Characterization of Overexpressed cDNAs Isolated from a Hormone-Autonomous, Radiation-Induced Tumor Tissue Line of *Arabidopsis thaliana*¹

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ABSTRACT

To investigate the molecular mechanisms of hormonal control of growth, we constructed a subtracted cDNA library enriched for sequences expressed more in a hormone-autonomous, radiationinduced tumor tissue line of Arabidopsis thaliana than in normal, hormone-dependent callus. Ten cDNA clones, which are expressed 1.3- to 10-fold more in the tumor line, were isolated and partially characterized. The clones differ greatly in their level of expression in tumor tissue and in their pattern of expression in plant organs. Southern blot hybridization and sequence analysis showed that this group contains three pairs of closely related clones. Northern blot analysis indicates that one pair of clones represents two members of a gene family that are expressed in different plant organs. One of the isolated sequences shows strong sequence similarity to a cDNA encoding a lipid transfer protein. Two sequences are highly similar to those of previously described membrane channel proteins but have different organ specificities. Two other cDNAs have significant sequence similarity to glycine-rich proteins and hydroxyproline-rich glycoproteins. When used to probe Southern blots, none of the cDNAs identified polymorphisms between tumor and callus DNA, which might be expected if their overexpression were due to local genome rearrangements induced by radiation. The diversity observed among these 10 clones suggests that some are likely to be involved in tumorous growth and not simply specific to a certain cell or tissue type present in the tumor.

When tissues of most angiosperm species are placed in culture, sustained growth occurs only if the medium is supplemented with both auxin and cytokinin. To study this hormone requirement and the nature of the hormonally regulated processes, we used radiation to induce tumors on plants and from these tumors established tissue lines that exhibit hormone-autonomous growth in culture. The isolation of these tumors and the characterization of the hormone physiology of some tumor tissue lines were described previously (1, 15).

To investigate the hormone-autonomous nature of these tumor lines at the molecular level, we have begun to isolate and characterize cDNAs complementary to transcripts that are expressed more in a hormone-autonomous tumor line than in normal, hormone-dependent cultured tissue of an otherwise similar phenotype. Such cDNA sequences could represent genes whose expression is responsible for hormone autonomy (genes involved directly in hormonal regulation or in downstream events in the pathway by which hormones regulate cell growth). A second, and probably more common, class of up-regulated cDNAs would represent genes whose expression is directly or indirectly regulated by the hormonal status of the tissue. Characterization of sequences in either category is expected to contribute to an understanding of the differences in the processes of growth control and gene regulation between normal and hormone-autonomous tissue.

In this report, we describe the use of a subtracted cDNA library to isolate cDNA clones representing genes that are overexpressed in a radiation-induced tumor tissue line (relative to normal callus). Preliminary characterization of these cDNA clones included partial sequencing, northern blot analysis to study the pattern of gene expression in vivo, and Southern blot analysis to search for restriction site polymorphisms associated with these genes.

MATERIALS AND METHODS

Plant Materials and Plant Tissue Culture

All plant material was derived from *Arabidopsis thaliana* L. Isolation of γ -radiation-induced tumor tissues was described previously (15). Tumor and callus tissues were maintained on Murashige and Skoog medium (12) modified to include 30 g·L⁻¹ of sucrose, 1 mg·L⁻¹ of nicotinic acid, 10 mg·L⁻¹ of thiamine-HCl, 1 mg·L⁻¹ of pyridoxine-HCl, and 4 mg·L⁻¹ of glycine and solidified with 9 g·L⁻¹ of agar. Tissue was grown under continuous cool-white fluorescent illumination (45–70 μ mol·m⁻²·s⁻¹) at 26°C and subcultured at intervals of no longer than 30 d. Normal callus was grown on medium supplemented with either 3 μ M NAA³ plus 1 μ M BA or 2.2 μ M 2,4-D plus 0.5 μ M kinetin, and tumor tissue was grown exclusively on hormone-free medium.

Plants used for nucleic acid extractions were grown at 22°C under continuous cool-white fluorescent illumination (35–60

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³ Abbreviations: NAA, α -naphthaleneacetic acid; GRP, glycinerich protein; HRGP, hydroxyproline-rich glycoprotein; SSC, standard sodium citrate (150 mM NaCl plus 15 mM trisodium citrate, pH 7.0); NB, callus grown on medium containing NAA plus BA; DK, callus grown on 2,4-D plus kinetin.

 μ mol·m⁻²·s⁻¹) in a mixture of peat, perlite, and vermiculite (1:1:1) and irrigated with *Arabidopsis* nutrient solution (18). Tissues for RNA extractions were harvested from plants at different developmental stages. "Rosettes" were whole shoots harvested from plants that had not begun to bolt and consisted mostly of leaf tissue. Stem tissue was obtained from bolting plants. "Inflorescences" included flowers and flower buds, as well as some stem and leaf tissue. "Immature" siliques were collected soon after flowering and before much seed growth had occurred. "Mature" siliques were swollen with seeds but still green when harvested. Roots were obtained from mature (flowering) plants.

Subtracted cDNA Library

A subtracted cDNA library was constructed as described by Duguid et al. (7) using the Librarian II and Subtractor kits (Invitrogen). Briefly, separate cDNA libraries were constructed from both starting and subtracting tissues (hormoneautonomous tumor tissue and normal callus grown on NAA plus BA, respectively). Randomly oriented cDNA inserts were cloned in phagemid vector pcDNAII and transformed into Escherichia coli strain $Inv1\alpha F'$. Single-stranded DNA rescue was performed on both libraries, and the DNA from the subtracting library was biotinylated. Single-stranded DNA from the starting library was hybridized with an excess of biotinylated subtracting DNA. Hybrids and excess subtracting DNA were removed by addition of streptavidin, followed by ammonium acetate precipitation. The remaining singlestranded DNA was precipitated and then rehybridized with excess biotinylated subtracting DNA. Residual starting DNA was made double stranded using T4 DNA polymerase and transformed back into E. coli. The resulting subtracted library was screened by the procedure of Hanahan and Meselson (9) using lower colony densities. Replicate filters were probed with ³²P-cDNA synthesized from mRNA isolated from both starting and subtracting tissues. Colonies of interest were picked, and the plasmids were used to probe northern blots of RNA from tumor and normal tissues.

RNA Extraction and Northern Blot Analysis

RNA was extracted by the method of Rochester et al. (16), with the addition of 3% (v/v) isobutanol to the extraction buffer. RNA was extracted from tumor and callus tissues 14 d after subculture, when the tissues were growing rapidly. Poly(A⁺) RNA was isolated by oligo(dT)-cellulose chromatography (17). Northern blots were prepared by blotting formaldehyde gels (17) to NitroPure membrane (Micron Separations, Inc., Westbore, MA) using 10× SSC. ³²P-labeled cDNA probes were synthesized from plasmid DNA by the random hexanucleotide primer method (8). All hybridizations were performed at 65°C in the buffer of Church and Gilbert (3). Filters were washed twice in $0.1 \times SSC$ plus 0.1% SDS at 55 to 60°C and exposed to x-ray film at -70°C with two intensifying screens. The degree of hybridization of cDNA clones to RNA from different tissues was estimated by densitometric scanning of autoradiographs of northern blots. Representative blots were also probed with pB1 mcr4, which contains most of the Arabidopsis β_1 -tubulin-coding sequence, to correct for differences in sample loading. At moderate stringencies ($0.5 \times SSC$ plus 0.2% SDS, 47°C), this probe hybridizes with total β -tubulin transcripts, which are present in approximately equal amounts in all tissues (13, D. Snustad, personal communication).

DNA Extraction and Southern Blot Analysis

Genomic DNA was extracted by the method of Cullis (5), omitting the ribonuclease and pronase digestions. Plasmid DNA was isolated by the alkaline lysis miniprep method (17). Southern blots were prepared from 0.8% (w/v) agarose gels after depurination of the DNA (19). Labeled probes were synthesized by the random hexanucleotide primer method (8). Filters were hybridized at 65°C in the buffer of Church and Gilbert (3), washed twice in $0.1\times$ SSC plus 0.1% SDS at 50°C, and exposed to x-ray film at -70°C with two intensifying screens.

DNA Sequence Analysis

DNA sequencing was performed by the dideoxy termination method, using Sequenase version 1.0 (United States Biochemicals) with -20 and reverse primers. A singlestranded template was prepared using helper phage R408 (Invitrogen). A double-stranded template was obtained from plasmid minipreps. The cDNA sequences obtained were compared with the GenBank data bases using the FASTA search routine (14).

RESULTS AND DISCUSSION

Construction and Screening of the Subtracted cDNA Library

A subtracted cDNA library was constructed using radiation-induced tumor tissue line 1.2A as the starting material and hormone-dependent callus as the subtracting tissue. Tumor line 1.2A consists of friable, relatively fast-growing tissue that forms shoots in culture. NB was chosen as the subtracting tissue because it is hormone dependent, but otherwise, it resembles line 1.2A. NB tissue produced shoots and roots and exhibited a growth rate similar to that of the tumor tissue. Furthermore, analysis of two-dimensional protein gels shows few differences in protein expression between the two tissues (J. Campanella and C. Town, unpublished data).

Separate cDNA libraries were constructed from tumor line 1.2A and from callus tissue. Each library consisted of approximately 300,000 clones, most of which contained inserts of 250 to 1000 bp, although larger inserts have also been detected. A subtracted library was constructed by hybridizing [³H]thymidine-labeled, single-stranded DNA from the tumor library with a 10-fold excess of biotinylated, single-stranded DNA from the callus library. After two rounds of subtraction, 1.5% of the labeled tumor cDNA remained. Second-strand synthesis followed by transformation into *E. coli* yielded a library of approximately 17,500 clones.

Approximately 1100 colonies from the unamplified library were transferred to nitrocellulose filters, and replicate filters were probed with cDNA synthesized from both tumor and callus $poly(A^+)$ RNA. Forty-seven clones that hybridized weakly with tumor cDNA but not with callus cDNA were selected, and their plasmids were used to probe northern blots of tumor and callus RNA. This secondary screening showed that eight clones contained sequences complementary to transcripts that are more abundant in tumor line 1.2A than in the subtracting callus tissue. An additional 14 clones that did not show detectable hybridization with cDNA from either tissue were picked at random and used for secondary screening. Three of these 14 clones contained sequences that are more strongly expressed in the tumor tissue. Of this total of 11 differentially expressed cDNA clones, three proved to contain sequences more abundant in DK tissue than in tumor line 1.2A. This additional comparison was made because NB and DK tissues have different phenotypes and patterns of gene expression, and we previously found that tumor cDNAs can be expressed strongly in one type of callus and very little in the other type (15). We reasoned that sequences overexpressed in the tumor relative to both callus types were most likely to be related to hormone autonomy, and therefore, the three clones that were strongly expressed in DK callus were not analyzed further. The remaining eight clones, along with two differentially expressed clones (A16 and A26) isolated from a previously constructed library subjected to only one round of subtraction, were characterized in more detail.

Patterns of Gene Expression

The criterion for the initial selection of these cDNA clones was their elevated expression in tumor line 1.2A relative to hormone-dependent callus of a similar phenotype (NB) derived from normal tissue. Each clone was further characterized by determining the insert size and estimating the size of the mRNA to which it hybridized. Relative abundance of the homologous mRNAs in callus tissue having an undifferentiated phenotype (DK), as well as in various plant organs, was also measured. Representative northern blots are shown in Figure 1 and the results are summarized in Table I. The levels of expression of these cDNA clones in tumor 1.2A showed considerable variation. The signal produced by clone B47 was several times stronger than that of pB1 mcr4, the probe for constitutively expressed β -tubulin genes (precise determinations of relative transcript abundance were impossible due to differences in probe length and specific activity). In contrast, clones B16 and B21 (which were among those that did not hybridize detectably with either cDNA probe in the primary library screening) produced a several fold weaker signal than pB1 mcr4.

The differentially expressed cDNAs showed several different patterns of expression in plants, which suggested that their overexpression in line 1.2A was not due simply to differences in cell types present in tumor tissue as compared with callus and might, therefore, be related to tumorous (i.e. hormone autonomous) growth. For example, clone B8 was expressed almost exclusively in leaves, whereas clones B14, B16, and B141 were expressed most in roots, which were produced by the subtracting tissue but not by the tumor tissue. Most clones were expressed in several organs of normal plants. Two cDNAs (B155 and B8) were complementary to transcripts that were more abundant in tumor line 1.2A than in any normal plant tissue. Most of the cDNAs that have been tested were also highly expressed in other radiation-induced tumor lines (not shown), further supporting the hypothesis that these clones are involved in tumorous growth.

Southern Blot Analysis

In an attempt to determine whether any of the cDNA clones are overexpressed in the tumor due to changes in genome structure in the vicinity of the gene, each clone was used to probe Southern blots of genomic DNA prepared from tumor, callus, and plants, and digested with three restriction enzymes. Representative results are shown in Figure 2. Some clones (B14, B16, B155) hybridized to single restriction fragments on these Southern blots, whereas the others hybridized to as many as four. These results suggest that some of the cDNAs isolated may represent members of small gene families. No polymorphisms between tumor and callus tissue were observed for any of the cDNAs, suggesting that no major rearrangements have occurred in the regions of the genome to which these probes hybridize. The only variation seen was with clone B21, which hybridized faintly with a restriction fragment of approximately 3.1 kb in the EcoRI digest of plant, but not tumor or callus, DNA (Fig. 2C). Whatever the reason for this additional fragment, it is presumably unrelated to the



Figure 1. Expression of cDNA clones in *Arabidopsis* tumor line 1.2A, callus, and plant organs. Northern blots of total RNA (20 μ g per lane) were hybridized with clones A26 (A), A16 (B), B14 (C), B35 (D), B155 (E), and B47 (F). 1.2A, Tumor line 1.2A; Ros, whole rosettes; Infl, inflorescences; Isil, immature siliques; Msil, mature (green) siliques. Exposure times ranged from 3 to 10 d to yield autoradiographs suitable for densitometry.

Table I. Characteristics of cDNA Clones Isolated from Subtracted Library

Clones are listed in approximate decreasing order of abundance of their complementary transcripts in tumor line 1.2A. Relative transcript abundance in different tissues was estimated by densitometric analysis of autoradiographs of northern blots probed with cDNA clones, defining the degree of hybridization to tumor line 1.2A RNA as 10. Autoradiographs of the same blots probed with pB1 mcr4 were used to correct for differences in RNA loaded per lane. Ros, Whole rosettes; Infl, inflorescences; Isil, Immature siliques; Msil, mature (green) siliques.

Clone	Insert Size	Transcript Size	1.2A	NB	DK	Root	Ros	Stem	Infl	Isil	Msil
	bp	kb					-				
B47	650°	0.80	10	2	<1	<1	11	5	16	10	<1
B155	220 ^b	0.80	10	3	<1	<2	<1	<1	<1	<1	<2
B8	350°	0.85	10	4	5	1	4	<1	<1	<1	1
A16	649 ^ь	1.25	10	3	<1	4	12	11	13	5	8
B35	750°	1.25	10	5	3	1	12	10	16	1	6
B14	339 ^b	1.10	10	7	2	15	<1	<1	<1	<1	<1
B141	550°	1.10	10	8	<1	20	<1	<1	<1	<1	<1
A26	450ª	1.10	10	1	<1	4	1	2	7	11	7
B16	412 ^b	0.90	10	5	6	10	5	2	8	2	2
B21	381 ^b	1.25	10	6	<1	10	5	2	14	2	2

overexpression of B21 in tumor versus callus, because both of these tissues gave the same hybridization pattern.

Relationships among Overexpressed cDNA Clones

Each differentially expressed cDNA insert was at least partially sequenced from both ends; therefore, the sequences obtained could be compared with one another and with the GenBank data base. The results indicate that the set of 10 overexpressed cDNAs includes three pairs of related clones, A16 and B35, B47 and B155, and B14 and B141. Clones A16 and B35 show 84% similarity over 300 bp of sequence overlap. These clones hybridized to sequences of comparable mol wt on northern and Southern blots, and their expression showed similar patterns of organ specificity (Table I). Sequence comparisons show that these clones are related to a family of genes encoding membrane channel proteins (see further).

Clones B47 and B155 also appear to be distinct members of a gene family. Southern blot analysis showed that these clones hybridize with restriction fragments of similar mol wt, although B47, which has a longer insert, hybridized with additional fragments. Clone B155 shows only 80% sequence similarity to clone B47, however, and northern blot analysis showed clear differences in the pattern of expression of these clones in plant organs (Fig. 1, E and F, Table I). Clone B47 is strongly expressed in leaves, stems, inflorescences, and immature siliques, whereas B155 is not highly expressed in any plant tissue. Genomic clones of these genes could prove useful for future studies of organ-specific promoters.

The third pair of clones, B14 and B141, appear to be independent isolates of a unique cDNA. Northern blot analysis showed that these two clones hybridize to transcripts of comparable length and have very similar patterns of expression in plants (Table I). These clones also hybridized to restriction fragments of equivalent size on Southern blots, although clone B141 (which has a longer insert) hybridized with more fragments than did B14. Finally, the sequences



Figure 2. Hybridization of cDNA clones with Southern blots of genomic DNA (10 μ g per lane) extracted from tumor, plant, and callus tissues. Blots were hybridized with clones B47 (A), A16 (B), and B21 (C).

obtained for these clones are identical over the region of overlap.

Comparison of Cloned Sequences with GenBank Data Base

Comparison of the overexpressed cDNA sequences with the GenBank plant data base showed that four clones, or pairs of clones, were similar to previously isolated sequences. Clone B47 shows 95% similarity to ATHSEQB⁴ over 244 bp. ATHSEQB is a highly expressed sequence of Arabidopsis that codes for a lipid transfer protein occurring mostly in the epidermis of all aerial organs (S. Thoma, U. Hecht, and C. Somerville, personal communication). The organ specificity of clone B47 expression is similar to that of ATHSEQB, further supporting this tentative identification. Clone A26 shows 92% similarity over 291 bp to ATGRP5, a previously described GRP gene of Arabidopsis (6). Northern blot analysis showed clear differences in transcript size and tissue specificity, however, suggesting that clone A26 also contains sequences that are distinct from ATGRP5 or that transcriptional regulation and transcript splicing of ATGRP5 is altered in the tumor tissue. Clone A26 also shows 58% similarity to a putative GRP cDNA from Arabidopsis, 2A12, which was recently isolated in this laboratory, and is strongly expressed in a number of our radiation-induced tumor lines (15). Some regions of clone A26 show significant sequence similarity to GRPs from bean and maize: 62% similarity to PHVGRP10 over a 117-bp overlap, 60% similarity to PHVGRP18 over 126 bp, and 60% similarity to MZEGRP over 123 bp.

Clones A16 and B35 show a high degree of similarity (71% identity over 534 bp [Fig. 3] and 72% over 175 bp, respectively) to ATHROOTSP, a root-specific mRNA from Arabidopsis that has putative homology to various membrane channel proteins (20), and to γ -TIP(At), a nearly identical homolog that encodes a tonoplast intrinsic protein expressed in all vegetative organs (11). Clones A16 and B35 clearly differ from the previously described genes, both in sequence and in organ specificity, but probably represent other members of this family of membrane proteins. The combined sequence obtained from clones B14 and B141 shows considerable similarity to several HRGPs from bean and tomato: 51% similarity to PHVHRGPC over 307 bp, 49% similarity to PHVHRGPA over 235 bp, 50% similarity to PHVHRGPB over 202 bp, and 48% similarity to TOMHRGP over 192 bp. To our knowledge, no HRGP sequences from Arabidopsis have yet been published.

The remaining clones, which did not show significant similarity to sequences in the plant data base, were compared with all other GenBank data bases. No significant sequence similarities were detected.

Possible Relation of Cloned Sequences to Hormone Autonomy

It is likely that clones A16 and B35 code for membrane channel proteins. Overexpression of membrane channel pro-

A16 CAGGUTCOCGUTCTCTCTCGCCGARGAGATGGARG ATHROOTSP AAATTCGCCACCGGAGGCTTGGCTGGCCCCCTTTGGTCTCTGTGTGGAGGAGAGGAGGGGGGGAGGGGCCGGTGGTCACCGCGGGAGGAGGGGGGCGGGTGGTCGGCGGCGTGGTCACCGCGGTGGAGGGGCGGGC					10	20	30
ATHROOTSP AAATTCGCCACCGGAGGCTTGGCTGGCCCCCTTTGGTGTGTGT	A16			CA .	GCGTTCCGG	TCTCTCTGCC	GGAGTCGGATCA
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280 290 300 310 320 A16 CTGGTCTCTTATGGTGGTGCCGCCGGAATTATCTACGACTTTGCTTCATCGATGATGATGA IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		650	660	670	680	690	700
A16 CTGGTCTCTTATTGGTGGTGCCGCCGGAATTATCTACGACGTTGTCTCTATCATCATACA ATHROOTSP CGGACCTCTCGTCGGCGGTGGAATCGCTGGACTATCTACGAAGTTTCTTCATC-AACA 710 720 730 740 750 760 330 340 350 360 370 380 A16 AAATGCCCACGACATTGCCTACCACCGATTACGAAGACGTCAAATTCAACGCTGTTA 1		280	290		300	310	320
ATHROOTSP CGGACCTCTCGTCGGCGGGTGGAATCCCTGGACCTACTCTACGAGAGTTTCTTCATC-AACA 710 720 730 740 750 760 330 340 350 360 370 380 A16 AAATGCCACGAGGAATATCCAACGCATTACTGAAGAGCTCAAACA 700 700 700 700 800 A16 CCACACACGAGCACATCCCAACACCAGACAACCGATACTGAAATTAATCTC-TTTTCT 770 780 790 800 810 A16 ATTCTGATG-AAATTTTTCGTGATTTGCTTATTTGGAATTTAATCTCCTGGCCG- 111 111 <td>A16</td> <td>CTGGTCTCTTAT</td> <td>TGGTGGTG-</td> <td>ccgccg</td> <td>GAATTATCI</td> <td>ACGACTTTGI</td> <td>CTTCATCGATGA</td>	A16	CTGGTCTCTTAT	TGGTGGTG-	ccgccg	GAATTATCI	ACGACTTTGI	CTTCATCGATGA
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330 340 350 360 370 380 A16 AARTGCCARGAGACATTGCTAACACCGATACTAAGGAGCGTGCTAACTACGAGAGCGTGCTACCACCGACCTACCACGACCTACCT		/10	/20	/30	740	750	/60
A16 AAATGCCCACGAGCAATGCCTACCACCCATTACTGAAGACGTCAAATCAAGGTGTTA i i i i i i i i i i i i i i i i i i i		330 34	0 3	50	360	370	380
ATHROOTSP 1	A16	AAATGCCCACGA	GCAATTGCC	TACCACCG	ATTACTGA	GACGTCAAAI	TCAACGTTGTTA
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A16 A50 A50 A10 A20 A30 A40 A16 ATCTGATG-AATTTTTCGTATTGGCTTTTTAATTTCGTATAGGGTTTCTGGCCG- i::::::::::::::::::::::::::::::::::::	ATHROOTSP	CCACACACACGA	GCAGCT-CC	AACCACAG	ACTACTGA	ATTAATC	TC-TCTTTTTCT
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ATHROOTSP CTCTTGTGTAATTTTATCGACTTGAATTTGGATTTTAATGTCTTTTTATTTCGGT 820 830 840 850 870 A16 TTGGATCATTTTAGATGAATCTTGGTC-ATGATGATCATTTATGGTCTTTG III IIII IIII IIIIIIIIIIIIIIIIIIIII				:: :			: : :::
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A16 TIGGATCATTITAGATGAATCATTGTCT-ATGATGATCATTTATGGATGGTCTTIG III IIII IIII IIIII IIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		450	460	47	0	480	490
ATHROOTSP TCGGTTGGTTTGGAGTGGAGTGGAGTGGAGTGGAGTGGA	A16	TTGGATCATTTI	TAGATGA	ATCTTTGI	CT-ATTGAT	TGATCATTT-	-ATGTGTCTTTG
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Al6GGTTTGTTGGAGTTGTAATA iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii		500	510	520			
ATHROOTSP TCCGTTGGTTTCAAGAGTGGCCATT	A16	GGTTTGTTG1	TGGAGTTGT	AATA			
	ATHROOTER	::: :::	IIII I	:: CATT			
940 950 960	ATHROOTSP	940	950	960			

Figure 3. Sequence comparison between cDNA clone A16 and ATHROOTSP, a root-specific mRNA of *Arabidopsis* (20).

teins could result in increased nutrient uptake or transport, allowing growth in the absence of the normal hormonal stimuli. Transformation of crown gall cells is accompanied by increased efficiency of substrate utilization, and it has been proposed that habituation (nontumorous, hormoneautonomous growth) involves perturbation of intracellular metabolic pathways (reviewed in ref. 10). The sequence of clone A26 is similar to GRPs of Arabidopsis and other species. It has been assumed that GRPs are structural proteins (2). Recent evidence shows that GRP1 of petunia is also an indicator of promeristematic cells (C.M. Condit, unpublished results) and that overexpression of GRP1 in transgenic plants can cause abnormal development characterized by supernumerary organ formation (S.-H. Cheng and C. Condit, personal communication). Therefore, it is possible that overexpression of A26 is a causal factor in tumorous growth. Clones B14 and B141 may code for HRGP(s). Because these are also thought to be structural components of the cell wall (2), for which no regulatory role has been reported, the elevated expression of these genes is more likely a result of tumorous growth than its cause. It is of interest to note that, in normal plants, each of the putative GRP and HRGP clones described here is expressed most in organs that are absent from the tumor tissue (roots, inflorescences, siliques). This phenomenon may indicate alterations in the regulation of gene expres-

 $^{^{4}\,\}text{All}$ sequence names are shown as listed in the GenBank data base.

sion or the proliferation of certain cell types in tissues in which they usually do not occur. Overexpression of the putative lipid transfer protein, clone B47, is unlikely to be the cause of hormone-autonomous growth. The presence of this clone in the subtracted cDNA library probably resulted from its extremely high level of expression and the leafy nature of the tumor tissue.

CONCLUSION

We used a subtracted cDNA library to isolate clones that are expressed more in a hormone-autonomous *Arabidopsis* tumor line than in hormone-dependent callus. The 10 cDNAs characterized thus far show considerable variation in degree of expression and in organ specificity. No polymorphisms suggestive of genome rearrangements in the vicinity of the overexpressed genes were found in the tumor line. Three pairs of related clones were isolated, and one pair was found to represent distinct members of a gene family that show different patterns of expression in normal plants. Sequence comparisons suggested possible functions for the proteins encoded by six of the overexpressed cDNA clones. Further study of genes that are overexpressed in radiation-induced tumors should aid in understanding the molecular cause(s) of hormone-autonomous tissue growth.

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