Research on mechanisms of auxin action: isolation and characterization of auxin-related mutants

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Abstract

Auxins are plant growth substances that control fundamental processes in plant growth, development, and environmental response. However, despite extensive research over the last century, their mechanisms of action remain unclear. Progress is most likely to come from complementary genetic, molecular, and functional studies. This review concentrates on recent results using the genetic approach to select and characterize auxin-response mutants.

Introduction

Growth and development of plants are directed by environmental signals that function in cooperation with a variety of internal factors. The internal factors include plant hormones. Among them auxins play a very important role.

The concept of translocation of chemical messengers in higher plants was proposed in the 19th century by the German botanist JULIUS VON SACHS and supported by WENT (1928). WENT discovered auxin, later shown to be indole-3-acetic acid (IAA) (HAAGEN-SMIT *et al.* 1941). Although IAA is by far the main auxin in most plants, other compounds that display auxin activity have been found such as PAA (phenylacetic acid) (WIGHTMAN & SIGHTY 1982) or 4-Cl-IAA (4-chloro-IAA) (PLESS *et al.* 1984). IAA is believed to be synthesized primarily in apical meristems, young leaves, but also in root meristems (DAVIES 1987).

Although it is accepted that the auxin affects diverse processes in the plant life cycle such as cell division and elongation, root formation and development, apical dominance, tropism and others, the precise molecular mechanisms of auxin action remain poorly understood (DAVIES 1995). These processes are controlled not only by the concentration of free auxin, but also by changes in the cellular auxin sensitivity (TREWAVAS & CLELAND 1993). In addition, it is known that the control of these processes is achieved by cooperation with other plant hormones such as cytokinins, which cooperate with auxins to control root and shoot formation (SKOOG & MILLER 1989). It is generally believed that auxin action is receptor-mediated. Early auxin action has been divided into three principal stages: perception of auxin, signal transduction and response. The pleiotropy of auxin responses suggests the existence of more than one system of auxin perception. An understanding of mechanisms of auxin action relies upon the development complementary molecular (the identification of the auxin receptors), functional (search for early auxin-induced cellular responses) and genetic (selection of auxin-response mutants) approaches. This review summarizes results of isolation and characterization of auxin-response mutants and mutants related to auxin polar transport.

Selection of auxin response and auxin polar transport mutants

A genetic approach allows to identify genes involved in auxin-signaling pathways without an *a-priori* assumption as to the biochemical and physiological processes involved. A classical method for studying the function of genes responsible for alterations in auxin responses is based on the comparison of morphology, physiology and biochemistry of single-gene mutants with wild-type plants, or on the study of interactions between different mutant loci in double mutants. Genetic and molecular analysis have been greatly facilitated in *Arabidopsis* whose genome is well characterized and where a large number of mutants have been isolated (KONCZ *et al.* 1992).

Auxin response mutants have been obtained by several selection systems including abnormal responses to auxin present in selection medium. Selection parameters included cell division, seed germination or elongation growth of roots, hypocotyls and seedlings; these are prominent physiological responses used in many labo-

ratories. Simultaneously, several auxin responses were used as selection criteria such as auxin resistance, sensitivity or dependence. For example, because auxin inhibits root growth (ESTELLE & SOMERVILLE 1987), most of the auxin response mutants have been obtained by using a positive selection screen based on the ability of mutants to grow on wild-type inhibitory auxin concentration. Selection criteria originally used for the isolation of mutants of different interest may also reveal auxin response mutants. For example, the *dgt* tomato mutant (KELLY & BRADFORD 1986) screened for diageotropic growth was found to be auxin resistant; also the *Arabidopsis agr3* mutant (BELL & MAHER 1990) selected for abnormal root gravitropism exhibited increased sensitivity to auxin. Finally, mutations in genes essential for plant viability may not be isolated by such a selection system.

Auxin mutants primarily screened for altered auxin responses as well as auxin-response mutants selected originally for other characteristics are mentioned in Table I and some of them will be discussed in detail.

Auxin response mutants

Selection and characterization of mutants affected in sensitivity to auxins are powerful tools for identifying genes involved in auxin perception or auxin signal transduction. Typically, auxin sensitivity mutants (Table I) have been isolated by screening chemically mutagenised populations. Much interest has been devoted to T-DNA insertional mutants in order to select auxin mutants, particularly those with a totally disrupted function of a gene. Most of the selected auxin sensitivity mutants in different plant species exhibit a reduced sensitivity to auxin or in an extreme case, auxin independence. Simultaneously, many of the isolated mutants with altered sensitivity to exogenous auxin also exhibited abnormal sensitivity to other plant hormones or growth regulators indicating interaction of auxin action with other hormones. This may be due to mutation in genes either encoding for a regulatory protein or for a protein involved in common steps of the transduction pathways. However, some auxin specific mutants in tobacco and *Arabidopsis* have been isolated and thus, they may be mutated in gene(s) involved in early steps of auxin action such as auxin perception. Interestingly, all the auxin resistant mutants are affected in their normal gravitropic response confirming involvement of auxin in that tropic response. A detailed description of some auxin response mutants focusing on supersensitive and resistant mutants follows.

Auxin supersensitive mutants

Two non-allelic monogenic recessive mutations, *aus1* and *aus2* have been isolated in *Nicotiana plumbaginifolia* resulting in auxin increased sensitivity mutants (DE SOUZA & KING 1991). The *aus1* mutant is larger than the wild-type and displays other morphological characteristics such as mild leaf epinasty, a short primary root, increased root branching and no root hairs. Detailed studies revealed that the *aus1* mutant displays increased sensitivity to exogenous auxin for hypocotyl and leaf rosette. Formation of lateral roots was induced at a lower auxin concentration than the wild-type. After culture at high auxin concentrations mutant seedlings died, even if transferred to an auxin free medium. *aus1* mutants exhibited higher sensitivity to ethylene and even higher to the ethylene precursor, ACC, suggesting that increased sensitivity to auxin may be a consequence of changes in the regulation of ethylene production or an increase in ethylene sensitivity. *aus1* also exhibited increased sensitivity to L-tryptophan, but not to D-tryptophan. Interestingly, these results may be related to auxin biosynthesis in these mutants. Specificity tests showed that *aus1* and wild type hypocotyls are equally inhibited in the presence of BA and ABA. However, *aus1* mutant exhibited a higher sensitivity to BA in cotyledon expansion.

In *Arabidopsis*, the *agr3* mutant selected for abnormal root graviresponse (BELL & MAHER 1990) was found to be more sensitive to auxin for root growth inhibition. Except for these traits, the *agr3* mutant phenotype appeared similar to the wild-type, and BELL & MAHER proposed that mutation in *AGR3* locus would affect only root gravitropism and auxin physiology. Because other gravitropic mutants affected in auxin sensitivity are more resistant to auxin than the wild-type, the existence of the auxin supersensitive mutant *agr3* may indicate a multifaceted role for auxin in gravitropism. SINCLAIR and collaborators (1996) found that IAA caused unexpectedly parallel inhibitions of growth and bending of *agr3* roots, as IAA is believed to regulate specifically the curvature processes by a gradient of auxin across the tip. Their results further suggest that the mutation at *agr3* alters either use or expression of calmodulin, a primary plant calcium receptor, known to be involved in gravitropic sensing and transduction. The *agr3* was found to be allelic to *agr1*, *agr2*, *wav6-52*, *eir1* and *pin2* (BELL & MAHER 1990; OKADA & SHIMURA 1990; LUSCHNIG *et al.* 1998; MÜLLER *et al.* 1998). UTSUNO *et al.* (1998) reported that *agr1* roots are sensitive to 2,4-D, but not to IAA and NAA. MÜLLER *et al.* (1998) found that *pin2* roots exhibit greater sensitivity towards the auxins 1-NAA > IAA > 2,4-D which is consistant with the known substrate specifically of the auxin efflux carrier (DELBARRE *et al.* 1996). The gene *PIN2* was cloned and the topology of PIN2 protein was found to be similar to members of the major facilitate superfamily of transport protein (MÜLLER *et al.* 1998). The authors showed that PIN2 protein was localised in membranes of cortical and epidermal cells in the meristematic and elongation zones revealing a polar localization, and its function was specific in roots. The authors suggest that PIN2 plays an important role in control of gravitropism regulating the redistribution of auxin from the stele towards the elongation zone of roots.

Tab. I.: Auxin response mutants in higher plants.

Locus/ mutant	Species (mutant library)	Auxin response used as selection criterion (selection parameter)	Observed auxin response (for)	Inheritance	Additional phenotypic traits and short description	References
AUXIN SUPERSENSITIVE MUTANTS						
aus1	*N. plumbaginifolia*	↑resistance	↑sensitivity	R	*aus1* : ↑sensitivity to auxin in sec. root formation; ↑sensitivity to ACC, ethylene and L-tryptophan; ↑sensitivity to BA; mild leaf epinasty, short prim. root, increased root branching, no root hairs.	DE SOUZA & KING 1991
aus2	"	"	"			
	(EMS)	(seedl. develop.)	(hyp., leaf)			
agr3	*A. thaliana* (EMS)	– (root gravitrop.)	↑sensitivity (root elong.)	R	Abnormal root gravitropism; inhibition of root elongation and gravitropism by IAA; phenotype~wt; resistant to ethylene and auxin-transport inhibitors; **allelic to *agr1,2, eir1, wav6-52, pin2*; gene cloned**.	BELL & MAHER 1990 MAHER & BELL 1990 OKADA & SHIMURA 1990 SINCLAIR *et al.* 1996 CHEN *et al.* 1998 LUSCHNIG *et al.* 1998 MÜLLER *et al.* 1998 UTSUNO *et al.* 1998
sax1	*A. thaliana* (EMS)	↑sensitivity (root elong.)	↑sensitivity (root elong.)	R	↑sensitivity to ABA in root elongation, seed germination and stomatal closing; ↑sensitivity to NPA and PBA in root elongat.; ↑resistance to ACC and GA_3 for hypocotyl growth; short root and hypocotyl; dwarf; dark-green leaves; rescued by brassinosteroids.	EPHRITIKHINE *et al.* 1995 FELLNER 1997 PAGANT *et al.* 1998
AUXIN RESISTANT MUTANTS						
rac	*N. tabacum*	↑resistance (cell division)	↑resistance (protopl. electrop. resp.)	D	Impaired in root development.	MÜLLER *et al.* 1985 EPHRITIKHINE *et al.* 1987 LUND *et al.* 1996
R1	*N. plumbaginifolia*	↑resistance	↑resistance	partially D	*R1*: plants short; *R15*: affected auxin-induced ethylene biosynthesis, root formation and hypocotyl graviresponse; *R25*: strongly affected many auxin-regulated processes, including short-term responses, plant dwarf; *R29*: affected auxin-induced root formation and cell elongation, plants lethal.	BLONSTEIN *et al.* 1991 STIRNBERG *et al.* 1995
R15	"	(hyp. elong.)		partially D		
R25	"			D		
R29	"			R		
	(EMS)					
iba1	*N. plumbaginifolia*	↑resistance	↑resistance	R	*iba1*: ↑resistance to ABA and paclobutrazol; early germination, reduced apical dominance.	BITOUN *et al.* 1990
iba2	"	(seed germin.)	(seed germin.)	R		
iba3	"			R		

Tab. I.: Continued.

Locus/ mutant	Species (mutant library)	Auxin response used as selection criterion (selection parameter)	Observed auxin response (for)	Inheritance	Additional phenotypic traits and short description	References
dgt	*L. esculentum*	– (root develop.)	↑resistance	R	↑resistance in several auxin responses (cell elongation, hypocotyl segment elongation, ethylene biosynthesis, petiole epinasty); ↑resistance to ethylene; ↑resistance to NPA; diageotropic growth; lack of lateral roots, abnormal vascular tissue, altered leaf morphology; lacks ABP in hypocotyl; inhibited induction of *LeSAUR*.	ZOBEL 1973; 1974 KELLY & BRADFORD 1986 HICKS *et al.* 1989a URSIN & BRADFORD 1989 DANIEL *et al.* 1989 MUDAY *et al.* 1995 MITO & BENNETT 1995
axr1	*A. thaliana* (EMS)	↑resistance (seedl. develop.)	↑resistance (root elong.)	R	↑resistance to cytokinin and ethylene; impaired root gravitropism; many morphological defects: roots longer and less branched, reduced hypocotyl and stem elongation, vascular tissue less developed, flowers poorly developed, plants short and bushy; deficiency in auxin-induced accumulation of *SAUR-AC1*mRNA; **gene cloned**.	ESTELLE & SOMERVILLE 1987 LINCOLN *et al.* 1990 ESTELLE 1992 LEYSER *et al.* 1993 ABEL *et al.* 1994 TIMPTE *et al.* 1995
tir1	*A. thaliana* (EMS, T-DNA)	– (root elong.)	↑resistance (root elong.)	SD	↑resistance to auxins and auxin transport inhibitors NPA and CPD; deficient in hypocotyl elongation and lateral root formation; **gene cloned**.	CERNAC *et al.* 1994 RUEGER *et al.* 1997 RUEGER *et al.* 1998
axr2	*A. thaliana* (EMS)	↑resistance (root elong.)	↑resistance (root elong.)	D	↑resistance to cytokinin, ABA and ethylene in root growth; impaired root and hypocotyl gravitropism; reduced root and hypocotyl length, lack root hairs, abnormal vascularization, decreased stem cell elongation, dwarf plants; deficiency in auxin-induced *SAUR-AC1* expression.	WILSON *et al.* 1990 KLEE & ESTELLE 1991 TIMPTE *et al.* 1992 KNEE & HANGERTER 1994 GIL *et al.* 1994
axr3	*A. thaliana* (EMS)	↑resistance (root elong.)	↑resistance (root elong.)	SD	↑resistance to ACC and cytokinin in root growth; reduced root elongation, no root gravitropism, increased adventitious rooting; small plants, enhanced apical dominance, small curled leaves; ectopic expression of *SAUR-AC1*; **gene cloned**.	LEYSER *et al.* 1996 ROUSE *et al.* 1998
axr4	*A. thaliana* (T-DNA)	↑resistance (root elong.)	↑resistance (root elong.)	R	Strong defect in root gravitropism; **allelic to *rgr1***;	HOBBIE & ESTELLE 1995
rgr1	*A. thaliana* (T-DNA)	– (root gravitrop.)	↑resistance (root elong.)	R	Reduced root gravitropism; ↑resistance to NPA, TIBA; shorter root exhibiting clockwise coiling, reduced lateral root formation; **allelic to *axr4***; ***rgr1*** cosegregates with T-DNA.	SIMMONS & SÖLL 1995 SIMMONS *et al.* 1995

Tab. I.: Continued.

Locus/ mutant	Species (mutant library)	Auxin response used as selection criterion (selection parameter)	Observed auxin response (for)	Inheritance	Additional phenotypic traits and short description	References
aux1	*A. thaliana* (EMS, T-DNA)	↑resistance (root elong.)	↑resistance (root elong.)	R	↑resistance to ACC, ethylene and cytokinin in root growth; impaired root gravitropism; short root hairs; retarded hypocotyl elongation; no expression of *AUX1* in root columella; modests effect on auxin-induced *SAUR-AC1* expression; **gene cloned**.	MAHER & MARTINDALE 1980 MIRZA *et al.* 1984 PICKETT *et al.* 1990 EVANS *et al.* 1994 HOBBIE & ESTELLE 1995 TIMPTE *et al.* 1995 BENNETT *et al.* 1995; 1996 YAMAMOTO & YAMAMOTO 1998
nph4	*A. thaliana* (T-DNA)	– (hyp. phototrop.)	↑resistance (hyp. growth)	R	Altered phototropism and gravitropism; impaired auxin-dependent gene expression; overal morphogenesis normal; **allelic to *msg1*, *tir5***.	LISCUM & BRIGGS 1995 LISCUM & BRIGGS 1996 STOWE-EVANS *et al.* 1998
msg1	*A. thaliana* (EMS)	↑resistance (hyp. growth curvature)	↑resistance (hy. growth)	R	↑leaf resistance to 2,4-D; rosette leaves epinastic or hyponastic; largely wt phenotype; **allelic to *nph4*, *tir5***.	WATAHIKI & YAMAMOTO 1997 WATAHIKI *et al.* 1999
dwf	*A. thaliana*	↑resistance (root elong.)	↑resistance (root elong.)	D	Impaired root and hypocotyl gravitropism; increased root and reduced hypocotyl length, reduced number of lateral and root hairs, dwarf plants with abnormal leaf shape; homozygous plants lethal.	MIRZA *et al.* 1980 MAHER & MARTINDALE 1980 MIRZA *et al.* 1984 MIRZA & MAHER 1985 MIRZA & MAHER 1987
agr	*H. vulgare*	– (root gravitrop.)	↑resistance (root growth)	R	↑resistance of roots to IAA but not to NAA or 2,4-D.	TAGLIANI *et al.* 1986

They proposed that the loss of PIN2 function impaires basipetal auxin transport. According the model of MORRIS *et al.* (1991) that auxin efflux carrier complex consists of at least three components: a transmembrane carrier protein, a NPA binding protein and a third component of high turnover, MÜLLER *et al.* (1998) propose that PIN2 represents likely the transmembrane component of the auxin efflux carrier. *PIN2* was independently cloned in other three laboratories (CHEN *et al.* 1998; UTSUNO *et al.* 1998; LUSCHNIG *et al.* 1998) and accordingly it was shown to encode a root-specific member of a novel membrane protein family, supporting the idea about its role in the transport of auxin.

An auxin supersensitive mutant *sax1* was isolated and characterized (EPHRITIKHINE *et al.* 1995; FELLNER 1997). Interestingly, *sax1* is the first mutant selected originally for its increased responsiveness to auxin. The increased sensitivity of mutant root elongation growth to auxin is consistant with fact that the root and hypocotyl are short and the cotyledons are epinastic. However, certain phenotypic traits of the mutant correspond rather to character of mutants resistant to auxin, for example the dwarf phenotype, less-branched roots and reduced fertility. Such controversial traits evoked the question whether *sax1* is specifically more sensitive to auxin. Consequently, further physiological experiments revealed that *sax1* is also more sensitive to ABA (sensitive to ABA and auxin) for seed germination, root elongation and stomatal closure. Further analysis revealed that the *sax1* plants were in other respects sensitive to GA_3 and the ethylene precursor ACC. The mutation appeared nuclear (mapped on chromosome 1, Ephritikhine and Vannini, unpublished results), recessive and monogenic. Very recently, it was shown that *sax1* phenotype could be partially rescued by brassinosteroids, and that *sax1* mutation defines a new locus involved in the brassinosteroid biosynthesis pathway (PAGANT *et al.* 1998).

Auxin resistant mutants

A relatively high number of auxin resistant mutants have been found compared to auxin supersensitive plants. However, only a few mutants exhibit specific auxin resistance. For example, the *rac*⁻ mutant has been selected for auxin resistance *in vitro* in populations of mesophyll protoplasts of *Nicotiana tabaccum* (MÜLLER *et al.* 1985). The mutants were completely blocked in primary root development resulting from nuclear, monogenic and dominant mutation. Derived protoplasts exhibited increased resistance to auxin (by about factor 10) compared with wild-type for the auxin-induced hyperpolarization (EPHRITIKHINE *et al.* 1987).

Other specific auxin resistant mutants were isolated by culturing M_2 *N. plumbaginifolia* seedlings on the auxin concentration significantly inhibiting hypocotyl elongation of the wild-type (BLONSTEIN *et al.* 1991). The auxin resistance of all the mutants was the result of a mutation at a single, nuclear locus. The mutant called *R29* was recessive, *R1* and *R15* showed partial dominance, and *R25* exhibited dominant inheritance. *R25* was shown to be strongly resistant to NAA compared with wild-type in inducing hypocotyl cell hyperpolarization (STIRNBERG *et al.* 1995).

The specific resistance of *rac*⁻ and *R25* mutants to auxin in their electrical responses suggests a defect at early steps in auxin action, which is consistent with recent results obtained on the *rac*⁻ mutant. LUND and collaborators (1996) showed that in *rac*⁻ formation of adventitious root meristems is completely blocked in stem cuttings even at high auxin concentrations, even though cell division remains responsive to auxin. In relation to previous results, the authors proposed that *rac*⁻ mutation either causes a higher threshold of auxin binding required for signaling in root initiation than for cell division, or perhaps it may be due to the existence of different auxin receptors for the two responses.

Some auxin resistant mutants were found that exhibited cross-resistance to other plant hormones. In *N. plumbaginifolia* auxin resistant mutants *iba1*, *iba2* and *iba3* were isolated as germinating on a selection medium containing high auxin and cytokinin concentrations (BITOUN *et al.* 1990). For each mutant, resistance was conferred by a single recessive mutation. Although the function of the three genes is not known, it was verified that the mutants did not show an alteration in the uptake or metabolism of applied NAA. In addition, homozygous *iba1* mutant seeds exhibited resistance to paclobutrazol and to ABA, and simultaneously, adult plants were supersensitive to water stress. Thus, the authors suggested that the *IBA1* gene may be involved in the control of ABA/GA balance. Because, simultaneously, *iba1* mutants are still sensitive to ABA and GA, BITOUN *et al.*(1990) assumed that *iba1* mutation affects the level of ABA or GA rather than the transduction of ABA or GA signal. Because *iba1* mutants exhibit a syndrome characteristic of defects in the biosynthesis, metabolism or location of ABA, and wild-type plants treated with GA exhibit the same syndrome, including auxin resistance, the authors proposed that auxin resistance of the *iba1* mutant may be a consequence of ABA--deficiency or overproduction of GA.

The tomato *dgt* mutant found to be resistant to auxin was originally selected for its abnormal orientation of root and shoot (horizontal growth, i.e. diagravitropism) (ZOBEL 1973). Mutation at *DGT* gene was nuclear, recessive, and led to other pleiotropic effects such as lack of lateral roots on primary and adventitious roots, abnormal vascular tissue (without large secondary xylem but with thick phloem fibers), altered leaf morphology (dark-green leaves with hyponastic segments) and open hypocotyl hook in dark-grown seedlings. The *dgt* mutant exhibited auxin insensitivity in several specific responses. It was blocked in auxin-induction of petiole epinasty (URSIN & BRADFORD 1989) and impaired in auxin-induced ethylene formation (ZOBEL 1973; KELLY & BRADFORD 1986). The mutant required only extremely low concentrations of ethylene for normal growth and development (ZOBEL 1974). Further it was shown that, despite the reduced sensitivity to auxins, *dgt*

hypocotyls elongated dramatically and synthesized ethylene rapidly to elongate in response to auxins. This suggested that the primary effect of the *dgt* mutation was to reduce the sensitivity of the tissue to auxin *via* an altered perception or action mechanism (altered regulation of ethylene synthesis being only one sympton) (KELLY & BRADFORD 1986). The suggestion that a defect in the *DGT* gene associates may be in a primary site of auxin perception or action was recently suspected by MUDAY *et al.* (1995). The authors reported that the *dgt* lesion appears unrelated to auxin transport, uptake, or binding of auxin-transport inhibitor, as these processes were all similar in mutant and wild-type roots. HICKS *et al.* (1989b) tested the idea that mutated *DGT* gene codes for a receptor protein. Using photoaffinity labeling, they showed that the photoaffinity auxin analog [^{3}H]5N$_3$-IAA specifically labeled the 40/42-kDa auxin-binding protein N$_3$-IBP (HICKS *et al.* 1989a) in hypocotyl and roots of wild-type but only in the roots of the *dgt* mutant. These data suggest that 40/42-kDa protein is part of a physiologically important auxin receptor system which is altered in the *dgt* mutant, probably in a tissue-specific manner. Because the *dgt* mutant is reduced in the above mentioned auxin-mediated responses, MITO & BENETT (1995) investigated the possibility of a *dgt* mutation affecting the expression of several auxin-regulated genes. They showed that the *dgt* mutation inhibited the induction of *LeAux* and *LeSaur* mRNA accumulation by NAA but had no effect on NAA-induced *Lepar* mRNA accumulation. These data suggest the presence of two auxin-dependent signal transduction pathways: one is interrupted by the *dgt* mutation, but the second one, regulating *Lepar* mRNA accumulation, is not.

In *Arabidopsis*, most auxin resistant mutants have been isolated by screening seedlings for root elongation on inhibitory concentrations of auxin. These resistant mutants, defining at least six loci (Table I) are resistant to all auxins, and the defects in each of these genes result more or less in a defect in root gravitropism. These results support evidence for a role of auxin in gravitropism. In addition, most of the mutants are cross-resistant to other plant hormones.

The *axr1* mutant was screened for reduced sensitivity of its roots to IAA, 2,4-D or NAA (ESTELLE & SOMERVILLE 1987). Later on it was shown that all the eight recessive alleles of *axr1* found also confer resistance to cytokinin and ethylene (LINCOLN *et al.* 1990). The *axr1* mutants display a number of morphological defects that might be explained by reduced auxin sensitivity. Mutant roots are longer and less branched, and hypocotyl and stem elongation is reduced. Plants have smaller rosette leaves, more branched and shorter inflorescence stems with less developed vascular tissue and flowers. The *AXR1* gene maps on chromosome 1 (LINCOLN *et al.* 1990) and has been cloned by chromosome walking (LEYSER *et al.* 1993). *AXR1* encodes a protein related to ubiquitin-activating enzyme E1, which catalyzes the first step in the biosynthesis of ubiquitin-protein conjugates. However, comparison of AXR1 and E1 proteins showed that AXR1 lacks several residues and domains, such as an active-site cysteine residue, necessary for E1 function. The existence of a relationship between AXR1 and E1, and the fact that some auxin-induced genes (*SAUR, PS-IAA4/5*) may be regulated by short-lived proteins suggest that through the action of AXR1, auxin may stimulate ubiquitin-mediated degradation of a putative repressor of auxin-regulated genes (ABEL *et al.* 1994). TIMPTE *et al.* (1995) showed that *axr1* plants display a significant deficiency in auxin-induced accumulation of *SAUR-AC1* mRNA in seedlings, rosette leaves and mature roots confirming that the *AXR1* gene is required very early in an auxin response pathway. CERNAC *et al.* (1997) provided new information about the *AXR1* gene. They identified a new locus, *SAR1* (suppressor of auxin resistance), and showed that the s,a,r1-1 mutation suppresses every aspect of the axr1 phenotype, and that this suppression is *axr1* specific. *sar1-1axr1* and *sar1AXR1* plants are indistinquishable, indicating that sar1 is epistatic to *axr1*. Therefore, CERNAC and collaborators (1997) propose that the *SAR1* gene product is a potential substrate of AXR1, i.e., it acts after AXR1, and that a major function of AXR1 is to relieve SAR1 mediated repression of auxin response. Recently, DEL POZO *et al.* (1998) identified sequence that encodes a protein ECR1 which function together with AXR1 as E1-like heterodimer which activates RUB1, member of the family of small ubiquitin-related proteins called RUBs (related to ubiquitin). This could show analogy with system in yeast where AOS1 protein (homologous to AXR1) forms a heterodimer with the second protein, Uba2p, which is homologous to C-terminal half of E1 (JOHNSON *et al.* 1997). By activation of RUB1, a pathway similar to the ubiquitination is activated in which RUB1 is covalently attached to a target protein. AXR1 was localized primarily to the nucleus of dividing and elongating cells, suggesting that the targets of RUB1 modification are nuclear. These results indicate that auxin response depends on RUB1 modification of one or more nuclear proteins (DEL POZO *et al.* 1998). The authors propose that activated RUB1 protein may modify, perhaps in response to auxin, activity of a ubiquitin-ligase complex called SCF that is required for degradation of negative regulator of auxin response. Possible targets for such modification include proteins involved in auxin-mediated regulation of the cell cycle, the Aux/IAA proteins, and other auxin signaling proteins (DEL POZO *et al.* 1998; HOOLEY 1998).

To identify additional genes involved in auxin physiology, CERNAC *et al.* (1994) have screened for *Arabidopsis tir* mutants that are resistant to the growth-inhibiting properties of the auxin-transport inhibitors NPA and CPD. From them, RUEGGER *et al.* (1997) identified recently seven new genes *TIR1* through *TIR7*, of which mutants *tir1* and *tir3* are best characterized. Results on *tir3* suggest that the *TIR3* gene functions in auxin transport (see below). Mutant *tir1* (RUEGGER *et al.* 1998) was also found to be deficient in hypocotyl elongation and lateral root formation, in addition to its re-

sistance to auxin transport inhibitors, indicating that *TIR1* also is required for normal response to auxin. Because auxin transport inhibitors affect root growth by increasing auxin level in the root tip, the authors suggest that resistance of *tir1* to NPA and CPD could result from a change in auxin transport or a reduction in auxin response. RUEGGER *et al.* (1998) later showed that polar auxin transport in *tir1* stem segments is similar to wild-type, and simultaneously that mutant seedlings were less sensitive to the growth-inhibiting effect of the auxin. Together, these results suggest that the primary effect of the mutation *tir1* is on auxin response and not auxin transport. A mutation in *TIR1* displays a synergistic interaction with mutation in *AXR1*, suggesting that the two genes function in overlapping pathways. Sequence comparisons indicated that *TIR1* encodes a F-box protein related to the yeast protein Grr1p and the human ubiquitin SKP2 (RUEGGER *et al.* 1998). F-box proteins have been shown to function in SCF complex. Thus, F-box protein TIR1 may be part of SCF complex that is required for the degradation of negative regulator of auxin response.

Dominant mutants at the locus *axr2* were found by screening an EMS-mutant library for seedlings resistant to high concentrations of auxin (WILSON *et al.* 1990). Mutant hypocotyls and roots were short and had defects in gravitropic response. Their seedlings lacked root hairs and were extremely dwarf. Mature *axr2* mutants were also very dwarf with agravitropic inflorescences, so the *axr2* phenotype was due to a dramatic decrease in stem cell elongation (TIMPTE *et al.* 1992). Besides auxin, the mutant roots were also resistant to ethylene and abscisic acid (WILSON *et al.* 1990) and cytokinin (KLEE & ESTELLE 1991). However interestingly, KNEE & HANGARTER (1994) found that dark-grown *axr2* seedlings had their root growth promoted at low IAA concentrations (0.001 to 0.1 mM) in contrast to wild-type root inhibition. In addition, at low IAA concentrations, the *axr2* mutant seedlings had more lateral roots than wild-type. Thus, the authors suggest that the *axr2* mutation does not affect sensitivity to IAA, but does effect the response to IAA in a manner that is yet to be explained. Like *axr1*, the *axr2* seedlings are defective in auxin regulation of *SAUR-AC1* expression (GIL *et al.* 1994), and it was found that no *SAUR-AC1* transcript was detected before or after auxin treatment (TIMPTE & ESTELLE, unpublished). These results indicate that the *axr2* mutation disrupts auxin action at an early step, such as perception or early transduction of the auxin signal.

The *axr3* mutant was originally isolated from an EMS-mutagenized population screened for resistance to the ethylene precursor, ACC (LEYSER *et al.* 1996). These mutants also exhibited strong resistance to auxin (1000-fold) and an unusual response to cytokinin: elongation of *axr3* roots was stimulated by concentrations which are inhibitory to wild-type root growth. The *AXR3* gene was defined by two semidominant mutations, each of which affects many auxin-regulated developmental processes. The phenotype of the *axr3* mutant has paradoxial aspects suggesting auxin supersensitivity rather than auxin resistance. The *axr3* phenotype is qualitatively opposite to the *axr1*: mutants show enhanced apical dominance, reduced root elongation, increased adventitious rooting (unusual root growth from the hypocotyl), no root gravitropism, and ectopic expression of the *SAUR-AC1* promoter. Thus, LEYSER *et al.* (1996) suggested that a primary defect in *axr3* mutant is in auxin action, particularly as the mutation did not affect auxin level. Further, *axr3* auxin-supersensitivity is consistent with root growth being stimulated by cytokinins, and with many traits of the mutant phenotype being rescued by exogenous cytokinin, probably as a result of restoration of wild-type auxin/cytokinin ratio. However, induction of *SAUR-AC1* transcript accumulation and the stimulation of callus growth occurred over the same range of auxin concentrations as in wild-type tissue demonstrating that the *axr3* phenotype does not result from uniform increase in an auxin resistance. A third possibility, that the mutation could result in a constitutive auxin-response, was raised from the fact that *SAUR-AC1* expression is an auxin dependent response. Lastly, on the basis of mutant callus proliferation, it was proposed that the mutation at *AXR3* affects the amplitude of the auxin response. In the *axr3* mutants, the modulation of the responsiveness to auxin is impaired such that the plants over-react to the auxin stimulus. Thus, the function of this gene in auxin signaling is still unknown. Recently, ROUSE *et al.* (1998) reported the sequence of the *AXR3* gene. They showed that AXR3 protein corresponds to one of the auxin-inducible *AUX/IAA* genes, *IAA17*, and the *axr3* mutations occur in one of domains conserved in this family of proteins. This finding provide strong evidence that AUX/IAA proteins can act as mediator of auxin signaling. Finally, the increased auxin response of *axr3* plants suggests that the mutations are hypermorphic and thus, the molecular characterization of the diverse *axr3* mutations offers an opportunity to understand better the molecular basis of *AUX/IAA* mediated auxin signaling (ROUSE *et al.* 1998).

The *axr4* mutant was recovered by screening T-DNA transformed lines for seedlings able to elongate roots on agar containing 0.1 µM 2,4-D, and genetic analysis revealed close linkage of T-DNA to the *axr4* mutation (HOBBIE & ESTELLE 1995). The *axr4* mutation is recessive and maps on chromosome 1. The *axr4* mutant was shown to be allelic to the polar transport inhibitor-resistant mutant *rgr1* (SIMMONS & SÖLL 1995) selected originally for its reduced root gravitropism (SIMMONS *et al.* 1995). The phenotype of mutant plants appeared to be largely of wild-type except for a strong defect in root gravitropism. Physiological analysis showed that the mutant is specifically less sensitive to auxin (about five-fold), the first auxin-specific mutant, thus pointing out the originality of *axr4*. Analysis of the double mutant *axr4axr1* revealed that the two mutations have additive (or synergistic) effects. The authors therefore proposed that the *AXR4* and *AXR1* gene products may have a more complex relationship: they may act in separate pathways of auxin response or perhaps perform partially redundant functions on a single pathway.

The *aux1* mutant was originally selected for its resistance to auxin of root elongation (MAHER & MARTINDALE 1980). In addition, it displayed reduction in sensitivity to ACC and ethylene (PICKET *et al.* 1990) and cytokinin (LINCOLN *et al.* unpublished results). On the basis of genetic analysis of ethylene signal transduction in *Arabidopsis*, ROMAN *et al.* (1995) suggest that *AUX1* gene may function in the interaction between ethylene and other plant hormones late in the signaling pathway. Like *axr4*, *aux1* plants have a less pleiotropic phenotype than other auxin-resistant mutants. *aux1* plants have shorter root hairs than wild-type (OKADA & SHIMURA 1994) and show dramatic reduction in root gravitropism (PICKET *et al.* 1990). In addition, *aux1* roots were shown to be deficient in touch-induced rotation of root tips (OKADA & SHIMURA 1990). Detailed studies of the *aux1* root structure and organelle movement during gravitropic stimulation revealed that starch-containing amyloplasts sediment more slowly than in wild-type (OLSEN *et al.* 1984). Nine independent recessive alleles were mapped to a single *AUX* locus on chromosome 2. Isolation of new alleles of locus *AUX1* tagged with T-DNA allowed the isolation and characterization of the *AUX1* gene (BENNETT *et al.* 1996). The AUX1 polypeptide exhibits sequence similarities to a family of plant and fungal amino acid permeases (proteins facilitating transport of amino acids), suggesting that AUX1 mediates the transport of an amino acid-like signalling molecule. IAA, which is structurally similar to the amino acid tryptophan, is thus a likely substrate. It was proposed that AUX1 may mediate proton-driven IAA uptake, as plant amino acid permeases mechanically function as proton-driven symporters (BUSH 1993). BENNETT and co-authors (1998) further showed that *AUX1* is expressed in root apical tissues that control the root gravitropic response and also in root epidermal cells. Gravitropism is often associated with auxin transport because auxin transport inhibitors can abolish gravitropic bending (MUDAY & HAWORTH 1994), and two auxin transport streams exist in roots (TSURUMI & OHWAKI 1978). These observations led to the idea of close association of *AUX1* expression and basipetal auxin transport (BENNETT *et al.* 1996). Recently, YAMAMOTO & YAMAMOTO (1998) discovered that sensitivity of *aux1* seedlings to various auxins correlates with suitability as substrates for the influx carrier (DELBARRE *et al.* 1996), e.g. *aux1* is resistant to IAA and 2,4-D, but sensitive to NAA since it altered agravitropic nature of *aux1* root growth. These results support the model that *AUX1* may encode the auxin influx carrier. On the basis of genetic analysis of an *aux1axr1* double mutant TIMPTE *et al.* (1995) proposed that mutations at *AUX1* and *AXR1* have additive effect on hormone sensitivity. This suggestion was supported by results of the cloning of the *AUX1* gene which confirmed that mutations at the *AUX1* and *AXR1* genes confer hormone resistance by different mechanisms. As to the relationship to the specifically auxin resistant *axr4* mutant, studies on the double mutant *aux1axr4* showed that the *aux1* mutation is epistatic to *axr4* with respect to auxin-resistant root elongation, whereas in lateral root formation, the effects of the two mutations are additive (HOBBIE & ESTELLE 1995). These results again suggest differences in the mechanism of auxin action during root elongation and the formation of lateral roots.

WATAHIKI *et al.* (1997) isolated recessive *msg1* mutant in *Arabidopsis* that exhibited resistance to IAA-induced hypocotyl growth curvature. The mutant hypocotyls were found to be resistant to 2,4-D in their growth and gravitropic response, but the roots responded normally to the auxin. The *msg1* hypocotyl growth did not differ in sensitivity to BAP, ABA, and ACC. The *msg1* leaves were also resistant to 2,4-D-induced chlorosis, and rosette leaves of mature plants and etiolated seedlings were either epinastic or hyponastic, dependently on allele, whereas the other phenotypic traits were generally wild-type in appearence. The authors reported that *msg1* is the first auxin-insensitive mutant in which its effects are mostly restricted to the hypocotyl and leaf, and it appears to be auxin specific. Recent results of WATAHIKI *et al.* (1999) suggest that *MSG1* acts independently from *AXR1* in separate pathways of the growth-curvature responses of hypocotyls. The authors showed that, in contrast, *MSG1* action is not affected by *AUX1* in any of the phenomena studied.

Earlier, *nph4* mutant in *Arabidopsis* was isolated by LISCUM & BRIGGS (1995) that exhibited, additionally to the altered phototropic responsiveness, also defect in hypocotyl gravitropic response and hook formation, suggesting that *NPH4* may function directly in the differential growth response (LISCUM & BRIGGS 1996). The *nph4* locus was mapped in chromosome 5 (STOWE-EVANS 1998) and similar map position has been reported for *msg1* (WATAHIKI & YAMAMOTO 1997) and *tir5*, identified by its resistance to auxin-transport inhibitors (RUEGGER *et al.* 1997). A complementation tests between *nph4* and *msg1*, and *nph4* and *tir5* have shown that these mutations occur at the same locus (STOWE-EVANS *et al.* 1998). Results from physiological studies demonstrate that NPH4 activity is conditionally required for a number of differential responses including phototropism, gravitropism, phytochrome dependent hypocotyl curvature, apical hook maintenance, and abaxial/adaxial leaf-blade expansion (STOWE-EVANS *et al.* 1998). Since the *nph4* exhibited severely impaired auxin-dependent gene expression, defects associated with differential growth have an origin in altered auxin responsiveness. Genetic and molecular studies indicate that NPH4 represents a temporally early-acting, concentration-dependent modulator of an auxin-response pathway(s) leading to differential growth (STOWE-EVANS *et al.* 1998).

Auxin polar transport mutants

Polar transport of IAA in plant tissue has been well demonstrated in a number of plant species and is believed to regulate a variety of developmental processes

Tab. II.: Auxin polar transport mutants.

Locus/ mutant	Species (mutant library)	Response used as selection criterion (selection parameter)	Observed auxin transport activity (in)	Inheritance	Additional phenotypic traits and short description	References
MUTANTS ALTERED IN AUXIN POLAR TRANSPORT						
pin1	*A. thaliana* (EMS)	– (flower develop.)	reduced (stem)	R	Reduced IAA content; abnormal vascularization, abnormal or none flowers, reduced fertility, abnormal leaf shape; **gene cloned**.	OKADA *et al.* 1991 GÄLWEILER *et al.* 1998
lop1	*A. thaliana* (T-DNA)	vascular patterning (leaf morphology)	reduced (stem)	R	Abnormalities in vascular development of leaves, abnormal pattern of root cell expansion and secondary root initiation.	CARLAND & MCHALE 1996
mp	*A. thaliana* (EMS)	– (root and hypocotyl phenotype)	reduced (inflorescence)	R	Reduced auxin transport capacity; lack basal body structures: hypocotyl, radicle, root meristem; alterations in octan stage embryo; reduced or none lateral flowers; reduction of vascular tissues and incomplete continuity within vascular strands; **gene cloned**.	MAYER *et al.* 1991 MAYER *et al.* 1993 BERTLETH & JÜRGENS 1993 PRZEMECK *et al.* 1996 HARDTKE & BERLETH 1998
pid	*A. thaliana* (EMS)	loss of flower production	reduced (stem)	R	Reduced auxin polar transport only after stems have ceased elongation; inflorescence defects, defects in floral organ developments, cotyledons and leaves.	BENNETT *et al.* 1995
La	*L. esculentum*	– (leaf morphology)	reduced (hypocotyl)	SD	Heterozygous: simple lanceolate leaves; homozygous: naked, tendril like hypocotyl without cotyledons and shoot apex; plants bushy; larger shoot apical cells.	MATHAN & JENKINS 1962 CARUSO 1968 LEVERONE *et al.* 1992 CARUSO *et al.* 1994 AVASARALA *et al.* 1996
AUXIN POLAR TRANSPORT INHIBITOR MUTANTS						
tir3	*A. thaliana* (EMS)	↑resistence to NPA (root elong.)	reduced (inflorescence)	R	↑resistance to auxin transport inhibitors NPA and CPD, reduced auxin polar transport activity; deficient in lateral root production; reduced apical dominance, decreased elongation of siliques, pedicels, roots, inflorescences.	CERNAC *et al.* 1994 RUEGGER *et al.* 1997
pis1	*A. thaliana* (EMS)	↑resistence to NPA (root growth)	?	R	↑sensitivity to auxin transport inhibitors NPA and TIBA in root elongation, root gravitropism, root phototropism, root curling.	FUJITA & SYÖNO 1997

Tab. II.: Continued.

Locus/ mutant	Species (mutant library)	Response used as selection criterion (selection parameter)	Observed auxin transport activity (in)	Inheritance	Additional phenotypic traits and short description	References
rcn	*A. thaliana* (T-DNA)	↑resistance to NPA (root curling)	?	R	↑resistance to NPA in root curling, ↑sensitivity to NPA in hypocotyl and root elongation and auxin accumulation; altered root and hypocotyl growth patterns; defect in apical hook formation; *RCN1* linked to T-DNA; **gene cloned**.	GARBERS *et al.* 1996
tis	*A. thaliana* (EMS)	altered sensitivity to auxin transport inhibitors	?	?	↑sensitivity to auxin transport inhibitors.	CERNAC *et al.* 1994
cmr	*A. thaliana*	↑resistance to CM	?	?	↑resistance to chlorfurenol-methyl (CM) but not to chlorfurenol and other auxin transport inhibitors.	RUEGGER *et al.* (unpublished)
agr1	*A. thaliana* (EMS)	see auxin supersensitive mutant ***agr3***				
sax1	*A. thaliana* (EMS)	see auxin supersensitive mutants				
tir1	*A. thaliana* (EMS, T-DNA)	see auxin resistant mutants				
rgr1	*A. thaliana* (T-DNA)	see auxin resistant mutants				
dgt	*L. esculentum*	see auxin resistant mutants				

(GOLDSMITH 1977). Chemiosmotic hypothesis of the auxin polar transport was independently proposed by RUBERY & SHELDRAKE (1974) and RAVEN (1975). This model assumes that auxin polar transport occurs through the action of cellular auxin influx and efflux carriers located in the plasma membrane of transporting cells. Explanation of polarity of transport was supported by JACOBS & GILBER (1983) who demonstrated basal location of a putative efflux carrier. The problems of auxin polar transport was recently reviewed (ESTELLE 1998; JONES 1998; BENNETT *et al.* 1998; LEYSER 1999).

Genetic approach aiming at the isolation of auxin polar transport mutants was developed during these last few years. The mutants sorted out can be subdivided in two classes, according to their transport properties (Table II).

Mutants deficient in auxin polar transport

In *Arabidopsis*, the recessive *pin1* mutant was originally identified because of abnormalities in the inflorescence (OKADA *et al.* 1991). Homozygous *pin1* mutants completely lack flower buds and polar auxin transport activity in their stems is reduced to about 7 to 14 % of the wild-type level. The authors also showed that treatment of *Arabidopsis* plants by auxin transport inhibitors (NPA, HFCA) led to a pin-formed phenotype, probably as a result of the reduction of auxin polar transport and the consequent auxin accumulation in the meristem. In addition, authors showed that treatment of wild-type plants by the anti-auxin PCIB has no effect on the inflorescence structure. These results were supported by the fact that *Brassica juncea* plants treated by auxin polar transport inhibitors developed embryos phenocopying the *pin1* mutant (LIU *et al.* 1993). Thus, OKADA *et al.* (1991) suggested that a primary function of the *PIN1* gene is the auxin polar transport in the inflorescence axis. Simultaneously, *pin1* had only about 8 % endogenous free IAA of wild-type. It is not known if the *PIN1* gene product is involved in both the polar transport system and IAA biosynthesis, or whether decreased level of IAA is a secondary effect of decreased auxin polar transport activity (OKADA *et al.* 1991). The *PIN1* gene was recently cloned by transposon tagging and was found to encode protein with similarity to bacterial and eucaryotic carrier proteins, and PIN1 protein was detected at the basal end of auxin transport-competent cells in vascular tissue of the shoot (GÄLWEILER *et al.* 1998). Thus, the authors suggest that PIN1 may act as a transmembrane component of the auxin efflux carrier. PIN1 is 64 % identical to PIN2 but, unlike PIN2 specifically functioning in roots, the polar localization of PIN1 in the inflorescence axis consistants with the proposed distribution of auxin efflux carriers that mediate shoot-basipetal auxin transport (GÄLWEILER *et al.* 1998).

Another mutant found in *Arabidopsis* to be affected in basipetal auxin transport was called *lop1* (CARLAND & MCHALLE 1996). The mutant was isolated from T-DNA mutant collection as potentially affected in vascular patterning in the leaves. The *lop1* mutant developed leaves whose midveins had disoriented axial growth, and bifurcated into twin veins rotated out of the normal dorsal/ventral axis. Mutant plants also showed abnormal patterns in cell expansion in the cortex and epidermis of the elongation zone of lateral roots. Mutant plants had a normal level of free IAA and did not show alterations in response to exogenous auxin. Basipetal auxin transport in mutant stems was reduced to about 10 % of the wild-type level. These defects may explain abnormalities observed in patterns of cell expansion and general disorganization of the *lop1* root elongation zone, as epidermal cells are known to be site of the basipetal auxin transport stream in roots (LOMAX *et al.* 1995). Thus, it appears that mutation at the *LOP1* gene has a rather specific effect on auxin polar transport in comparison to the *pin1* mutant which is strongly affected in the level of free IAA, so that auxin transport is the primary lesion of *lop1* mutant.

The monopteros, *mp*, mutant was isolated on the basis of its seedling phenotype folowing EMS mutagenesis (MAYER *et al.* 1991). The *mp* mutant seedlings lack basal body structures such as hypocotyl, radicle and root meristem. Cells of the embryo fail to establish division patterns that would normally generate these structures (BERLETH & JÜRGENS 1993). The results of Berleth and Jürgens support the idea that the *MP* gene is required for organizing the basal body region in the developing embryo, rather than for making the root. PRZEMEK *et al.* (1996) used the capacity of *mp* mutant seedlings to form adventitious roots and they showed that mutants form normal rosettes and root systems, but inflorescences failed to form lateral flowers or these flowers were greatly reduced. The auxin transport capacity of inflorescence axis was impaired and the vascular strands in all analyzed organs were distorted. The *MP* gene was isolated by using positional cloning (HARDTKE & BERLETH 1998) and shown to encode a transcriptional factor, IAA24, which binds to auxin response elements, AREs (ULMASOV *et al.* 1997). IAA24 is similar to ARF1, the functionally tested ARE-binding factor. The *MP*mRNA accumulates subepidermally throughout globular-stage embryos, and is gradually resctricted to central domains of later stage. The IAA24 appears to allow a canalization of auxin flow and thus to define the embryo axis (NELSON 1998).

Another polar auxin transport affected mutant in *Arabidopsis*, *pid*, was isolated and characterized (BENNETT *et al.* 1995), and it is briefly described in Table II.

The tomato *La* (lanceolate) mutant was isolated by MATHAN & JENKINS (1962), exhibiting a simple lanceolate shaped leaves, as result of the action of a semi-dominant gene in the heterozygous condition. The homozygous mutant displayed a naked, tendril-like hypocotyl without cotyledons or shoot apex. Mature plants were bushy with small lanceolate leaves and tendrils in the inflorescence. This mutant was found to have reduced rate of IAA transport in hypocotyl segments

(LEVERONE *et al.* 1992), as observed for the *pin1* mutant. CARUSO (1968) observed that in the mutant shoot apical region cells were relatively large, and later on, it was found that *La* mutant hypocotyls contained more free IAA (CARUSO, unpublished) and that hypocotyl segments accumulated more exogenously applied auxin than did wild-type (CARUSO *et al.* 1994). Thus, the *La* gene may cause an impairement of auxin transport, giving a local accumulation. Consequently, the higher level of auxin leads to the enlargement of shoot apical cells. Young wild-type tomato seedlings treated by polar auxin transport inhibitors NPA and HFCA developed a phenocopy of the heterozygous and homozygous lanceolate mutant (AVASARALA *et al.* 1996). Together, these results suggest that the *LA* gene in tomato impairs polar auxin transport.

Mutants affected in sensitivity to auxin polar transport inhibitors

A second class of auxin transport mutants is represented by plants affected in their sensitivity to auxin polar transport inhibitors.

RUEGGER *et al.* (1997) analyzed of the *tir3* mutant, which had been selected for its reduced response to auxin transport inhibitors NPA and CPD. Mutant plants were also reduced in polar auxin transport capacity, and exhibited a variety of morphological defects that could be ascribed to changes in IAA distribution. *tir3* seedlings were strongly deficient in lateral root production, they displayed a reduction in apical dominance and decreased elongation of siliques, pedicels, roots and inflorescences. RUEGGER *et al.* (1997) found that *tir3* plants had a fewer of NPA binding sites and suggested that the *TIR3* gene is required for expression, localization, or stabilization of the NPA binding protein (NBP) associated with the efflux carrier. Alternatively, the *TIR3* gene may encode the NBP. Because the *tir3* mutants have a substantial defect in NPA binding, their phenotype provides genetic evidence for a role for the NBP in plant growth and development.

Polar auxin transport inhibitor sensitive mutant *pis1* in *A. thaliana* has been recently isolated and characterized by FUJITA & SYONO (1997). This NPA supersensitive mutant was screened on medium containing 0.3 µM NPA which slightly inhibits root growth and root gravitropism in wild-type seedlings. The authors showed that this mutant is supersensitive to NPA and TIBA over the broad spectrum of its effects such as growth of seedlings, root elongation, root gravitropism, root phototropism and root curling. The fact that the *pis1* is normally sensitive to phytohormones indicates that the *pis1* mutant is specifically affected in the polar auxin transport system. The authors propose that *PIS1* gene product might be specifically involved in the response pathway of NPA and TIBA, leading to interference with auxin efflux carriers, and might act as a negative regulator of Finally, hormones do interfere with each other in a common final response such as in stem elongation where the action of NPA and TIBA. DELBARRE *et al.* (1996) have reported that NPA can influence efflux and influx carrier activities within tobacco suspension cells. Thus, possibly, *PIS1* gene may act as a negative regulator of both the efflux and influx carriers (FUJITA & SYONO 1997; BENNETT *et al.* 1998).

A mutant designated *rcn1* was isolated by screening a collection of *Arabidopsis* lines generated by T-DNA insertional mutagenesis on NPA containing medium for root curling seedlings (GARBERS *et al.* 1996). Although NPA blocks root curling in wild-type plants, *rcn1* mutants exhibited increased curling in the presence of NPA. They found that *rcn1* plants were more sensitive to NPA in hypocotyl elongation and in auxin accumulation, but mutant root elongation in the presence of NPA was identical to the wild-type. Simultaneously, mutants exhibited identical sensitivity to 2,4-D and TIBA in root and hypocotyl elongation response. Dark-grown *rcn1* seedlings also had a defect in apical hook formation, a morphological process requiring polar auxin transport (ROTHENBERG & ECKER 1993). Genetic analysis showed that the *rcn1* mutation is recessive and nuclear encoded, and that it is tightly linked to T-DNA. Cloning the *RCN1* gene revealed that T-DNA insertion disrupts a gene for the regulatory A subunit of protein phosphatase 2A, a serine/threonine phosphatase (PP2A-A) (GARBERS *et al.* 1996). Protein phosphatases are known to play important roles in enzyme regulation, gene expression, and signal transduction (MUMBY & WALTER 1993). Thus, protein phosphatase 2A appears to be involved in the regulation of auxin transport in *Arabidopsis*. GARBERS and co-authors (1996) proposed that the *rcn1* mutation alters the level of phosphorylation and thus the activity of a component involved in the regulation of auxin transport.

Finally, other *Arabidopsis* mutants were isolated with altered response to polar auxin transport inhibitors. The transport inhibitor sensitive *Arabidopsis* mutants *tis* (CERNAC *et al.* 1994), and *cmr1* (RUEGGER *et al.* unpublished) resistant to chlorfurenol-methyl were found, and their detailed genetic and physiological characterization is in progress.

Conclusion and perspectives

Auxin-related mutants have been isolated from diverse selection strategies. Most of the mutants were not specifically affected in their sensitivity to auxin, but exhibited more or less cross-sensitivity to other plant hormones. These facts are not surprising for several reasons. First, a hormone like auxin is well known for its pleiotropic effects on plant growth and development. It stimulates cell elongation, cell division and cell differentiation (such as secondary root formation). There is redundancy between hormones, for example auxin and gibberellin both stimulate cell elongation in the stem. besides the hormones with positive action (auxin and gibberellin) two other hormones act with a negative

fashion (ABA and ethylene). In addition to these characteristics of phytohormone action, environmental stimuli, such as light, also interact with hormone signalling pathways. In these conditions, an altered response to auxin may be a consequence of defects in a specific step of auxin signalling, but more likely in a common step to different transduction pathways.

The results of different selection systems raise the question of what would be ideal selection procedure to search for auxin-response mutants. An optimal screen should be based on a cellular response, be specific, fast, and easy to measure, i.e. the opposite of a growth response which is too integrated. A good example of such a successful screening strategy is illustrated by the ethylene response mutants (BLEECKER *et al.* 1988), isolated on the basis of a specific, and relatively rapid response (3 days). The knowledge of the cellular mechanisms for auxin action is still limited and it is difficult to imagine building a selection system directly based on an auxin specific cellular response like the stimulation of the H^+-ATPase activity. However, at the moment, at least two examples illustrate some new promising screening strategies. An interesting strategy was developed by P. HOOYKAAS and his group for the investigation of the signal transduction pathway components which are involved in the regulation of the expression of auxin inducible genes. A construct made of an auxin-inducible promoter linked to the GUS reporter gene was introduced in *Arabidopsis*. The selection was applied to transformed plants treated by EMS and consisted in searching for down or up-regulated gene fusions (VAN DER KOP *et al.* 1996). Similarly, targeted genetic screen aimed at further elaborating the role and regulation of early auxin-responsive genes yielded the *age1* and *age2* mutants exhibiting tissue-specific enhanced auxin sensitivity (OONO *et al.* 1998). Interestingly, the *age* mutants show phenotypic traits that are characteristic of specific defects in auxin signalling, short and bushy stature and altered root and leaf morphology. The mutant map localization indicates an existence of new auxin-related mutants (OONO *et al.* 1998). Important fact is that those mutants would not have been clearly detected in screens for auxin inhibition of root growth (HOOLEY 1998). Alternative screens built on suppression of already identified mutant or transgenic plants have recently been developed (CERNAC *et al.* 1997). Such screens provide the possibility of isolation of mutants which may be a wild-type phenotype, or they may have a phenotype that would not easily be recognized as being auxin related.

The spectrum of mutants mentioned in this report illustrates the success of the genetic approach in identifying genes involved in auxin action. The phenotypic traits of these mutants support many theories originally developed using biochemical and physiological approaches. Future analysis of such mutants should be based on cloning their gene products to obtain maximum information. Simultaneously, the mutants integrated into the experimental programmes and approaches can finally provide excellent tools for investigation of the role of auxin, and the hormones generally, in the plant kingdom.

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Abbreviations

ABA – cis-abscisic acid; ABP – auxin binding protein; ACC – 1-aminocyclopropane-1-carboxylic acid; BAP – 6-benzylaminopurine; CFM – methyl-2-chloro-9-hydroxy-fluorene-9-carboxylate; CPD – 2-carboxyphenyl-3--phenylpropane-1,2-dione; EMS – ethylmethane sulfonate; GA – gibberellin; GUS – β-glucuronidase; HFCA – 9-hydroxyfluorene-9-carboxylic acid; IAA – indole-3-acetic acid; NAA – 1-naphthaleneacetic acid; NBP – NPA binding protein; NPA – N-1-naphthyl--phthalamic acid; PBA – 2-(1-pyrenoyl)benzoic acid; PCIB – 2-(*p*-chlorophenoxy)-isobutyric acid; TIBA – 2,3,5-triiodobenzoic acid; T-DNA – transferred DNA; wt – wild-type; 2,4-D – 2,4-dichlorophenoxyacetic acid.

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