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Two distinct subforms of the retrotransposable DRE element in NC4 strains of *Dictyostelium discoideum*

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ABSTRACT

Approximately 2% of the *Dictyostelium discoideum* genome consists of multiple copies of a retrotransposable element termed DRE (*Dictyostelium* Repetitive Element). These elements have always been found integrated in a position and orientation-specific manner 50 ± 4 nucleotides upstream of the coding region of tRNA genes (tDNAs). An intact DRE is 5.7 kb long. It carries an extensive coding region flanked by non-identical long terminal repeats (LTRs), composed of three distinct modules A, B and C. The left LTR proximal to the tRNA gene contains one or several A-modules followed by a single B-module (A_nB). By contrast, the right LTR is composed of a B-module followed by a C-module (BC). Approximately 50% of the DRE elements in NC4 derivatives of *D.discoideum* are structurally different from the 5.7 kb DRE described above. They carry the following alterations: a) a 3.1 kb deletion in the coding region; b) two small deletions of 8 and 29 nucleotides in the B-module of the right LTR; c) a 72 bp deletion in the B-C junction; and d) three distinct point mutations within the A-module of the left LTR. The deletion in the open reading frame encompasses the putative coding regions for reverse transcriptase and integrase. At least 60 copies of this smaller 2.4 kb DRE subtype are found in the genome of *D.discoideum* NC4 strains associated with tRNA genes. Thus, inspite of their lack in reverse transcriptase and integrase those 2.4 kb elements are presumably transposable and at least all isolated copies are found exclusively in the proximity of tRNA gene loci. The enzymes needed for their replication and transposition are likely to be provided by the intact 5.7 kb DREs.

INTRODUCTION

Retrotransposons are amplified in a single cell through a mechanism resembling retroviral replication (1). The template copy itself is not mobilized but remains at its original site. It is copied into RNA which is subsequently reconverted into DNA by reverse transcriptase. Although integration of retrotransposons was initially thought to occur at random positions, recent studies described regular integration specificities for several retrotransposons (2, 3, 4, 5, 6, 7) and retroviruses.

Most remarkable examples for position specific integration of retrotransposons are the Ty3 elements of *Saccharomyces cerevisiae* (5, 7, 8) and the DRE elements of *Dictyostelium discoideum* (9, 10, 11). Both elements are found exclusively at the 5'-side of tRNA genes with relatively constant distances from the tRNA coding regions. Ty3 inserts are found in either orientation 16 to 19 nucleotides upstream of different tRNA genes. DREs have always been found in only one orientation 50 ± 4 nucleotides upstream of different tRNA genes. Since 5'-flanking regions are unique for every eukaryotic tRNA gene a mechanism other than sequence specific integration has to be postulated.

While the structural organization of Ty3 strongly resembles that of typical retroviruses with identical long terminal repeats (LTRs), also known as σ elements (1, 12), DRE comprises non-identical LTR structures. Both DRE LTRs are composed of two distinct modules. The 5'-LTR proximal to the tRNA gene carries an A- and a B-module. The A-module consists of one to several core units of 199 nucleotides plus a direct repeat of nucleotides 1–72. Two subforms of the core unit of the A-module (A_a and A_b) have been identified differing in three point-mutations at positions 7, 103 and 178 (9, 10). The 290 bp B-module is separated from the A-module by a homopolymeric stretch of 39 adenylate residues (A_{39}).

The 3'-LTR distal to the tRNA gene is composed of a B- and a C-module. The C module is 149 bp long and has been operationally defined as the sequence following the B-module of 3'-LTRs that terminates DRE elements.

These LTRs flank an extensive coding part with two overlapping open reading frames. ORF1 has coding potential for 443 amino acids. The second open reading frame (ORF2) contains 3456 nucleotides. The first 105 codons of ORF2 specify no methionyl residues and ORF1 and ORF2 overlap out of phase by 22 nucleotides. A putative translation product derived from ORF1 would be a polypeptide with distant similarities to some

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+X68231, X69170, X69171 and X69101

retroviral *gag* proteins. A minor product is probably a read-through protein, that resumes the proper reading frame of ORF2 either due to an alternative splicing event, ribosomal 'hopping' or −1 frameshifting (9). This situation would then be analogous to several other retrotransposons and retroviruses (12−18). ORF2 has the coding potential for a hypothetical protein with significant similarity to reverse transcriptase (RT), and for a second polypeptide containing three cysteine motifs (amino acid positions 894−1037) which may constitute metal binding domains functionally involved in RNA or DNA binding. Therefore the putative product of ORF2 could specify both, a functionally active reverse transcriptase and an integrase.

Here we describe the identification of a novel class of 2.4 kb DRE elements, the so called DREb elements. From Southern data we estimate that these elements occur in NC4 strains in at least 60 copies in addition to approximately 60 copies of the 5.7 kb DRE elements (DREa elements). DREb elements carry an extensive internal deletion encompassing both the putative reverse transcriptase and metal binding domains. They also contain several deletions within the right LTR and A_b-type modules in their left LTRs. By contrast, 5.7 kb DREa elements always carry A_a-modules. Both elements are present in NC4 derivatives in almost equal numbers, and therefore it is tempting to speculate that the deleted version is amplified by enzymatic activities provided in trans by the 5.7 kb DREs. After transactivation the 2.4 kb DREs retain their integration specificity. The more than 19 characterized DREb elements are found integrated in a position- and orientation-specific manner in front of tRNA genes.

MATERIALS AND METHODS

Strains and plasmid DNAs

The wild type *D.discoideum* strain NC4 as well as the axenic derivatives AX2 and AX3 were obtained from Dr. G.Gerisch, Max-Planck-Institut für Biochemie, Martinsried. Axenic strains were grown in HL5 medium (20) at 21°C, whereas NC4 strains were grown on SM plates on lawns of *Klebsiella aerogenes* (21).

Recombinant clones carrying internally deleted DRE elements and tRNA genes were isolated from plasmid libraries constructed by ligating *Hin*dIII- or *Bgl*II restricted genomic DNA into pUC18 plasmids. Recombinant plasmids were transformed into the *E.coli* SURE™ strain (Stratagene) and were screened for the presence of DREs using LTR-specific oligonucleotide probes.

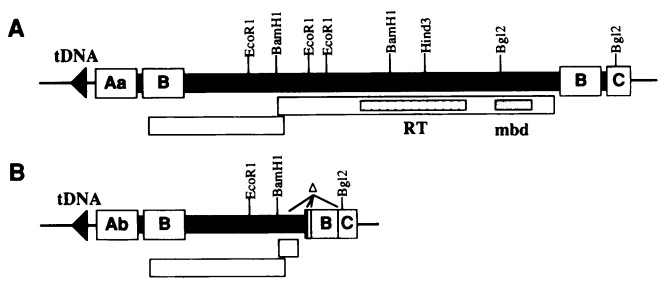


Figure 1. Schematic structures of DREa (**A**) and DREb (**B**) elements.The tRNA gene is indicated by an arrow head pointing in the direction of transcription. The boxes below the elements indicate the two overlapping open reading frames. RT and mbd show the coding regions for putative reverse transcriptase and metal binding proteins, respectively.

Sequence analysis of genomic tDNA clones

Plasmids containing DRE elements were sequenced with a set of 36 different oligonucleotides designed from the known DRE sequence. Oligonucleotides relevant for this communication are Rep1: 5'-GAGATTGTTGTTGTTGTTTGTT-3' (34−12, 233−211), Rep9: 5'-TCTTCGACTTCACCAGCCC-3' (1280−1298), Rep13: 5'-AGAAAGACTATGGAGAC-3' (2396−2413), Rep15: 5'-CCTCTTTTAGAGACCCTG-3' (5551−5568), Rep17: 5'-TTAAATTGGCTACTAGTGC-3' (4839−4821), Rep19: 5'-GATGATTTGTGACACCC-3' (1952−1936), Rep21: 5'-CCACTCAAAATGGTCCTC-3' (3516−3533), Rep28: 5'-GGGATTGTTGTGTTCCAG-3' (3154−3147), Rep44: 5'-TTATAGAAAGTACTGAGTGAA-3' (5154−5134), RepS1: 5'-GTTCTATTGCG::GGGTGTTG-3' (5105−5096::1962−1955). Numbers in brackets correspond to sequence coordinates (9).

Southern, Northern and dot blot analysis

Southern and dot blot analyses were carried out as described (9). Genomic DNA fragments were transferred to a GeneScreen Plus™ membrane and hybridized with suitable radio-labeled oligonucleotides at stringent temperatures. Isolated RNA was separated on agarose gels containing formaldehyde and after transfer to a Nylon membrane filters were hybridized with nick-translated DNA fragments or labeled, strand-specific oligonucleotides.

Nomenclature

The entire DRE element (A_nB----BC) is approximately 5.7 kb in length. Nucleotides were numbered from left to right, the left side being the end proximal to the tRNA gene. Nucleotides 1−199 constitute the 'core unit' of the A-module proximal to the tRNA gene. This 'core unit' occurs frequently in multiple, tandemly organized copies or may even be missing (9, 10). The two known subforms of A-modules (9, 10) are termed A_a and A_b. The core units of both subforms are of identical length, but are distinguished by three point mutations at positions 7 (Aa = T ; Ab = no nucleotide), 103 (Aa = G; Ab = A) and 178 (Aa = no nucleotide; Ab = T). Due to the missing nucleotide at position 7 in the A_b subform, the direct terminal repeats of this A-module comprise 71 nucleotides, as opposed to the A_a subform, in which these repeats comprise 72 nucleotides. Therefore monomeric Aa-modules contain 199 + 72 = 271 nucleotides, whereas monomeric Ab-modules are 199 + 71 nucleotides long.

Nucleotide +1 of tRNA genes is conventionally defined as the first nucleotide of the coding region of mature tRNAs.

RESULTS

Identification of two distinct DRE subtypes

After the sequence analysis of the 5.7 kb DRE element (Figure 1A) (9) additional clones were isolated from a *D.discoideum* library constructed from genomic DNA digested to completion with *Hin*dIII. These clones were obtained by screening with probes specific for either the 5'-LTR (Rep 1) or the 3'-LTR (Rep 15), respectively. All clones isolated with oligonucleotide Rep 15, specific for the C-module, contained only right half DRE elements with B- and C-modules (Figure 2, line 5). This result was to be expected, because the 5.7 kb DREs contain a single *Hin*dIII restriction site. Surprisingly, clones

isolated with oligonucleotide Rep 1 specific for the A-module contained either only the expected left-half DRE elements with just A- and B-modules (Figure 2, line 4), or a second plasmid type with both, the left and right parts of DRE elements (Figure 2, lines 1 and 2). All plasmids that carried an A-module also contained a tRNA gene.

Dot blot mapping of five clones, which were isolated from a genomic *Hin*dIII library and which contained both DRE ends, revealed an extensive internal deletion spanning the single *Hin*dIII restriction site (Figure 2, lines 1 and 2). Furthermore, Rep 15, the oligonucleotide specific for the C-module in the right LTR, did not bind to these clones (Figure 2, dots 1g and 2g). Sequence analysis of these aberrant DRE elements confirmed this interpretation derived from dot blot analysis. They were only 2.4 kb long since all these elements carried an identical deletion in their coding region from nucleotides 1963 to 5095 of the 5.7 kb DRE element. Consequently these clones were able to bind the oligonucleotide Rep S1 designed to be complementary to nucleotides 5105−5096::1962−1955 and thus to span the internal deletion (Figure 2, dots 1h and 2h). The 2.4 kb DREs also carried deletions between coordinates 5219−5226 and 5245−5273 in the B-module of the right LTR and lacked 72 nucleotides in the B-C-module of the right LTR (nucleotides 5489−5560). Furthermore, they all carried b-type A-modules in their left LTRs, and therefore were termed DREb elements as opposed to 5.7 kb DREa elements which carry a-type A-modules. And finally, they were associated with a tRNA gene residing 47−50 nucleotides upstream of the element. The structures of a DREb in comparison with a DREa element is depicted in Figures 1A, B and 2B, C.

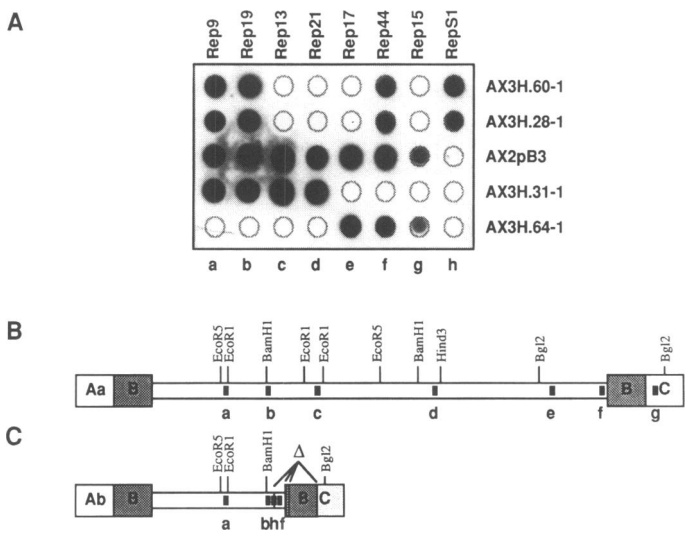
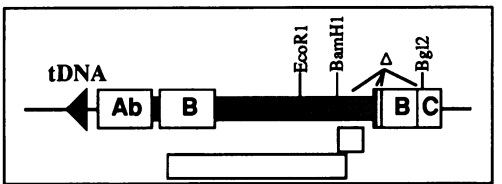
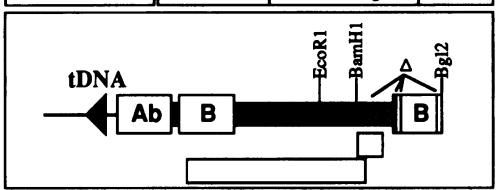


Figure 2. Dot blot analysis (**A**) of different plasmid types (**B, C**). The insert of the only available plasmid (AX2pB3) which carries a nearly intact DRE element (9) is represented by dots in line 3. The inserts of plasmids isolated from a *Hin*dIII library carrying 5′-half (AX3H.31-1) or 3′-half DRE elements (AX3H64-1) are represented by dots in lines 4 and 5, respectively. The inserts of two examples of plasmids carrying complete DREb-type elements (AX3H.60-1 and AX3H.28-1) are given by the dots in lines 1 and 2. Individual filter strips (lanes a−h) were incubated with [^{32}P]-end-labelled oligonucleotides binding to different regions (indicated on the maps **B** and **C** below the dot blot analysis) of DRE elements. The oligonucleotide Rep S1 (lane h) was designed to span the internal deletion of DREb-type elements. DREb elements were also unable to bind the oligonucleotide Rep 15 (lane g), which is complementary to the 5′ part of the C-module in the right LTR.

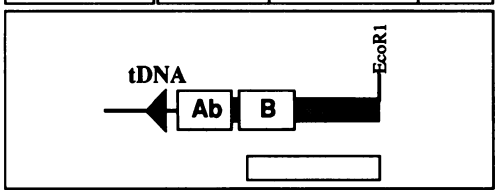
From a genomic *Bgl*II library 7 elements were identified which also hybridized with Rep S1. A detailed analysis revealed the DREb-typical mutations within the A-module, the large deletion



tRNA gene(s)	distance tDNA-DRE	isolated clones	cited in ref.
arg UCU 2	47bp	AX2H.135-gt	11
glu UUC 11	50bp	AX2H.27-gt AX2H.36-gt	11
leu UAA 1	47bp	AX3H.57-1 AX3H.60-1	9, 11
ser AGA 1	47bp	AX3H.42-1	11
ser UGA 1	47bp	AX3H.28-1 AX2H.40-gt	9, 11



tRNA gene(s)	distance tDNA-DRE	isolated clones	cited in ref.
gln UUG 1	46bp	AX3Bg.9-1	11
gln UUG 2	47bp	AX3Bg.10-1 AX3Bg.72-1	9, 11
lys UUU 1	47bp	AX3Bg.12-1 AX3Bg.74-1	9, 11
lys UUU 2	46bp	AX3Bg.21-1 AX3Bg.68-1	9, 11
lys UUU 3	50bp	AX3Bg.17-1 V12E.304-6	9, 11
ser GCU 1	47bp	AX3Bg.70-1	11
tyr AUA 1	50bp	AX3Bg.26-1	11



tRNA gene(s)	distance tDNA-DRE	isolated clones	cited in ref.
glu UUC 1	54bp	AX2GluUUC1	9,10
glu UUC 3	54bp	AX2GluUUC3	9,10
glu UUC 7	50bp	AX2GluUUC7	9,10
glu UUC 13	48bp	V12E.213-15	11
leu UAA 2	48bp	DD61E.6-1	9, 11
lys UUU 4	50bp	AX2E.14-1 AX2E.20-1	9, 11
trp CCA 2	50bp	AX2TrpCCA2	11

Figure 3. Characterized clones carrying DREb elements. The first group of clones contains intact DREb elements, since these clones were isolated from a genomic *Hin*dIII library. The second and third group of clones contain 3′-truncated DREb elements since these clones were isolated either from a genomic *Bgl*II library (group II) or from a genomic *Eco*RI library (group III). In addition to the clone designations also the associated tRNA genes (9−11), the distances separating the tRNA genes and the DRE elements and a reference is shown.

within the coding region and the typical deletions within the right LTR. Also these elements were associated with tRNA genes residing 46 to 50 nucleotides upstream of the elements (9–11).

Finally, it is likely to assume, that also formerly characterized DRE elements (9–11) with b-type A-modules must be classified as DREb subtypes. Since these elements were isolated from *Eco*RI libraries the other b-type typical mutations are not contained on those clones. But all 7 characterized elements of this type are associated with a tRNA gene, as well.

All clones relevant to these conclusions are summarized in Figure 3.

DREb subtypes are flanked by a 13–15 bp target site duplication and are associated with tRNA genes

Since plasmids carrying DREb elements contained both LTRs, is was possible to reevaluate the duplication of target sites created by the integration of DREs. Three of the four independently isolated DREb elements were flanked by 14 identical nucleotides whereas one DREb element contained a 15 bp target site duplication (Figure 4). These results are in very good agreement with an earlier observation made on DREa elements, which had been integrated into the so called H3-repeat and with the $tRNA^{Val}$(UAG)1 gene. All these elements contained either a 14 or a 15 bp target site duplication (9). Finally, a DREa element associated with the tRNA gene *val*AAC13 in AX2 and AX3 (9) generated a 13 bp target site duplication (Figure 4).

The characteristic duplication of the target site is a strong indication that DREb elements also retained the characteristic integration specificities known for DREa elements, although they lack substantial parts of their coding region, including the reverse transcriptase domain (RT in Figure 1A) as well as parts of the putative metal binding domain (mbd in Figure 1A). Specific integration characteristics of DREb elements were further confirmed by the finding that all four isolated complete DREb elements were associated with a tRNA gene (Figure 4).

tRNA gene(s)	distance tDNA-DRE	targetsite duplication	#	DRE subtype	isolated clones
glu UUC 11	50bp	ACTATAATTTTTTT	14	DREb	AX2H.27-gt AX2H.36-gt
ser UGA 1	47bp	CTATTAGAAATAGG	14	DREb	AX3H.28-1 AX2H.40-gt
ser AGA 1	47bp	TTGTATGAAATTTAA	15	DREb::DREa	AX3H.42-1
leu UAA 1	47bp	TGAACGCATTCATT	14	DREb	AX3H.57-1 AX3H.60-1
val UAC 1	47bp	AATCGCAATTTTAA	14	DREa	AX2pDT206 AX3e.21-17
val AAC 13	48bp	CTTTTTTTCAAAA	13	DREa	AX3H.63-1 AX2H.90-gt
lys UUU 5	47bp	CTTTATAAATATATA	15	DREa	V12E.209-15
arg UCU 1	49bp	CTTTATAAATATATA	15	DREa	AX3E.1-1 AX3E.12-1
arg ACG 2	47bp	TAAAATTAATAGAT	14	DREa	AX3H.31-1 AX3Bg.18-1
gln UUG 2	47bp	TAAAATTAATAGAT	14	DREb	AX3Bg.10-1 AX2Bg.72-1

Figure 4. Target site duplications identified on plasmids carrying DREb elements. The individual clones, the associated tRNA gene and the sequences flanking the DREb elements are shown. Three DREb elements generated a 14 bp target site duplication while one DREb element generated a 15 bp target site duplication. This finding is in good agreement with 14 bp or 15 bp target site duplications found after integration of DRE elements into H3-repeats in case of *lys*UUU5, *arg*ACG2, *arg*UCU1 and *gln*UUG2 (9, 11) or with 14 bp target site duplications generated by DRE elements associated with $tRNA^{Val}$(AAC)13 genes and $tRNA^{Val}$(UAC)1 genes in AX2 and AX3 (9).

Verification of DRE subtypes in the genome of *D.discoideum* strains AX2, AX3 and their wild type parent strain NC4

After elucidation of the structure of normal DREa and internally deleted DREb elements it became possible to verify these results by genomic Southern blot analyses. In three independent sets of experiments genomic DNAs from strains AX2, AX3 and NC4 were digested with either *Bam*HI or *Eco*RV. Both enzymes cut DREb elements only once but DREa elements at least twice. In addition, genomic DNAs were doubly digested with either *Bam*HI/*Bgl*II or *Eco*RV/*Bgl*II. These digests should generate distinct fragments also from DREb elements (Figure 5). After size fractionation and transfer to a GeneScrene Plus membrane blots were probed with a suitable oligonucleotide (Rep 19). As predicted from single digest experiments, DREa elements were revealed as a unique restriction fragment (a 1522 bp *Bam*HI fragment or a 1583 bp *Eco*RV fragment) whereas DREb elements apparently gave rise to a set of fragments with different sizes. Based on the determined structure of DREb elements these fragments were generated by a single cut within the truncated elements and a second cut elsewhere in the genome. After a

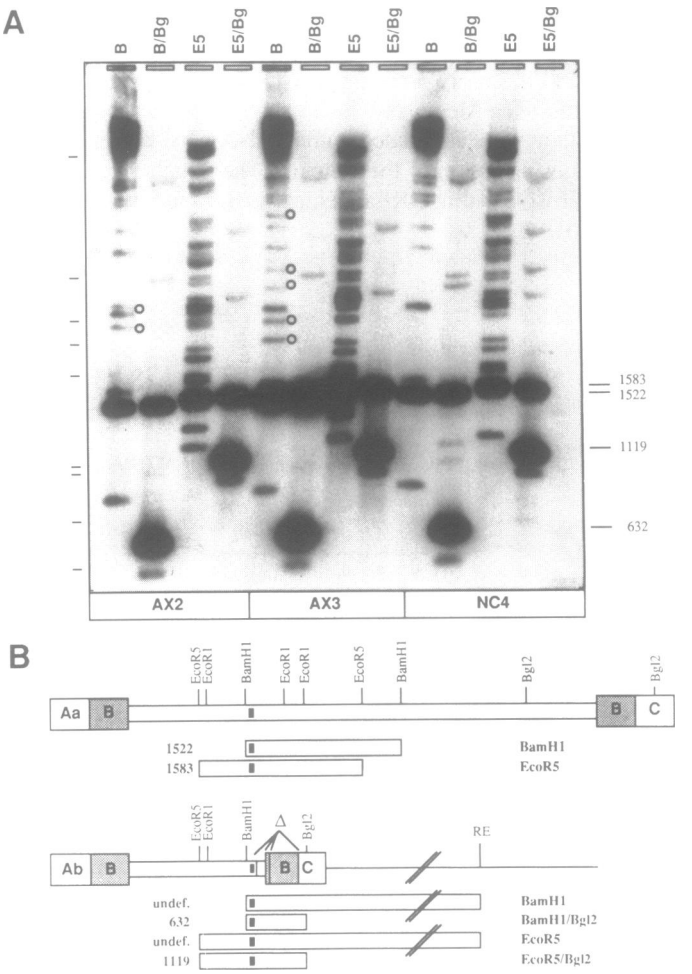


Figure 5. Southern blot analysis (**A**) of genomic DNAs from *D.discoideum* strains AX2, AX3 and NC4. B = *Bam*HI; E5 = *Eco*RV; Bg = *Bgl*II. Fragments indicated by dots in the resolved regions of *Bam*HI digests indicate DREb elements unique to a particular strain. Single copy fragments in *Bam*HI/*Bgl*II or *Eco*RV/*Bgl*II double digest represent DRE elements with structures that do not correspond to those shown in panel **B**.

combined digestion with *Bgl*II and either *Bam*HI or *Eco*RV, a defined fragment of predicted size was also revealed for internally deleted DREb elements (a 632 bp *Bam*HI/*Bgl*II fragment and a 1119 bp *Eco*RV/*Bgl*II fragment). These results confirmed DREa and DREb elements as the predominant DRE structures in *D.discoideum* NC4 strains. Only very few fragments of sizes other than the predicted once were detected in double digest experiments. These resulted probably from 5'-truncated DRE elements (unpublished observation).

Estimated from the signal intensities the number of DREb elements was approximately the same or even slightly greater than the number of normal DREa elements in *D.discoideum* NC4 strains.

Interestingly, the fragment patterns of DREb elements in all these three highly related strains were similar but clearly polymorphic (Figure 5). This indicates that in NC4 derived strains DRE elements are not only transcribed (see below) but may also able to jump.

Correlation of the two different DRE subtypes with expression data

Although expression of DRE elements is very low, a reproducible pattern of at least six different RNAs was observed when cellular RNA is probed with an oligonucleotide (Rep 19) binding to the ORF1 region (9). A putative primary transcript of about 5.7 kb and smaller transcripts of 3.9 kb and 3.1 kb constitute a minor fraction of the RNAs derived from DREs. RNA species of 2.8 kb, 2.4 kb and 2.1 kb occurred with significantly greater abundance (Figure 6). With oligonucleotide Rep 28 which is complementary to the central part of the second open reading frame only the 5.7 kb and the 3.1 kb RNAs can be detected (Figure 6). This observation could be explained if the higher abundant but shorter RNAs represented either processed derivatives of a primary transcript that had lost increasing fractions of the second ORF or transcripts of DREb elements (9).

Subsequently, several independent cDNA clones were isolated. One cDNA clone contained the entire open reading frame thus representing a copy of a DREa transcript. Three cDNA clones carried precisely the extensive deletion of the open reading frame in DREb elements. This is very good indication that also DREb elements are actively transcribed, probably with higher efficiency than normal DREa elements.

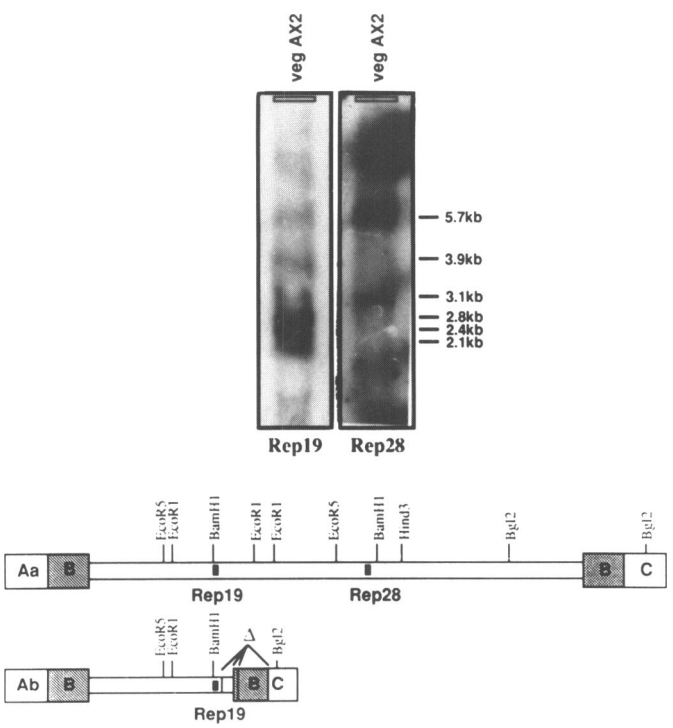


Figure 6. Northern blot analyses of DRE transcripts isolated from growing AX2 cells. (**A**) Hybridization with Rep 19, (**B**) hybridization with Rep 28. In the lower part of the figure the binding sites of oligonucleotides Rep 19 and Rep 28 in DREa and DREb elements are indicated.

DISCUSSION

DREs are highly abundant retrotransposable elements in *D.discoideum*. Here we demonstrate the presence of two main subsets of DRE elements, DREa and DREb, with almost identical copy numbers in NC4-derived strains. DREa elements are probably capable of autonomous transposition because they contain coding information for polypeptides with significant similarity to known reverse transcriptases, integrases and probably *gag*-like proteins. In addition they contain two non-identical long terminal repeats. The other element lacks large portions of the coding region, and contains a variant A-module as well as several small deletions in the right LTR.

The fact that several elements with identical internal deletions have been isolated is strong indication that these elements were amplified form a single prototype, probably through partial transactivation of products of 5.7 kb DREa elements. If amplification occurs via reverse transcription it has to be postulated that DREb elements must contain a functional promoter. This appears to be indeed the case because a major fraction of DRE encoded transcripts seems to be derived from DREb elements. This was first suggested from transcript size (2.4 kb and 2.1 kb RNAs) and has subsequently been supported by sequence analysis of isolated cDNAs which contained the identical extended deletion of ORF2 as DREb-type elements. Interestingly, these transcripts were significantly more abundant than transcripts of the 5.7 kb DREa. Whether this difference is due to a higher stability of the RNA or more efficient transcription is currently being investigated.

Despite their internal deletions the nearly 20 independently characterized DREb elements were found at the same remarkable, specific integration sites as DREa elements, i.e. they occured exclusively in a position- and orientation-specific association with tRNA genes. This proves deleted parts of the 2.4 kb DREs dispensable for their maintenance at these specific sites.

Finally, DREb elements have confirmed preliminary observations concerning the extent of target site duplications subsequent to DRE integration. Evidence for a 13 to 15 bp target site duplication was originally derived from DREa elements which had been inserted into H3-repeats, e.g. upstream of tRNA genes *lys*UUU5, *arg*UCU1, *arg*ACG2, and *gln*UUG2 (9, 11). Depending on the orientation of the H3-repeat DRE elements were flanked either by the sequence 5'-CTTTATAAATATAT-A-3' (in case of *lys*UUU5, *arg*UCU1) or by the sequence 5'-TAAAATTAATAGAT-3' (in case of *arg*ACG2, and *gln*UUG2). These latter 14 nucleotides were also detected downstream of the right LTR in the two independently isolated clones, H.37−1 and H.64−1 (9). This observation was basically confirmed by the identification of a 13 bp target site duplication

around a DREa element associated with *val*AAC13 from AX2 (9). No clones carrying an entire DREa element are available. Therefore this result was obtained from an allelic *val*AAC13 gene isolated from the wild-type strain V12, which was not associated with a DRE element.

Apparently, both types of DRE elements are still transpositionally active in NC4 derived strains. This was suggested indirectly from the polymorphic restriction patterns given in Figure 5. In addition to quantitative differences in the restriction patterns of the three analyzed strains qualitative differences with respect to fragment intensity were also observed. This result suggested that genomic DNAs were prepared from heterogeneous populations with respect to DRE elements.

In view of the uniquely specific integration pattern of DRE elements a forward selection system for DRE transposition requires the presence of a selectable marker in front of a target tRNA gene. Such a system is currently under construction.

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