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°C 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 brule 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.

REFERENCES AND NOTES

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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-
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 thin 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 phototropistic 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 isoforms (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 asparagine for Glu97 (13), corresponding to Glu524 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 hypocotyl phenotype of dark-grown det2 seedlings was rescued (Fig. 4B 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.

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Swimming 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 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 bacterio
dophage 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.

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