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Jordan, an Active Volvox Transposable Element Similar to Higher Plant Transposons

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We have isolated a 1595-bp transposable element from the multicellular green alga *Volvox carteri* following its insertion into the nitrate reductase (*nitA*) locus. This element, which we have named *Jordan*, has short (12-bp) terminal inverted repeats and creates a 3-bp target site duplication, like some higher plant transposons of the classic type. Contained within the first 200 bp of one end of the element are 55-bp inverted repeats, one of which begins with the terminal inverted repeat. Revertants of the transposon insertion into the *nitA* locus were obtained at a rate of $\sim 10^{-4}$ per Volvox embryo per generation. In each revertant examined, all transposon sequences were completely excised, but footprints containing both sets of duplicated bases, in addition to three to nine extra bases, were left behind. *Jordan* contains no significant open reading frames and so appears to be nonautonomous. DNA gel blot analysis indicates that *Jordan* is a member of a large family of homologous elements in the Volvox genome. We have isolated and characterized several of these homologs and found that they contain termini very similar to those of *Jordan*. Efforts to utilize *Jordan* and its homologs as tools to tag and clone developmentally interesting genes of Volvox are discussed.

INTRODUCTION

Because of their ability to move from site to site within the genome, frequently causing insertion mutations of genes in which they happen to land, transposable elements can be powerful tools for tagging and cloning genes of eukaryotic organisms. Because a transposon inserted within a gene is flanked by portions of that gene, probes derived from the transposon can be used to retrieve portions of the gene that it has inactivated. Many genes from a wide variety of organisms have already been isolated in this manner, including the *pallida* locus of snapdragon (Martin et al., 1985), the *bronze* locus of maize (Fedoroff et al., 1984), the *unc-22* locus of *Caenorhabditis elegans* (Moerman et al., 1986), and the *vestigial* locus of Drosophila (Williams et al., 1988). In theory, any gene whose inactivation leads to a discernible phenotype may be tagged and cloned by using transposons.

Transposable elements of higher plants, especially maize and snapdragon, have been particularly well characterized, and they are now being used to tag and clone genes in heterologous plants. *Activator/Dissociation* (*Ac/Ds*) elements from maize have been shown to transpose in tobacco (Baker et al., 1986), tomato (Yoder et al., 1988), potato (Knapp et al., 1988), rice (Izawa et al., 1991), flax (Ellis et al., 1992), Arabidopsis, and carrot (Van Sluys et al., 1987). Similarly, *Enhancer/Supressor-mutator* (*En/Spm*) of maize can also transpose when introduced into tobacco (Masson and Fedoroff, 1989) and potato (Frey et al., 1989), and *Tam3* of snapdragon can transpose in tobacco and petunia (Haring et al., 1989). When it works, this strategy has the advantage that the heterologous transposon constitutes a unique sequence in its new host. But not all plants are amenable to the methods of DNA transfer commonly utilized, and in some plants foreign transposons may not be functional. In several such organisms, endogenous transposons have been identified and isolated by probing for homologs of previously characterized elements (Voytas and Ausubel, 1988). But not all organisms will be amenable to this approach; homologs might not exist, they might not be sufficiently similar to be detected by hybridization or polymerase chain reaction (PCR), or when found they might not be mobile. The most generalizable method for obtaining active transposons is to select for insertions into a previously cloned gene whose inactivation causes a recognizable phenotype; such genes are referred to as "transposon traps." Potential transposon insertions are then identified by screening the mutant strains for restriction fragment length polymorphisms (RFLPs), using the cloned selectable marker as a probe. This strategy recently led to the successful isolation of the *Tnt1* element of tobacco (Grandbastien et al., 1989), the *Tag1* element of Arabidopsis (Tsay et al., 1993), and the *Fot1* element of the fungal plant pathogen Fusarium (Daboussi et al., 1992), and in two of these instances, the nitrate reductase gene was used as the trap.

Volvox carteri f *nagariensis* is a multicellular green alga that has a simple developmental program amenable to genetic analysis (Harper and Kirk, 1986; Schmitt et al., 1992). Volvox consists of two cell types, large reproductive cells, or gonidia, and small, Chlamydomonas-like somatic cells, organized in a sphere (Starr, 1969). Mutants in which the normal pattern

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of cellular differentiation is disrupted are easily isolated, including a class, regenerator (Reg), in which somatic cells dedifferentiate and redifferentiate as gonidia (Starr, 1970; Huskey and Griffin, 1979) and a class, gonidialess (Gls), that lacks any normal gonidia (Kirk et al., 1991). Until now there have been no efficient means of cloning the genes defined by such mutations; therefore, we began efforts to isolate Volvox transposons using the recently characterized *nitA* (nitrate reductase–encoding) gene of Volvox (Gruber et al., 1992) as a transposon trap. Because Volvox is haploid, and loss of nitrate reductase activity confers resistance to chlorate (Huskey et al., 1979), *nitA* mutants of Volvox are readily isolated as chlorate-resistant colonies.

Recently, two families of *copia*-like retrotransposons were identified in Volvox (Lindauer et al., 1993). Here, we report the isolation from Volvox of an active transposable element that resembles some higher plant transposons. This transposon, which we have named *Jordan*, contains 12-bp imperfect terminal inverted repeats (TIRs) and causes a 3-bp target-site duplication upon insertion. There appear to be many homologs of *Jordan* in the Volvox genome, and we have cloned several of these in an attempt to identify those most promising as agents for tagging and cloning of developmentally important genes.

RESULTS

Isolation of CR Mutants

In an attempt to recover transposable elements from Volvox, we used *nitA*, the gene encoding nitrate reductase, as a transposon trap. Based on the knowledge that Volvox mutants with depressed nitrate reductase activity tolerate much higher concentrations of chlorate ion than wild-type strains (Huskey et al., 1979), we collected more than 80 chlorate-resistant (CR) mutants of wild-type strains HK10 and EVE in the hope that some of these would contain insertions at the *nitA* locus. But because it is known that mutations of the nitrate reductase structural gene constitute only one of several categories of mutations that can result in a CR phenotype (Sosa et al., 1978; Huskey et al., 1979; Fernandez and Matagne, 1984; Aguilar et al., 1992), the first 32 CR mutants isolated were tested for their ability to utilize nitrate as sole nitrogen source. Because 29 of these 32 CR mutants were unable to utilize nitrate as sole nitrogen source, we assumed that most CR mutants were candidates to contain insertions at the *nitA* locus.

Induction of CR Mutants by Heat Shock

It had previously been observed that subjecting cleaving Volvox embryos to a heat shock increased the rate at which certain morphological mutants arose (D. Kirk, unpublished observations). Therefore, we tested the effect of heat shock on the rate of occurrence of CR mutants. As shown in Table 1, embryos subjected to heat shock (40 min at 42.5°C followed by 20 min at 45°C) (Kirk and Kirk, 1986) gave rise to significantly more CR colonies than did embryos plated without heat shock. Furthermore, the cleavage stage at which the heat shock was applied appeared to be important: early-cleavage embryos (2 to 16 cells) were affected minimally, midcleavage embryos (~128 to 512 cells) were affected maximally, and late-cleavage embryos were affected somewhat less. The CR mutants described elsewhere in this study were isolated from both non-heat-shocked embryos and from embryos heat shocked at various stages of cleavage.

Characterization of CR Mutants

DNA samples from 57 CR mutants were examined on DNA gel blots (Maniatis et al., 1982) that were hybridized to a *nitA* probe. Genomic DNAs were digested with SalI, which cuts the wild-type *nitA* gene into fragments of 0.8, 1.3, 2.1, and 3.0 kb, as shown in Figure 1B, electrophoresed on agarose gels, and transferred to nylon membranes. The membranes were probed with plasmid pVcNR1, which contains the entire coding region of the Volvox *nitA* gene (Gruber et al., 1992). Of the 57 mutants, 13 contained RFLPs that could have been caused by insertion of a new DNA fragment into one of the four SalI fragments. In each of these cases, one of the four wild-type fragments was missing and was replaced by one or more SalI fragments of greater combined size than the missing fragment. Seven of the 13 mutants contained a small insertion of apparently the same size (~1.6 kb). As shown in Figure 1, in strains CRH1, CRH2, CRH8, CRH17, CRH22, and CRH28, the wild-type 2.1-kb SalI *nitA* fragment is missing and is replaced by a 3.7-kb fragment (lanes containing CRH1, CRH8, CRH2, CRH28, CRH22, and CRH17), and in strain CRH46, the wild-type 3.0-kb fragment is missing and is replaced by a 4.6-kb fragment (lane containing CRH46). The remaining six mutants contained insertions of heterogeneous sizes, ranging from ~0.6 to more than 11 kb (Figure 1 and data not shown).

DNAs from the six strains containing ~1.6-kb insertions in the 2.1-kb SalI fragment and of CRH46, which contains an

Table 1. Effect of Heat Shock on Accumulation of CR Mutants

	CR Mutants Recovered per 10^5 Embryos Plated	
Embryo Stage	Control	Heat Shock
Early cleavage (~2 to 16 cells)[a]	1.0	2.0
Mid–late cleavage (~128 to 512 cells)[a]	1.5	20
Late cleavage to early postcleavage	2.0	11

[a] Average of values from two separate experiments.

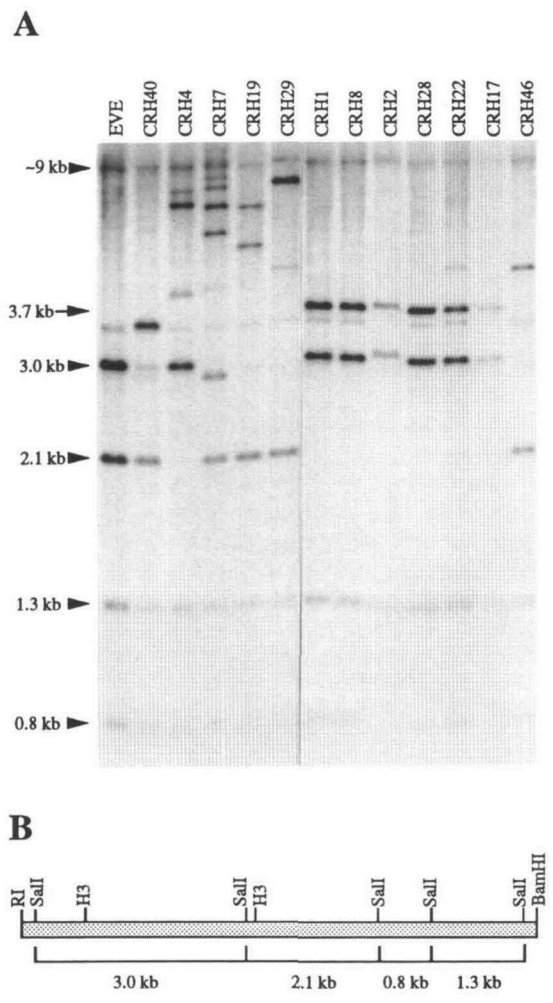

Figure 1. SalI RFLP Analysis of CR Mutants.

(A) DNA gel blot showing RFLPs at the *nitA* locus among CR mutants. **(B)** Restriction map of the cloned *nitA* fragment used as the probe in **(A)**. DNAs were cleaved with SalI, electrophoresed on a 0.8% agarose gel, and transferred to nylon membranes; the resulting DNA gel blot was probed with plasmid pVcNR1, which contains the 7.6-kb EcoRI-BamHI restriction fragment that encompasses the entire coding region of *nit*A, along with several hundred base pairs upstream and downstream of it (Gruber et al., 1992). The numbers and arrowheads at the left edge of **(A)** indicate the lengths of the SalI fragments of the wild-type gene *nitA*. The arrow in **(A)** indicates the position of the 3.7-kb RFLP appearing in strains CRH1, CRH2, CRH8, CRH17, CRH22, and CRH28. RI, EcoRI; H3, HindIII.

insertion of ~1.6 kb in the 3.0-kb fragment (Figure 1), were further tested by DNA gel blot analysis following digestion with the enzymes HindIII and HpaI. In the six strains having the insertion in the 2.1-kb SalI fragment, the wild-type 6.5-kb HindIII fragment was replaced by two smaller fragments, as shown in Figure 2 (lanes containing CRH1, CRH8, CRH2, CRH28, CRH22, and CRH17). In each case, the sum of the lengths of the two new HindIII fragments was ~7 kb, ~1 kb shorter than expected for a 1.6-kb insertion in a 6.5-kb fragment. This indicated that all six of these strains possess a 1.6-kb element containing at least two HindIII sites located ~1 kb apart. In CRH46, the wild-type 2.6-kb HindIII fragment was replaced by a single fragment of ~3.0 kb (Figure 2), suggesting that the novel DNA found in the *nitA* gene of this strain was inserted near a HindIII site and contains at least one HindIII site, ~0.4 kb from one of its ends. DNA gel blot analysis of HpaI-digested DNAs indicated that CRH1, CRH2, CRH8, CRH17, CRH22, and CRH28 all possess at least two HpaI sites separated by ~1.5 kb (data not shown). The *nitA* insertion in CRH46 also possesses at least one HpaI site, but it could not be determined whether multiple sites exist, as in the other insertions (data not shown).

Four strains (CRH2, CRH17, CRH22, and CRH28) were similar in terms of the sizes of these novel HindIII and HpaI fragments (Figure 2 and data not shown), indicating that they contain insertions at similar positions within the *nitA* gene.

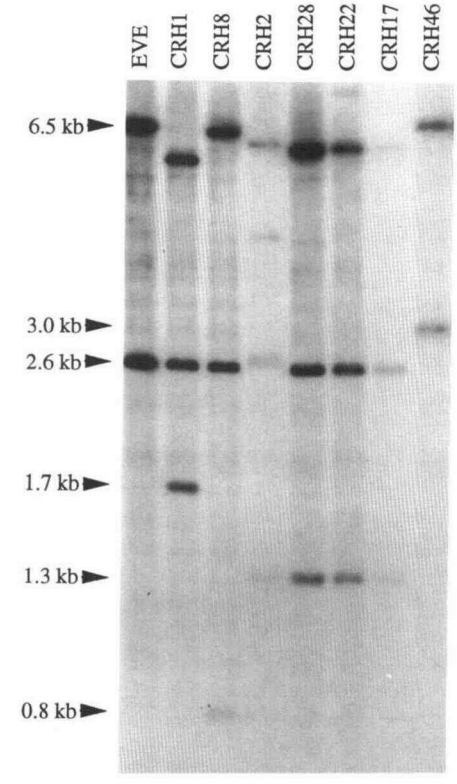

Figure 2. HindIII RFLP Analysis of CR Mutants with ~1.6-kb Insertions at *nitA*.

DNAs were cleaved with HindIII, electrophoresed on a 0.8% agarose gel, and transferred to nylon membranes; the resulting DNA gel blot was probed with plasmid pVcNR1 as given in Figure 1. The numbers and arrowheads at left indicate the lengths of the wild-type HindIII fragments and of the smaller polymorphic HindIII fragments.

While it is possible that these four strains arose independently as the result of insertions at identical or nearly identical positions within the *nitA* gene, it is more likely that they represent clonal descendants of a single CR strain that was either present in the population at the time chlorate selection was applied, or that was mechanically disrupted following heat shock and before plating. Consistent with this interpretation is the fact that all four of these strains were isolated during the same plating experiment. Therefore, the above gel blot analyses indicate that of the 57 CR strains initially studied, at least three or four contain different insertions of the same 1.6-kb element at the *nitA* locus.

Reversion Analysis

A hallmark of mutations caused by transposon insertions is that at least a subset of them may be highly revertible (McClintock, 1948, 1951). We therefore sought to determine if any of the 13 CR mutants with suspected transposon insertions at the *nitA* locus might be capable of reverting to the wild-type condition (nitrate autotrophy). Initially, we chose four mutants for this study, two containing large insertions (CRH4, >6 kb; CRH7, >11 kb) and two with the 1.6-kb insertion (CRH1 and CRH22) (Figure 1). Cultures of all four strains were grown nonselectively for several generations to improve the likelihood of detecting reversion events. Cleaving embryos were then isolated, subjected to the heat shock regimen described above, and plated in agarose overlays on standard Volvox medium containing nitrate as the only nitrogen source (NSVM); ~50,000 embryos were tested from each strain. There were no survivors among the plated embryos from strains CRH4, CRH7, and CRH1, but the embryos from strain CRH22 gave rise to 38 colonies. Spheroids were picked from each colony and retested in liquid medium for their ability to utilize nitrate as sole nitrogen source, and all were able to grow in NSVM.

To determine whether heat shock increased the rate of reversion of strain CRH22 from *nitA*$^-$ to *nitA*$^+$, mid-to-late cleavage embryos of strain CRH22 were isolated and plated on NSVM either following the heat shock treatment or with no heat shock. Of ~32,000 heat-shocked embryos plated, 29 gave rise to growing colonies on NSVM plates, whereas ~53,000 non-heat-shocked embryos gave rise to only 18 colonies. In duplicate experiments, the absolute rate of phenotypic reversion varied somewhat, but the ratio of revertants obtained following heat shock versus control conditions was always about the same. Since embryos at the time of heat shock contained an average of 10 gonidial initials with reproductive potential, the rates of reversion for the heat-shocked versus control embryos in this experiment were 0.89×10^{-4} and 0.34×10^{-4}, respectively. Therefore, heat shock appeared to have a modest effect on increasing the rate of phenotypic reversion.

To test whether the polymorphism at the *nitA* locus had reverted along with the selectable phenotype, we performed DNA gel blot analysis of genomic DNAs isolated from six of the revertants and from the parental mutant strain, CRH22. A control strain lacking an insertion in the 2.1-kb SalI restriction fragment of *nitA* (CRH19) was also included. The SalI-digested DNAs were probed with a plasmid containing the 2.1-kb SalI restriction fragment from the wild-type gene (the fragment in which the insertion had occurred in strain CRH22). In all six phenotypic revertants, the polymorphism in the SalI fragment detected by this probe also reverted, indicating that the insertion element had departed, as is shown in Figure 3. In light of this evidence for the mobility of the 1.6-kb element, we set about to clone and characterize it.

Isolation of the 1.6-kb Element

The 1.6-kb insertion element was cloned by PCR amplification of the appropriate region of genomic DNA isolated from strain CRH22. Oligonucleotides were designed to hybridize to regions of the *nitA* gene flanking the 2.1-kb SalI restriction fragment, and PCR products obtained with these primers and CRH22 DNA were cloned into plasmid pBluescript KS+. Colonies containing candidate clones were screened by hybridization to the 2.1-kb SalI restriction fragment of the *nitA*

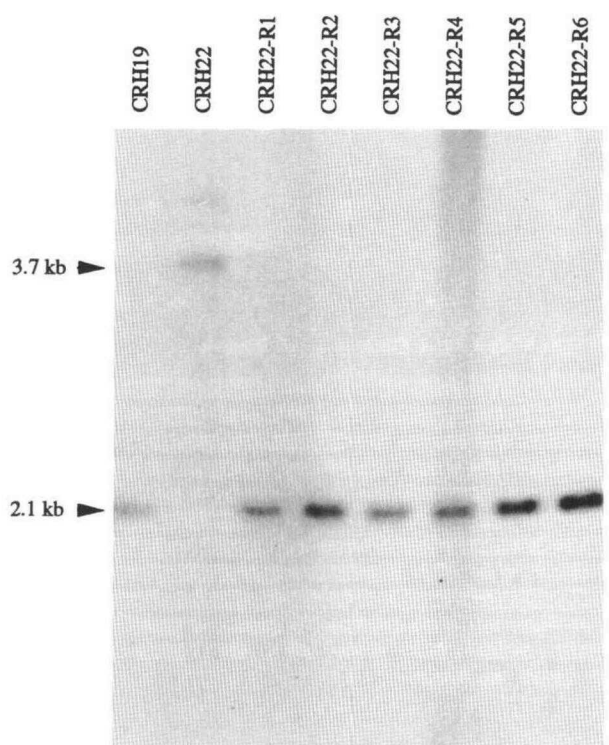

Figure 3. Reversion of the CRH22 *nitA* RFLP.

DNAs from CRH22, six phenotypic CRH22 revertants, and a control strain (CRH19) were cleaved with SalI and electrophoresed on an 0.8% agarose gel. The resulting DNA gel blot was probed with a plasmid containing the 2.1-kb SalI restriction fragment from pVcNR1 (Figure 1B). Molecular length markers are given at left.

gene. Plasmids were isolated from several colonies that gave strong hybridization signals and were shown by restriction analysis to contain a 1.6-kb insertion within the 2.1-kb SalI *nitA* fragment. SalI-digested DNA from one of these recombinant plasmids (pLV6-14) and SalI-digested CRH22 genomic DNA were compared side by side on a DNA gel blot filter that was probed with the 2.1-kb SalI restriction fragment, and the probe hybridized to fragments of 3.7 kb in both DNAs (data not shown). This was further evidence that the correct fragment had been amplified and cloned.

Sequence Characteristics of the Element

A restriction map was made of the insert, and based on this information, several sequence primers were chosen to find the points within the PCR-amplified insert where the *nitA* sequence was interrupted by foreign DNA. Beginning at the position corresponding to base pair 1352 of the 2592-bp *nitA* coding region (within the seventh exon of the gene) (Gruber et al., 1992), the sequence of the plasmid insert diverged from the wild-type *nitA* sequence for 1598 bp. The final three of these 1598 bp are a direct repeat of the 3 bp preceding the point of divergence, and the remaining 1595 bp contain symmetry motifs common to certain eukaryotic transposable elements. These symmetry features are highlighted in Figure 4, where the complete nucleotide sequence of the element is shown. At the left terminus of the element is the 12-bp sequence 5′-CCCTATGGCATA-3′, and the element terminates with the imperfect inverted repeat of this sequence, 5′-TATGCCATTGGG-3′. Within 160 bp of the left terminus is a cluster of five regularly spaced repeats of the 12-bp sequence 5′-GTTGGGTTAACG-3′, each separated from the next by the 4-bp sequence 5′-PuPyCPu-3′. Five shorter versions of this motif are scattered within 160 bp of the right TIR as the sequences 5′-GGTTAACG-3′ (once), 5′-GTTAACT-3′ (once), and 5′-GGTTACC-3′ (three times). There are no significant open reading frames within the 1595 bp of the element (data not shown).

Eukaryotic transposable elements that transpose via a DNA intermediate create short target site duplications upon insertion into a new site, contain short TIRs, and often contain near their termini short direct repeats that serve as transposase binding sites (Gierl et al., 1988; Kunze and Starlinger, 1989; Gierl and Saedler, 1992). Because the 1.6-kb insertion element has all of these properties, in addition to the demonstrated ability to be inserted into and excised out of the *nitA* locus, it would appear to be a transposon belonging to the class of elements that transpose by means of a DNA intermediate. We have named this new transposon *Jordan* in recognition of its exceptional ability to jump.

Jordan has one structural feature that distinguishes it from other transposons of its class. The first 55 bp of the left end of the element are a near-perfect inverted repeat (IR) of the 55 bp extending from position 198 to 144, and four of the 12-bp direct repeats are located between this pair of long IRs; the fifth direct repeat is part of the internal IR (Figure 4). No

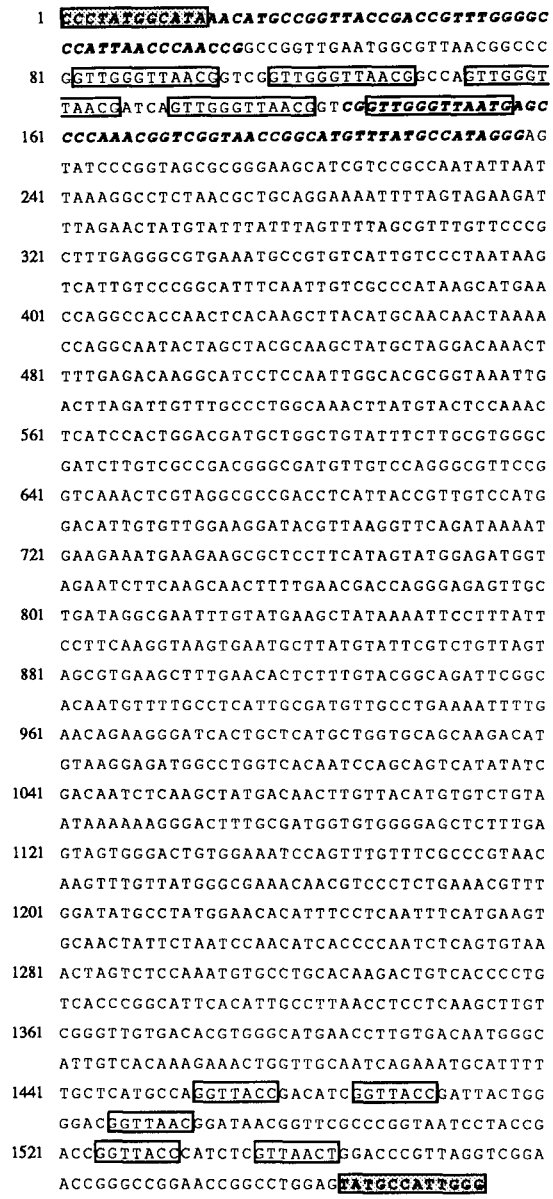

Figure 4. Complete Nucleotide Sequence of the 1595-bp Transposon *Jordan*.

Highlighted for emphasis are the 12-bp imperfect TIRs (stippled boxes), the 12- and 7-bp subterminal repeats (open boxes), and the 55-bp internal IRs (bold italics). The GenBank accession number is L20411.

such extended pair of inverted repeats is found near the right terminus nor anywhere else in the element.

As noted above, *Jordan* caused a 3-bp target site duplication when it inserted into the *nitA* gene. To determine whether revertants of this transposon-induced mutation contained a *nitA* gene that was restored to the wild-type sequence, retained the 3-bp duplication, or sustained some other nondetrimental

sequence alteration, we isolated empty donor sites from 11 revertants. These sites were amplified from genomic DNA by PCR, cloned, and sequenced. In nine of the 11 revertants, an extra hexanucleotide, 5′-CCTAGG-3′, remained at the insertion site following excision of the transposon; in one revertant, the nonanucleotide 5′-CCTCCTAGG-3′ remained; and in the final revertant, the 15-bp sequence 5′-CCTAGCTCTCCTAGG-3′ was left at the site of excision, as shown in Table 2. Note that in each of these cases the right end of the footprint left behind constitutes the trinucleotide target site duplication that was produced during insertion, and in most cases the additional trinucleotides represent inverted repeats of this duplication. In none of the revertants examined was any remnant of *Jordan* sequence left behind.

DNA Gel Blot Analysis with *Jordan*

To determine the copy number and distribution of sequences homologous to *Jordan* in the Volvox genome, we used a cloned fragment of the transposon as a probe in DNA gel blot analysis of genomic DNAs from different wild-type Volvox strains. Our culture collection includes strains derived from three geographically distinct pairs of isolates of *V. c.* f *nagariensis*, two native to Japan and the third to India. Strains ADM (male) and EVE (female) were derived from strains isolated at one of the two Japanese sites, NIES male and NIES female came from the other Japanese site, and Poona male and female from the Indian site. With most probes that have been tested, RFLPs between strains derived from the same country are quite rare, while RFLPs are much more common when comparing Indian to Japanese isolates (Adams et al., 1990; C. Adams and D. Kirk, unpublished observations). DNAs isolated from each of the six wild-type strains were digested with HindIII and probed with a plasmid containing the first 422 bp of the transposon, as shown in Figure 5B. In each strain, the probe hybridized to more than 40 HindIII fragments (Figure 5A), indicating that *Jordan* is probably a member of a large family of homologous transposons in Volvox. Numerous RFLPs were detected when comparing the Poona strains to any of the Japanese strains, and a smaller number were seen when comparing the more closely related pairs of Japanese strains (see bands in NIES strains at 2.2 and 3.0 kb that are not present in ADM or EVE in Figure 5A). A few RFLPs were also detected, however, between strains derived from the same site (compare ADM and EVE at 5.2 kb in Figure 5A). These observations are consistent with our mutation and reversion analysis, which indicated that *Jordan* is mobile.

Table 2. Analysis of Empty Donor Sites following *Jordan* Excision

Strain	*nitA* Sequence at Site of *Jordan* Insertion/Excision
HK10[a]	TGGAGCTAGGAGAGCGCGGT
CRH22	TGGAGCTAGGccctat.....attgggaggAGAGCGCGGT
CRH22-R1	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R2	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R3	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R4	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R5	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R6	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R7	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R8	TGGAGCTAGGcctaggAGAGCGCGGT
CRH22-R10	TGGAGCTAGGcctcctaggAGAGCGCGGT
CRH22-R11	TGGAGCTAGGcctagctctcctaggAGAGCGCGGT
CRH22-R12	TGGAGCTAGGcctaggAGAGCGCGGT

[a] Wild type.

Isolation and Characterization of *Jordan* Homologs

We next sought to isolate and characterize some of the sequences in the Volvox genome that have homology to *Jordan* to determine whether these sequences represent transposons with structural features similar to those found in *Jordan*. In the process, we hoped to isolate an active transposon homologous to *Jordan* that would contain sequences present in much lower copy number, which might make it more suitable for use in gene-tagging experiments. Approximately 40,000 plaques from a previously constructed Volvox genomic library (Harper and Mages, 1988), made in the vector λ Charon 30, were screened with a plasmid containing the internal 466-bp HindIII restriction fragment from *Jordan*. More than 40 positive plaques were identified, and phages were purified from 10 of the most strongly hybridizing of these. Initial restriction analysis of the DNAs obtained from these phages established that four of the phages were unique. These four—λJ2, λJ3, λJ4, and λJ5—were subjected to further characterization.

To determine which sequences within the λ inserts were homologous to *Jordan* sequences, we mapped the λ clones more extensively and probed fragments of them with plasmids bearing restriction fragments containing the left-terminal 417 bp and the right-terminal 243 bp of *Jordan*. Two of the phages, λJ2 and λJ3, contained sequences hybridizing strongly with both *Jordan* termini, whereas the other two appeared to contain sequences highly homologous to only the right terminus. (One of these, λJ5, also contained restriction fragments hybridizing weakly to the left terminal restriction fragment of *Jordan*, however.)

Restriction fragments hybridizing with the *Jordan* termini were subcloned and sequenced. The sequence data revealed that each of the restriction fragments cross-hybridizing to the *Jordan* termini did indeed contain sequences identical or nearly identical to a *Jordan* TIR, as shown in Table 3. This indicated that the *Jordan* homologs that we had cloned might also be transposons, or portions of transposons, closely related to *Jordan*, and not simply repetitive elements containing some sequences common to *Jordan*. Additional DNA gel blot and sequence analysis of the elements residing on phages λJ4 and λJ5, however, revealed that they are incomplete. The left end of *Jordan-4* appears to have been lost during construction of the phage library, and the same thing might have

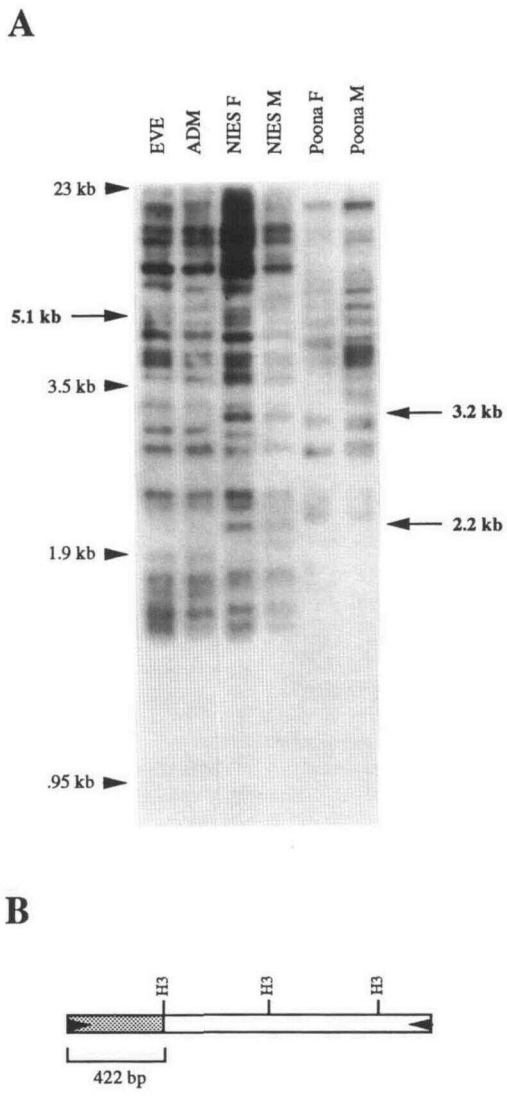

Figure 5. Copy Number and Distribution of *Jordan* Homologs in the Volvox genome.

(A) DNA gel blot comparing distinct *V. carteri* isolates.
(B) Fragment of *Jordan* used as the probe in **(A)**.
Wild-type DNAs from three pairs of geographically distinct *V. carteri* isolates were digested with HindIII and electrophoresed on a 0.8% agarose gel. The resulting DNA blot was probed with plasmid pLV11-3, which contains the left-terminal 422 bp of *Jordan* (box at left in **[B]**), along with ~900 bp of *nitA* sequence. The *nitA* fragment alone would detect only one 6.5-kb fragment on such a blot. Length markers are indicated with arrowheads, and positions of representative bands representing RFLPs are indicated with arrows. M, male; F, female; H3, HindIII.

occurred to the left end of *Jordan-5*. Restriction maps comparing regions of homology of *Jordan-4* and *Jordan-5* with *Jordan* are shown in Figure 6.

Jordan homologs *Jordan-2* and *Jordan-3*, residing on phages λJ2 and λJ3, respectively, were also subjected to DNA gel blot and sequence analyses. *Jordan-2* and *Jordan-3* are 3.6 and 1.8 kb in length, respectively, and like *Jordan-4* and *Jordan-5*, they appear to possess an extensive region of sequence similarity to *Jordan* at the right-hand end (Figure 6). *Jordan-2* and *Jordan-3* are also nearly identical to *Jordan* in sequence for >120 bp starting from their left termini, as is shown in Figure 7. However, *Jordan-2* contains nine copies of the 12-bp direct repeat 5′-GGTTGGGTTAAC-3′, whereas *Jordan* has five copies and *Jordan-3* has only three. Neither *Jordan-2* nor *Jordan-3* has a long internal IR within its left end, as does *Jordan*.

Whereas the 12-bp TIRs found in *Jordan-3*, *Jordan-4*, and *Jordan-5* match the corresponding TIRs in *Jordan* exactly, the right TIR of *Jordan-2* differs from that of *Jordan* at the most terminal base pair (Table 3). It would therefore appear that *Jordan-2* has sustained a mutation altering this last base pair, either before or during cloning. Further evidence that a mutation has affected this region of the transposon is the fact that there is no target site duplication flanking the element. *Jordan* caused a 3-bp direct repeat upon its insertion into the *nitA* gene in strain CRH22, and *Jordan-3* also appears to have caused a 3-bp direct repeat of its target sequence (Table 3), but no such repeat exists adjacent to the *Jordan-2* termini.

Copy Number and Distribution of *Jordan-2*

Jordan-2 is about 2 kb larger than *Jordan*, and to determine whether some of its sequences might be present in lower copy number in the Volvox genome than *Jordan* sequences are, we used a cloned internal restriction fragment from it to probe DNA gel blots of wild-type DNAs digested with a variety of restriction enzymes. The plasmid used to probe the DNA gel blots contained a 1.0-kb PstI restriction fragment of *Jordan-2* that lies within the 2.2-kb HindIII restriction fragment of *Jordan-2*, as indicated in Figure 8B. In each of the strains tested, the probe recognized a 2.2-kb HindIII fragment, which by far constitutes the major hybridizing band in these lanes, as shown in Figure 8A. This suggests that this portion of the transposon might be strongly conserved in all of these strains. EVE and NIES also contain larger, more weakly hybridizing HindIII fragments, which could represent related sequences.

Table 3. TIRs and Insertion Sites of *Jordan* and Cloned Homologs[a]

Element	TIRs and Insertion Sites
Jordan	agct**agg**CCCTATGGCATA.....TATGCCATTGGG**agg**agag
Jordan-2[b]	agagcttCCCTATGGCATA.....TATGCCATTGG*atggggt
Jordan-3	agggt**gt**CCCTATGGCATA.....TATGCCATTGGG**tgt**ggtg
Jordan-4	.....TATGCCATTGGGaagaccg
Jordan-5	.....TATGCCATTGGGatggatgt

[a] Underlined dots represent transposon sequences internal to the TIRs; left TIRs were not found in *Jordan-4* or *Jordan-5*.
[b] Asterisk indicates a missing base in the TIR of *Jordan-2* relative to the TIRs of the other elements.

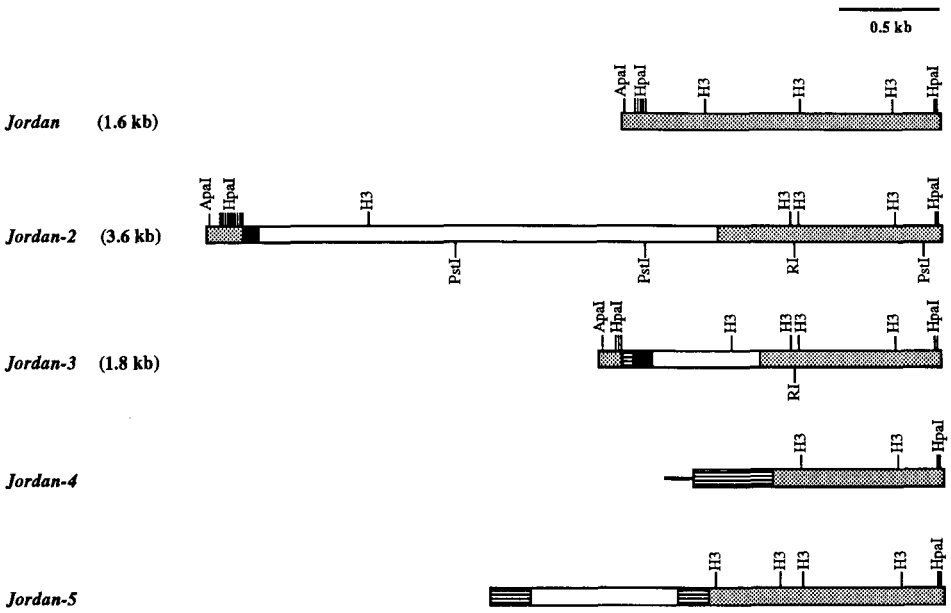

Figure 6. Regions of Homology among *Jordan* and Four Related Elements.

Four elements that cross-hybridized with *Jordan* were cloned, restriction mapped, and partially sequenced. Stippling indicates regions of near identity with *Jordan*, as determined by DNA gel blot analysis and by sequencing. The right-terminal 800 bp or more of the five elements are nearly identical. Black boxes indicate regions of sequence identity in two of the variants. Striped boxes indicate regions of "unique" sequence (not found in any of the other elements yet analyzed). White boxes indicate regions not yet characterized in detail. The left end of *Jordan-4* abuts the end of the one phage on which it was isolated (thin line), and the same may be true for *Jordan-5*. We could not find left TIRs in the *Jordan-4* or *Jordan-5* clones, even after attempting to sequence them with primers designed to hybridize to the left TIR of *Jordan*. Only the terminal ApaI site appearing in *Jordan-2* is indicated; additional sites exist but have not been mapped. H3, HindIII; RI, EcoRI.

EcoRI digests proved particularly informative, because there is only one EcoRI site within the transposon (outside the region detected by the probe). The probe hybridized to four fragments in EVE, three in ADM, five in NIES female, three in NIES male, and three in each of the Poona strains, indicating that *Jordan-2* is present in very low copy in all of these strains. In addition, the positions of the more slowly migrating bands in ADM and EVE are different (Figure 8A, EVE and ADM cleaved with EcoRI). Similarly, ADM and EVE DNAs cleaved with SalI (which also cuts only once within *Jordan-2*, and outside the region detected by the probe) also contained hybridizing fragments of different mobilities when probed with the 1.0-kb PstI fragment (Figure 8A). These results suggest that *Jordan-2* is mobile in these strains.

DISCUSSION

Jordan Is a Classical Eukaryotic Transposon Similar to Some Higher Plant Elements

Here, we report the isolation, from the multicellular green alga Volvox, of a new transposable element with features commonly found in elements that transpose via a DNA intermediate. *Jordan* was isolated from CRH22, a chlorate-resistant strain containing a revertible 1.6-kb insertion mutation at the *nitA* locus. Sequence analysis of this insertion element and of its site of insertion within the *nitA* locus revealed it to have 12-bp TIRs (5′-CCCTATGGCATA-3′) and to have caused a 3-bp target site duplication. Eukaryotic transposons that transpose via a DNA intermediate have short TIRs and cause target site duplications, and among plant transposable elements of this class, *Jordan* most closely resembles *En/Spm* and members of the so-called "CACTA" family of elements. *En* and *Spm* are functionally and structurally homologous maize transposons first identified in the early 1950s (Peterson, 1953; McClintock, 1954). They are archetypes of a family of related higher plant transposons: *Mpi1* in maize (Weydemann et al., 1987), *Tgm1* in soybean (Vodkin et al., 1983), *Pis1* in pea (Shirsat, 1988), and several of the *Tam* elements in snapdragon (Bonas et al., 1984a, 1984b; Upadhyaya et al., 1985); all members of the family cause 3-bp target site duplications and have TIRs nearly identical to the TIRs of *En/Spm*. The 13-bp TIRs of *En/Spm* have the sequence 5′-CACTACAAGAAAA-3′ (Pereira et al., 1985), which matches four of the first five bases in the *Jordan* TIR, but only two of seven bases thereafter.

The *cis* requirements for transposition of *En/Spm* are, in addition to its 13-bp TIRs, 14 copies of the 12-bp sequence 5′-CCGACATCTTA-3′, eight of which occur within ~200 bp of the left end and six within ~300 bp of the right end (Peterson and Salamini, 1986; Tacke et al., 1986; Schiefelbein et al., 1988).

These repeats are recognized by an *En/Spm*-encoded product, TNPA, and the resulting interaction is believed to bring the two ends of the transposon together, facilitating excision (Gierl et al., 1988). *Ac/Ds* of maize, also an archetype for a large family of higher plant transposons, and *Tam1* also contain subterminal repeats that are essential for transposition and that appear to interact with transposon-encoded products (Kunze and Starlinger, 1989; Nacken et al., 1991; Gierl and Saedler, 1992). Although *En/Spm* and *Tam1* are otherwise closely related, the sequences of their subterminal repeats are not (Gierl et al., 1988; Nacken et al., 1991). *Jordan* contains five copies of the 12-bp repeat sequence 5′-GTTGGGTTA-ACG-3′ within 150 bp of its left terminus and four copies of the similar sequence 5′-GGTTA/CC-3′ within 150 bp of its right terminus. The right-end subterminal repeats of *Jordan* are randomly situated, as are all the subterminal repeats in *En/Spm*, *Tam1*, and *Ac/Ds*. However, the left-end repeats of *Jordan* are positioned with striking regularity, each repeat motif in this end being spaced exactly 4 bp from the next one by the conserved sequence 5′-PuPyCPu-3′ (Figure 4).

Perhaps the most unusual structural feature of *Jordan* is the pair of 55-bp internal IRs located within the left-terminal 200 bp of the element. One of the pair begins with the left TIR, and the other begins at base pair 198 and contains the last of the five 12-bp direct repeat motifs in this region (Figure 4). *Mutator* elements of maize have long TIRs of ~200 bp, and some nonplant transposons, such as the Drosophila fold-back elements (Truett et al., 1981) and the *Tc4* transposon of *C. elegans* (Yuan et al., 1991), possess long TIRs of ~500 to 800 bp. But *Jordan* differs from these in that one of its long IRs is internal. Whether these unusual long IRs are functionally significant remains to be determined.

Jordan encodes no open reading frames longer than 300 bp and so appears to be a nonautonomous transposon that

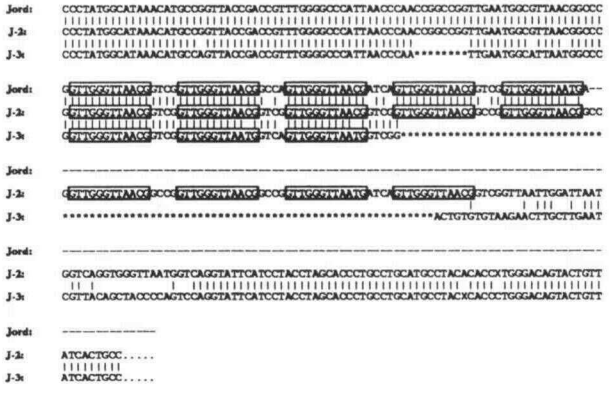

Figure 7. Comparison of the Left Ends of *Jordan*, *Jordan-2*, and *Jordan-3*.

Alignment of the first 200 to 300 bp of sequence from *Jordan* (Jord), *Jordan-2* (J-2), and *Jordan-3* (J-3) indicates that the elements are nearly identical up to the region of subterminal repeats (boxed), of which there are three in *Jordan-3*, five in *Jordan*, and nine in *Jordan-2*. Note the strikingly regular spacing of the repeats. Following the repeats, the sequences of the three elements diverge from each other, but the *Jordan-3* sequence returns to that of *Jordan-2* after an 85-bp gap and 44 divergent bases. Asterisks indicate gaps, dashes indicate divergence of *Jordan* sequence from that of the other two elements, X's indicate unreadable sequence, and vertical bars indicate identical bases. *Jordan-2* and *Jordan-3* identity probably continues (dots), but no more sequence is yet available.

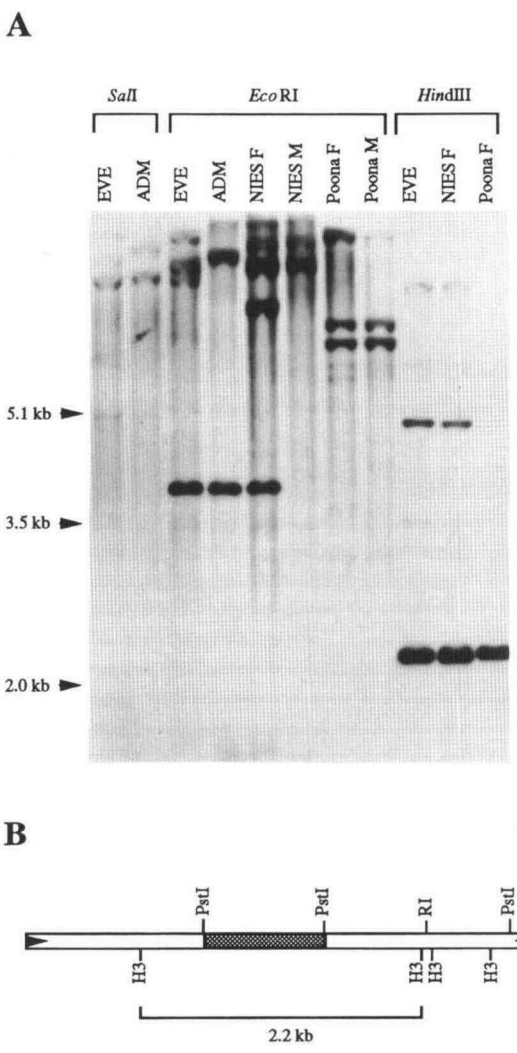

Figure 8. Copy Number and Distribution of *Jordan-2* in the Volvox Genome.

(A) DNA gel blot comparing distinct *V. carteri* isolates.
(B) Fragment of *Jordan-2* used as the probe in **(A)**.
Wild-type DNAs from the indicated strains were cleaved with SalI, EcoRI, or HindIII and electrophoresed on an 0.8% agarose gel. The resulting DNA gel blot was probed with plasmid pLV93-2, which contains the 1.0-kb PstI restriction fragment (area flanked by left-most PstI sites in **[B]**) of *Jordan-2* cloned into pBluescript KS+. H3, HindIII; RI, EcoRI; M, male; F, female.

must rely on other elements to supply it with *trans*-acting factors, such as transposase, to activate it. In this regard, it might be analogous to the *Inhibitor* and *Ds* elements of maize and the *Tam2* element of snapdragon, which are nonautonomous, truncated derivatives of *En/Spm, Ac,* and *Tam1*, respectively (McClintock, 1948; Fincham and Sastry, 1974; Pereira et al., 1985; Upadhyaya et al., 1985). As an algal transposon with properties resembling those of a family of higher plant transposons, *Jordan* joins the Chlamydomonas element *Gulliver*, which has features in common with the *Ac/Ds* family (Ferris, 1989).

Footprints Found in *nitA*$^+$ Revertants of CRH22 Are Similar to Those Generated by Higher Plant Transposons

On rare occasions, the original sequence of the insertion site invaded by a plant transposon is restored following its excision, but usually the target site duplication created during integration, or some alteration of it, is left behind when the transposon departs (Sachs et al., 1983; Bonas et al., 1984b; Schwarz-Sommer et al., 1985); these modifications of the original insertion site are sometimes called footprints. Analysis of footprints has inspired several transposition models in plants, including one by Saedler and Nevers (1985) that invokes participation of the cell's DNA repair system in excision to explain the alterations of target site duplications that are commonly observed. One such class of alterations consists of the addition of extra nucleotides that represent partial inversions of one or both copies of the target site duplication, accounted for in the model by template switching at one or both termini of the element during excision. The model predicts that in the extreme cases excision should generate one or two inverted copies of the entire duplication sandwiched between the duplicated bases.

We found that all 11 footprints left by *Jordan* in phenotypic revertants of CRH22 contained, between the members of the target-site duplication, extra bases that resemble inverted repeats of bases in the adjacent *nitA* genomic sequence (Table 2). Nine of the 11 footprints were identical, consisting of the sequence 5′-CCTAGG-3′, in which the duplicated bases are separated by one inverted copy of the duplication. One footprint contained two copies of the CCT inversion. These 10 footprints fall neatly into the two subclasses predicted by the model of Saedler and Nevers (1985) to occur with template switching, the first nine (CCTAGG) when switching occurs at one end of the transposon and the last (CCTCCTAGG) when switching occurs at both ends. The remaining footprint (found in CRH22-R11) is more complex; in this case, the inverse of the eight base pairs of *nitA* sequence immediately adjacent to the left end of the insertion site plus the inverse of the four base pairs immediately adjacent to the right end of the insertion site were inserted between the sets of bases duplicated during the insertion event. This footprint can also be explained by the model if it is assumed that in this case the staggered nicks that initiated excision were misplaced and occurred five bases to the left of the left set of duplicated bases and one base to the right of the right set of duplicated bases. While the footprint data presented above are entirely consistent with the model of Saedler and Nevers (1985), and as interpreted provide strong support for it, other interpretations are possible, and the data do not necessarily preclude other models.

Clearly, the footprints analyzed here may represent a biased sample. Because of the phenotypic selection that was used to recover candidates for excision analysis, our study was necessarily confined to strains in which the transposon had excised in such a way that the function of the *nitA* locus was restored. Moreover, since the *Jordan* insertion in strain CRH22 turned out to be within the coding region of *nitA*, it is not at all surprising that all of the footprints we recovered from CRH22 contained multiples of three base pairs. It is possible that a more diverse array of footprints are generated when *Jordan* undergoes excision, but that some of these footprints preclude restoration of function when the insertion site is within the coding region, because they shift the reading frame. Recovery and analysis of such footprints would require starting with a strain carrying a *Jordan* insertion in some site other than within a coding region.

Jordan Is a Member of a Family of Volvox Transposons

Gel blot analysis of DNAs from three distinct pairs of geographical isolates of *V. carteri* indicated that all of our wild-type strains contain 40 or more copies of a sequence that cross-hybridizes to a probe derived from the left end of *Jordan* (Figure 5). To determine the nature of some of these sequences, we isolated several of them from a Volvox genomic library. Two elements containing both TIRs and two partial elements (missing one TIR) were recovered. The two partial elements are missing left-end sequences, and perhaps internal regions, which may have been lost during construction of the genomic library. Based on DNA gel blot, restriction map, and sequence data, all five elements appear to be very similar over a stretch of more than 800 bp in their right-terminal regions (Figure 6). This is not surprising, considering that a 466-bp HindIII fragment derived from the right half of *Jordan* was the hybridization probe used to retrieve the other elements. All TIRs present in the four elements are identical to the *Jordan* TIRs, except for the right TIR of the largest element, *Jordan-2*, which is missing the terminal G/C base pair (Table 3). Unlike *Jordan* and *Jordan-3* (the other full-length element), *Jordan-2* is not flanked by a 3-bp duplication; therefore, it is likely that the right TIR region of *Jordan-2* has suffered some sequence alteration, either in the genome or during cloning. The otherwise-absolute identity of the TIRs of all of these elements to the *Jordan* TIRs suggests that they are members of a family of higher plant–like transposons in Volvox.

The three complete elements, *Jordan*, *Jordan-2*, and *Jordan-3*, are nearly identical over the first ~130 bp, but thereafter they differ significantly. Where *Jordan* has five 12-bp

repeats, *Jordan-2* has nine and *Jordan-3* has only three. After the last of these repeats, all three sequences diverge (Figure 7). It is noteworthy that the unusual 55-bp internal IR observed in *Jordan* is not present in either *Jordan-2* or *Jordan-3*.

Comparisons of restriction maps and available sequence data led us to believe that the internal 1.0-kb PstI restriction fragment of *Jordan-2* (Figure 6) might contain a comparatively unique sequence, relative to the more terminal sequences. This proved to be the case. When a plasmid containing the 1.0-kb PstI restriction fragment was used as a hybridization probe in DNA gel blot analysis of three independent sets of Volvox isolates, it detected only three to five fragments in each strain (Figure 8A). The fact that this probe detects RFLPs both among and within isolate pairs raises the possibility that one or more of these elements with homology to *Jordan-2* is also a mobile element. Whether any of the fragments hybridizing to the probe derived from *Jordan-2* correspond to an autonomous transposon remains to be determined by genetic studies.

Potential Use of *Jordan* to Tag and Clone Developmentally Important Genes in Volvox

Mutational analysis has led to the working hypothesis that a relatively small number of genes, most notably the *gls*, *regA*, and *lag* (late gonidia) loci, are responsible for programming germ versus somatic differentiation in *V. carteri* (Kirk, 1988). Many additional, independent strains with mutations at one of these loci—which arose either spontaneously or following heat shock—have recently been isolated. The possibility that some of these strains may carry *Jordan* insertions at one of the loci of major developmental interest is now being explored.

As a gene-tagging tool, *Jordan* has three advantages: it is highly mobile, it can cause revertible mutations, and it appears that its rate of transposition and excision can be increased by heat shock. However, the many cross-hybridizing sequences in the genome (Figure 5A) make it difficult to recognize novel *Jordan* insertion events and could greatly complicate retrieval of flanking sequences. We are exploring three ways to alleviate this problem. First, we have identified a 164-bp HpaI restriction fragment at the left end of *Jordan* that hybridizes to only about half of the sequences that hybridize to the 422-bp fragment used in Figure 5 (data not shown). Second, *Jordan-2* appears to be mobile, and a probe derived from it hybridizes to only a few sequences in the Volvox genome (Figure 8); novel insertions of *Jordan-2* should be far easier to identify and recover than those of *Jordan* itself. Third, because *Jordan* is apparently nonautonomous and, therefore, must be activated in *trans*, it should be possible to construct derivatives of *Jordan* that contain unique internal sequences. This third strategy has been made possible with the recent development of a transformation system for Volvox (D. Kirk, B. Schiedlmeier, W. Müller, M. Kirk, W. Mages, and R. Schmitt, manuscript in preparation). We have constructed and are currently testing a plasmid-rescue transposon that should greatly simplify the cloning of flanking sequences.

METHODS

Volvox Strains and Cultivation Conditions

All Volvox strains used in this study were derived from one of three sets of geographically and temporally distinct isolates of *Volvox carteri* f *nagariensis*. HK9 (male) and HK10 (female) were obtained from a rice paddy in Japan in 1967 (Starr, 1969). The NIES strains (male and female) were isolated from a different location in Japan and much more recently, and the Poona male and female strains were isolated from a pond in India. HK10, 69-1b (an F_1 male derived from an HK9 × HK10 cross), and the Poona strains were provided by the University of Texas Culture Collection of Algae (UTEX, Austin, TX), and the NIES male and female strains were obtained from the National Institute for Environmental Studies in Japan (Ibaraki). Strains ADM and EVE were derived in our laboratory as randomly selected clones of 69-1b and HK10, respectively (Harper et al., 1987). In addition, an HK10 strain that had originally been obtained from UTEX, but that had been in cultivation in Germany for many years, was obtained from R. Schmitt at the University of Regensburg and was used in some of these studies. Where not otherwise specified, Volvox cultures were maintained in standard Volvox medium (SVM) under standardized culture conditions (Kirk and Kirk, 1983), except that a 16-hr-light/8-hr-dark cycle was used to synchronize growth.

Isolation of Chlorate-Resistant Mutants and Revertants

Chlorate-resistant (CR) mutants were selected by plating cleaving embryos in a 0.375% agarose (Seaplaque, FMC Bioproducts, Rockland, ME) overlay, containing SVM supplemented to 8 mM with $KClO_3$ (CSVM) on CSVM agar (1% Bactoagar, Difco) plates. In a typical experiment, ~100,000 embryos were collected and distributed among 10 plates. When the effect of heat shock on mutation rate was to be tested, isolated embryos were inoculated into 300 mL of SVM in a 500-mL bubbler flask, incubated for 40 min at 42.5°C followed by 20 min at 45°C (Kirk and Kirk, 1986), and then allowed to recover at 32°C for 2 days before plating. Control samples of embryos in these experiments were treated similarly, but without heat shock. For the first 30 CR mutants isolated, several spheroids from each colony were inoculated into 2 to 3 mL of NSVM (SVM lacking the usual urea and containing nitrate as the only nitrogen source) to test for the ability to utilize nitrate.

Restoration of the ability to grow on nitrate as a sole nitrogen source was used as the basis for selecting revertants from CR mutant strains. When the objective was merely to determine whether a CR strain was capable of reversion, embryos derived from cultures that had been maintained on nonselective medium were used. However, when the objective was to measure reversion rate, the strain to be analyzed was first grown for at least six asexual generations in SVM supplemented with 2 mM $KClO_3$ to eliminate any preexisting revertants; then cleaving embryos were isolated and plated on agar-agarose plates containing NSVM. (We found that it was necessary to prewash the agar that was to be used as the base layer in such plates exhaustively with water for several days in order to extract reduced nitrogen contaminants that would otherwise have interfered with the selection.) In both selection protocols, the number of embryos plated was determined by counting the number of embryos, or juveniles derived from them, present on a representative sample of plates in each category 1 to 2 days after plating.

DNA Isolation and Analysis

Volvox genomic DNA was prepared either as described by Harper et al. (1987) or by a "miniprep" method (adapted from Rogers and Bendich, 1988), which is faster and easier than the standard method but which yields less DNA. For the miniprep method, spheroids that had grown to stationary phase in a 500-mL bubbler flask were collected by filtration and transferred to a 15-mL Corex tube containing 0.6 mL of resuspension buffer (0.05 M Tris-HCl, pH 8; 0.01 M EDTA, pH 8; 1.7% sarcosyl). Approximately 1.5 mL of glass beads (425 to 600 μm; Sigma) was added, and the sample was vortexed five times at high speed for 1 min, with 1-min incubations on ice between pulses. The tube was then frozen and thawed at 37°C five times. The resulting slurry was decanted from the glass beads and transferred by Pasteur pipette to 1.5-mL Eppendorf tubes in aliquots no larger than 500 μL. Cell debris and any contaminating beads were pelleted by centrifuging 10 min in a microcentrifuge, and the supernatant was then transferred to a clean Eppendorf tube with a Pasteur pipette. An equal volume of 2 × CTAB buffer (2% hexadecyltrimethylammonium bromide [CTAB], 100 mM Tris-HCl, pH 8; 20 mM EDTA, pH 8; 1.4 M NaCl, 1% polyvinylpyrrolidone), prewarmed to 65°C, was added and the sample mixed; the tube was then filled with $CHCl_3$, mixed again, and spun 5 min in a microcentrifuge. The aqueous layer was transferred to a clean Eppendorf tube, one-tenth volume 10% CTAB buffer (10% CTAB, 0.7 M NaCl) was added, the tube was mixed, and the sample was extracted with $CHCl_3$ as before. Once again the aqueous layer was transferred to a new Eppendorf tube, and nucleic acids were precipitated by adding an equal volume of CTAB precipitation buffer (1% CTAB, 50 mM Tris-HCl, pH 8; 10 mM EDTA, pH 8), mixing, and allowing to stand at room temperature for 30 min. Nucleic acids were pelleted by spinning the tube for 10 min in a microcentrifuge, the supernatant was discarded, and the pellet was resuspended in high-salt TE (10 mM Tris-HCl, pH 8; 1 mM EDTA, pH 8; 1 M NaCl). Nucleic acids were then precipitated with ethanol, dissolved and precipitated again, washed with 70% ethanol, dried, and dissolved in 100 to 200 μL of TE (10 mM Tris-HCl, pH 8; 1 mM EDTA, pH 8).

For DNA gel blot analysis, ~25 μL of the above-mentioned preparations was digested with the appropriate restriction endonuclease in the presence of 20 μg/mL RNase A before gel electrophoresis; electrophoresis, transfer to membranes, and hybridization to labeled probes were as previously described (Harper et al., 1987). Probes were labeled with ^{32}P-dCTP as previously described (Harper et al., 1987) or with digoxygenin-conjugated dUTP (DIG), using the Boehringer Mannheim random-hexamer labeling kit according to the directions of the manufacturer. Chemiluminescent detection of DIG-labeled probes bound to membranes was also performed according to the directions of the manufacturer (Boehringer Mannheim), except that following incubation with the conjugated antibody, the filter was washed four times with buffer A (0.1 M Tris-HCl, pH 7.5; 0.15 M NaCl) instead of two times.

Plasmid Preparations, DNA Sequencing, and Polymerase Chain Reaction

The alkaline lysis method (Maniatis et al., 1982) was used for large-scale bacterial plasmid preparations. Small-scale preparations of bacterial plasmids were prepared by the boiling method (Holmes and Quigley, 1981), and plasmid DNA was prepared for sequencing with a Magic Miniprep kit (Promega). Dideoxynucleotide DNA sequencing was performed by the method of Sanger et al. (1977), using α-^{35}S-dATP (Du Pont–New England Nuclear) and a Sequenase kit (U.S. Biochemical Corp.).

The portion of the nitrate reductase (*nitA*) gene of strain CRH22 containing the *Jordan* insertion was amplified by polymerase chain reaction (PCR) using the primers UpSal2 NR (5′-CTTAAGCTTGCCGTACACCATGCGCGGATACGCGTACGC-3′) and DnSal2 NR (5′-CTTAAGCTTGACCTTCTGGCGAGGGTTGAGGACC-3′), which hybridize just upstream and downstream, respectively, of the 2.1-kb SalI restriction fragment of the *nitA* gene (Gruber et al., 1992). The parameters of the thermocycle program were as follows: 5 min at 94°C (once); 1.5 min at 94°C, 2 min at 47°C, 4 min at 72°C (twice); 1.5 min at 94°C, 2 min at 62°C, 3 min at 72°C (28 times). Amplifications were performed in 100-μL reactions as described by Saiki (1990), except that the final concentration of $MgCl_2$ in the reactions was 0.75 mM. Amplified PCR products were cleaved with SalI and cloned into the SalI site of plasmid pBluescript KS+ (Stratagene).

Screening of a Volvox Genomic Library

A previously constructed λ Charon 30 clone bank of genomic Volvox sequences (Harper and Mages, 1988) was screened according to the protocol of Maniatis et al. (1982) with a ^{32}P-labeled probe derived from *Jordan* sequences. *Escherichia coli* strain NM538 was used as the host for all λ infections, and phages were purified by standard methods.

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