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**The Role of Small Signal Transducing GTPases in
the Regulation of Cell Wall Deposition Patterns in
Plants**

D.P. Delmer, D. Johnson, A. Levine

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The role of small signal transducing GTPases in the regulation of cell wall
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Investigators:
(Principal first)

Deborah P. Delmer
Douglas Johnson
Alex Levine

Affiliated Institution:

University of California Davis
University of Vermont
The Hebrew University

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Principal Investigator

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Attachments:

Figure 1 of Unpublished Results

Reprints/preprints resulting from this grant

I. Abstract

The combined research of the groups of Delmer, Levine and Johnson has led to a number of interesting findings with respect to the function of the small GTPase Rac in plants and also opened up new leads for future research. The results have shown: 1) The Rac13 protein undergoes geranylgeranylation and is also translocated to the plasma membrane as found for Rac in mammals; 2) When cotton Rac13 is highly-expressed in yeast, it leads to an aberrant phenotype reminiscent of mutants impaired in actin function, supporting a role for Rac13 in cytoskeletal organization; 3) From our searches, there is no strong evidence that plants contain homologs of the related CDC42 genes found in yeast and mammals; 4) We have identified a rather unique Rac gene in Arabidopsis that has unusual extensions at both the N- and C-terminal portions of the protein; 5) New evidence was obtained that an oxidative burst characterized by substantial and sustained production of H_2O_2 occurs coincident with the onset of secondary wall synthesis in cotton fibers. Further work indicates that the H_2O_2 produced may be a signal for the onset of this phase of development and also strongly suggests that Rac plays an important role in signaling for event. Since the secondary walls of plants that contain high levels of lignin and cellulose are the major source of biomass on earth, understanding what signals control this process may well in the future have important implications for manipulating the timing and extent of secondary wall deposition. 6) When the cotton Rac13 promoter is fused to the reporter gene GUS, expression patterns in Arabidopsis indicate very strong and specific expression in developing trichomes and in developing xylem. Since both of these cell types are engaged in secondary wall synthesis, this further supports a role for Rac in signaling for onset of this process. Since cotton fibers are anatomically defined as trichomes, these data may also be quite useful for future studies in which the trichomes of Arabidopsis may serve as a model for cotton fiber development; the Rac promoter can therefore be useful to drive expression of other genes proposed to affect fiber development and study the effects on the process; 7) The Rac promoter has also been shown to be the best so far tested for use in development of a system for transient transformation of developing cotton fibers, a technique that should have many applications in the field of cotton biotechnology; 8) One candidate protein that may interact with Rac13 to be characterized further in the future is a protein kinase that may be analogous to the PAK kinase that is known to interact with Rac in mammals.

II. Objectives of the Original Research Proposal; Background

This grant proposed to initiate studies using both genetic and biochemical approaches to analyze the function of the small, signal-transducing GTPase Rac. In developing cotton fibers, the gene is expressed at the time the cytoskeleton undergoes rearrangement, gene expression patterns change, and synthesis of secondary wall cellulose is initiated. Since Rac in animals is known to be involved in signaling for changes in cytoskeletal organization, we hypothesized that it might play a similar role in cotton fibers, and proposed to begin dissection of signal transduction pathways involving Rac. We also proposed that it might play a role in regulation of signaling pathways related to an oxidative burst, since Rac in animals is involved in such a process. We hoped to gain evidence for the involvement of Rac in any of the above signaling pathways, and also to attempt to identify upstream and downstream partners in these signal transduction pathways. The PI on the original grant was D.P. Delmer at The Hebrew University, with Co-PI being Douglas Johnson at the University of Vermont. At the end of the second year of funding, two changes were made. The PI, DPD, relocated to the Section of Plant Biology, University of California Davis, and Dr. Alex Levine of The Hebrew U. joined the project.

Background; The Ras Superfamily of Small GTPases. This family of small GTP-binding proteins has, and continues to be, a major focus for studies on signal transduction pathways in animals (Bokoch and Der, 1993). All of these proteins, of molecular weight in the range of 20-25 kD, share the common feature of possessing GTPase activity. Cycling between the active GTP-bound and inactive GDP-bound state provides the means by which these proteins function in signal transduction. This superfamily of genes is generally subdivided into 4 major families: the *Ras* family involved primarily in pathways relating to cell proliferation; the *Rab/YPT* members that are involved in regulation of the protein secretory pathway; the *Ran* members that function in secretion in and out of the nucleus; and the *Rho* family that has several diverse functions including regulation of actin organization, of the oxidative burst in leukocytes, and in complex interactions with members of the *Ras* family. Plants have homologs of genes that encode members of the *Ran* and *Rab/YPT* families that function in ways quite analogous to those studied in animals, and a few members of the *Rho* family(see below). Curiously, no members of the *Ras* family have been found in plants.

The *Rho* Family of GTPases. In animals, the major members of the *Rho* family are those of the *Rho*, *Rac*, and *CDC42* subfamilies. Proteins from all of these

subfamilies play some role in regulation of actin organization and cell shape, and the interaction between their signaling pathways, and also with that of *Ras*, are very complex (Hall, 1994; Symons, 1996; Tapon and Hall, 1997). Microinjection of dominant positive (always "on") and dominant negative (always "off") recombinant forms of Rac, Rho, or CDC42 proteins into fibroblasts showed clear effects on actin organization (Ridley and Hall, 1992; Ridley et al., 1992; Nobes and Hall, 1995). Mammalian targets of CDC42 and Rac include a number of recently-identified proteins, of which the best characterized are the PAK kinases (Lim et al., 1996; Sells et al., 1997; Sells and Chernoff, 1997) and the Wiskott-aldrich syndrome protein WASP (Symons et al., 1996). Both effectors may play some further role downstream in actin organization that is still not well understood. PAK undergoes autophosphorylation on multiple sites and is activated upon binding with the active GTPases, and PAK function *in vivo* probably also includes activation of JNK/SAPK and p38 MAP kinase pathways (Zhao et al., 1998).

In animals, Rac proteins play another important role as activators of the leucocyte NADPH oxidase involved in production of superoxide, that leads to generation of other reactive oxygen species (ROS) such as H_2O_2 and hydroxyl radicals, a process called the "oxidative burst" that is important in defense against pathogens. The NADPH oxidase is activated by signals generated by the presence of foreign pathogens in a complex process in which cytoplasmic subunits called p67phox and p47phox are recruited to the membrane along with Rac that interacts with p67phox; these all interact with the membrane-associated gp91phox/cytochrome b558 component, leading to activation of the enzyme (see Diekmann et al., 1994). Plants also exhibit an oxidative burst in response to elicitors and other pathogenic signals (Low and Merida, 1996), and the production of ROS may also play an additional role in programmed cell death (Lamb and Dixon, 1997). There are a number of potential sources of ROS in plants (Bolwell, 1995; Bolwell et al., 1998; Alan and Fluhr, 1997), one of which is the NADPH oxidase that is analogous to that of leucocytes and may be one of the major cell-regulated sources of ROS (e.g., Xing et al., 1997). A plant homolog of the gp91phox subunit gene has been identified; the gene encodes a related protein that also has an additional unique calcium-binding domain, suggesting a possible role for calcium influx in ROS production (Keller et al., 1998).

A *Rho* gene was first described in plants by Yang and Watson (1993) who identified a homolog in pea they called *Rho1p*, although analyses of distinct signature motifs for this gene clearly indicate it encodes a protein closer to a Rac than to a Rho. A similar gene

is highly-expressed in pollen tubes of garden pea and may be important in tip growth (Lin and Yang, 1997). Databank searching reveals a minimum of 7 distinct Racs in Arabidopsis (Collins and Johnson, 1997; Winge et al., 1997), while another two have been identified in *Lotus japonicum* (Borg et al., 1997). Most of these have very high sequence homology to two cotton *Rac9* and *Rac13* sequences (see below and Delmer et al., 1995), with *Ara-rac2* (Winge et al 1997) being the closest to the cotton Racs. Two recent studies have implicated a role for plant Racs in oxidative signaling. Xing et al. (1997) using tomato and Kieffer et al. (1997) with tobacco have shown that a protein that reacts with an antibody against animal Rac2 shows changes in intracellular localization in response to elicitor treatment. Since the elicitor treatment leads to an oxidative burst, these results suggest that Rac may function to modulate an NADPH oxidase as it does in leucocytes.

The Rac Genes of Cotton. The work proposed herein is based upon two Rac genes discovered by Delmer et al., (1995) that are highly-expressed in cotton fibers just at the time when fibers undergo a transition from primary to secondary wall synthesis. Cotton fibers are single cells that develop synchronously with a boll; beginning on the day of anthesis, about 1 in 4 epidermal cells initiates elongation which continues for about 3 weeks. At about 15-18 days post-anthesis (DPA), the fibers undergo a number of interesting changes that relate to the initiation of secondary wall cellulose synthesis. The cortical microtubule network that somehow sets the patterns of deposition of the cellulose microfibrils, undergoes a rearrangement from transverse to helical arrays (Seagull, 1990), there is an increase in levels of tubulin mRNA (D. Pear and D. Stalker, personal communication) and tubulin protein (Seagull, 1992), a transient deposition of callose begins that is suggestive of elevation in intracellular Ca^{2+} since the cotton fiber callose synthase is activated by this cation (Hayashi et al., 1987), the *Ce1A* genes encoding the catalytic subunit of cellulose synthase begin to be highly-expressed (Pear et al., 1996), and the rate of cellulose synthesis increases over 100-fold (Meinert and Delmer, 1977). Seagull (1990) showed that treatment of fibers with cytochalasin that disrupts actin organization leads to a change also in the cortical microtubule patterns suggesting that the organization of these two cytoskeletal networks is interrelated.

III. Relevant Data, Methodology, Results and Discussion

General Comments. The progress on this grant was slowed by some delays associated with the relocation of the PI, and by numerous unexpected difficulties encountered by the Johnson and Delmer groups in attempts to prepare and screen a

cotton fiber cDNA library designed for use in the yeast two-hybrid system to study protein-protein interactions. Both groups spent considerable effort in 3 different attempts to prepare such a cDNA library (one attempt by the Johnson group, two by companies; screening efforts in both the Delmer and Johnson groups), but , in each case, the quality of the library was not sufficiently good to allow detection of any promising cDNAs for this project.

In spite of these unexpected delays, the project got "back on track", and we have recently generated a significant number of new findings, most of which are just now being prepared for publication. In particular, we have made the truly novel and unexpected finding that an oxidative burst may regulate the initiation of secondary wall synthesis in plants, and that Rac may play a role in this signal transduction pathway (see attached manuscript of Potikha et al., submitted for publication). Since the secondary walls of plants represent huge allocations of fixed carbon in form of cellulose, hemicellulose and lignin, this finding opens up the long-term potential for modification of patterns of deposition. In addition Dr. Doron Holland, a scientist from the Volcani Institute, has spent a year in the lab of DD on sabbatical and also was partially funded by this grant, during which time he has become quite interested in the Rac project and is responsible for generating a significant amount of as-yet-unpublished data that have helped the project move forward.

Specific Results and Discussion.

a) Prenylation and Localization of Rac13. We have showed (Trainin et al. 1996; reprint attached) that recombinant cotton fiber Rac13 is an excellent substrate for geranylgeranylation, but not farnesylation, a result consistent with its possessing the CXXL motif at its C-terminal. The ability to undergo prenylation is recognized as important for membrane localization, and in subsequent and still unpublished work, the lab of DD has shown that Rac is indeed localized to the plasma membrane fraction and the level of the protein increases substantially as fibers enter into the phase of secondary wall development (see also Fig. 1B of unpublished data attached).

b) Cotton Rac13 Alters Cell Shape in Yeast. The lab of Co-PI D. Johnson placed cotton *Rac13* under control of a galactose-inducible promoter and expressed it in yeast. When *Rac13* was induced to high levels, it was observed that the yeast cells enlarged greatly and possessed bizarre shapes reminiscent of mutants impaired in actin function (see Fig. 1A of unpublished data attached). This suggests that Rac may function in pathways relating to cytoskeletal organization and also relates to a study by

Xia et al (1996) in which a random Arabidopsis cDNA expressed in yeast also showed altered cell shape and, when sequenced, proved to be a *Rac*.

c) Search for CDC42 Genes in Plants. Co-PI Doug Johnson's laboratory are known for their work on the small GTPase *CDC42* in yeast. This gene is closely related to *Rac*'s, although can be distinguished by certain specific differences in sequence. In yeast, *CDC42* is involved in the control of polarized cell growth (Johnson and Pringle, 1995), while in animals, one role is, like *Rac*, is to participate in signaling leading to reorganization of the actin cytoskeleton (Tapan and Hall, 1997). However, in animals, *Rac* and *CDC42* are definitely distinct genes, and yeast lack *Rac* genes. We were interested to see if plants have *CDC42* genes in addition to *Racs*. An extensive searching both by screening of the cotton fiber cDNA library and by PCR approaches did not uncover any evidence for *CDC42*, and thus higher plants may well be more like yeast in this regard, possessing only one such GTPase, *Rac* (in plants) or *CDC42* (in yeast). During these searches, we did discover a rather novel Arabidopsis *Rac*, that is different from most others by virtue of having a 45 amino acid N-terminal extension and another of 21 amino acids at the C-terminal (Collins and Johnson, 1997, reprint enclosed). The function of this particular *Rac* in plants is not yet clear.

d) The Role of Rac in Regulation of Secondary Wall Synthesis and Programmed Cell Death. Another major finding has been that cotton fibers initiate production of H_2O_2 coincident with the onset of secondary wall synthesis (see Fig.1 and 2 of appended preprint of Potikha et al.). Scavenging of the H_2O_2 with tempo or hydroxytempo or inhibiting its production by addition of the oxidase inhibitor DPI, prevents the onset of secondary wall cellulose deposition. Addition of H_2O_2 to young fibers leads to premature induction of secondary wall cellulose synthesis (Figs. 3, 4 of preprint). These results, taken together, indicate that an oxidative burst, and in particular the H_2O_2 produced, may be a developmental signal for the onset of secondary wall synthesis in plants. (Although H_2O_2 is not a direct product of the NADPH oxidase, it can be rapidly generated either spontaneously or through the action of SOD working on superoxide). Two other studies also now suggest a role for similar signaling events in cell wall synthesis. Maddalena et al. (1998) have shown that oligogalacturonide elicitors stimulate cellulose deposition in the pericycle cell walls of tobacco explants, while DeMarco and Roubelakis-Angelakis (1996) showed that H_2O_2 generation is important for proper cell wall regeneration of protoplasts.

The Johnson lab has constructed mutant forms of *Rac13* that are either dominant positive (gly 15 changed to val; called V15) or dominant negative (thr 20 changed to asn; called N20; see Ridley et al., 1992). When these forms were transformed by the AL lab into cultured soybean or Arabidopsis cells, they lead to either enhanced (V15) or reduced (N20 or antisense of V15 *Rac*) production of H_2O_2 (see Fig. 5, preprint). Thus, a role for *Rac* in signaling for the production of reactive oxygen species (ROS) is certainly implicated. Of further interest is our additional finding relating to a possible role for *Rac* in programmed cell death (PCD). Briefly, transformations with the dominant-negative *N-20-Rac* or with the antisense form of the *V15-Rac* reduced the amount of cell death (compared to the dominant-positive *V-15-Rac*) in cultures stimulated with exogenous H_2O_2 (Fig. 1E of attached unpublished results). These results imply that *Rac* activity is necessary not only for H_2O_2 generation but also for the downstream portion of the signal transduction pathway that ultimately leads to PCD. When viewed together with an earlier report which showed that H_2O_2 induced PCD could be prevented by kinase inhibitors (Levine et al., 1996) these results suggest that *Rac* may regulate kinases that are important for PCD execution.

e) Patterns of Cotton *Rac* Expression in Arabidopsis. While on sabbatical as a visiting scientist in the lab of DD, Doron Holland, has addressed the question of regulation of *Rac* gene expression. One approach was to couple the promoter region from cotton *Rac13* (provided by D. Stalker, Calgene Inc.) to the reporter gene *GUS* and to use this construct to transform Arabidopsis plants. The results, show a striking pattern of expression that is under tight developmental regulation, and the major site of *GUS* activity was in developing trichomes (see Fig. 1 D of unpublished results attached). Since cotton fibers are anatomically defined as trichomes, and the activity of the *Rac* promoter in Arabidopsis trichomes appears during the early-to-mid phase of trichome development, this suggests that one can use the Arabidopsis trichome as an additional model system to study *Rac* function as it relates to secondary wall formation. This is also supported by previous work in the lab of DD (Potikha and Delmer, 1995) that shows that trichomes undergo a preliminary phase of primary wall deposition and shape determination, followed by a rapid phase of secondary wall cellulose deposition. Furthermore, that study identified a mutant called *tbr* that was blocked in the ability to produce secondary wall cellulose. The finding of additional *Rac* promoter activity in portions of the developing vascular system (not shown) could well relate to the timing of

deposition of the thick secondary walls of the xylem elements, although this has not yet been explored.

f) Development of a Technique for Transient Foreign Gene Expression in Cotton Fibers. Doron Holland while visiting scientist in the Delmer lab has also used the cotton *Rac13* promoter to develop a system for driving transient foreign gene expression via an *Agrobacterium*-mediated transient transformation process in cotton fibers. This was a surprising achievement to many in the cotton world and may have quite a few practical applications. He has found that the cotton *Rac* promoter that is highly-expressed in fibers of cotton is by far the best of all by far for this purpose, and general constitutive promoters such as 35S or a dual promoter are barely active. UCD is filing a patent on this invention (RARD was informed of this), and Calgene/Monsanto will also support Doron for another year to optimize this system further. Further details of the invention can be provided as soon as the patent is filed.

g) Interacting Partners for Rac. The involvement of Rac in the downstream events stimulated by the oxidative stress was further studied by the AL lab by analyzing Rac-binding proteins in soybean suspension cultures. A number of proteins were found to be induced by oxidative stress, some of which were shown to bind to Rac using GST-Rac beads (Fig. 1F, unpublished results). One or more of these proteins had a strong kinase activity, the binding of which to Rac depended upon the presence of GTPγS (Fig. 1G, unpublished results). Preliminary studies with antibodies against mouse PAK1 using soybean cell cultures showed that a cross-reacting protein of a similar molecular weight was bound to the GST-Rac beads. These results are very promising since an animal PAK was shown to be involved in both responses to stress (Pombo, 1996) and the reorganization of peripheral actin (Manser et al., 1997; Sells et al., 1997; Zhao et al., 1998).

IV. Description of the Cooperation

The various partners to this grant kept in communication regularly via e-mail and exchange of ideas and results. In addition, Delmer visited the laboratory of Johnson in the first year of this grant. The Johnson laboratory has interacted in a very specific way with the other groups by creating by site-directed mutagenesis, both the dominant-positive and dominant-negative versions of the *Rac13* gene that have been used subsequently by Levine and Delmer as described above. The manuscript of Potikha et al. that is being submitted and enclosed here, is therefore a genuine collaboration between all 3 laboratories, since the results represent our combined efforts. The paper

was written jointly by Levine and Delmer, the *Rac13* constructs were made by the Johnson group, some of the experiments were performed in the Levine laboratory and one was done in Delmer laboratory at UC Davis.

V. Evaluation of the Research Achievement

The combined research of the 3 groups of Delmer, Levine and Johnson has led to a number of interesting findings with respect to Rac function in plants and also opened up new leads for future research. In doing so, we believe we have met many of our original goals. We have obtained evidence that indicates that Rac in plants may function in several ways that are strikingly analogous to its function in mammals and yeast. Thus, we have evidence that supports a role for Rac in actin organization as Rac does in animals, and we also have other evidence that strongly supports a role for cotton fiber Rac in signaling for an oxidative burst that signals the onset of secondary wall formation. Since Rac in leucocytes also plays a similar role, here again, there is an overlap between plants and animals in function. This latter finding may well have importance in a practical sense because the secondary walls of plants that contain high levels of lignin and cellulose are the major source of biomass on earth. Therefore, understanding what signals control this process may well in the future have important implications for manipulating the timing and extent of secondary wall deposition. Another very practical result has been our development of a system for transient transformation of cotton fibers using the *Rac13* promoter. Since it is very tedious to carry out stable transformation of cotton, time consuming, and expensive, being able to test certain constructs directly in the fiber by transient transformation has already attracted the interest of a major plant biotechnology company who want us to optimize this system further. Finally, we are also beginning to find partner proteins that may interact with Rac in vivo, and therefore are in a position to further explore the entire signal transduction pathway in which Rac functions in plants.

VI. Conclusions

The results obtained in this project were: 1) The prenylation motif found in the *Rac13* protein was predicted to specify for geranylgeranylation of the protein; our results support this hypothesis and indicate that *Rac13* is indeed prenylated and is also translocated to the plasma membrane as it is in mammals; 2) The observation that when cotton *Rac13* is highly-expressed in yeast, it leads to an aberrant phenotype reminiscent of mutants impaired in actin function, support a role for *Rac13* in this process; 3) There is no strong evidence that plants contain homologs of the related

CDC42 genes that are found in yeast and mammals; 4) We have identified a rather unique *Rac* gene in *Arabidopsis* that has unusual extensions at both the N- and C-terminal portions of the protein; the function of this particular *Rac* remains to be explored; 5) New evidence was obtained that an oxidative burst characterized by substantial and sustained production of hydrogen peroxide occurs coincident with the onset of secondary wall synthesis in cotton fibers. Further work indicates that the hydrogen peroxide produced may be a signal for the onset of this phase of development and also strongly suggests that *Rac* plays an important role in signaling for this oxidative phase in development; 6) We have used the *Rac13* promoter from cotton to study patterns of *Rac* expression in the heterologous system of *Arabidopsis*. When this promoter is fused to the reporter gene *GUS*, expression patterns indicate very strong and specific expression in developing trichomes and in the vascular tissues (most likely xylem). Since both of these cell types are engaged in secondary wall synthesis, this further supports a role for *Rac* in signaling for onset of this process. In addition, since cotton fibers are anatomically defined as trichomes, these data may be quite useful for future studies in which the trichomes of the simple *Arabidopsis* plant may serve as a model for cotton fiber development; the *Rac* promoter can therefore be useful to drive expression of other genes proposed to affect fiber development and study the effects on the process; 7) The *Rac* promoter has also been shown to be the best so far tested for use in development of a system for transient transformation of developing cotton fibers, a technique that should have many applications in the field of cotton biotechnology; 8) We are also beginning to identify proteins that interact with the cotton fiber *Rac13*, and one candidate which can be further characterized in the future is a protein kinase that may be analogous to the PAK kinase of mammals that is known to interact with *Rac*.

VII. List of Publications Resulting from this Grant

Trainin, T., Shmuel, M., and Delmer, D.P. *In vitro* prenylation of the small GTPase *Rac13* of cotton. *Plant Physiol.* 112: 1491-1497 (1996)

Collins, C.C., and Johnson, D.I. An *Arabidopsis thaliana* EST cDNA that encodes a *Rac*-like protein (Accession No. U88402) (PGR97-056) *Plant Physiol.* 113: 1463 (1997)

Potikha, T.S., Collins, C.C., Johnson, D.I., Delmer, D.P., and Levine, A. The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Submitted for publication

The following additional manuscripts are in various states of preparation at the moment, and we anticipate submission of these within the next 3-6 months:

Holland D, Delmer DP. Agrobacterium-mediated transient transformation of developing cotton fibers. (this work was also partially supported by a grant from the US Department of Energy).

Holland D, Delmer DP. A Rac13 promoter showing enhanced expression in cotton fibers also directs high expression in trichomes of Arabidopsis.

Levine A, Potikha T, Solomon M. Identification of a protein in cotton fibers that interacts with the small GTPase Rac13.

VIII. Patent

U.S. Patent Application, University of California Case 98-250-1 "Method for assaying genetic attributes in cotton fiber cells" in the names of Deborah P. Delmer and Doron Holland (This is a procedure for transient transformation of cotton fibers that makes use of the cotton Rac13 promoter; patent is currently in process of being filed).

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X. Legends to Attached Figure 1 that Shows Some Unpublished Results

- a) Phenotype of yeast expressing the cotton fiber Rac13 gene under control of galactose-inducible promoter. A. Induced by growth on galactose; B. Non-induced growing on glucose
- b) Western blotting of cotton fiber proteins with Anti-Rac13 antibody. Results show that the majority of Rac13 is found in the plasma membrane and the level increases when fibers enter secondary wall synthesis
- c) Not relevant to this report
- d) Expression of GUS (blue color) in transgenic Arabidopsis young developing trichomes when GUS is placed under control of the cotton fiber Rac13 promoter
- e) Effects of dominant-positive, dominant-negative or anti-sense constructs of Rac13 when expressed in cultured plant cells (35S promoter) and subjected to varying levels of oxidative stress. Note that the dominant-negative and anti-sense constructs tend to suppress programmed cell death induced by H2O2.
- f) Proteins that are induced in soybean cell cultures in response to H2O2; identification of those that may interact with GST-Rac. Details are on figure.
- g) Kinase activity of protein extracts from cells treated with H2O2; identification of induced kinase that also bound to GST-Rac in presence of GTP γ S.

B Western Blotting of Cotton Fiber Proteins with Anti-Rac13 Antibody

Kinase activity of protein extracts from cells treated with H₂O₂ and preincubated in the presence of γ-GTP or β-GTP. C: Control untreated cells. 5: Five minutes after induction; 15: Fifteen minutes after induction.