zone. For example, I used census data to calculate relative aphid abundance (A) for each zone, (that is, from Fig. 2, stem mothers are 119 times more abundant per kilometer in the hybrid zone than in the narrowleaf zone). In addition, aphid transfer experiments showed that survival rates (S) were different for each zone (that is, from Fig. 3, stem mother survival averaged 60.7% in the hybrid zone compared to 38.5% in the pure narrowleaf zone). Finally, by using maps of the drainage system I estimated the linear size (K) of each riparian zone (that is, 13 km of hybrid and 430 km of pure narrowleaf zone). Thus, the relative contribution of each zone to the aphid population is the product of $A \times S \times K$ (that is, gall abundance per kilometer of habitat × survival × the river kilometers of each habitat).

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Transfer RNA Genes: Landmarks for Integration of Mobile Genetic Elements in *Dictyostelium discoideum*

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In prokaryotes and eukaryotes mobile genetic elements frequently disrupt the highly conservative structures of chromosomes, which are responsible for storage of genetic information. The factors determining the site for integration of such elements are still unknown. Transfer RNA (tRNA) genes are associated in a highly significant manner with different putative mobile genetic elements in the cellular slime mold *Dictyostelium discoideum*. These results suggest that tRNA genes in *D. discoideum*, and probably tRNA genes generally in lower eukaryotes, may function as genomic landmarks for the integration of different transposable elements in a strictly position-specific manner.

THE MAJOR FUNCTION OF TRANSFER RNA genes (tDNA) is to provide genetic information for tRNAs, molecules that act as adaptors for amino acids to ensure the faithful translation of the genetic information into proteins. However, other functions of tRNA genes or tRNA-like genes are known. On the compact mitochondrial genomes of mammals, tRNA genes separate other genes and mark positions for processing (*1*), and tRNA gene-like structures on retroviral genomes act as primers to copy the genetic information of these viruses (*2*). In *Saccharomyces cerevisiae*, tRNA genes are frequently associated with mobile genetic elements (transposons) (*3, 4*). The reason for this tight association is unclear.

We have isolated and cloned 24 genomic

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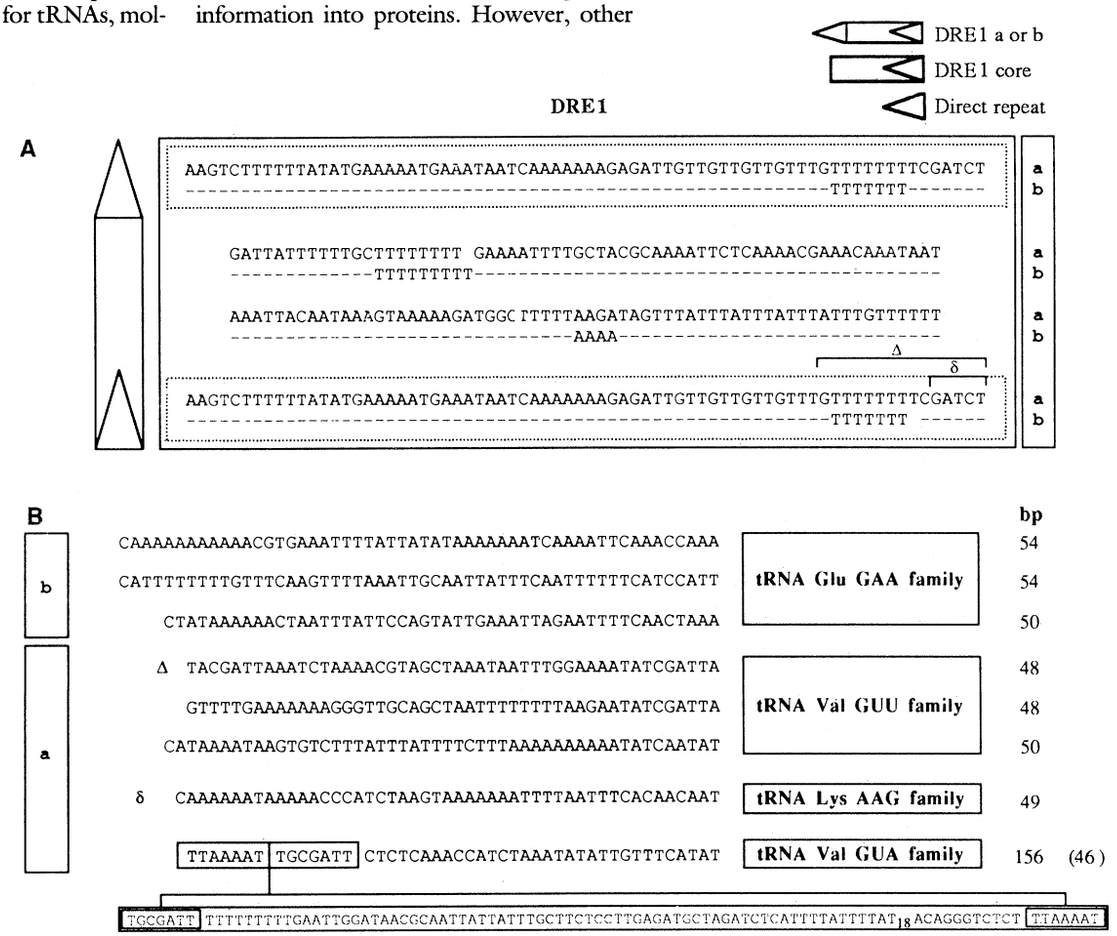

Fig. 1. Nucleotide sequence of DRE1 elements (**A**) and their position relative to different *D. discoideum* tRNA genes (**B**). (A) The nucleotide sequence (written in 5′→3′ direction toward the tRNA genes) of variants a and b of the DRE1 element is shown. They consist of a core element of 199 bp in addition to a direct repeat of nucleotides 1 to 72 in case of DRE1a or 1 to 71 in case of DRE1b. Slightly truncated forms are associated with $tRNA^{Val}(GUU)1$ with nucleotides deleted marked with Δ, and with $tRNA^{Lys}$-(AAG)1 with nucleotides deleted marked with δ. (B) Nucleotides that separate *D. discoideum* tRNA genes and associated DRE1 elements.

Eco RI fragments from the axenic *Dictyostelium discoideum* strain AX2 that contain different tRNA genes. So far 13 individual $tRNA^{Val}$(GUU) genes, one $tRNA^{Val}$(GUA) gene, two $tRNA^{Trp}$(UGG) genes, one $tRNA^{Lys}$(AAG), and eight $tRNA^{Glu}$(GAA) genes were isolated and analyzed (*5, 6*). Additionally, two $tRNA^{Met}$ genes were identified on available sequence data described by Poole and Firtel (*7*). Genomic fragments containing these tRNA genes were routinely characterized by sequence analysis with a set of sequencing primers that recognize different regions of the tDNAs, as well as with sequencing primers complementary to terminal regions of the cloning vector (pUC8). These studies uncovered regions of extreme similarity at conserved positions relative to the tRNA genes. Three elements, named DRE1, DRE2, and Tdd3, were identified. DRE1 and DRE2 represent elements that have not been encountered before; Tdd3 has been described (*7*).

DRE1 consists of a core of 199 bp (Fig. 1A) and contains, in addition, nucleotides 1 to 72 as a terminal direct repeat. The 199-bp core could also be found in multiple tandem copies; it is present as a duplicate in $tDNA^{Val}$(GUU)6, $tDNA^{Val}$(GUU)13, and $tDNA^{Glu}$(GAA)1 or as a quadruplicate in $tDNA^{Val}$(GUA)1 (Fig. 2). In two cases, DRE1 was slightly truncated at its tRNA-proximal end. The element associated with $tDNA^{Val}$(GUU)1 carries a deletion of 15 nucleotides, whereas the DRE1 element associated with a $tDNA^{Lys}$(AAG) lacks 5 nucleotides (Fig. 1B). Very few sequence microheterogeneities were identified among different DRE1 copies. On the basis of these differences two classes (a and b) of DRE1 elements can be defined (Fig. 1A). DRE1a elements contain two strings of $d(T_8)$ residues at relatively distal positions and a d(AAGA) sequence at a central position; DRE1b elements contain strings of $d(T_9)$ and $d(T_7)$, respectively, at corresponding positions and have a $d(A_4)$ sequence instead of d(AAGA). Therefore, the terminal repeats of DRE1b elements are only 71 nucleotides in length (Fig. 1A). DRE1a elements are associated with $tDNA^{Val}$(GUU), a $tDNA^{Val}$(GUA), and with the $tDNA^{Lys}$, while DRE1b elements occur in association with $tRNA^{Glu}$(GAA) genes (Fig. 1B). The reason for this obvious association of distinct DRE1 elements with distinct tRNA gene families is not yet understood. Data obtained from the analysis of many independent *D. discoideum* wild-type isolates, in which a constant number of tRNA genes was seen on fragments exhibiting large restriction fragment length polymorphisms, exclude the possibility that these specific associations could have resulted from duplication of the tRNA gene and their flanking DRE1 elements (*8, 9*).

The second identified element, DRE2, was always found associated with DRE1; the elements were separated by 39 dA-dT base pairs (Fig. 2). DRE2 is composed of at least 1018 nucleotides spanning from the end of the $(dA)_{39}$ homopolymer to an Eco RI recognition site. Since all our clones were isolated from genomic Eco RI libraries, we suspect that only a part of this element has been characterized. There are minor differences in nucleotide structure among different DRE2 elements. However, all characterized DRE2 elements are identical in length. An open reading frame spans more than 939 nucleotides. The close association of DRE1 and DRE2 suggests that both elements establish one functional unit, with two DRE1 sequences acting as long terminal repeats (LTRs) of a composite element. Additional evidence to support this suggestion is a recently isolated clone characterized from a partial Mbo I genomic library. This clone hybridizes twice with a DRE1-specific probe, and both elements are separated by about 3 kb carrying DRE2-specific nucleotides.

Both elements were extremely unstable even when cloned in *Escherichia coli recA*$^-$ strains. We frequently observed changes during various cloning steps, and, in several cases, we had to go back to the original phage isolate to obtain reliable sequence information. We therefore believe that the

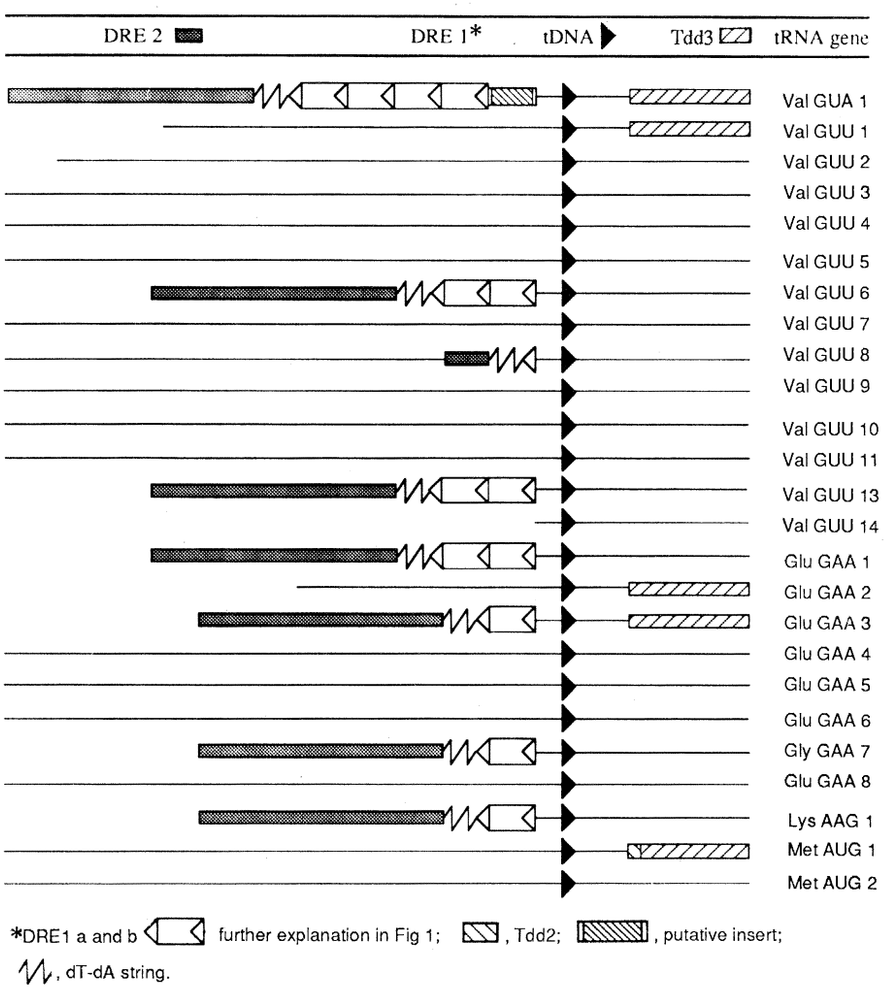

Fig. 2. Genomic organization of the region surrounding 27 *D. discoideum* tRNA genes. Tdd3 has been described (*7*). All Tdd3 elements on our clones are incomplete because of an internal Eco RI restriction site, since recombinant clones carrying tRNA genes were generated after complete Eco RI digestion of genomic DNA. The Tdd3 element associated with $tRNA^{Met}$(AUG)1 has been inserted into another repetitive element termed Tdd2 (*7*). DRE1a and b consist of at least one core unit of 199 nucleotides in addition to one direct repeat of nucleotides 1 to 72 and 1 to 71, respectively. DRE2 represents, like Tdd3, a partial repetitive element, since it also contains an internal Eco RI site. At the moment 1018 nucleotides of this element are characterized. So far it always occurs in association with DRE1, separated from this by a $(dT\text{-}dA)_{39}$ string. DRE elements associated with $tDNA^{Val}$(GUU)8 have abnormal structures most likely due to a cloning artifact. Another putative insertion element associated with $tDNA^{Val}$(GUA)1 is shown.

remnant structure associated with tDNA^{Val}-(GUU)8 most likely represents a cloning artifact (Fig. 2).

The third element, Tdd3, was originally described by Poole and Firtel (7). We have found it associated with five different tRNA genes. In contrast to DRE1 and DRE2, Tdd3 always occured downstream from mature tRNA coding regions (Fig. 2). In the case of tRNA^{Glu}(GAA) genes 2 and 3, Tdd3 was located 95 nucleotides and 124 nucleotides, respectively, downstream of the tRNA gene. The tRNA genes Val(GUA)1 and Val(GUU)1 were separated from their Tdd3 elements by 97 nucleotides and 80 nucleotides, respectively, while the Tdd3 element associated with the tRNA^{Met}-(AUG) gene was found 107 nucleotides downstream from the tDNA. It thus appears that Tdd3 occurs approximately 100 nucleotides downstream of several *D. discoideum* tRNA genes. We have no indication for a sequence-specific integration of any of the different elements; however, they were integrated at specific sites relative to the tRNA gene. This is supported by the analysis of recently isolated clones. They were selected from a genomic library with a DRE1-specific probe and always carried tRNA genes about 50 nucleotides apart from the DRE1 element. This observation strongly implies that tRNA genes mark genomic regions for integration of different mobile genetic elements.

A summary of all our results is presented in Fig. 2. Clones coding for tRNA^{Val}-(GUA)1 and tRNA^{Glu}(GAA)3 contain all three elements; plasmids coding for tRNA^{Val}(GUU)1, tRNA^{Glu}(GAA)2 and for a tRNA^{Met}(AUG) (7) contain just a Tdd3 element; genes for tRNA^{Val}(GUU)6, tRNA^{Val}(GUU)13, tRNA^{Glu}(GAA)1, tRNA^{Glu}(GAA)7 and tRNA^{Lys}(AAG)1 are associated with DRE1 and DRE2 but not with Tdd3; and tRNA^{Val}(GUU)8 is located on a clone containing truncated DRE1 and DRE2 elements.

More intriguing than their association with different tRNA genes per se is the characteristic position of the three different elements relative to the tRNA genes. With one exception, DRE1 always occurs at approximately position −50 of eight different tRNA genes (Fig. 1B). The exception concerns tDNA^{Val}(GUA)1. In this particular case, it seems very likely that another integration event might have occurred. Next to DRE1, a DNA sequence of 99 bp is located that is flanked by perfect direct repeats of 14 nucleotides. If such a 14-bp direct terminal repeat represents the target site of DNA integration, then subtraction of the presumably inserted DNA leaves a spacer of 46 nucleotides between the 5′ end of the tDNA^{Val}(GUA) and the start of DRE1 (Fig. 1B).

On the basis of the regularities described above, we detected two additional tRNA genes. One clone containing the tRNA^{Glu}-(GAA)5 gene hybridized with DRE1- and DRE2-specific oligonucleotides, although sequence analysis of the 5′ flanking region of this tRNA gene did not uncover the expected elements. However, sequence analysis around the DRE1 element on the plasmid containing the tRNA^{Glu}(GAA)5 gene revealed a second tRNA gene coding for a tRNA^{Lys}(AAG). This tRNA gene was located exactly 50 nucleotides downstream of DRE1 (Fig. 1B). In the second case, a tRNA gene was detected on a genomic fragment characterized by Poole and Firtel (7). This fragment contains a Tdd3 element and, according to our rules, a tRNA gene should be located about 100 bp upstream of such an element. We identified two 72-bp inverted repeats, described as such by Poole and Firtel (7), as two tRNA^{Met}(AUG) genes. One of these genes resided, as predicted, 100 nucleotides upstream of the Tdd3 element.

Tdd3 has been found in different locations in related strains causing a 9- to 10-bp duplication of the target site DNA (7), which is characteristic of mobile genetic elements. Similarly DRE1/DRE2 seems to be mobile (Fig. 3). Two *D. discoideum* strains (AX2 and HU1628) that were derived from the wild-type isolate NC4 show a defined restriction fragment length polymorphism after Eco RI digest when probed with a tRNA^{Glu} gene–specific oligonucleotide. Us-

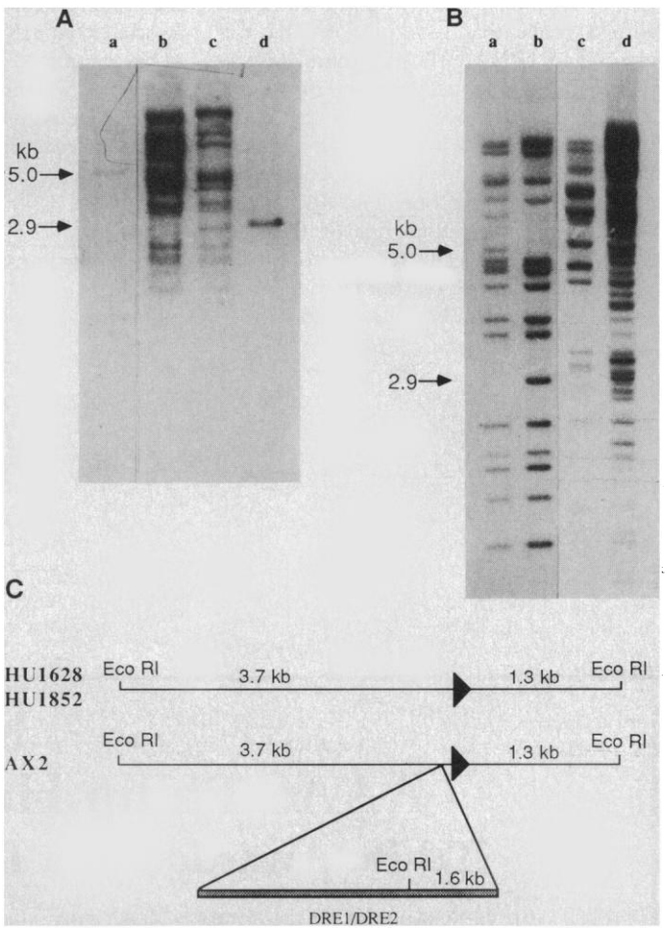

Fig. 3. Evidence for transposition of DRE1/DRE2 elements. (**A**) Genomic DNAs isolated from the NC4 derivatives HU1628 (lanes a and b) and AX2 (lane c) and plasmid yGlu-(GAA)1 (lane d) were digested with Eco RI, size-fractionated on an agarose gel, and transferred to a nitrocellulose membrane. The filter containing lanes b, c, and d was incubated with a radioactively labeled synthetic oligonucleotide that specifically recognizes tRNA^{Glu}-(GAA) genes. The filter containing lane a was incubated with a radioactively labeled fragment isolated from the 3′ flanking region of the tRNA-^{Glu}(GAA)1 gene. (**B**) Genomic DNAs isolated from the NC4 derivatives HU1628 (lanes a and c) and AX2 (lanes b and d) were digested with Eco RI, and aliquots were size-fractionated on the same agarose gel before transfer to a nitrocellulose membrane. The filter containing lanes a and b was incubated with a radioactively labeled synthetic oligonucleotide that specifically recognizes tRNA^{Glu}(GAA) genes. The filter containing lanes c and d was incubated with a radioactively labeled synthethic oligonucleotide that specifically recognizes DRE2 elements. Polymorphic Eco RI fragments of 5.0 and 2.9 kb in strains HU1628 and AX2, respectively, are indicated. The 2.9-kb tRNA^{Glu}(GAA)1-containing fragment of AX2 comigrates with an Eco RI fragment of AX2 that is recognized by the DRE2-specific probe. The 5.0-kb tRNA^{Glu}(GAA)1-containing fragment in strain HU1628, however, is not recognized by the DRE2 probe. (**C**) Schematic representation of the genomic organization of the tRNA^{Glu}(GAA)1 gene in *D. discoideum* strains HU1628 (and HU1852) and AX2. All strains are closely related and were derived from the wild-type isolate NC4. In strain HU1628 (and HU1852), the tRNA^{Glu}(GAA)1 gene resides on a 5.0-kb Eco RI fragment. In AX2, a DRE1/DRE2 integrated about 50 nucleotides upstream of the tRNA^{Glu}(GAA)1 gene. This element contains an Eco RI restriction site at 1.6 kb. Consequently, Eco RI generates a tRNA^{Glu}(GAA)-containing fragment in AX2 that is 2.9 kb in length, instead of the 5.0-kb fragment in HU1628 (and HU1852).

ing a single-copy probe isolated from the 3′ flanking region of the $tRNA^{Glu}$(GAA)1 gene, we were able to identify two corresponding polymorphic fragments of 2.9 kb in strain AX2 while the same gene resides on a 5.0-kb Eco RI fragment in strain HU1628 (Fig. 3A). This result can be explained if one assumes that the $tRNA^{Glu}$(GAA)1 allele resides on a 5-kb genomic Eco RI fragment in HU1628 and is not associated with DRE1/DRE2 elements. The corresponding allele in *D. discoideum* strain AX2 is known to contain these elements 54 nucleotides upstream of the tRNA gene. Since the DRE2 element contains an internal Eco RI site at a distance of about 1.6 kb from the integration site, digestion of genomic AX2 DNA with Eco RI generates a fragment of 2.9 kb (Fig. 3C). Results obtained from hybridizing the same filters containing size-fractionated genomic Eco RI fragments from AX2 and HU1628 with a probe that recognizes DRE2 elements and $tRNA^{Glu}$(GAA) genes, respectively, strongly support this interpretation. According to this analysis, a 2.9-kb Eco RI fragment from AX2 hybridizes as well with the $tRNA^{Glu}$(GAA)-specific probe as with the DRE2-specific probe (Fig. 3B, lanes b and d), while the 5.0-kb Eco RI fragment from HU1628 is only recognized by the tRNA-specific probe but not by the DRE2-specific probe (Fig. 3B, lanes a and c).

Our observations described here provide a plausible explanation for the relative ease with which tRNA genes from *D. discoideum* were mapped from restriction fragment length polymorphisms (*6, 8*). Clearly tRNA genes in *D. discoideum* are preferentially targeted by mobile genetic elements.

Our results are analogous to those obtained in yeast and may therefore implicate more general mechanisms for transposition or retrotransposition. In yeast, sigma elements are almost exclusively associated with tRNA genes, located at position −19 to −16 relative to the 5′ end of different tRNA coding regions (*3*). Although most sigma elements characterized to date are isolated insertions, a few of these elements occur relatively closely spaced to a Ty3 and function as an LTR of this element (*4*). Another repetitive element termed tau (*10*) has been encountered in yeast that is so far exclusively associated with tRNA genes (*11*). It remains to be determined for yeast as well as for *D. discoideum* whether the association of a tRNA gene with a mobile genetic element may interfere with the expression of the tRNA gene.

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