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62. Least-squares regression equations for each plot are: $y = 2.082 + 0.005x$, $P = 0.552$, $SE_{slope} = 0.0074$ for S-C, Fig. 1A; $y = 2.524 - 0.003x$, $P = 0.88$, $SE_{slope} = 0.0224$, P/P, Fig. 1B; $y = 0.395 + 0.002x$, $P = 0.289$, $SE_{slope} = 0.0021$, TF, Fig. 1C; $y = 0.452 - 0.0004x$, $P = 0.816$, $SE_{slope} = 0.0018$, IF, Fig. 1D; $y = 0.153 - 0.002x$, $P = 0.024$, $SE_{slope} = 0.0008$, BF, Fig. 1E.
63. Trophic aggregation was performed on 41 webs listed in Table 1. The number of steps to the reduced, aggregated web across all webs ranged from 2 (*33, 48, 50, 51*) to 44 (*47*). Webs listed in Table 1 with ten or fewer taxa have lower percentages of actual taxa (65%), and aggregation in these smaller systems, if attempted, would give trivial results; for example, webs with fewer than seven taxa are trivially rigid. If the smallest webs are included, the trivial bias would make the properties. S-C, fraction intermediate, and fraction basal appear to vary systematically with web size.
64. One should be mindful that this procedure of trophic aggregation is not equivalent to lumping based on taxonomic similarity.
65. Supported by NSF grant BSR88-07404. We thank R. Beaver for assistance in the compilation of data sets. We are grateful to J. Cohen, J. Enright, R. May, R. Paine, and S. Pimm for comments on an earlier draft.

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The *Diageotropica* Mutant of Tomato Lacks High Specific Activity Auxin Binding Sites

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Tomato plants homozygous for the *diageotropica* (*dgt*) mutation exhibit morphological and physiological abnormalities which suggest that they are unable to respond to the plant growth hormone auxin (indole-3-acetic acid). The photoaffinity auxin analog [^{3}H]5N$_3$-IAA specifically labels a polypeptide doublet of 40 and 42 kilodaltons in membrane preparations from stems of the parental variety, VFN8, but not from stems of plants containing the *dgt* mutation. In roots of the mutant plants, however, labeling is indistinguishable from that in VFN8. These data suggest that the two polypeptides are part of a physiologically important auxin receptor system, which is altered in a tissue-specific manner in the mutant.

THE PLANT GROWTH HORMONE AUXin [indole-3-acetic acid (IAA)] appears to activate cellular responses, such as the promotion of cell elongation, by binding to specific receptor proteins. Evidence for auxin binding to both membrane-bound and soluble proteins has been reported (*1*). However, no direct experimental connection has been made between any plasma membrane auxin-binding protein and a known molecular or cellular response to auxin (*2*). Obtaining such evidence in conjunction with the isolation of the receptor would be important in elucidating the molecular mechanism of auxin action.

One way to investigate the physiological relevance of putative auxin receptors is through the use of mutant plants that are insensitive to auxin or which exhibit abnormalities likely to be influenced by IAA. The *diageotropica* (*dgt*) mutant of tomato (*Lycopersicon esculentum*, Mill.) is a recessive mutant of the parental variety, VFN8, and appears to have arisen spontaneously at a single locus. Tomato plants homozygous for the *dgt* mutation have diagravitropic shoot growth, abnormal vascular tissue, altered leaf morphology, and no lateral root branching (*3, 4*). Although the endogenous levels of auxin are the same in *dgt* and VFN8 shoot apices (*5*), *dgt* mutants are insensitive to exogenously applied auxin in ethylene production (*4, 6*) and stem elongation (*7*). The morphological abnormalities exhibited by *dgt* plants, in addition to their inability to elongate in response to auxin, suggest that the *dgt* lesion is associated with a primary site of auxin perception or action. We report here on experiments that were designed to test the hypothesis that the mutated *dgt* gene codes for a receptor protein which is present at abnormally low levels or has a greatly reduced ability to bind auxin.

To label and identify potential auxin receptors, we used a radioactively labeled photoaffinity auxin analog, [^{3}H]5N$_3$-IAA (azido-IAA). Azido-IAA is an active auxin in several different bioassays and its uptake and transport characteristics in stems are similar to those of auxin (*8*). Thus, one would expect azido-IAA to bind to auxin receptors with an affinity similar to that of auxin. In an earlier study on the binding of the azido-IAA to plasma membrane proteins from zucchini hypocotyls, the vesicles were exposed to azido-IAA and photolyzed with ultraviolet light (300 nm) at −196°C (*9*). Subsequent SDS–polyacrylamide gel electrophoresis (SDS-PAGE) followed by fluorography revealed that azido-IAA was associated at high specific activity with a polypeptide doublet of 40 and 42 kD. These polypeptides are of low abundance, as they cannot be visualized in silver-stained gels until they have been electroeluted and concentrated approximately tenfold. Competition experiments with various auxin analogs suggest that this doublet binds auxin specifically (*9*).

We have now performed a similar experiment with microsomal membrane preparations from the hypocotyls (stems) or roots

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of 10-day-old etiolated VFN8 and *dgt* tomato seedlings (Fig. 1). The silver-stained gel indicates that *dgt* and VFN8 microsomes do not display any obvious differences in their pattern of polypeptides, suggesting that *dgt* is indeed isogenic with VFN8 (Fig. 1A). Both the roots and stems of VFN8 have a polypeptide doublet of 40 and 42 kD, which is labeled to high specific activity with the azido-IAA (Fig. 1B). The molecular weight of the tomato polypeptides agrees with those observed in zucchini. These labeled polypeptides were not detected in membranes from *dgt* stems after our usual fluorographic exposure time of 4 days, although the labeled doublet was present in *dgt* roots at an intensity equal to that in VFN8 roots (Fig. 1B). Prolonged exposure (17 days) of these fluorographs produced a faint signal from *dgt* shoot preparations (Fig. 1C). These data indicate that the 40- and 42-kD auxin-binding polypeptides are greatly diminished or have much reduced auxin-binding capacity in the mutant stems. In either case, the alteration is developmentally regulated.

The presence of Triton X-100 (0.1%) during photolysis increases the intensity of polypeptide labeling by the azido-IAA in VFN8 stem microsomes (Fig. 2). As the data shown in Fig. 1 were obtained in the presence of Triton X-100, it was important to demonstrate that the detergent does not preferentially remove the 40- and 42-kD doublet from *dgt* membrane preparations before gel electrophoresis. A selective removal of the proteins from the *dgt* membranes cannot explain the difference between the mutant and wild-type tissues in binding azido-IAA, since membranes exposed to azido-IAA in the absence of Triton X-100 also fail to yield bands that label with high specific activity in *dgt* stems (Fig. 2). The fact that the labeled polypeptides in VFN8 remain with the sedimented membrane fraction after treatment with a relatively high concentration of detergent suggests that the polypeptides specifically labeled with azido-IAA may be intrinsic membrane proteins. We have obtained similar results with zucchini plasma membrane vesicles (*9*).

The unusual phenotype of *dgt* plants and their insensitivity to auxin strongly suggest that this mutant has an altered auxin receptor (*6, 7*). Our results are consistent with this hypothesis. Microsomes from *dgt* shoots have greatly reduced amounts of the 40- and 42-kD polypeptides that efficiently bind azido-IAA. If this is an auxin receptor, the polypeptides should be (i) ubiquitous in plant tissues that respond to IAA, (ii) of low abundance, (iii) saturable with increasing concentrations of IAA, and (iv) capable of binding specific analogs that are also active auxins or specific antagonists. Azido-IAA labels a polypeptide doublet of this molecular weight range in various auxin-responsive tissues in many plant species (*10*). Increasing concentrations of auxin protect the polypeptides from photoaffinity labeling in a corresponding manner (Fig. 3, inset). Densitometer scans of the fluorography (Fig. 3) reveal an apparent half-maximal saturation (K_{Da}) at about $10^{-5}M$ IAA. Similar K_{Da}'s for auxin binding have been previously reported (*1, 2*). These results suggest that the photoaffinity labeling is saturable and, thus, probably reflects a specific interaction of the azido-IAA with a receptor molecule. Studies of zucchini microsomes and plasma membrane vesicles demonstrate that competition for the auxin binding exhibits a high degree of specificity for active auxins and auxin analogs (*9*).

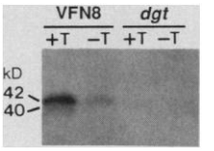

Fig. 2. The effect of Triton X-100 on the azido-IAA photoaffinity labeling of polypeptides in VFN8 and *dgt* microsomes. Fluorography (4-day exposure) showing labeled 40- and 42-kD polypeptides from VFN8 and *dgt* stems in the presence (+T) and absence (−T) of 0.1% Triton X-100. Photoaffinity labeling was as described for Fig. 1.

The *dgt* plants lack lateral roots, a known developmental abnormality thought to be auxin-related (*11*); yet membrane preparations from roots appear to have the 40- and 42-kD receptor with normal auxin-binding capacity. We have not resolved this apparent paradox. However, it has been reported that *dgt* roots produce a normal branching pattern when grafted to VFN8 shoots, whereas the reverse graft has no effect (*12*). Thus, we suggest that auxin-sensitive stems produce a diffusible or transported factor that influences root branching. As *dgt* stems are auxin-insensitive, they would not be expected to produce this factor. This hypothesis remains to be tested as does the question of whether *dgt* roots show some wild-type responses to exogenous IAA (for example, inhibition of elongation), as would be expected if they have a growth-specific auxin receptor.

There are several ways to explain the reduced levels of detectable azido-IAA labeling in *dgt* stems that are consistent with the finding that the azido-binding polypeptides are present in *dgt* roots. Perhaps the most straightforward explanation is that the structural gene (or genes) for the polypeptides is intact, but because of an alteration of cis- or trans-acting regulatory factors it is expressed at a diminished level in the stem. It is also possible that there are separate genes expressed in root and stem with the latter being defective in *dgt*. Alternatively, the *dgt* lesion may affect post-translational process-

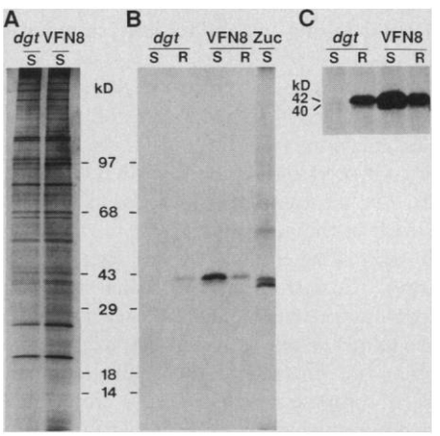

Fig. 1. Differential high specific activity labeling of 40- and 42-kD polypeptides in microsomes of *dgt* and VFN8. Microsomes were prepared from stems or roots of 10-day-old etiolated seedings of both tomato varieties. Material was homogenized with a Polytron for 15 s (level 8) in an equal amount (w/v) of ice-cold buffer I (10 m*M* tris-HCl, *p*H 7.5, and 0.25*M* sucrose, 1 m*M* disodium-EDTA, 1 m*M* dithiothreitol, 0.1 m*M* $MgSO_4$, 0.2 m*M* phenylmethylsulfonyl fluoride, 1 μg of pepstatin per milliliter and 1 μg of leupeptin per milliliter). The homogenate was filtered through four layers of cheesecloth, and the remaining material was reground with the same amount of buffer I. The combined filtrate was centrifuged at 4°C for 20 min at 3000*g*, and the pellet was discarded. The supernatant was centrifuged at 100,000*g* for 30 min at 4°C, and the resulting microsomal pellet was suspended in buffer I, aliquoted, frozen in liquid N_2, and stored at −80°C. For photoaffinity labeling (performed under red light), a quantity of microsomes equivalent to 100 μg of total protein [assayed according to (*14*)] was diluted to a final volume of 50 μl with buffer II (10 m*M* morpholinoethanesulfonic acid/bis[tris(hydroxymethyl)-methylamino]propane, *p*H 6.5, and 0.25*M* sucrose), $5 \times 10^{-7}M$ [^{3}H]5N_3-IAA (16 Ci/mmol), and Triton X-100 (final concentration 0.1%). Thirty seconds after addition of microsomes, samples were transferred to glass cover slips, which were then placed on an aluminum block surrounded by liquid N_2, and the samples were irradiated for 10 s with 300-nm ultraviolet light. Irradiated samples were stored overnight in the dark at −20°C, washed by dilution into 1 ml of buffer II, and centrifuged at 200,000*g* for 5 min at 4°C. The pellets were resuspended in 20 μl of SDS loading buffer (62.5 m*M* tris-HCl, *p*H 6.8, 2% SDS, 10% glycerol, 0.2*M* dithiothreitol, and 0.25% bromphenol blue), and the proteins were separated by electrophoresis through 7.5% to 15% polyacrylamide gradient gels at 4°C. After being stained with Coomassie brilliant blue, gels were treated with a fluorographic enhancer, dried, and exposed to Kodak XAR-5 film. (**A**) Silver-stained (*15*) gel comparing unlabeled stem (S) microsomal polypeptides from *dgt* and VFN8. (**B**) Fluorograph (4-day exposure) showing polypeptide labeling in microsomes of stems (S) and roots (R) from *dgt* and VFN8. Labeled microsomal polypeptides of zucchini stems (Zuc) are included for comparison. (**C**) Fluorograph resulting from a 17-day exposure of a portion of the gel shown in (B).

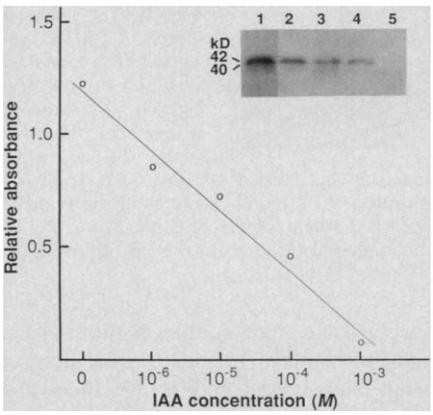

Fig. 3. Reduction of 40- and 42-kD polypeptide labeling in microsomes of VFN8 stems by IAA. Inset: Fluorograph displaying polypeptide labeling in the presence of (1) zero, (2) $10^{-6}M$, (3) $10^{-5}M$, (4) $10^{-4}M$, or (5) $10^{-3}M$ added IAA. Polypeptides of VFN8 stem microsomes were labeled as described in Fig. 1. Line drawing shows results of a densitometer scan of the inset fluorograph. Relative absorbance (in arbitrary units) of the 42-kD band was plotted as a function of the molar concentration of added IAA.

ing, which prevents normal membrane insertion or folding of the polypeptides or reduces the affinity of the binding site for auxin in stem tissue.

It is believed that there are at least three plasma membrane–bound auxin receptors; an uptake symport, an asymmetrically distributed efflux carrier, and a receptor associated with an outwardly directed proton pump thought to be involved in elongation growth (*1*). Present data do not allow us to distinguish between these possible receptor types. On the one hand, zucchini hypocotyl plasma membrane vesicles show a specificity for competition of azido-IAA labeling by auxin analogs that is similar to the specificity demonstrated for auxin uptake into both membrane vesicles and hypocotyl segments via the symport (*9*). In addition, the ability of VFN8 shoots to normalize *dgt* roots could indicate that the *dgt* lesion alters either the uptake or efflux of auxin during cell-to-cell transport. However, other experiments indicate that the rate of polar auxin transport (for example, from shoot apex to base) is unimpaired in *dgt* stems as compared to that in VFN8 (*13*). In addition, since *dgt* hypocotyl sections do not grow in response to externally applied auxin (7), the receptor responsible for auxin-stimulated growth may be affected by this lesion. The identification of the two polypeptides that appear to be affected by the *dgt* lesion may make it possible to dissect the mechanism of auxin action.

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Influence of Interior Packing and Hydrophobicity on the Stability of a Protein

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Protein interiors contain many tightly packed apolar atoms in a nearly crystalline state. Both shielding of apolar atoms from solvent and efficient interior packing arrangements affect protein stability, but their relative importance is unclear. To separate these effects, the stabilities of wild-type and mutant gene V proteins from bacteriophage f1 were studied by measuring resistance to denaturation. The effects of subtle interior packing changes, both separate from and combined with changes in buried side chain hydrophobicity, were measured. For the interior apolar-to-apolar substitutions studied, the two effects were of the same magnitude and alteration of packing without accompanying hydrophobicity changes substantially destabilized the protein.

PROTEINS ARE ONLY MARGINALLY stable, with their active, folded forms favored by as little as 5 to 15 kcal/mol as compared with their denatured forms (*1, 2*). Apolar amino acid side chains that are buried in the interiors of proteins are likely to affect protein stability in two general ways. The first effect is that their shielding from the external aqueous solvent stabilizes the folded protein through the hydrophobic effect (*3*). The magnitude of this effect has been compared with scales of amino acid hydrophobicity derived from partitioning of small apolar molecules between water (representing the unfolded protein) and apolar solvents or the vapor phase (representing the interior of the folded protein) (*4–6*). These studies suggest that burying larger apolar side chains should increase protein stability.

Protein interiors have some properties unlike those of apolar liquids or the vapor phase, however (*7, 8*). Protein interiors are more densely packed than apolar liquids, and their packing has been likened to that of crystals of small molecules (*7*). This packing can be quite fixed; for example, in a lysozyme mutant in which an interior methyl group was replaced by a hydrogen, all other atoms were still in the same positions as the wild type and a cavity remained in the place of the methyl group (*6*). Moreover, not all interior atoms in proteins are nonpolar, and water molecules are sometimes buried inside proteins (*1*). A second class of effects, then, are those due to differences between protein interiors and, for example, an apolar liquid. Because these effects are generally related to the rigidity and tight packing of protein interiors, we refer to them as "packing" effects. As used here, packing encompasses the combined effects of close packing, distortion of the remainder of the protein, and the polarity of the protein interior on protein stability, and thus differs slightly from the traditional usage of the term (*8*). For example, close packing is expected to increase favorable van der Waals interactions, but disruption of the remainder of the protein from an otherwise optimal conformation might decrease its stability (*6, 7*).

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