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5. In situ hybridization histochemistry was performed with procedures previously employed for localization and measurement of neuropeptide mRNA's [W. S. Young III, E. Mezey, R. E. Siegel, *Neurosci. Lett.* **70**, 198 (1986); W. S. Young III, T. I. Bonner, M. R. Brann, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9827 (1986)]. We modified the published procedures by increasing the hybridization temperature from 22°C to 37°C and by quantitating autoradiograms from x-ray film under bright-field illumination with the same image analysis system as that used to quantitate immunoreactivity. Background values were determined by comparison of inner layers of the retina to sections incubated with an equivalent concentration of tyrosine hydroxylase cDNA probe. This probe was prepared in the same way as the T_α cDNA probe and was complementary to bases 1441 to 1488 of rat tyrosine hydroxylase [B. Grima *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 617 (1985)]. These values were equivalent and always less than 10% of those obtained with specific labeling. On each section, the optical density over three randomly selected domains, selected without knowledge of section grouping, were averaged to reduce sampling error. Standards were prepared by mixing known amounts of [^{35}S]deoxyadenosine in brain paste. These standards were subsequently sectioned and exposed to film with the retinal sections. Optical densities were converted to copies per cubic micrometer with nonlinear regression of the data from the standards (*7*). Due to the moderate resolution of our autoradiographic technique and the rarity of cones in the rat retina, we were not able to determine if cones were labeled in addition to rods. Recent work [C. L. Lerea *et al.*, *Science* **234**, 77 (1986)] indicates the sequence of our cDNA probe is restricted to rods in the bovine retina, and that cones have a different transducin-like mRNA.
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Site-Specific Nick in the T-DNA Border Sequence as a Result of *Agrobacterium vir* Gene Expression

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The T-DNA transfer process of *Agrobacterium tumefaciens* is activated by the induction of the expression of the Ti plasmid virulence *(vir)* loci by plant signal molecules such as acetosyringone. The *vir* gene products act in trans to mobilize the T-DNA element from the bacterial Ti plasmid. The T-DNA is bounded by 25–base pair direct repeat sequences, which are the only sequences on the element essential for transfer. Thus, specific reactions must occur at the border sites to generate a transferable T-DNA copy. The T-DNA border sequences were shown in this study to be specifically nicked after *vir* gene activation. Border nicks were detected on the bottom strand just after the third or fourth base (± one or two nucleotides) of the 25–base pair transfer-promoting sequence. Naturally occurring and base-substituted derivatives of the 25–base pair sequences are effective substrates for acetosyringone-induced border cleavage, whereas derivatives carrying only the first 15 or last 19 base pairs of the 25–base pair sequence are not. Site-specific border cleavages occur within 12 hours after acetosyringone induction and probably represent an early step in the T-DNA transfer process.

Agrobacterium tumefaciens HARBORing a Ti (tumor-inducing) plasmid induces tumors in plants by transferring and integrating a specific DNA segment, T-DNA, into the plant nuclear genomes [reviewed in (*1*)]. The T-DNA transfer process is mediated by products of the Ti plasmid virulence *(vir)* (*2*) and chromosome virulence *(chv)* (*3*) loci. While *chv* expression is constitutive, *vir* expression is tightly regulated (*2, 4*). Activation of the *vir* genes is the direct result of the recognition by *Agrobacterium* of signal molecules produced by wounded plant cells (*5*), and this activation initiates the transfer process.

The T-DNA is bounded by imperfect 25–base pair (bp) direct repeats (*1*). While deletion of the left border repeat has no significant effect on oncogenicity (*6*), deletion of the right repeat totally abolishes it (*7–9*). When the orientation of the right border is reversed with regard to its natural orientation, the efficiency of transfer or integration (or both) of the T-DNA is greatly attenuated (*7, 9*). Since only the T-DNA element is found integrated in the plant nuclear genome, specific reactions are expected to occur at the T-DNA borders (especially at the right border) to generate a transferable T-DNA copy. Recent experiments have shown that *vir* induction by acetosyringone (AS), a purified plant signal molecule, results in the production of S1 nuclease–sensitive structures at the T-DNA borders (*10*). The data described below indicate that this S1 sensitivity is due to AS-induced specific single-stranded cleavage within the 25-bp repeat sequence.

Various constructs (Fig. 1A), including a 3.3-kbp Hind III fragment overlapping the right T-DNA border of the nopaline-type Ti plasmid as well as several derivatives of the transfer-promoting 25-bp border sequence (*7*), were tested for their sensitivity to S1 nuclease after AS induction. Border nicks began to occur within 4 hours and reached a maximal level by 12 hours, after the addition of AS to bacterial cultures grown in Murashige and Skoog medium, with sucrose and phosphate (MSSP) (*10*). Thus, the different *Agrobacterium* strains were induced with AS for 12 hours, and total DNA was prepared

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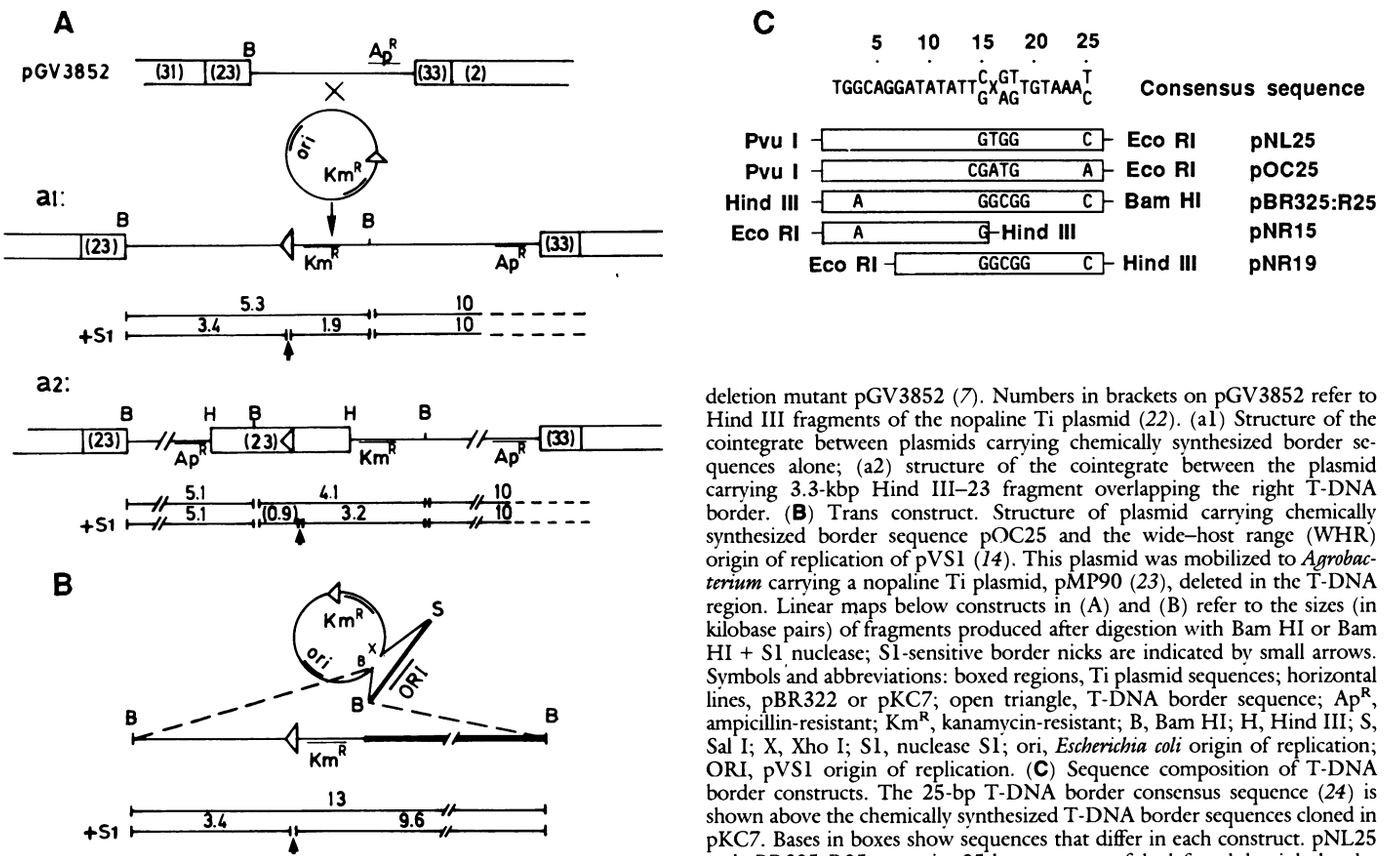

Fig. 1. Constructs used to detect acetosyringone-induced nicking. (**A**) Cis constructs. The T-DNA border sequences cloned in pKC7 (*20*) were mobilized (*21*) to *Agrobacterium* and cointegrated into the right border deletion mutant pGV3852 (*7*). Numbers in brackets on pGV3852 refer to Hind III fragments of the nopaline Ti plasmid (*22*). (a1) Structure of the cointegrate between plasmids carrying chemically synthesized border sequences alone; (a2) structure of the cointegrate between the plasmid carrying 3.3-kbp Hind III–23 fragment overlapping the right T-DNA border. (**B**) Trans construct. Structure of plasmid carrying chemically synthesized border sequence pOC25 and the wide–host range (WHR) origin of replication of pVS1 (*14*). This plasmid was mobilized to *Agrobacterium* carrying a nopaline Ti plasmid, pMP90 (*23*), deleted in the T-DNA region. Linear maps below constructs in (A) and (B) refer to the sizes (in kilobase pairs) of fragments produced after digestion with Bam HI or Bam HI + S1 nuclease; S1-sensitive border nicks are indicated by small arrows. Symbols and abbreviations: boxed regions, Ti plasmid sequences; horizontal lines, pBR322 or pKC7; open triangle, T-DNA border sequence; Ap^R, ampicillin-resistant; Km^R, kanamycin-resistant; B, Bam HI; H, Hind III; S, Sal I; X, Xho I; S1, nuclease S1; ori, *Escherichia coli* origin of replication; ORI, pVS1 origin of replication. (**C**) Sequence composition of T-DNA border constructs. The 25-bp T-DNA border consensus sequence (*24*) is shown above the chemically synthesized T-DNA border sequences cloned in pKC7. Bases in boxes show sequences that differ in each construct. pNL25 and pBR325:R25 are native 25-bp sequences of the left and the right border of nopaline pTiC58; pOC25 is the base-substituted (G17 → T17) 25-bp sequence of the left border of octopine TR-DNA from pTiA6; pNR15 and pNR19 are deletion derivatives of the right border of nopaline pTiC58.

and digested with restriction enzymes and S1; after electrophoresis the fragments overlapping the T-DNA border regions were detected by hybridization (Fig. 2A). After S1 treatment, novel fragments were detected with all the constructs containing the 25-bp border sequence (Fig. 2A, lanes b, e, and g), and the molecular weights of these fragments corresponded to those predicted to arise if cleavage occurs at the 25-bp sequences (Fig. 1). Without AS induction no border cleavage fragments were detected (Fig. 2A, lane c; control). Thus, the 25-bp sequence is sufficient to provide an effective substrate for the AS-induced nicking reaction.

A single base change in the variable region of a naturally occurring 25-bp sequence (pOC25, G to T change at position 17 shown in Fig. 1C) does not significantly alter its efficiency in the border cleavage reaction (Fig. 2A, lane g; compare to pNL25 in lane e, the native nopaline left 25-bp segment). However, deleted derivatives carrying only the first 15 (pNR15) or last 19 (pNR19) base pairs of the 25-bp sequence are not effective substrates for the border cleavage reaction (lanes h and i). The ability of the different constructs to serve as substrates for the border cleavage reaction correlates with their ability to form tumors when they are inoculated onto tobacco plantlets—that is, all strains are active in tumor formation except those carrying pNR15 or pNR19 (*11*).

Comparison of the relative intensities of the signals representing the S1 cleavage products to the signal representing their uncleaved restriction fragment reveals the efficiency of different constructs in the border nicking reaction. The efficiency of the 25-bp sequence alone (Fig. 2A, lanes e or g; compare the uncleaved 5.3-kbp band with the cleaved 3.4-kbp and 1.9-kbp bands) is approximately the same as when it is contained in the complete right border fragment (Fig. 2A, lane b; compare the uncleaved 4.1-kbp band to the cleaved 3.2-kbp band). Recently, a sequence designated "overdrive," just rightward (within 60 bp) of several 25-bp repeats has been reported to greatly stimulate transfer of adjacent T-DNA's to plant cells (*12*). Our data suggest

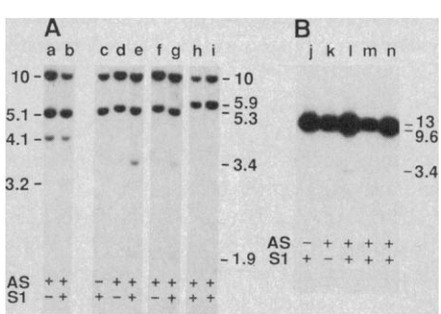

Fig. 2. S1 endonuclease sensitivity of T-DNA border sequences after *vir* induction with AS. Numbers refer to fragment sizes in kilobase pairs. AS (+/−) indicates that *Agrobacteria* were induced or not induced by AS; S1 (+/−) indicates that total DNA was digested with or without S1 nuclease. (**A**) Cis T-DNA constructions: (lanes a and b) pGV3852::pH23-1; (lanes c, d, and e) pGV3852::pNL25; (lanes f and g) pGV3852::pOC25; (lane h) pGV3852::pNR15; and (lane i) pGV3852::pNR19. The low level of AS-induced border cleavage fragments detected without S1 treatment (lanes d and f) likely reflects mechanical breakage during sample preparation. Note that the Bam HI fragment for pGV3852::N15 (and NR19) (lanes h and i) is 5.9 kbp, since these constructs are not deleted between the Pvu I and Eco RI sites of pKC7 (*20*). (**B**) Trans T-DNA constructions: (lanes j, k, and l) pOC25-WHR/pMP90; (lane m) control pKC7/pMP90 (no insertion of border sequence in pKC7); (lane n) pOC25-WHR in C58C1 (no Ti plasmid). For experimental procedures, see (*25–27*).

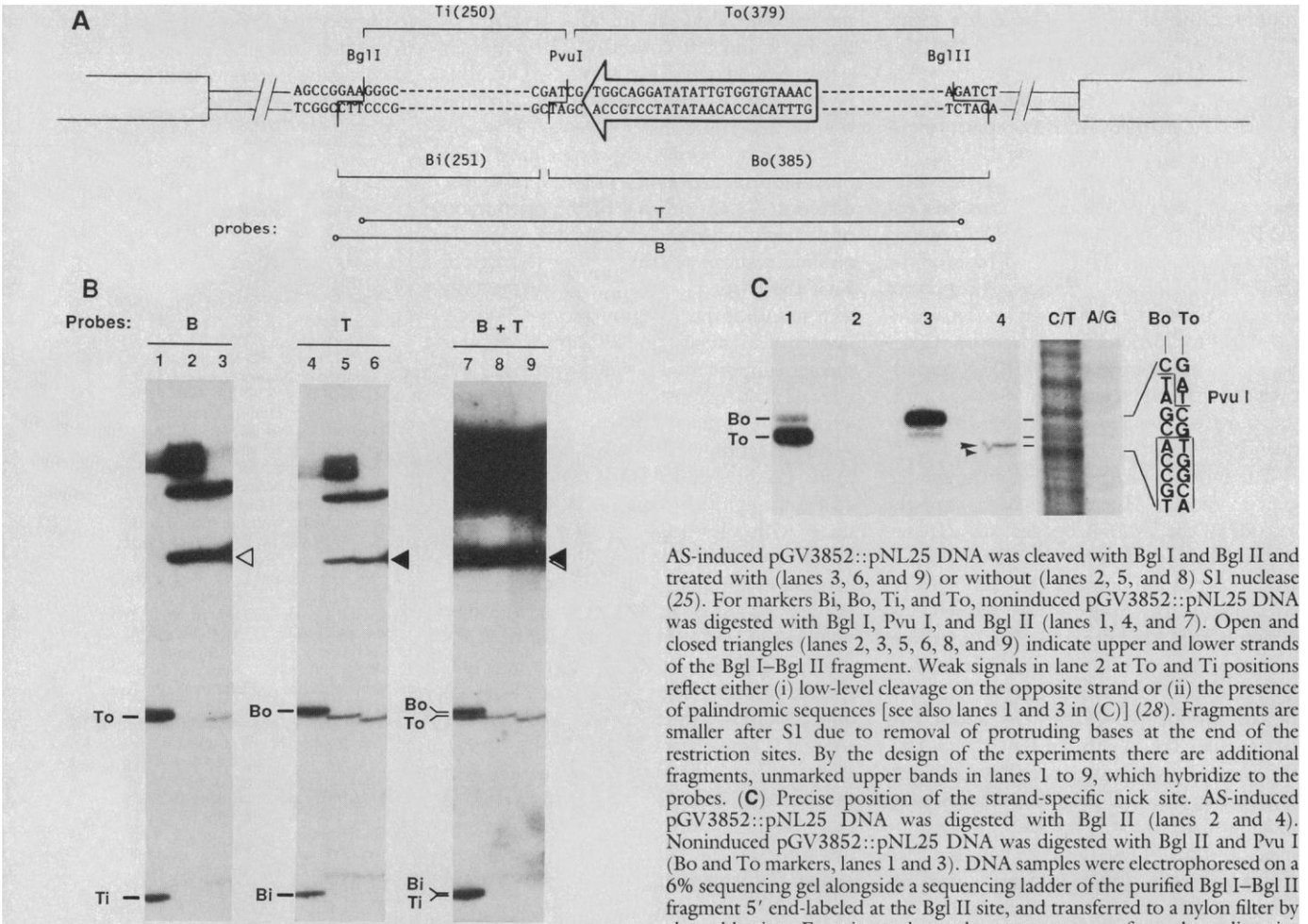

Fig. 3. Determination of AS-induced cleavage site within the 25-bp T-DNA border sequence. (**A**) Nopaline left 25-bp border sequence (arrow) and flanking restriction sites in pGV3852::pNL25. Ti, To, Bi, and Bo indicate top (T) or bottom (B) strands inside (i) or outside (o) the 25-bp sequence after restriction with Bgl I, Pvu I, and Bgl II; numbers in brackets indicate the lengths of each strand in nucleotides. T, top strand probe; B, bottom strand probe. (**B**) Detection and localization of the strand-specific nick site. AS-induced pGV3852::pNL25 DNA was cleaved with Bgl I and Bgl II and treated with (lanes 3, 6, and 9) or without (lanes 2, 5, and 8) S1 nuclease (*25*). For markers Bi, Bo, Ti, and To, noninduced pGV3852::pNL25 DNA was digested with Bgl I, Pvu I, and Bgl II (lanes 1, 4, and 7). Open and closed triangles (lanes 2, 3, 5, 6, 8, and 9) indicate upper and lower strands of the Bgl I–Bgl II fragment. Weak signals in lane 2 at To and Ti positions reflect either (i) low-level cleavage on the opposite strand or (ii) the presence of palindromic sequences [see also lanes 1 and 3 in (C)] (*28*). Fragments are smaller after S1 due to removal of protruding bases at the end of the restriction sites. By the design of the experiments there are additional fragments, unmarked upper bands in lanes 1 to 9, which hybridize to the probes. (**C**) Precise position of the strand-specific nick site. AS-induced pGV3852::pNL25 DNA was digested with Bgl II (lanes 2 and 4). Noninduced pGV3852::pNL25 DNA was digested with Bgl II and Pvu I (Bo and To markers, lanes 1 and 3). DNA samples were electrophoresed on a 6% sequencing gel alongside a sequencing ladder of the purified Bgl I–Bgl II fragment 5′ end-labeled at the Bgl II site, and transferred to a nylon filter by electroblotting. Experimental samples were cut away from the radioactive sequencing ladder before hybridization with Bo probe (lanes 1 and 2) or To (lanes 3 and 4). The nucleotide sequence of the Pvu I site used to generate the markers Bo and To was used to align the two parts of the filter after autoradiography. The major and possibly secondary nick products (lane 4) are indicated by the long and short arrowheads, respectively. The corresponding sequence is shown at the right. For experimental procedures, see (*29, 30*).

that overdrive is not essential for border nicking; however, recent results indicate that this sequence can act at a long distance (*13*). Possibly the border nicking seen here might require an overdrive sequence that is located elsewhere on the cointegrate Ti plasmids.

The 25-bp sequence also is nicked after AS induction when it is carried on a small replicon in trans to the Ti plasmid (Figs. 1B and 2B), and this nicking is dependent on the presence of an intact *vir* region (Fig. 2B, lane l versus lane n). This trans plasmid is identical to that used to measure border nicking in cis in Fig. 2A, lane g, except for the origin of replication of pVS1 (*14*). However, the efficiency of the border cleavage reaction is reduced approximately fivefold in the trans construct [compare the uncleaved and cleaved bands in lane g of Fig. 2A (5.3 kbp, 3.4 kbp, and 1.9 kbp) with those in lane l of Fig. 2B (13 kbp, 9.6 kbp, and 3.4 kbp)]. This result may be a consequence of the higher copy number of this trans plasmid (six or seven copies per *Agrobacterium*) (*14*) if the AS-induced molecular reactions of the T-DNA are regulated to occur to a limited extent (*10*)—that is, the number of events per AS-induced cell does not increase with larger numbers of substrate molecules.

The experiments shown in Fig. 3 precisely localize the S1-sensitive border nick within the 25-bp border sequence of pNL25 (identical results are obtained with plasmids carrying pOC25 or pBR325:R25). DNA isolated from AS-induced bacteria was cleaved with Bgl I and Bgl II, and half of each sample was further digested with S1 nuclease. The samples were electrophoresed in triplicate on a denaturing polyacrylamide gel and hybridized with probes homologous to the bottom, the top, or both bottom and top strands of the Bgl I–Bgl II fragment overlapping the 25-bp border sequence (Fig. 3B).

The border nick is confined to the bottom strand of the 25-bp border sequence since the nick cleavage products are detected with the top strand probe in the absence of S1 treatment (Fig. 3B; compare lanes 2 and 5). This result is also reflected in the intensities of the uncleaved Bgl I–Bgl II fragment; the top strand (open triangle, lanes 2 and 3) gives a stronger hybridization signal than the bottom strand (closed triangle, lanes 5 and 6). After S1 treatment, cleavage products are detected with either top or bottom strand probes (lanes 3 and 6).

The size of the fragments representing the bottom-strand nick—that is, the fragments detected without S1 treatment by means of the top-strand probe (lane 5)—can be used to estimate the position of the nicked site within the 25-bp sequence. Lanes 1, 4, and 7 in Fig. 3B represent molecular weight markers for the fragments immediately sur-

rounding the 25-bp sequence either inside (Ti and Bi) or outside (To and Bo) this sequence (Fig. 3A). One of the two AS-induced cleavage products migrates just below the To marker band (compare lane 4 with lane 5 and lane 7 with lane 8). Since the AS-induced band corresponds to the bottom strand, and there are four extra bases at the Bgl II site on this strand, the AS-induced cleavage occurs after the third base of the 25-bp sequence. The smaller AS-induced cleavage product migrates at a size corresponding to 258 or 259 nucleotides. Comparison with the Bi marker (251 nucleotides) places the AS-induced cleavage after the third or fourth base of the 25-bp sequence.

The product of AS-induced nicking adjacent to the Bgl II site (that is, toward the outside of the T-DNA border) was electrophoresed alongside a nucleotide sequencing ladder corresponding to the same region overlapping the 25-bp border sequence to position the AS-induced border nick just after the third or fourth base of the bottom strand of the 25-bp sequence (Fig. 3C). The nicked product is smaller (by about two bases) than To on this gel (lanes 3 and 4), confirming a result suggested by its migration in Fig. 3B. The product of AS-induced nicking adjacent to the Bgl I site (toward the inside of the T-DNA border) has also been electrophoresed alongside a nucleotide sequencing ladder of the corresponding region to confirm the nick position.

Because of the experimental design, the assignment of the nick site is accurate only within one base (or possibly two bases); nevertheless, the data suggest that AS induction most likely leads to the generation of a nick, rather than a gap, in the 25-bp border sequence. Interestingly, there is a faint signal below the major border cleavage product in lane 4 (Fig. 3C). This result suggests that the border-nicking enzyme activity may sometimes cleave the 25-bp border sequence at one or more secondary sites a few bases downstream from the major nick site. Multiple nick sites have also been observed at the origin of transfer (oriT) site which is used during F plasmid conjugal DNA transfer (*15*).

The detection of the AS-induced border nick within the 25-bp sequence provides important data for understanding the T-DNA transfer process. Recently, experiments have shown that AS induction leads to the generation of a free single-stranded linear T-DNA copy, the T-strand, corresponding to the bottom strand of the T-DNA region on the Ti plasmid (*10*). The T-strand has been proposed to be the molecule that is transferred and integrated into the plant cell chromosome by a conjugative mechanism. Nicks on the bottom strand of the right and left copies of 25-bp border sequences on the Ti plasmid would thus provide sites for the initiation and termination of T-strand transfer.

The 25-bp border sequences have been observed to function in a polar, right to left, direction (*7, 9*); reversal of the orientation of the right copy of this sequence on the Ti plasmid severely restricts tumor formation. That the T-strand corresponds to the bottom strand of the T-region and the T-DNA border nicks are also in the bottom strand is further support that T-strand synthesis, and hence T-DNA transfer, occurs in a right to left (5′ to 3′) direction.

AS induction also leads to the production of free double-stranded T-DNA circles (*4, 5, 16*). Although the frequency of detection of these molecules suggests they are not a major component of the T-DNA transfer process, the presence of nicks at the T-DNA borders provides an explanation for their occurrence: nicks could lead to the generation of T-DNA circles by inducing site-specific recombination within the 25-bp sequences (*10*). Furthermore, T-DNA circles contain a single copy of the 25-bp border sequence, and the nucleotide sequence of this junction 25-bp sequence (*16*) indicates that recombination must have occurred to the right of the third base of the 25-bp sequence; the present results on the position of the nick site confirm this hypothesis.

Several T-DNA–plant DNA junctions have been analyzed to date (*17*). There is no right junction beyond the third base of the 25-bp sequence; one junction ends after the first base, three junctions after the second base, and one junction after the third. On the left a larger portion of the 25-bp sequence is included in the integrated T-DNA copies; junctions occur after the 1st, 7th, 12th, 15th, or 18th base to include the last 24, 18, 13, 10, or 7 bases of the 25-bp sequence, respectively. These data on the ends of the T-DNA following integration agree with the positioning of border nicks within the 25-bp sequence. The one left border that contains 24 of the 25 bases of the border repeat may be fortuitous and reflect homology to the 25-bp sequence at the plant insertion site.

The generation of nicks at the T-DNA borders is probably the first T-DNA–associated molecular reaction in the transfer process. Recently, the border endonuclease activity has been identified to be specified by the proximal two cistrons of *vir* D (*18, 19*). That mutants of these genes do not produce single-stranded T-strand molecules (*19*) strongly suggests that the border nicks provide sites for the generation of the T-DNA transfer intermediates. It will be of interest to identify the AS-induced proteins involved in the later steps of T-strand production and to determine precisely how border nicks are used in this synthesis.

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tween the Pvu I and Bgl II sites, which may account for the weak intrastrand hybridization signals seen in lane 2 of Fig. 3B or lanes 1 and 3 of Fig. 3C.

29. The DNA samples were denatured and loaded in triplicate on a 5% sequencing gel (*30*). DNA was transferred to nylon filter by electroblotting after equilibration of the gel in electroblot buffer (10 m*M* tris, 5 m*M* sodium acetate, and 0.5 m*M* EDTA, *p*H 7.8) (Bio-Rad Trans-blot instruction manual) for 30 minutes. To prepare ^{32}P-labeled single-stranded DNA probes, pNL25 plasmid DNA was digested with Bgl II and subsequently with alkaline phosphatase. To label the 5′ end of the bottom strand (B probe), we incubated the DNA with [γ-^{32}P]dATP and T4 kinase. To label the 3′ end of the top strand (T probe), we filled in the DNA with [α-^{32}P]dATP by using the Klenow fragment of *E. coli* DNA polymerase (*27*). After the labeling period, the samples were digested with Bgl I and the fragments corresponding to T or B probes were isolated from 1.2% low-melting agarose gel. After phenolization and precipitation the specific activity of the end-labeled probes was 1×10^6 to 5×10^6 cpm/μg. The B probe was used in lanes 1, 2, and 3, and the T probe in lanes 4, 5, and 6. The entire pNL25 plasmid DNA was used to make the nick-translated probe (homologous to both B and T) for hybridization shown in lanes 7, 8, and 9. Probes were boiled before hybridization, and hybridization was carried out at 65°C for 16 hours. Filters were washed with 4× SSC (1× SSC is 0.15*M* NaCl and 0.015*M* sodium citrate) and 0.1% sodium dodecyl sulfate for 30 minutes at 60°C by changing the washing solution every 10 minutes.

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31. We thank J. Gielen, R. Villarroel, A. Lenaerts, and J. Coppieters for sequence synthesis and analysis; R. Deblaere for providing the pVS1 derivative; P. Dhaese and L. Herrera-Estrella for critical reading of the manuscript; and M. De Cock, S. Elia, S. Van Gijsegem, and K. Spruyt for preparation of this manuscript and figures. Supported by a Ph.D. fellowship of the Rockefeller Foundation (to K.W.), by grants from the "A.S.L.K.-Kankerfonds," by grant 3.001.82 from "Fonds voor Geneeskundig Wetenschappelijk Onderzoek," and by grant O.O.A. 12.0561.84 of the Services of the Prime Minister (to M.V.M. and J. Schell).

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A G1 Glycoprotein Epitope of La Crosse Virus: A Determinant of Infection of *Aedes triseriatus*

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Arthropod-borne viruses (arboviruses) have specific vector-vertebrate host cycles in nature. The molecular basis of restriction of virus replication to a very limited number of vector species is unknown, but the present study suggests that viral attachment proteins are important determinants of vector-virus interactions. The principal vector of La Crosse (LAC) virus is the mosquito *Aedes triseriatus*, and LAC virus efficiently infects the mosquito when ingested. However, a variant (V22) of LAC virus, which was selected by growing the virus in the presence of a monoclonal antibody, was markedly restricted in its ability to infect *Ae. triseriatus* when it was ingested. Only 15% of the mosquitoes that ingested V22 became infected and 5% of these developed disseminated infections. In contrast, 89% of the mosquitoes that ingested LAC became infected and 74% developed disseminated infections. When V22 was passed three times in mosquitoes by feeding, a revertant virus, V22M3, was obtained that infected 85% of *Ae. triseriatus* ingesting this virus. In addition, V22M3 regained the antigenic phenotype and fusion capability of the parent LAC virus. These results suggest that the specificity of LAC virus-vector interactions is markedly influenced by the efficiency of the fusion function of the G1 envelope glycoprotein operating at the midgut level in the arthropod vector.

ARTHROPOD-BORNE VIRUSES ARE typically restricted in their range of vector species. This vector specificity, which is a major feature of arbovirus cycles in nature, is dictated in part by the biological and behavioral attributes of the vector population. The interaction of the virus and the vector at the midgut level is another major determinant of vector specificity. When the virus has been ingested in a blood meal, it must first infect the vector's midgut epithelial cells. Progeny virus must then disseminate from midgut cells into the hemocoel, to infect the salivary glands if the virus is to be transmitted to further vertebrate hosts or to infect the ovaries if the virus is to be transovarially transmitted to further vectors (*1*). In nonpermissive vector species, arboviruses frequently are incapable of infecting the midgut cells (*1*). The molecular determinants of this midgut barrier are unknown.

One of the more attractive hypotheses to explain the midgut barrier is the presence of specific virus receptors on vector midgut cells. Virion surface proteins may act as ligands and bind to cellular receptors and may mediate early steps in infection, such as fusion of the viral envelope with cellular membranes. There is little direct evidence to suggest that such receptors function in vivo in the vector midgut; however, Western equine encephalomyelitis virus apparently penetrates mesenteronal cells by fusion (*2*).

We have used LAC virus, the causal agent of California (La Crosse) encephalitis, to investigate the midgut barrier in *Aedes triseriatus* mosquitoes. LAC virus (Bunyaviridae) is transmitted mainly by *Ae. triseriatus*, by way of the salivary glands; chipmunks and squirrels are the principal vertebrate hosts. LAC virus can also be transmitted transovarially and venereally by *Ae. triseriatus* (*3*).

The development of monoclonal antibodies to specific epitopes on the LAC virus G1 glycoprotein has made it possible to assess the role of the major envelope protein in midgut infection of *Ae. triseriatus*. These monoclonal antibodies neutralize LAC virus and inhibit hemagglutination, suggesting that the G1 protein functions as a ligand for cellular receptors (*4*). These antibodies also inhibit the fusion function of LAC virus, which is demonstrable at *p*H 6.2 or lower (*5*).

A panel of the monoclonal antibodies was used to select from the parent LAC stock virus (a mouse brain preparation, passage 6) a corresponding series of variant viruses that were monoclonal antibody–resistant (MAR). A few of the MAR variants showed reduced fusion capability and reduced virulence for mice (*5*). One such variant, V22, showed the greatest reduction in fusion efficiency and mouse neuroinvasiveness. We investigated the ability of V22 (selected with monoclonal antibody 807-22) to infect and to replicate in the La Crosse strain of *Ae. triseriatus*, which was originally collected in La Crosse, Wisconsin, in 1980.

Mosquitoes were permitted to ingest blood meals composed of equal parts of washed human red blood cells, the virus preparation, and a mixture of 50% sucrose and fetal calf serum. The virus titer in the meal preparations ranged from 6.6 to 6.8 $\log_{10}$ $TCID_{50}$ per milliliter. After 14 days of extrinsic incubation at 24°C, mosquitoes were examined by immunofluorescence for the presence of viral antigen (*6*). Heads and abdomens were severed from mosquitoes and squashed on glass slides. After fixation in acetone, tissues were stained with mouse antibodies to LAC virus conjugated with fluorescein isothiocyanate. Detection of viral antigen in the abdominal tissues indicated that the virus infected the midgut cells, while the presence of viral antigen in head

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