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A repetitive and apparently transposable DNA sequence in *Dictyostelium discoideum* associated with developmentally regulated RNAs

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ABSTRACT

Dictyostelium discoideum AX3 genomic DNA contains about 40 copies of a 4.5 Kb sequence. Each has the same restriction map, suggesting that they are all very similar. The sequences are scattered throughout the genome, and each of the six representative copies we cloned have different flanking sequences. Partial fragments of the 4.5 Kb sequence also are scattered throughout the genome. The DNA sequences flanking the 4.5 Kb repetitive sequences are different in different strains of Dictyostelium suggesting that the 4.5 Kb sequence is a transposable element. Each sub-region of this 4.5 Kb sequence is associated with a large number of mRNAs, all of which are developmentally regulated, but whose function is not known.

INTRODUCTION

Transposable elements have been found in many organisms, including bacteria, yeast, Drosophila, and maize (1, 2, 3). In vertebrates, retroviruses may function as transposable elements (4). Transposable elements generally have a well-defined sequence flanked by terminal repeats; these repeats are often found independently of the remainder of the transposable element. Transposable elements can be present in up to several hundred copies per genome. Unlike other repetitive sequences such as ribosomal RNA genes, transposable elements usually are not linked tandemly to each other. Their locations in the genome often varies from strain to strain, and can even differ between different individuals within a population.

We have identified a repetitive sequence in Dictyostelium that possesses some of the characteristics of a transposable element. Interestingly, this repetitive sequence is associated with a large set of developmentally regulated mRNAs. Perhaps a better understanding of the function and the coordinate regulation of these RNAs will give us a clue to the function of these mobile elements.

Previously we showed that the cloned Dictyostelium genomic EcoRI-generated DNA fragment pB41-6 (2.5 Kb long) hybridizes to a large set of

cytoplasmic polyadenylated RNAs, all of which are developmentally regulated (5). This sequence is repetitive, and hybridizes to a large number of differently sized genomic DNA fragments generated by digestion with EcoRI. Interestingly, it hybridizes very strongly to a fragment 4 Kb in length, a result suggesting that there are a large number of genomic EcoRI fragments 4 Kb long that share some homology with this 2.5 Kb repetitive sequence. Here we show that each of the 40 copies of the "4 Kb" element has a similar restriction map extending to ~ 200 bp on either side of the EcoRI sites. The sequences flanking each are different. Furthermore, the sequences flanking this element are different in different strains of Dictyostelium. Thus, this 4.5 Kb repetitive sequence has features characteristic of a transposable element. We call this sequence DIRS-1, for Dictyostelium intermediate repeat sequence 1. Since about 2.3 Kb of the 4 Kb element is similar in sequence to the 2.5 Kb EcoRI fragment cloned in pB41-6, it was not surprising to find that this region of the cloned "4 Kb" elements also hybridizes to a large class of developmentally regulated mRNAs. Interestingly, the remaining 1.5 Kb sequence of the "4 Kb" element also hybridizes to a set of developmentally regulated mRNAs.

MATERIALS AND METHODS

Dictyostelium discoideum strains

AX3, NC4 and V12 were obtained from Dr. Richard Kessin (Harvard University).

Growth and differentiation of Dictyostelium cells

Conditions for axenic growth and synchronous development of Dictyostelium strains were described by Blumberg and Lodish (6).

Preparation of Dictyostelium DNA

Axenic growth of AX3 and preparation of DNA from this strain has been described (6). To grow NC4 or V12 for DNA extraction, four 100 mm SM plates (10 gm dextrose, 10 gm bactopeptone, 1 gm yeast extract, 1 gm $MgSO_4$ and 10^{-3} M phosphate buffer, pH 6.4, per liter of 2% agar) were prepared, each containing 10^5 cells plated on a lawn of Klebsiella aerogenes. When the cells exhibited confluent clearing of the bacterial lawn, they were harvested by scraping and were resuspended in 20 ml of 0.2% NaCl. They were recovered by centrifugation at 1000 g for 2 min. The pellet was resuspended in 20 ml of 0.2% NaCl and the centrifugation and resuspension was repeated twice to remove most of the bacteria. DNA was prepared as described in reference 6.

Preparation of Dictyostelium RNA

Cytoplasmic polyadenylated RNAs were extracted from cells at different stages of development according to reference 6.

Restriction mapping

Restriction maps were generated by the combined analysis of the products of the complete single, double and triple digests of the clones in question. In cases where the results were ambiguous, the genomic fragments were subcloned into plasmid vectors, mapped and then the complete maps were reconstructed by Southern blotting analysis. Conditions for digestion by restriction enzymes were according to the suggestions of the enzyme's manufacturer. Restriction enzymes were obtained from BRL Inc. and Biolabs Inc. The gel systems are described in the figure legends. Size markers were λ HindIII and ØX 174 Hae III digests.

DNA and RNA blot hybridizations

Labeling of DNA (nick-translation), gel fractionation of RNA and DNA, and blotting onto nitrocellulose filters were performed as described in references 5 and 7. For analysis of genomic DNA, complete digestion of the DNA by restriction enzymes was confirmed by probing one of the lanes of the gel with a cloned single copy sequence (not shown). Filters containing DNA or RNA were prehybridized in hybridiation buffer (50% formamide, 0.75 M NaCl, 0.075 M NaCitrate, 0.08% polyvinylpyrollidone, 0.08% Ficoll, 5 mM EDTA, 20 mM sodium phosphate, pH 6.5) for 24 hrs at 40°C. For the hybridizations, buffer was removed and replaced with hybridization buffer containing 1-2 x 10^7 cpm of denatured labeled DNA. Filters were incubated for 48 hrs at 40°C, and then washed for several hours at 55°C in 15 mM NaCl, 1.5 mM NaCitrate, 0.15% SDS.

Isolation of genomic clones

The Dictyostelium EcoRI genomic library in lambda gt-λWES has been described previously (7). The charon 28 genomic library was constructed by ligating partially digested Sau 3A genomic fragments to the Bam H1 site of the vector (8), and will be described in detail elsewhere (R. Chisholm and H.F. Lodish, in preparation). Genomic clones carrying sequences complementary to DIRS-1 were isolated by the plaque hybridization assay of Benton and Davis (9) using labeled pB41-6 DNA as probe (5).

RESULTS

Cloning of DIRS-1

In previous studies, we constructed a Dictyostelium genomic DNA library by replacing the B fragment of λgtWES-B with a mixture of completely and par-

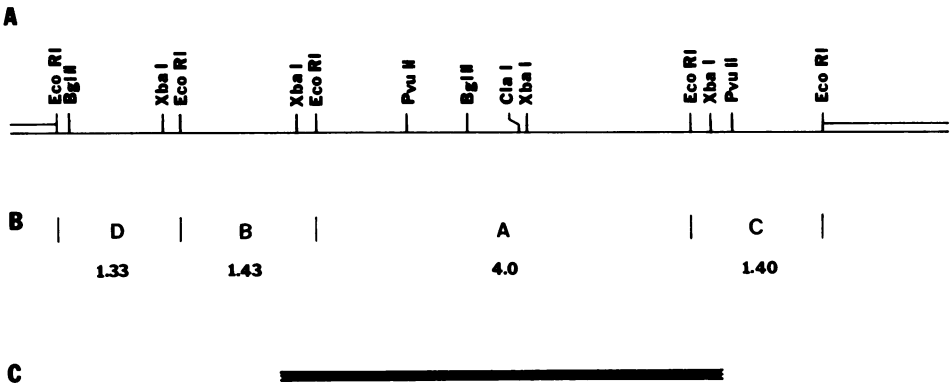

Figure 1: GM45-A contains a DIRS-1 sequence.
A: The restriction map of GM45.
B: Subclones of GM45. The four EcoRI fragments were inserted into the EcoRI site of plasmid pMB9.
C: The solid bar indicates the minimum length of DIRS-1.

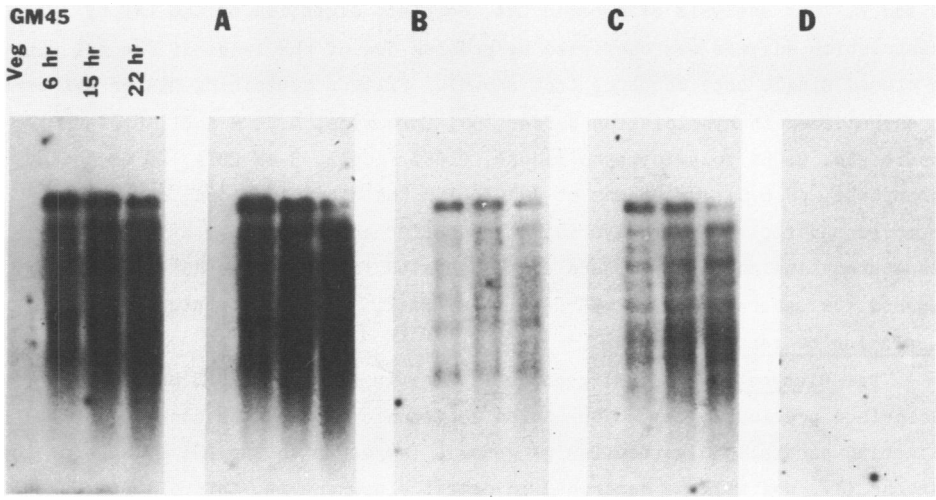

Figure 2: Transcription of the four subregions of GM45. 2 µg of cytoplasmic polyadenylated RNA from vegetitive cells, 6 hr cells (preaggregation), 15 hr cells (post aggregation) and 22 hr cells (culmination) were applied to the lanes as indicated. The RNAs were separated by electrophoresis on denaturing formaldehyde-1.5% agarose gels and blotted onto nitrocellulose filters as previously described (10). The filters were then hybridized to 10^7 [^{32}P] labeled DNA from GM45, GM45A, B, C, and D, as indicated. About 10^7 cpm of labeled DNAs were added to each hybridization mix. The filters were washed and exposed to X-ray film for 48 hrs.

tially EcoRI-digested Dictyostelium DNA fragments (7). In the process of selecting clones that encode developmentally regulated mRNAs, we isolated several that resemble pB41-6 in that they hybridize to a large set of developmentally regulated RNAs (5). One of these clones, GM45 (7), contains 4 EcoRI fragments, called GM45A, B, C, and D, of 4.0, 1.43, 1.4 and 1.33 Kb respectively. The order of these fragments is indicated in Fig. 1A. Each fragment was subcloned into the EcoRI site of pMB9. GM45A, B and C each hybridize to a large set of developmentally regulated RNAs much like pB41-6, while GM45D does not hybridize at to any RNA a detectable level (Fig. 2). The following studies show that GM45A is a representative of the many 4 Kb EcoRI-generated genomic fragments that hybridize to pB41-6. We call this segment DIRS-1.

DIRS-1 is an integral unit in Dictyostelium DNA

GM45A hybridizes very strongly to the 4 Kb region of EcoRI-digested Dictyostelium DNA, and to a large number of other sized DNAs (Fig. 3). To determine whether all of these 4 Kb EcoRI genomic fragments, in fact, have the same restriction map as does GM45A, we digested genomic Dictyostelium DNA with both EcoRI and a second restriction enzyme that cuts GM45A only once (Fig. 1A). The digested DNA was analysed by Southern blot hybridization using labeled GM45A as a probe. If all the genomic 4 Kb EcoRI fragments have the same sequences as GM45A, one would expect the 4 Kb band to be replaced by two smaller bands. This indeed was the case with the pairs EcoRI-ClaI and EcoRI-BglII (Fig. 3; also PvuII-EcoRI and HindIII-EcoRI, not shown). The molecular weights of the two bands are those expected from the map of GM45A, and add up to 4.0 Kb, thus supporting the idea that they are the products of a single cut at the same site within all of the 4 Kb genomic EcoRI fragments. Digestion with EcoRI and XbaI generated 3 major bands that hybridize to GM45A. The two larger bands (2.2 Kb and 1.9 Kb) apparently derive from the 4 Kb EcoRI fragments. The origin of the third band (1.5 Kb) is unknown, and is discussed below.

Although this experiment would not reveal small variations among the different genomic 4 Kb EcoRI sequences that hybridize to GM45A, it clearly indicates that the majority are very similar. We call these units DIRS-1 to distinguish them from EcoRI fragments not 4 Kb in length that also share homology with GM45A.

Is the entire GM45 sequence (D-B-A-C) found in all DIRS-1 sequences? The answer is negative, since when an EcoRI digest of Dictyostelium DNA is probed with labeled GM45B (1.43 Kb) or GM45C (1.40 Kb) multiple fragments are visua-

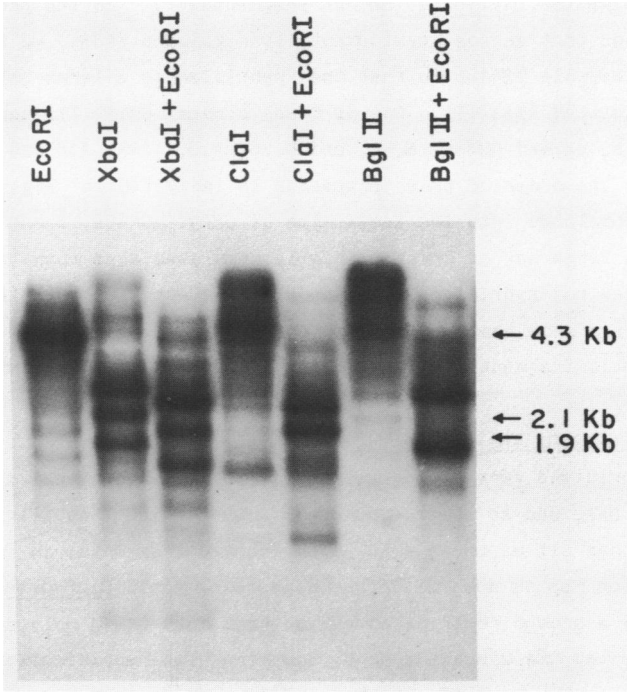

Figure 3: Southern blot of digested genomic DNA hybridized to labeled GM45A DNA. Dictyostelium DNA was digested with the restriction enzymes indicated on the top of each lane. After fractionating the DNAs on a 1% agarose gel, the DNAs were transferred to nitrocellulose paper (5) and hybridized to [^{32}P]labeled GM45A DNA. The filter was exposed to X-ray film for 12 hrs at -70°C with an intensifying screen. The arrows on the side indicate the position of molecular weight markers.

lized, but the size region 1.43 Kb or 1.4 Kb does not hybridize more strongly than any other fragment size (data not shown).

Arrangement of DIRS-1 sequences in the genome

The DIRS-1 sequences are not linked tandemly in the genome. This is shown by the study in Fig. 3 in which Dictyostelium DNA was digested with ClaI or BglII, enzymes that cut GM45A once. Probing Southern blots of this digest with GM45A revealed no major band at 4 Kb (Fig. 3). Were the DIRS-1 sequences tandemly linked, such a band, or one slightly larger, would be expected. This conclusion is supported by the fact that at least one of the DIRS-1 sequences, that in clone GM45, is adjacent to a single-copy sequence, GM45D (Fig. 1).

When a Southern blot of XbaI-digested genomic DNA is probed with GM45A, three major bands hybridize at about equal intensity (Fig. 3). A comparison

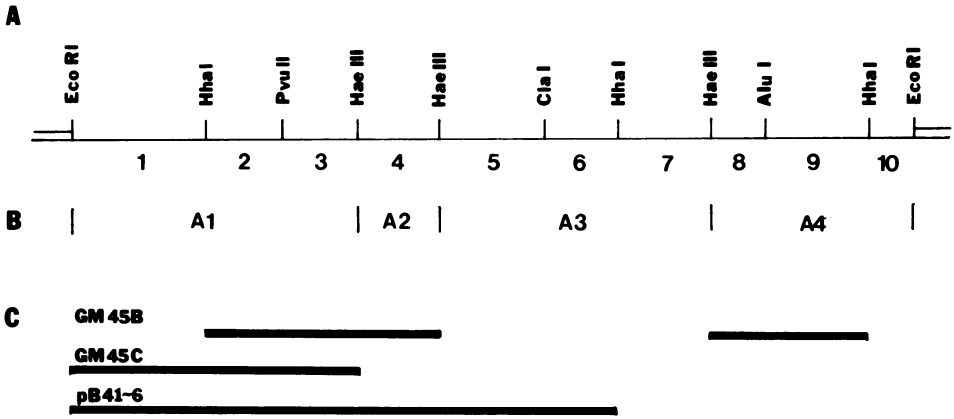

Figure 4A: Restriction map of GM45A. The restriction sites define the 10 fragments of GM45A used for hybridization in figures 5 and 9. These fragments were generated by the digestion of the subclones GM45A1, A2, A3 and A4.
B: Subclones of GM45A. The four HaeIII fragments of GM45A were inserted into the EcoRI site of plasmid pMB9 using synthetic EcoRI linkers to join the DNAs.
C: GM45A DNA was digested with HhaI and HaeIII. A Southern blot of the digested DNA was hybridized to labeled DNA inserts from clones GM45B, GM45C, and pB41-6. The solid bars indicate the regions on GM45A that hybridized with each of the DNA probes.

of the molecular weights of these bands (1.7, 2.0. 2.5 Kb) with those of XbaI digested GM45 fragments showed that the two larger bands correspond to the two XbaI fragments (2.0 and 2.5 Kb) that encompass GM45A (Fig. 1). Additionally, digestion of genomic DNA with EcoRI as well as XbaI resulted in shortening of each of the two larger XbaI fragments by 0.23 Kb, exactly as obtained by digesting GM45 itself with both nucleases (Fig. 1a). Thus, we conclude that the two XbaI sites flanking GM45A represent the minimum length of DIRS-1, about 4.5 Kb (Fig. 1C); DIRS-1 is then defined by the two flanking XbaI sites (4.5 Kb) rather than the EcoRI sites (4.0 Kb).

We do not know the origin of the smallest of the three major XbaI bands. However, it is repeated as many times as the 4 Kb EcoRI sequence. It is likely that this sequences is not an integral part of DIRS-1. This is reflected in the structure of GM45 since this cloned sequence does not carry such a fragment (see section on genomic clones below).

There are no extensive internal repeats within GM45A

We subcloned the four HaeIII fragments of GM45A into the EcoRI site of plasmid pMB9 by using synthetic EcoRI linkers to join the DNAs. These subclones were named GM45A 1, 2, 3 and 4 (Fig. 4B). These HaeIII fragments do

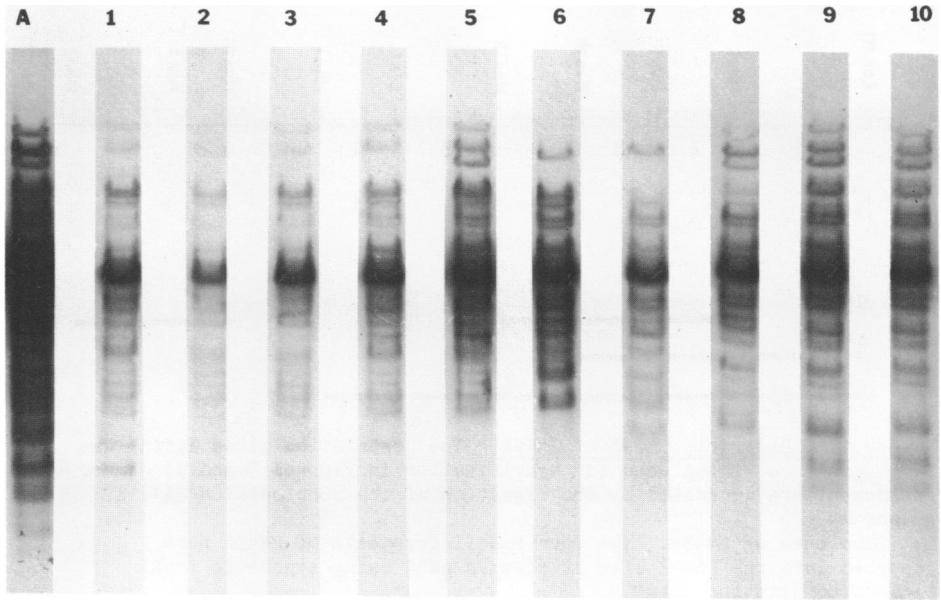

Figure 5: Southern hybridization of genomic DNA digested with EcoRI. Dictyostelium genomic DNA was digested with the restriction enzyme EcoRI and the fragments were size fractionated on a 1% agarose gel (5). After Southern transfer to nitorcellulose strips, the filter in lane A was hybridized with [^{32}P] GM45A DNA. Lanes 1 through 10 were hybridized to fragments of GM45A DNA. The number on top of each lane corresponds to the fragment shown in Fig. 4A. 1 X 10^7 cpm of labeled DNA were used to hybridize each filter and all filters were exposed to X-ray film for 18 hrs.

not cross hybridize with each other, even at low stringincies, indicating that there is no extensive internal repeat sequences within the 4 Kb EcoRI sequence of DIRS-1 (data not shown).

Relationship between DIRS-1 and the genomic EcoRI fragments not 4 Kb in size that cross-hybridize with it

Cloned DNAs pB41-6, GM45B and GM45C all hybridize to a large set of DNA and mRNA sequences much like GM45A. They are examples of the "non 4Kb" repeat sequences. To understand the relationship between DIRS-1 and these "non 4Kb repeat sequences" we digested GM45A into smaller pieces by nucleases HhaI and HaeIII, and hybridized Southern blots of digested GM45A DNA with labeled pB41-6, GM45B and GM45C DNA. The results are summarized in Fig. 4C. Clones GM45C and pB41-6 hybridize to fragments of GM45A that are contiguous with each other, while GM45B hybridizes to parts of GM45A that are not adjacent to each other. Thus pB41-6, GM45B, and GM45C could be generated from GM45A by single

deletion events.

To determine whether all regions of GM45A are represented in these "non 4KB' genomic sequences, we digested the four subclones of GM45A - GM45A1, 2, 3 and 4 - with restriction enzymes as indicated in Fig. 4A, and separated the fragments by electrophoresis in agarose gels. The 10 fragments thus generated (Fig. 4A) were eluted from the gel, labeled with [^{32}P], and hybridized to a Southern blot of EcoRI-digested Dictyostelium DNA. As expected, each of the 10 fragments hybridizes most intensely with a 4 Kb genomic fragment (Fig. 5). Each also hybridizes to a set of other genomic fragments. Some fragments of genomic DNA hybridize to many fragments of GM45A while some only hybridize to one or two. This indicates that some "non 4 Kb" genomic fragments contain large portions of DIRS-1, while some only contain a small part of it. It is not possible to deduce a simple model as to how all "non 4 Kb" repeat sequences might be generated.

The number of copies of DIRS-1

It is clear that within the Dictyostelium genome there are integral units of the 4.5 Kb DIRS-1 sequence as well as segments that contain fragments of DIRS-1. Therefore, it is impossible to determine the number of copies of intact DIRS-1 by solution hybridization. Instead, we used quantitative Southern hybridization to estimate the number of 4 Kb EcoRI genomic fragments that hybridize to GM45A. A Southern blot of Dictyostelium DNA, digested with EcoRI, was hybridized to labeled GM45A DNA (Figure 6). In parallel, similar Southern blots were hybridized with a labeled cloned DNA fragment that occurs only once in the genome (clone SC 253; ref. 10) and with a cloned ribosomal DNA fragment that is present at about 200 copies per genome (11). By scanning the radioautograms (Fig. 6) we estimate that there are about 40 copies of intact DIRS-1 sequences per genome. DNA hybridizations using total genomic DNA indicated that DIRS-1 related sequences (i.e. complete and incomplete) are present at about 250 copies per genome (not shown).

Distribution of DIRS-1 in a collection of genomic clones

The data presented above clearly indicated both that integral copies of DIRS-1 are scattered throughout the Dictyostelium genome and that their minimum size, 4.5 Kb, is defined by two flanking Xba I sites. In order to further characterize the size and location of DIRS-1 elements, we screened a Dictyostelium genomic library and isolated several clones which hybridized to pB41-6. Many such clones were restriction-mapped and a representative set which contains complete or almost complete copies of DIRS-1 is presented here. Figure 7 shows the restriction maps and location of DIRS-1 in six genomics

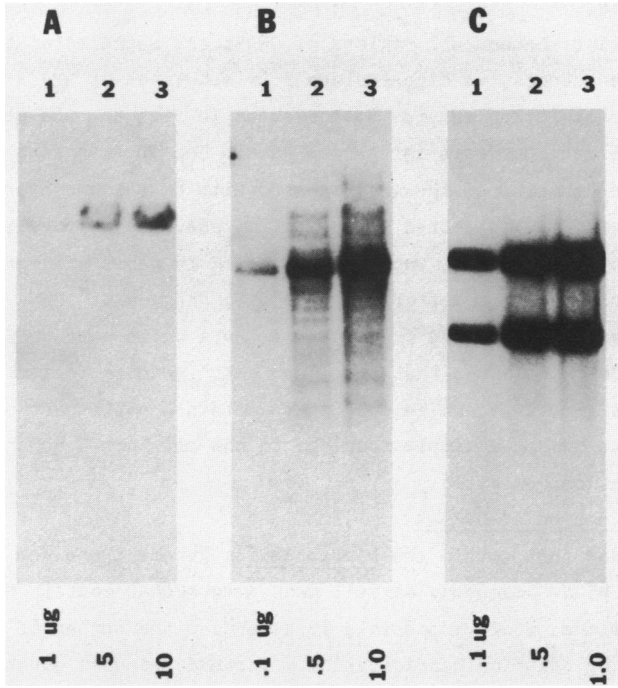

Figure 6: Quantitative Southern hybridization. Dictyostelium DNA was digested with EcoRI, fractionated on a 1% agarose gel and transferred to nitrocellulose paper as described in reference 5. The amount of DNA in each lane is shown at the bottom. The filter in panel A was hybridized to cloned DNA SC253, a single copy DNA sequence (10). The filter in panel B was hybridized to GM45A DNA and the filter in panel C was hybridized to a cloned ribosomal DNA, present at about 200 copies per genome (11). This clone contains the 5 Kb and 3 Kb EcoRI fragments of the ribosomal RNA genes (unpublished results). 1×10^4 cpm of labeled DNA were used for each of the hybridizations. A lower exposure of the filters in panel B and C were used for densitometer scanning to compare the amount of hybridization.

clones, including GM45.

Clones GM45, SB41, SB63 and SB68 were isolated from a λgt-λWES EcoRI genomic library, and clones CP19-1 and CP19-5 from a charon 28 Sau 3A genomic libarary. All clones except CP19-5 contain complete, identical copies of the DIRS-1 element. Clone CP19-5 is lacking the EcoRI-PvuII fragment of DIRS-1, most likely due to the presence of a Sau 3A site to the left of the BglII site (unpublished data). All six clones contain flanking sequences very different from each other. Therefore, DIRS-1 elements are indeed scattered throughout the genome. One point to be noted is the presence of XbaI sites flanking the

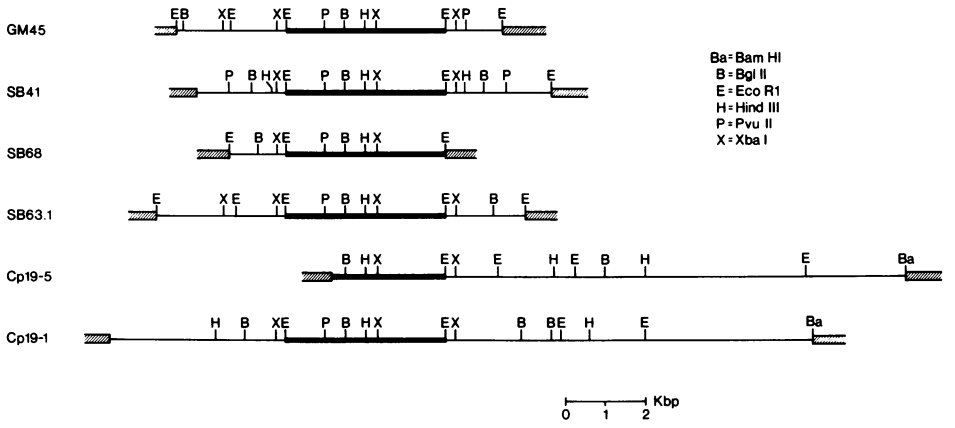

Figure 7: Restriction map of genomic clones containing DIRS-1. Dictyostelium genomic libraries were screened by the plaque hybridization assay of Benton and Davis (9) with labeled pB41-6 DNA. Positive clones were isolated and restriction mapped as described in Materials and Methods. Only those that contain complete or almost complete copies of DIRS-1 are shown here. Hatched lines represent the λ vector, and the solid black line denotes the 4.0 Kb DIRS-1 sequence. The XbaI sites flanking the 4 Kb EcoRI fragment define the terminal repeats of DIRS-1.

EcoRI sites of all six cloned DIRS-1 elements. Thus, consistent with the genomic digest presented before (Figure 3), DIRS-1 is defined by two XbaI sites flanking the EcoRI sites. None of the six clones contained the 1.5 Kb XbaI fragment observed upon digestion of Dictyostelium genomic DNA with XbaI and probing with GM45 (Figure 3). Clearly, this fragment, although present in as many copies as the 4.5 Kb DIRS-1 element, is not found flanking most DIRS-1 sequences in the genome. Rather, it is interspersed with other sequences.

Finally, we note that the XbaI-EcoRI fragments found at both termini of all DIRS-1 sequences not only are identical in size (0.23 Kb) but also cross-hybridize to each other at very high stringincies (not shown). In fact, we have sequenced the EcoRI-XbaI flanking sequences and found that they represent inverted repeats (Zuker, Chung and Lodish, in preparation).

DIRS-1 in other Dictyostelium strains

Thus far we have shown that in AX-3 DNA, DIRS-1 sequences are integral units present in about 40 copies per genome, and that portions of it are also scattered throughout the genome. One possible explanation is that DIRS-1 is a transposable element. If this were true, one would expect that some of the DIRS-1 sequences are situated in different locations in the genomes of distantly related Dictyostelium strains. Thus, we compared the locations of

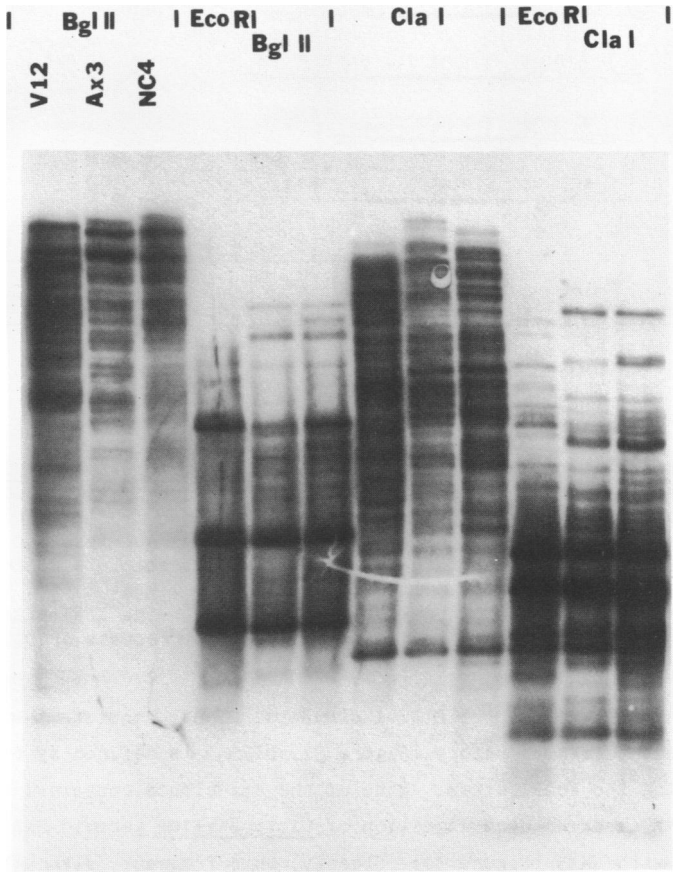

Figure 8: Southern blot hybridization of DNA from V12, AX3 and NC4. Nuclear DNA from V12, AX3 and NC4 was isolated as described in Materials and Methods. Approximately 1 µg of DNA was subjected to restriction endonuclease digeston and then size fractionated on a 0.9% agarose gel (5). The restriction enzymes used are indicated on the top of the gel. After transfer to nitrocellulose filter, the DNAs were hybridized with 1.5×10^7 cpm of nick translated GM45A DNA. Exposure was for 18 hr at -70°C with an intensifying screen.

DIRS-1 in AX3, the strain used in the above studies, to its parent strain NC4, and to an independent Dictyostelium isolate, V12 (12).

First, it was necessary to show that DIRS-1 itself is conserved in all three strains. To this end, Southern blots of double digests of the three DNAs, with EcoRI/BglII and with EcoRI/ClaI, were probed with labeled GM55A DNA (Fig. 8). All three DNAs revealed the same characteristic major bands (Fig. 8). Since these derive from the 40 genomic DIRS-1 copies (Fig. 3), this

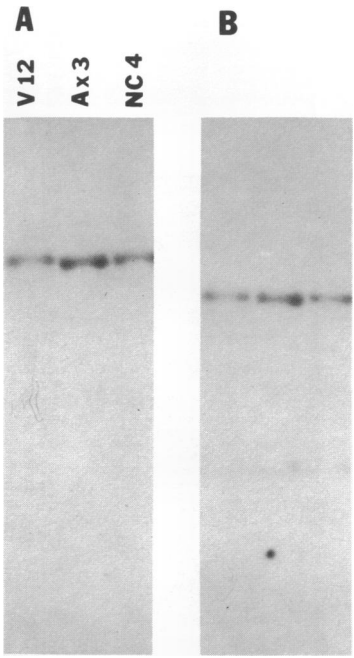

Figure 9: Southern blot hybridization of DNA from different strains of Dictyostelium. The DNAs were the same as those used in Fig. 8. All DNAs were digested with EcoRI. The filter in panel A was hybridized to SC253 DNA, and that in panel B was hybridized to SC211. Both clones contain a single EcoRI-generated genomic fragment (10; unpublished data).

indicates that the sequence between the two EcoRI sites near the ends of DIRS-1 is well conserved in the genome of the three strains.

We then digested the DNA of different Dictyostelium strains with either ClaI or BglII alone, restriction enzymes that cut DIRS-1 only once. Since the other cuts would be in the sequences flanking DIRS, the size of the fragments that hybridize to GM45A reflect the genomic locations of DIRS-1 (Fig. 8). The majority of the DNA fragments that hybridized to GM45A are the same in NC4 and AX3. However, the hybridization pattern to V12 DNA is very different from that to AX3 or NC4 DNA, suggesting that the sequences flanking DIRS-1 in V12 are very different from those flanking DIRS-1 in AX3 or NC4.

As a control, we probed Southern blots of EcoRI-digested DNA from the three strains with two cloned single-copy genomic sequences (Fig. 9). Clone SC253 encodes an mRNA which is synthesized throughout development, while SC211 encodes an mRNA that is present only late in differentiation (10; and unpub-

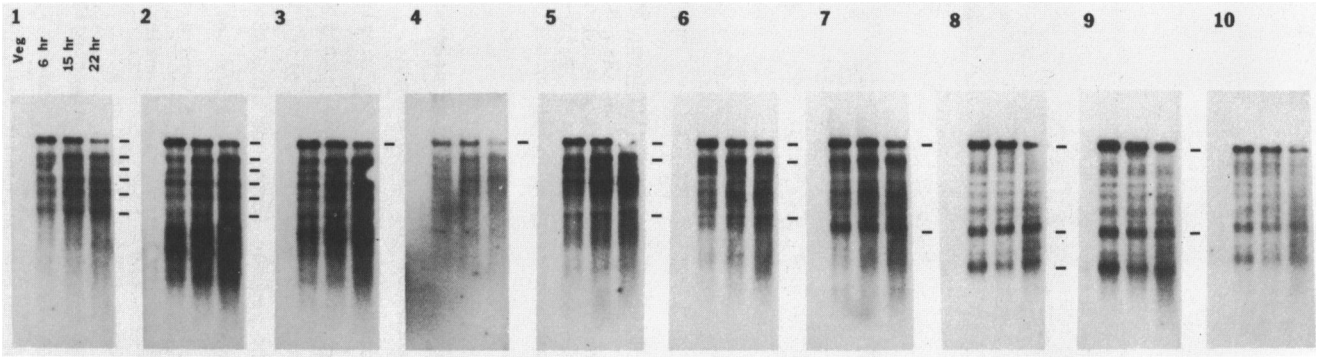

Figure 10: Each of the 10 subfragments of GM45A hybridizes to a set of developmentally regulated RNAs. RNA blot hybridizations are similar to those in Fig. 2. Each filter contains gel separated mRNA from the four stages detailed in the legend to Figure 2, and was hybridized to one of the 10 subfragments of GM45A. Hybridizations were carried out at 40°C for 48hrs, with 1 X 10^7 cpm of labeled DNA. Exposure was for 48 hr at -70°C with an intensifying screen. The number on top of each panel indicate the fragment number of GM45A, as shown in Fig. 4A. The bars indicate the RNA species that hybridize to two DNA fragments that are adjacent to each other in GM45A.

lished data). The three strains were indistinguishable. Thus, there appears to be little sequence variation between the three DNAs except for the regions flanking DIRS-1. It is likely that the variation in the flanking sequences reflects different chromosomal locations of DIRS-1.

RNA complemetary to DIRS-1 subregions

DIRS-1 hybridizes to a large set of RNAs that are developmentally regulated (Fig. 2). We wanted to know whether the entire sequence of DIRS-1 is transcribed or whether only part of it is represented in RNA. In an attempt to answer this question, we digested GM45A into 10 fragments (Fig. 4A). Each DNA fragment was labeled with [^{32}P] and hybridized to blots of gel-separated cytoplasmic polyadenylated RNAs isolated from vegetative cells, preaggregation cells (6 hr), post-aggregation cells (15 hr) and culminating cells (22 hr; Fig. 10). All 10 fragments hybridize to a large number of RNAs, all of which are developmentally regulated. Several discernable RNA species, indicated by the bars, hybridize to more than one adjacent GM45A fragment, indicating that they contain transcripts of a large portion of DIRS-1. We do not know whether these RNAs are transcribed from the entire length of intact DIRS-1 and then processed to smaller pieces, or whether they are transcribed from several promotors within DIRS-1. Yet another possibility is that they are transcribed from the smaller fragments of DIRS-1 that are scattered throughout the genome. The largest RNA band, about 4-6 Kb in length, appeared to hybridize to all 10 fragments of GM45A. This is a possible candidate for an end-to-end transcript of DIRS-1.

DISCUSSION

We have described a repetitive sequence in Dictyostelium discoideum DNA that is at least 4.5 Kb long. There are about 40 copies dispersed throughout the genome, and each of these has an identical pattern of digestion with several restriction endonucleases. Apparent deletions or fragments of this sequence are also found scattered in the genome. This repetitive sequence, called DIRS-1, is well conserved in different strains of Dictyostelium. On the other hand, the sequences flanking DIRS-1 in different strains of Dictyostelium show great variations, suggesting that the chromosomal locations of DIRS-1 are different in different strains. These observations are characteristics of transposable elements. However, we have no direct evidence that DIRS-1 actually is transposable.

AX3 is a mutant of NC4 that can grow in axenic medium (13). It is remarkable that even between these closely related strains there are several

different DNA restriction fragments that hybridize to GM45A (Fig. 8), a result that suggests that a few DIRS-1 elements are located in different sites in these two strains. Unfortunately, we do not have a record of the maintenance of these two strains and so we cannot estimate the number of generations that separated them.

It is interesting that the large set of RNAs associated with DIRS-1 are all developmentally regulated. These RNAs are not found in the cytoplasm of vegetative cells. They accumulate at the onset of the developmental program and persist throughout the later stages of development. These RNAs are retained by oligo dT columns, indicating that they contain polyadenylic acid sequences. At all stages of differentiation these transcripts are present predominantly in polysomes (Zuker and Lodish, manuscript in preparation), suggesting that they serve as messenger RNAs. However, we do not have direct evidence that they encode protein.

Clone pB41-6 (2.5 Kb) shares sequence homology with half of the DIRS-1 sequence (Fig. 4). We have shown that transcripts of only one strand of pB41-6 are found in cytoplasmic RNA, and, by implication, only one strand of DIRS-1 is transcribed (Zuker and Lodish, in preparation) pB41-6 has been sequenced (Zuker, et al. submitted). The "coding" strand contains three overlapping open reading frames of 789, 621 and 726 bases. If spliced together, a pB41-6 RNA transcript could encode a protein of approximately 50,000 molecular weight.

The more extensively studied transposable elements like the Tyl sequence in yeast (14) and copia (15) and other mobile dispersed genetic elements in Drosophila (16, 17) all appear to code for RNA. A common feature among these elements is that each hybridize to a large RNA that appears to be an end-to-end transcript of the whole element. In addition to the end-to-end transcript they also hybridize to one or two smaller RNAs that are transcribed from an internal promotor. In the case of Tyl sequences, the synthesis of the large RNA is regulated by the mating type locus while the smaller RNA is present in cells of all mating types (14). The RNAs coded by copia are specific for the larval stage (15). It is not clear if these RNAs play any functional role in the development of these organisms.

DIRS-1 is different from these transposable elements in that it hybridizes to a large set of RNAs that are coordinately regulated. The largest RNA may to be an end-to-end transcript of the whole element. The origin of the smaller RNAs is not known. One possibility is that they are all transcribed from promotors within DIRS-1. In this case, the different species of RNA

could represent different processing products of larger RNAs, or they could be transcribed from different promotors within DIRS. All ten subfragments of the cloned DIRS-1 sequence hybridize to a large, overlapping set of cytoplasmic polyadenylated RNAs. If these RNAs are all transcribed from DIRS-1, then there must be numerous internal promotors, or a few large primary transcripts must be spliced in many different combinations. A more likely alternative is that some of the RNAs are transcribed from promotors within fragments of DIRS-1 such as pB41-6 that are scattered throughout the genome.

Only small fragments of pB41-6 DNA, 300-500 bp, are protected against endonuclease S1 digestion by developmentally regulated RNAs (5). There are at least three possibilities to explain this result: First, the RNAs are spliced, and colinearity between transcript and coding sequence is interrupted every 300 bp or so. Second, the 250 different genomic copies of pB41-6-related sequences might contain the sequences in rearranged order; thus RNAs derived from such regions, although complementary to pB41-6, would not be colinear with pB41-6. Third, the RNAs might contain single copy (or other non-repetitive sequences) linked to pB41-6-related sequences. Whatever the answer(s) turn out to be, we note that most RNAs complementary to pB41-6 (or DIRS-1) are induced in a coordinated fashion during development; some are induced "early" (0-5 hrs) while some later (10-15 hrs)(5; Zuker *et al.* submitted). Thus the sequences responsible for their developmental induction must be conserved.

Experiments are underway to determine if transcripts of single copy DNA sequences are associated with RNA that contain DIRS-1-related sequences. These could result from transcription units that initiate within DIRS-1 (or fragments of DIRS-1) and extend into adjacent DNA sequences. It is possible that some developmentally regulated genes might be expressed through their linkage with these DIRS-1-related sequences. Clearly, understanding the function of the RNAs associated with the transposable elements might lead us to understand the origin of these elements.

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