION

The *Dictyostelium* DIRS-1 genomic family has several structural features in common with well-characterized prokaryotic and eukaryotic transposable elements. (*i*) There are multiple copies (≈40) of intact DIRS-1 sequences in the genome. (*ii*) The elements are inserted at genomic locations having no apparent homology with each other. (*iii*) The genomic locations of the elements vary in different *Dictyostelium* genetic stocks (1). (*iv*) The elements carry terminal inverted repeats. (*v*) Elements that are inserted at different chromosomal locations have the same inverted repeats.

There are many DIRS-1 related sequences in the genome that are not part of an intact 4.7-kbp DIRS-1 element. Most of these vary in size and pattern of restriction enzyme digestion (1). It is possible that many, or all, such fragments were derived by deletions of DIRS-1, or as a result of transposition events that generate incomplete DIRS-1 sequences. To date, we have been unable to find DIRS-1 deletion fragments containing both "left" and "right" repeats. This is in contrast to TY-*1* elements of yeast and retroviruses, where precise excision of the internal sequences between the long terminal direct repeats does occur (16, 17).

DIRS-1 is an unusual eukaryotic transposon in that it has long inverted terminal repeats (330 nucleotides). The great majority of eukaryotic transposons and transposable-like elements (e.g., retroviruses) has direct terminal repeats (18, 19). Only four eukaryotic transposable elements are known to bear inverted terminal repeats; the P and FB elements of *Drosophila* (18, 20), the σ element of yeast (21), and the Tc*1* element of *Caenorhabditis elegans* (22). The inverted repeats flanking P and σ elements are very short (31 or 15 bp and 8 bp, respectively), whereas those at the ends of FB contain periodic tandem repeats of a 10-, a 20-, and a 31-bp sequence (23). The inverted terminal repeats of Tc*1* are 54 bp in length (22). DIRS-1 is also unusual in that the lengths of the left and right repeats differ by 28 bp. To our knowledge, no other tranposon has left and right repeats of different lengths.

Any model for transposition of DIRS-1 must explain two features of the terminal repeats. All right repeats contain a 28-bp A+T-rich extension of the canonical (left) repeat. Also, both the left and right repeats flanking a single DIRS-1 element frequently contain nucleotide sequence alterations that are found in no other repeats sequenced; this suggests that the left and right repeats of a DIRS-1 element may be copied or corrected from a single DNA sequence during transposition. The direct repeats of TY-*1* and *copia* elements may be copied or corrected by an analogous process (24). Because two of the nine repeats carry a duplication of the terminal A+T-rich sequence, it is possible that an internal duplication of this region is a consequence of or an intermediate step in the process of transposition.

DIRS-1 hybridizes to a large number of differently sized cytoplasmic polyadenylylated RNAs that accumulate in a coordinated fashion during both development and heat shock (5). We show here that DIRS-1 carries heat shock-like promoter sequences in each of its terminal repeats. We have shown that the right terminal repeat of DIRS-1 is transcriptionally functional by introducing clone pB41-6, which contains the right repeat of SB41 (Fig. 6), into yeast cells. This heat shock promoter present in the repeat directs heat-inducible transcription of the DNA region equivalent to the flanking genomic sequences (6). Thus, the heat shock promoters present in the terminal repeats of DIRS-1, shown in Figs. 5 and 6, are oriented so that they are able to direct transcrip-

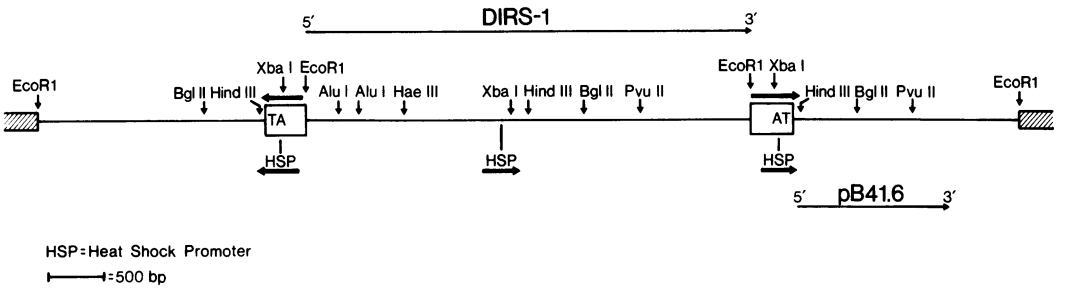

FIG. 6. Structure and transcription of DIRS-1 in genomic clone SB41. The diagram shows the physical map of genomic clone SB41. The center 4.0-kbp *Eco*RI fragment corresponds to DIRS-1 and the two open boxes at each of its ends correspond to the terminal inverted repeats. The arrows on top of the repeats indicate the direction of the repeats, and "TA" stands for the A+T-rich termini of the repeats. The 2.5-kbp *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. 5′ → 3′ refers to the direction of transcription of DIRS-1 and pB41-6 sequences, respectively. HSP refers to sequences that resemble heat shock promoters, and the arrows indicate their orientation.

tion outward into the flanking sequences. However, internal sequences of DIRS-1 are induced by heat shock as well (5). Thus, either DIRS-1 also carries internal heat shock promoters or the heat shock-like promoters present in the terminal repeats induce transcription in both directions. Because the sequence of the heat shock promoter in the repeats forms part of a palindrome, the opposite strand contains 11 nucleotides of the 14 that make up the consensus *Drosophila* promoter (Fig. 5). In this way, the promoter might represent two smaller, divergent, heat shock promoters with a center of symmetry at the *Xba* I restriction site, T-C-T-A-G-A. S1 nuclease mapping of DIRS-1 transcripts in *Dictyostelium* has shown that a major initiation of rightward transcription during heat shock and development occurs near the junction of the left repeat and the internal DIRS-1 sequences (unpublished data). This result is consistent with a bidirectionality for the heat shock promoter in the terminal repeats of DIRS-1. Partial nucleotide sequence of DIRS-1 suggests that it carries at least one internal heat shock promoter (data not shown; see Fig. 6).

The origin and possible function of DIRS-1 remain unclear. However, these sequences are expressed in a coordinated fashion during development, as well as after heat shock, and appear to code for mRNAs. It is possible that DIRS-1 encodes a protein that mediates its own transposition and that DNA transposition is induced during the sporulation developmental cycle or during heat shock.

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