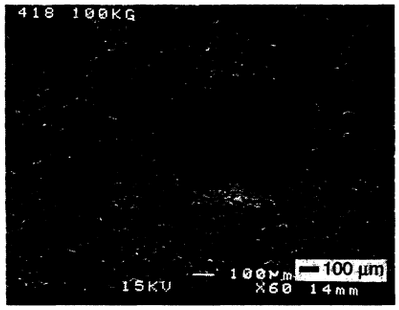

Fig. 5. Rockwell indentation tests conducted on diamond films on a TiN-coated 418-steel substrate that had been morphologically surface modified.

ing of the coating. The diamond film on WC–10% Co substrates did not exhibit any signs of visible delamination for indentation loads of up to 150 kg, thus qualitatively confirming excellent adhesion of the film.

Qualitative scratch tests were also conducted on these samples. In these tests, the coating adhesion is related to the minimum load at which stripping of the diamond film occurs. The stripping of the diamond film is attributed to film fracture, which results in propagation of the crack along the interface. In our scratch tests, no catastrophic failure of the film at the interface was observed. However, the substrate was found to deform and fracture before failure of the diamond film at the interface (Fig. 3). The film thickness was ~5 μm, so complete filling of valley features was not achieved. The tip regions of the microrough bulk materials were removed by the scratch tests. However, no catastrophic failure of the diamond film was observed. No cracks or visible marks of delamination were observed in the diamond film, attesting to the good adherence of the film.

The surface modification method also was found to increase the adhesion of diamond films on type 418-steel substrates substantially. Steel remains an elusive substrate for the deposition of adherent diamond thin-films. Besides large thermal stresses, graphitization of depositing carbon species and rapid diffusion of carbon into the steel substrates prevents growth of diamond films. To overcome some of these barriers, we adopted a three-step process. First, LIMS were created on the stainless steel surface. For steel, the samples were processed in a vacuum to reduce the shock wave–related effects that can limit the formation of microrough structures. LIMS in steel have a periodicity and roughness of ~100 μm. After the creation of the LIMS, the surface was coated with a TiN diffusion barrier that prevents graphitization and diffusion of carbon species. Finally, the diamond films were deposited on these substrates with the ECR-CVD technique at temperatures in the range of 525° to 550°C. To compare the effectiveness of the surface roughness, we also deposited diamond films on unmodified TiN-coated 418-steel substrates. The surface morphology of the diamond films deposited on laser-modified and smooth surfaces is shown in Fig. 4. Although the surface morphology of the diamond film in both samples was approximately the same, diamond films deposited on the unmodified steel showed substantial cracking and debonding.

The adhesion of the diamond film on the steel substrate was quantified by Rockwell indentation tests conducted with a brale indenter. Figure 5 shows a micrograph of indentation tests conducted on diamond samples deposited on a laser-modified surface. Partial delamination of the film was already observed in the diamond film deposited on unmodified TiN-coated steel substrates (Fig. 4A). Although the microrough steel substrate plastically deforms with the application of the load, the diamond film did not catastrophically fracture even when a load of 150 kg was applied by the indenter. The surface roughness of diamond can be minimized by controlling film nucleation. Thus, this method shows immense promise for the fabrication of adherent coatings in large thermal-expansion mismatched systems.

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16. Supported by the University of Florida, NSF, the Engineering Research Center for Particle Science and Technology at the University of Florida, NSF grant EEC 94-02989, NSF grant DMR 91-12574, and NSF grant DDM 93-14270.

8 November 1995; accepted 12 February 1996

A Role for Brassinosteroids in Light-Dependent Development of *Arabidopsis*

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Although steroid hormones are important for animal development, the physiological role of plant steroids is unknown. The *Arabidopsis DET2* gene encodes a protein that shares significant sequence identity with mammalian steroid 5α-reductases. A mutation of glutamate 204, which is absolutely required for the activity of human steroid reductase, abolishes the in vivo activity of DET2 and leads to defects in light-regulated development that can be ameliorated by application of a plant steroid, brassinolide. Thus, *DET2* may encode a reductase in the brassinolide biosynthetic pathway, and brassinosteroids may constitute a distinct class of phytohormones with an important role in light-regulated development of higher plants.

Plant growth and development are governed by complex interactions between environmental signals and internal factors. Light regulates many developmental processes throughout the plant life cycle, from seed germination to floral induction (*1*), and causes profound morphological changes in young seedlings. In the presence of light, hypocotyl growth is inhibited, cotyledons expand, leaves develop, chloroplasts differentiate, chlorophylls are produced, and many light-inducible genes are coordinately expressed. It has been suggested that plant hormones, which affect the division, elongation, and differentiation of cells, are directly involved in the response of plants to light signals (*2*, *3*). However, the interactions between photo-

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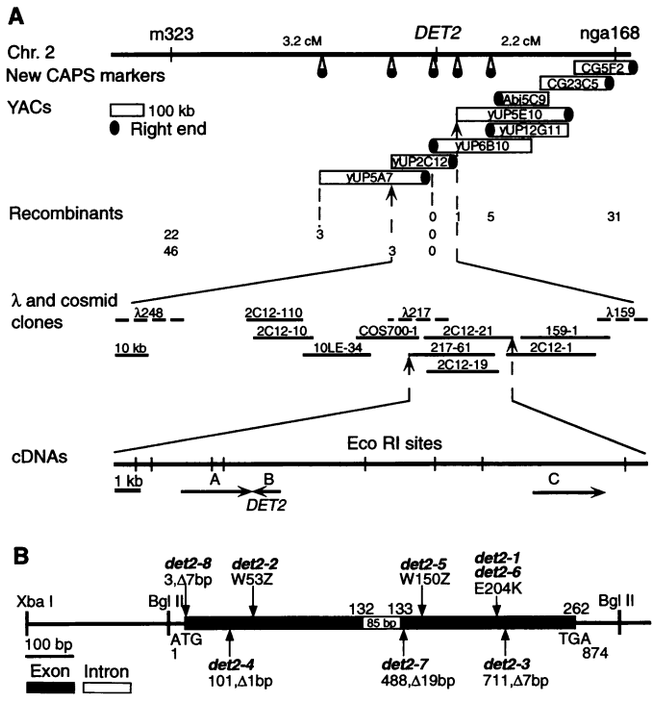

Fig. 1. Cloning and sequence analysis of the *DET2* gene. (**A**) Summary of positional cloning. Molecular marker nga168 was used as the starting point for identifying eight overlapping YAC clones covering ~800 kb of *Arabidopsis* genomic DNA (*8*). Restriction fragment length polymorphism analysis delimited the *DET2* locus to a 150-kb region between the left ends of yUP2C12 and yUP5E10 (*8*). A cosmid contig was assembled within this region, and three cosmids, 2C12-19, 2C12-21, and 217-61, rescued *det2* mutant phenotypes (*9*). Three classes of cDNA were identified from an *Arabidopsis* cDNA library with cosmid 217-61 as a probe (*10*), and their relative positions and transcriptional directions (5′ → 3′) are indicated. (**B**) Gene structure of *DET2* and mutations in the *DET2* gene. Thick lines indicate exons, and the open box denotes an intron. Positions of mutations are relative to the initiation codon. Z, stop codon.

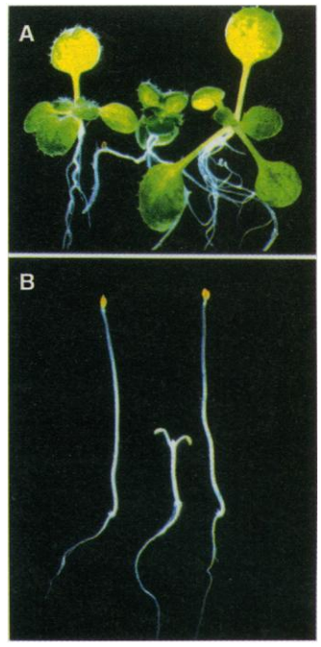

Fig. 2. Complementation of *det2* by the wild-type *DET2* gene. (**A**) Light-grown 12-day-old seedlings. (**B**) Dark-grown 10-day-old seedlings. (From left to right in each panel) Wild-type Col-0, *det2-1*, and transgenic *det2-1* containing cosmid 217-61.

transduction pathways and plant hormones are not well understood.

To study how light interacts with endogenous developmental programs in young plants, we previously isolated *Arabidopsis* mutants that have characteristics of light-grown plants even when grown in the dark. At least 11 such genetic loci, known as *det*, *cop*, and *fus*, have been identified (*4*). Double-mutant analyses with several photoreceptor mutants suggest that DET1, COP1, and COP9 act in one signal transduction pathway, whereas DET2 acts in a different pathway (*4*). *DET1*, *COP1*, and *COP9* all encode nuclear-localized proteins whose mode of action is not yet understood (*4*).

Loss-of-function mutations in *DET2* have pleiotropic effects (*5*). In the dark, *det2* mutants are short, have thick hypocotyls, accumulate anthocyanins, have open, expanded cotyledons, and develop primary leaf buds. These morphological changes are accompanied by a 10- to 20-fold derepression of several light-responsive genes. In the light, *det2* mutants are smaller and darker green than wild type, show reduced cell size (*6*) in the tissues examined (hypocotyl, cotyledons, and leaves), and have reduced apical dominance and male fertility. *det2* mutations also affect photoperiodic responses and cause a delay in flowering, a shortening of the circadian period of *CAB* (chlorophyll a/b–binding proteins) gene expression, inappropriate day and night regulation of gene expression, and a delay of leaf and chloroplast senescence (*5*, *7*). Such phenotypic differences show that DET2 plays an important role throughout *Arabidopsis* development.

We mapped the *DET2* gene to a 150-kb interval on *Arabidopsis* chromosome 2 (Fig. 1A) (*8*). Within this interval, we identified a 20-kb genomic fragment that can rescue the *det2* phenotypes (Fig. 2) (*9*). This fragment gives rise to at least three transcripts (Fig. 1A) (*10*), one of which is altered in all *det2* alleles analyzed and is derived from the *DET2* gene (Fig. 1B). The *DET2* transcript contains a single, long open reading frame that encodes a 262–amino acid protein. We determined the corresponding genomic sequences of eight *det2* alleles (*11*), all of which have similar mutant phenotypes. Four alleles contain frameshifting deletions, and another two mutations cause premature termination of the DET2 protein. The two remaining alleles have a nonconservative substitution of lysine for glutamate at position 204 (Fig. 1B).

The deduced amino acid sequence of the *DET2* gene is similar to that of mammalian steroid 5α-reductases, with 38 to 42% sequence identity (Fig. 3A) (*12*). The sequence similarity increases to 54 to 60% when conservative substitutions are taken into account. Two isozymes (types 1 and 2) of steroid 5α-reductase have been isolated in rats and humans (*13*). Phylogenetic analysis shows that DET2 is at least as closely related to type 2 enzymes as type 2 enzymes are related to type 1 enzymes (Fig. 3B) (*12*). Eighty percent of the absolutely conserved residues in mammalian enzymes are found in the predicted DET2 protein. Mammalian steroid 5α-reductases catalyze the nicotinamide adenine dinucleotide phosphate (reduced) (NADPH)–dependent conversion of testosterone to dihydrotestosterone, which is a key step in steroid metabolism and is essential for the embryonic development of male external genitalia and prostate (*13*). The importance of this reaction is evident from certain forms of hereditary male pseudohermaphroditism in humans that are caused by steroid 5α-reductase deficiency. Sequence analysis of the steroid 5α-reductase type 2 gene from affected families has identified a missense mutation that causes a conservative substitution of aspartate for Glu^{197} (*13*), corresponding to Glu^{204} in DET2. In the *det2-1* and *det2-6* alleles, this glutamate is changed to lysine, indicating that this glutamate has a similar critical function as in the human 5α-reductase. Because even a conservative substitution at this position causes inactivation of the human enzyme (*13*), we predict that the nonconservative glutamate-to-lysine change completely abolishes DET2 activity, which explains the severe phenotypes of the two missense alleles. Together, these data suggest that the DET2 enzyme may catalyze a

biochemical reaction similar to that catalyzed by the human enzyme.

In plants, many steroids have been identified (*14*), but only brassinosteroids (BRs) have wide distribution throughout the plant kingdom and unique biological activity on plant growth when applied exogenously (*15*). A pathway for the biosynthesis of brassinolide, the most active BR, has recently been proposed on the basis of evidence from cell suspension cultures and whole-seedling experiments (Fig. 4A) (*16*). Although the biosynthesis of brassinolide involves many oxidation steps, only two steps involve reduction. One occurs early in the pathway where a double bond in campesterol is reduced to form campestanol. This reaction is similar to that catalyzed by the mammalian steroid 5α-reductases, suggesting that DET2 could catalyze the conversion of campesterol to campestanol. Because the *Arabidopsis* genome does not contain any other sequences that are closely related to *DET2* (6), one possibility is that the *det2* phenotypes are due to reduction or elimination of BR biosynthesis. To test this hypothesis, we treated *det2* seedlings with exogenous brassinolide (*17*). Although addition of brassinolide at 10^{-6} M to the growth medium had no effect on wild-type seedlings in the dark, the short hyocotyl phenotype of dark-grown *det2* seedlings was rescued (Fig. 4, B and D). Similarly, when added at 10^{-7} M, brassinolide had no effect on the petioles and leaves of wild-type seedlings but fully suppressed the dwarf phenotypes of these organs in light-grown *det2* plants (Fig. 4C). In contrast, neither applied gibberellins (GA1 or GA4, 10^{-8} to 10^{-5} M) nor auxin (IAA, 10^{-6} and 10^{-5} M) rescued the *det2* defects (6). Brassinolide treatment reversed the inhibition of hypocotyl elongation caused either by *det1* mutation or light (Fig. 4D), but it did not complement the mutant phenotypes of either dark- or light-grown *det1* seedlings (6), supporting our previous genetic studies that DET1 and DET2 act on separate pathways controlling light-regulated processes.

Our results suggest that *DET2* encodes a reductase involved in BR biosynthesis in *Arabidopsis*. The pleiotropic effects of *det2* mutations on *Arabidopsis* development suggest the involvement of BRs in several processes in *Arabidopsis* regulated by light or other hormones, including the expression of light-regulated photosynthetic genes, the promotion of cell elongation, normal leaf and chloroplast senescence, and the promotion of floral induction. Moreover, our studies suggest that light acts by modulating this hormone signal transduction pathway in target cells, perhaps by regulating the biosynthesis of BRs or by altering responsiveness of these cells to BRs. Given the well-described phenotypes of *det2* mutants and the various effects of applied BRs (*15*), we suggest that brassinosteroids be recognized as an important class of plant hormones.

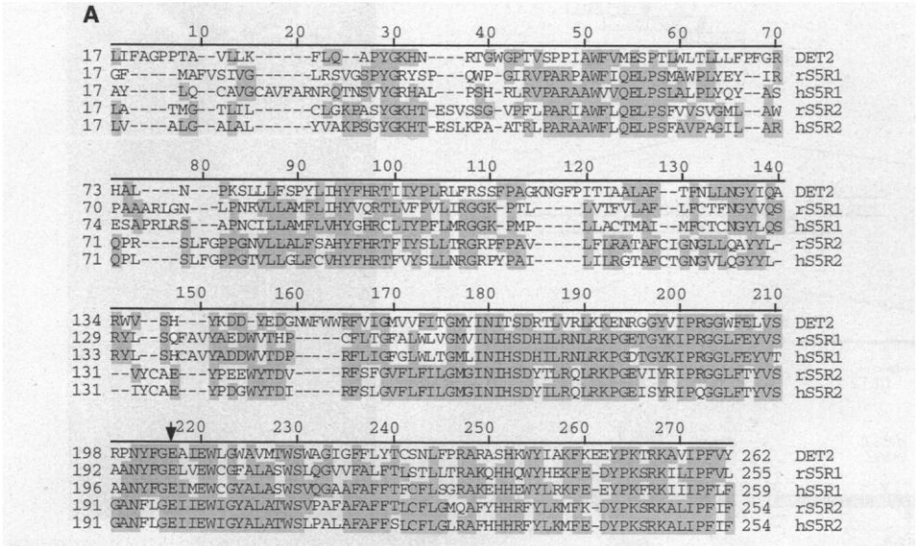

Fig. 3. Sequence comparison of DET2 with mammalian steroid 5α-reductases. (**A**) The deduced amino acid sequence (*18*) of the *DET2* gene is aligned with the steroid 5α-reductases from rat (rS5R1 and rS5R2) and human (hS5R1 and hS5R2) (*12*). Dashes indicate gaps introduced to maximize alignment, and residues conserved in at least two of the five sequences are shaded. Arrow indicates the glutamate mutated in *det2-1* and *det2-6* alleles. (**B**) Phylogenetic analysis of relationship between DET2 protein and mammalian steroid 5α-reductases (*12*). The scale measures the relative distance between sequences.

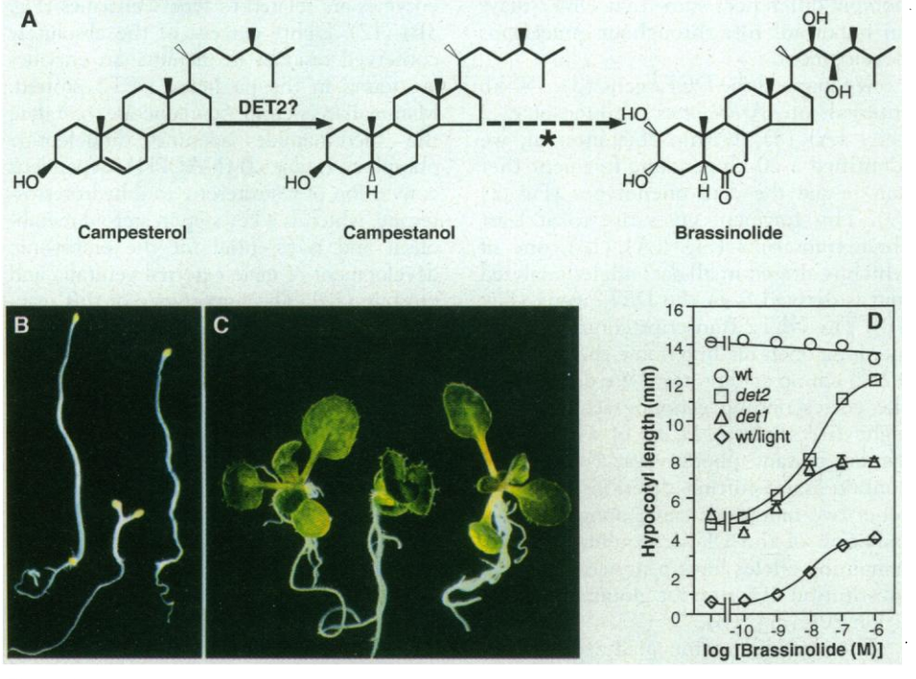

Fig. 4. (**A**) Proposed function of DET2 protein in the BR biosynthetic pathway. Asterisk (*) indicates six intermediate steps (*16*). (**B** and **C**) Rescue of *det2* phenotypes by brassinolide. (B) Dark-grown 10-day-old seedlings and (C) light-grown 12-day-old seedlings. (From left to right in each panel) Wild-type, *det2-1*, and brassinolide-treated *det2-1* plants (*17*). (**D**) Dose-response of brassinolide-induced hypocotyl elongation of dark-grown seedlings and light-grown wild-type plants (*17*). Data represent the mean ± SE obtained from triplicate determinations, each with an average sample size of 12 seedlings.

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8. Homozygous *det2-1* mutant (Col-0) was crossed to either wild-type No-0 or La-*er*. DNAs from F_2 *det2* seedlings were prepared [S. Dellaporta, J. Wood, J. Hicks, *Plant Mol. Biol. Rep.* **1**, 19 (1983)] for simple sequence length polymorphisms (SSLPs) [C. J. Bell and J. R. Ecker, *Genomics* **19**, 137 (1994)] and cleaved amplifed polymorphic sequences (CAPS) analysis [A. Konieczny and F. M. Ausubel, *Plant J.* **4**, 403 (1993)]. Overlapping yeast artificial chromosome (YAC) clones were isolated from three separate YAC libraries of *Arabidopsis* [E. R. Ward and G. C. Jen, *Plant Mol. Biol.* **14**, 561 (1990); J. R. Ecker, *Methods* **1**, 186 (1990); E. Grill and C. Somerville, *Mol. Gen. Genet.* **226**, 484 (1991)]. Fine RFLP analysis was performed with F_2 *det2* plants with recombination break points either in the m323-*DET2* region (68 recombinants, two mapping populations) or in the *DET2*-nga168 interval (31 recombinants), and new CAPS markers were converted directly from YAC insert ends or derived from phage clones of an *Arabidopsis* genomic library isolated with YAC end probes (*9*).
9. Cosmid and phage clones were isolated from two *Arabidopsis* genomic libraries [N. Olszewski, F. Martin, F. Ausubel, *Nucleic Acids Res.* **16**, 10765 (1988); the lambda genomic library was provided by R. W. Davis (Stanford University)] by hybridization with yUP2C12, yUP6B10, YAC end probes, or cosmid-derived probes. Cosmid DNAs were transformed into *det2-1* plants by a modified vacuum infiltration method [N. Bechtold, J. Ellis, G. Pelletier, *C. R. Acad. Sci. Paris* **316**, 1194 (1993); A. F. Bent *et al.*, *Science* **265**, 1856 (1994)] to identify cosmids containing the *DET2* gene.
10. Labeled Eco RI fragments of cosmid 217-61 were used as probes to screen ~2×10^6 clones of an *Arabidopsis* complementary DNA (cDNA) library constructed in lambda ZAPII [J. J. Kieber, M. Rothenburg, G. Roman, K. A. Feldmann, J. R. Ecker, *Cell* **72**, 427 (1993)]. Positive clones were converted to plasmids by in vivo excision according to the manufacturer's protocol (Stratagene) and sequenced with gene-specific primers .
11. The transcribed region of the *DET2* gene was amplified by polymerase chain reaction (PCR) from genomic DNAs of wild-type Col-0 and eight *det2* alleles, subcloned into pGEM-T vector (Promega), and sequenced. To minimize PCR errors, we pooled at least four different clones from two independently amplified fragments for sequencing.
12. Database searches were performed at the U.S. National Center for Biotechnology Information with the BLAST program [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. The sequence alignment and phylogenetic analysis were performed with the Megalign program (DNAStar) by the method of J. Hein [D. G. Higgins and P. M. Sharp, *Comput. Appl. Biosci.* **5**, 151 (1989)].
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17. Seeds were germinated on moist Whatman papers placed on MS medium [0.5× MS salts (Gibco), 1× Gamborg's B5 vitamins (Sigma), 0.8% phytagar (Gibco) and 1% sucrose], pH 5.7, for 2 days and transferred to fresh plates supplemented with various concentrations of auxin (IAA, 0 to 10^{-5} M), brassinolide (0 to 10^{-6} M), and gibberellins (GA1 or GA4, 0 to 10^{-5} M). Hormones were sterile-filtered into the cooling MS medium. For dark-grown seedlings, seeds were exposed to 2-hour light treatment before their plates were wrapped with three layers of aluminum foil, and the seedlings were transferred under a green safe-light. The hypocotyl lengths of 10-day-old etiolated seedlings and 12-day-old light-grown wild-type plants were measured.
18. Single-letter abbreviation for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, The; V, Val; W, Trp; and Y, Tyr.

19. We thank K. Hanson for technical assistance; S. Clouse (North Carolina State University) for helpful discussions; and D. Weigel, P. Doerner, S. Worland, and members of our lab for critical reading of the manuscript. Supported by grants to J.C. from USDA (93-373019125) and the National Science Foundation (DIR-9116923).

22 November 1995; accepted 25 January 1996

Switching from Cut-and-Paste to Replicative Tn7 Transposition

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The bacterial transposon Tn7 usually moves through a cut-and-paste mechanism whereby the transposon is excised from a donor site and joined to a target site to form a simple insertion. The transposon was converted to a replicative element that generated plasmid fusions in vitro and cointegrate products in vivo. This switch was a consequence of the separation of 5′- and 3′-end processing reactions of Tn7 transposition as demonstrated by the consequences of a single amino acid alteration in an element-encoded protein essential for normal cut-and-paste transposition. The mutation specifically blocked cleavage of the 5′ strand at each transposon end without disturbing the breakage and joining on the 3′ strand, producing a fusion (the Shapiro Intermediate) that resulted in replicative transposition. The ability of Tn7 recombination products to serve as substrates for both the limited gap repair required to complete cut-and-paste transposition and the extensive DNA replication involved in cointegrate formation suggests a remarkable plasticity in Tn7's recruitment of host repair and replication functions.

Transposable elements are DNA segments that can move from one DNA site to another in the absence of homology between the two sites. Many elements, including the bacterial transposon Tn7, move through a cut-and-paste mechanism in which the transposon is first excised from the donor site by double-strand breaks at each end of the transposon and is then joined to the target site (*1*–*3*) (Fig. 1). This joining occurs through the linkage of the 3′ ends of the transposon to staggered positions on the top and bottom strands of the target DNA, resulting in small (several nucleotide) gaps flanking the newly inserted transposon. Repair of these gaps by host functions generates the small flanking direct repeats characteristic of transpositional recombination.

Other transposons, including bacteriophage Mu and Tn3, can move via a replicative pathway which involves extensive DNA replication (*1*, *2*). In Mu, only one strand at each end of the element is cut, exposing the 3′ transposon end. Transfer of this end to the target DNA results in a fusion product between the donor and target molecules termed a Shapiro intermediate or strand transfer intermediate, in which one strand at each end of the transposon remains joined to the donor DNA, and the other is joined to the target DNA (*4*, *5*). Repair of this transposition product by host functions involves much more extensive DNA replication than the repair of simple insertions (up to tens of kilobases compared to several nucleotides) and generates a circular cointegrate molecule in which the donor and target sequences are joined by two copies of the transposon.

A fundamental difference between the cut-and-paste and replicative transposition pathways lies in the cleavage of the 5′ ends of the transposon (*2*). In standard Tn7 recombination the break is double-stranded, that is, both 5′ and 3′ strands are cleaved in the cut-and-paste transposition. We now show that, with a single point mutation in a Tn7-encoded protein, 5′ end cleavage is blocked and the transposition mechanism is switched; Tn7 now transposes replicatively to produce a Shapiro intermediate, which can be subsequently processed into a cointegrate transposition product.

Tn7 transposition requires an elaborate array of Tn7-encoded proteins: TnsA, TnsB, TnsC, TnsD, and TnsE (*3*, *9*, *10*). TnsA and TnsB constitute the heart of the transposition machinery that executes the DNA breakage and joining reactions that

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