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Molecular Analysis of racE Function in *Dictyostelium*

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ABSTRACT *Dictyostelium* has long proven to be a valuable system for studying various aspects of the cytoskeleton and cell motility. In this review we describe the isolation of a novel gene, *racE*, and how we have used multiple approaches to learn how the product of this gene is involved in cytokinesis. The *racE*gene was isolated in a screen designed to identify genes specifically required for cytokinesis. The use of GFP fusion proteins, coupled with mutational analysis, allowed us to determine that racE exerts its function at the plasma membrane throughout the entire cell cycle. Measurements of cortical tension and observations of live cells in suspension culture revealed that racE is involved in the regulation of cortical tension and actin organization at the cortex. We postulate that in the absence of proper cortical tension, cytokinesis cannot proceed normally. *Microsc. Res. Tech. 49:145–151, 2000.*

INTRODUCTION

Several years ago we developed a molecular genetic screen to identify genes essential for cytokinesis in *Dictyostelium discoideum*. Our thought was that through the identification of essential genes, we might be able to shed some light on the regulation of cytokinesis. Using this screen, we found a gene, named *racE* (Larochelle et al., 1996), that encodes for a novel member of the ras-related family of small GTPases. In this review we discuss the studies of racE, as well as some of the innovative methods we have devised to understand the function of this protein during cytokinesis.

Dictyostelium has long proven to be a useful model system for the study of cell motility and the cytoskeleton (Egelhoff et al., 1991; Mann et al., 1994). In part, this is because of the dynamic nature of the amoeboid vegetative cells that move in a manner similar to mammalian macrophages. *Dictyostelium* cells also contain similar cytoskeletal components as those in higher cells. One of the greatest attributes of *Dictyostelium* as a model system is the relative ease with which the genome can be manipulated. These cells are haploid in the wild and undergo homologous recombination at a high frequency, allowing gene targeting through reverse genetics. De Lozanne and Spudich (1987) were the first to demonstrate homologous recombination in *Dictyostelium* by disrupting the gene that encodes for the myosin II heavy chain (*mhcA*) protein. This provided an elegant demonstration of the requirement for myosin II in cytokinesis. Cells deficient in this protein became extremely large and multinucleated when grown in suspension culture. However, if these cells were grown as an attached culture they were found to propagate through a mechanism referred to as "traction-mediated cytofission" (Fukui et al., 1990). In fact, it is this phenotype (ability to propagate as an attached culture but becoming large and multinucleated when grown in suspension) that has served as the basis for our screen to isolate genes specifically required for cytokinesis. In the absence of traction-mediated cytofission, mutations in such genes would otherwise be lethal. However, because these cells can continue to propagate, we can raise cells that harbor mutations in cytokinesis-specific genes. This screen was first undertaken by Vithalani et al. (1996) to search for cytokinesis-specific genes.

In essence, there are two components to the screen. The first relies on restriction enzyme-mediated integration (REMI) (Kuspa and Loomis, 1992) as a means of random mutagenesis by gene disruption. Genes disrupted in this manner can then be identified by isolating and sequencing genomic DNA that flanks the plasmid used in the disruption Thus, REMI is a very powerful tool for identifying genes involved in specific processes (providing one can screen for defects in those processes). The second component of the screen is the isolation of cell lines that are defective in cytokinesis due to mutations induced by REMI. By virtue of selectable markers in the plasmids used for REMI, we are able to isolate clonal populations of transfected cells. These cell lines are then assayed for defects in cytokinesis as follows. Samples are transferred to duplicate 24-well plates where one plate is placed on a shelf, as a stationary culture, and the other placed on an orbital shaker, as a suspension culture. We search for cell lines that can propagate on the stationary plate (through traction-mediated cytofission) but not on the shaking plate. Such cell lines are passed from the stationary culture to additional plates for further screening. Cells that are defective in cytokinesis will not be able to propagate in the shaking culture but instead become large and multinucleate. Such cells will ultimately become so large that they can no longer withstand the sheer force produced by shaking and consequently will lyse (Fig. 1). As was noted by Chisholm (1997), this

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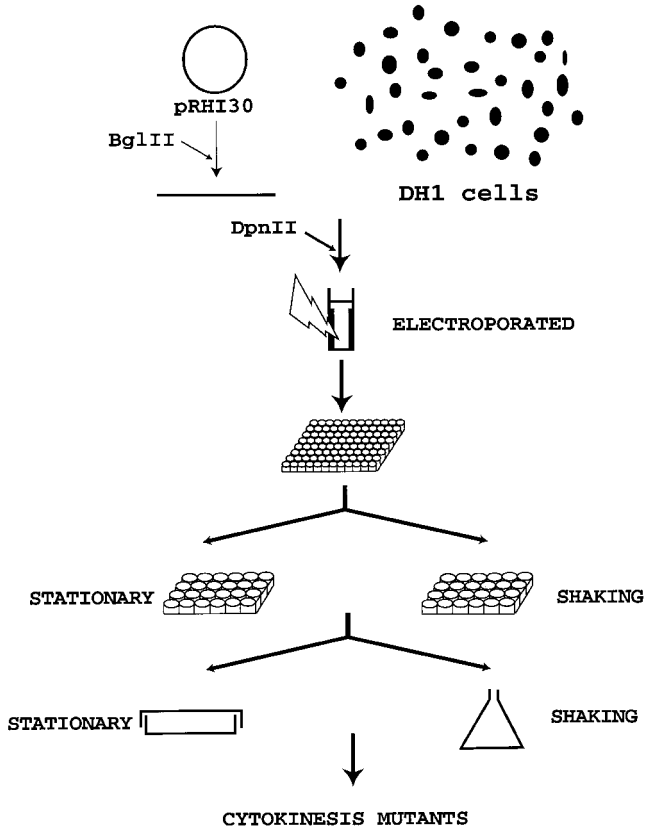


Fig. 1. The screen for cytokinesis mutants. DH1 cells (an axenic cell line derived from AX3 cells; Insall et al., 1996) are electroporated in the presence of the plasmid pRHI30 which has been linearized with BglII and the restriction enzyme DpnII, which generates the same cohesive ends as BglII. Selection for clonal populations of cells that have incorporated the plasmid takes place in 96-well plates. Colonies from these plates are replicated onto two 24-well plates; one is kept stationary whereas the other is placed on an orbital shaker in order to maintain the cells in suspension. Cell lines that were able to grow in the stationary plate but not the shaking plate were often screened two or more times in the same fashion. Cell lines that continued to demonstrate this phenotype were then grown in larger petri dishes and shaking flasks in order to obtain more cells for additional phenotypic analysis, as well as for the generation of growth curves. Only cell lines that maintained the inability to grow in suspension culture throughout all of this screening were considered cytokinesis mutants.

screen will not identify mutations in essential proteins or those that are required for both cytokinesis and traction-mediated cytofission.

Genomic DNA is then collected from the isolated cell lines and assayed by Southern blot analysis for the presence of the transfecting plasmid. This technique also allows us to identify restriction endonucleases that may be useful in cloning the flanking genomic DNA along with the plasmid during plasmid rescue. Once this flanking DNA has been cloned it can be sequenced to identify the disrupted gene. To confirm that the REMI-induced gene disruption is actually responsible for the cytokinesis-deficient phenotype, the mutation is recreated in wild-type cells by homologous recombination. These newly created mutant cell lines are then assayed for recapitulation of the defect in cytokinesis. In this way we can confirm that the observed cytokinesis-deficient phenotype is actually due to the REMI-induced gene disruption and not some spurious mutation that occurred elsewhere in the genome.

Following this procedure we identified the *racE* gene. Sequence analysis revealed racE to be a novel member of the ras-related superfamily of small GTPases. RacE is most similar to proteins in the rac/rho family of proteins. Although there have been several other rac-related proteins previously isolated in *Dictyostelium* (Bush et al., 1993), apparently none of these can complement for the loss of racE in cytokinesis. Initial phenotypic analysis of *racE* mutant cells revealed them to be fairly normal with the exception of their defect in cytokinesis. Their acto-myosin cytoskeleton remained intact, as they were able to cap ConA, an acto-myosin dependent process (Fukui et al., 1990). They were also able to phagocytose bacteria at normal rates, they underwent myosin II-dependent cortical contractions, and they were able to complete the developmental life cycle, forming functional spores. These results suggested that the cytokinesis-deficient phenotype observed in the *racE*-mutant cells was not due to an overall disruption of the acto-myosin cytoskeleton (an integral part of the cytokinetic machinery). But, as will be discussed later, we have observed some differences in the integrity of the actin cytoskeleton in *racE* mutant cells.

To understand the function of *racE* we have taken multiple approaches and had to develop new methods that may be applicable to the study of other proteins in cytokinesis. In the following sections, we discuss the mutational analysis of *racE*, the use of green fluorescent protein (GFP) to localize *racE* protein in live and fixed cells, the analysis of cleavage furrow formation in cells grown in suspension culture, the analysis of cortical tension and actin content in *racE* mutant cells, and the search for proteins that interact with racE as a means of establishing the signal transduction pathway through which racE functions.

MUTATIONAL ANALYSIS OF *racE*

Making use of the ease with which one can conduct reverse genetics in *Dictyostelium*, we carried out a mutational analysis of *racE* to try to elucidate its role in cytokinesis (Larochelle et al., 1997). Because of the high degree of homology between *racE* and the other members of the rho family of small GTPases, we were able to design point mutations resulting in single amino acid substitutions that convert the racE protein into constitutively active or inactive forms (Chung et al., 1993; Diekmann et al., 1991; Kozma et al., 1995; Ridley and Hall, 1992; Ridley et al., 1992). This allowed us to ask the question of whether or not racE acts as a molecular switch (as is common for many small GTPases) and if so, is that switch important in determining the timing of contractile ring formation during cytokinesis? To address these questions, we generated constructs that allowed for the exogenous expression of wild-type and mutant forms of *racE* and transfected these constructs into both wild-type cells and racE null cells. By transfecting into racE null cells, we could test if any of these constructs were able to rescue the cytokinesis defect. Because the exogenous expression of wild-type *racE* did rescue the cytokinesis defect in racE

null cells, we could determine the effect of locking racE in a constitutively active or inactive form.

Substituting valine for glycine at the 20th amino acid of racE (V20racE) results in a GTP-bound protein which remains constitutively active (Garrett et al., 1989). Expression of this form of the protein, at approximately wild-type levels, was able to rescue the cytokinesis defect of racE null cells. On the other hand, substituting asparagine for threonine at the 25th amino acid of racE (N25racE) results in a GDP-bound form that remains constitutively inactive (Feig and Cooper, 1988). Expression of this form of the protein was not able to rescue the cytokinesis defect of racE null cells. Furthermore, neither form of the protein (constitutively active or inactive) behaved as a dominant-negative mutation when expressed in wild-type cells. Taken together, these results suggested that although cytokinesis requires an active form of racE, it does not demand that racE turn off. This implies that racE must not be functioning as a molecular switch in the regulation of cytokinesis. Because cells expressing the constitutively active form of racE grow at wild-type rates in suspension culture, racE cannot be the signal that determines the timing of cytokinesis in cells.

Because seven other rac proteins had already been described in *Dictyostelium* (Bush et al., 1993), we were curious to determine what was unique about racE that allowed it to function specifically in cytokinesis. The most outstanding difference between racE and the other *Dictyostelium* rac proteins, and in fact rac proteins from a variety of other organisms, is the extended C-terminus, just before the terminal CAAX box. Wishing to test the importance of this region, we constructed a chimerical protein in which this region was substituted for the C-terminus of racC (racE's closest relative based on sequence homology). If this region of racE is the defining region in terms of racE's function in cytokinesis, we should be able to convert racC into the functional equivalent of racE by the substitution of the racE C-terminus for racC's. To then test for this we simply needed to express this chimera in racE null cells and look for rescue of the cytokinesis defect when such cells are grown in suspension culture. Because this construct did not rescue racE null cells, we know that there must be other domains unique to racE that, either alone or perhaps in conjunction with the extended C-terminal domain, allow racE to function in cytokinesis.

LOCALIZATION USING GFP

One important aspect of trying to understand how racE functions in cytokinesis is to know where it is found in cells, particularly during cell division. Using the construct mentioned above for the exogenous expression of racE, we inserted the coding sequence for GFP at the 5' end of the racE coding sequence, resulting in the expression of a GFP-racE chimera (Larochelle et al., 1997). By doing so we hoped to be able to follow the distribution of racE in live and fixed cells by monitoring the distribution of GFP. We first demonstrated that the GFP-racE fusion protein was able to rescue the cytokinesis defect of racE null cells. Consequently, the distribution of GFP-racE must reflect the normal distribution of racE. We found that GFP-racE localized uniformly to the plasma membrane in both live cells and fixed cells (Fig. 2). More importantly, we observed that this distribution did not change as cells underwent cytokinesis.

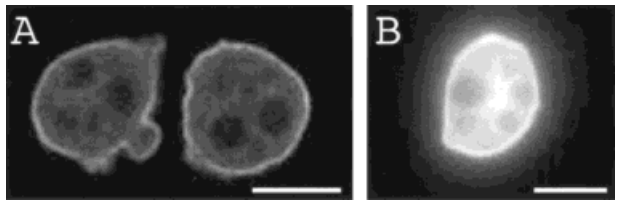


Fig. 2. Distribution of GFP-racE in live and fixed cells. RacE-null cells expressing the GFP-racE fusion protein were examined by either confocal microscopy (A) or conventional epifluorescence (B) for the distribution of GFP as a means of determining the localization of racE in cells. **A:** Live cells. **B:** Fixed cell prepared according to Larochelle et al. (1997). In both cases GFP-racE localizes primarily to the plasma membrane. Bar = 10 μm.

The uniform distribution of racE throughout the plasma membrane did not preclude the possibility that, although uniformly distributed, racE may be selectively activated in a specific region of the plasma membrane during cytokinesis. For example, we hypothesized that racE may be specifically activated in the cleavage furrow or at the poles of a cell as it is about to undergo cell division. From the results of the previous section we knew that racE was not the signal that determined the timing of cytokinesis, but perhaps it was the signal that determined the placement of the contractile ring during cytokinesis. We could test for this possibility by determining the localization of the V20racE mutant protein. Since this form of the protein rescues the cytokinesis defect of racE null cells, its distribution must reflect the distribution of activated racE during cytokinesis. We created a GFP chimera using the V20racE construct and expressed GFP-V20racE in racE null cells. This protein also rescued the cytokinesis defect of racE null cells. Surprisingly, it showed the same plasma membrane distribution as GFP-racE (Fig. 3), suggesting that there is no cytokinesis-specific distribution of activated racE. Although it is possible that a sub-population of racE may be selectively activated at the time of cytokinesis, the process of cytokinesis is not impaired by having all of the racE in an activated state. Because of this, we believe that racE is not the signal that determines the placement of the contractile ring during cytokinesis.

In addition to wild-type racE and V20racE, we were able to use GFP to localize N25racE, racC, and GFP fused solely to the extended C-terminus of racE. Interestingly, all of these localized uniformly to the plasma membrane. This is most likely due to the presence of the C-terminal CAAX box, which serves as a prenylation site (Moores et al., 1991). Thus, even though racC colocalizes with racE at the plasma membrane, it cannot complement the cytokinesis defect in racE null cells. This implies that these two small GTPases must interact with different partners at the membrane.

We have found GFP to be invaluable to our studies on racE, yet several adjustments had to be made when visualizing GFP in *Dictyostelium* cells. Firstly, since the GFP-fusion proteins we have generated tended to be expressed at relatively low levels, we had to contend

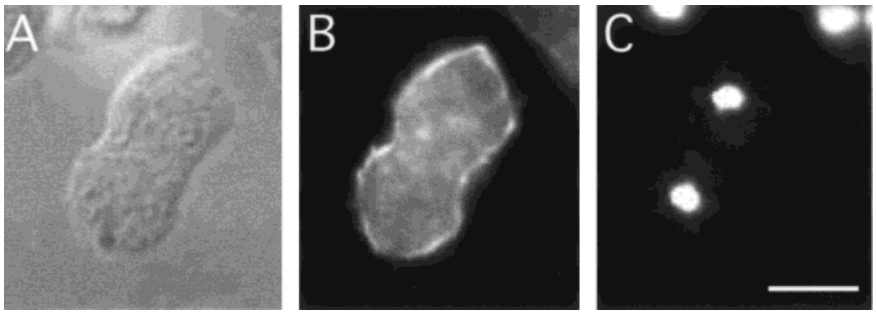


Fig. 3. Distribution of GFP-V20racE in a dividing cell. RacE-null cells expressing GFP-V20racE were fixed and stained with DAPI as described in Larochelle et al., 1997. **A:** DIC image. **B:** Visualization of GFP demonstrating a uniform distribution throughout the plasma membrane. **C:** Visualization of DAPI revealing that this cell was in the process of cytokinesis at the time of fixation. Bar = 10 μm.

with the high levels of background fluorescence seen in cells grown in HL5 media. This background fluorescence was often significant enough to obscure the signal from our GFP-fusion proteins. This situation, however, was easily remedied by incubating the cells in starvation buffer for one to several hours immediately before making observations. This was not necessary if the cells were to be fixed prior to observation but was necessary for observing live cells. As a control for the intracellular localization of the GFP-fusion proteins, we also engineered a construct that allowed us to express GFP alone (not as part of a fusion protein). GFP alone showed a uniform distribution throughout the cytosol and was not found in the plasma membrane. This allowed us to rule out the possibility that the fluorescence we were observing was due to the nonspecific distribution of GFP. For observing live cells, we have found that they were most easily kept viable during observations through the use of a Dvorak-Stotler Controlled Environment Culture Chamber (Nicholson Precision Instruments Inc., Gaithersburg, MD) that was periodically perfused with starvation buffer. We have also observed that high intensity fluorescent light (particularly at higher magnifications) was deleterious to *Dictyostelium* amoebae. Consequently, it is advised to minimize the time the cells are exposed to fluorescent light by searching for interesting cells (in our case, cells that were in the process of dividing) using either DIC or phase contrast optics at lower magnifications. Once such cells are located, they can then be observed under fluorescence illumination. This was found to be true whether using conventional fluorescence microscopy or confocal microscopy. Finally, because *Dictyostelium* amoeboid cells can assume many shapes we found it imperative to examine fixed cells that, in addition to expressing GFP-fusion proteins, were also stained with DAPI to allow us to visualize the nuclei. Only in this way could we be absolutely certain of the stage the cells were at in the cell cycle.

MICROSCOPIC ANALYSIS OF GROWTH IN SUSPENSION

From our initial screen, which identified racE, we knew that racE null cells became large and multinucleate in suspension, while remaining uninucleate with normal growth rates on a substrate.

To gain insight into the function of racE, we used video light microscopy to observe racE null cells as they divided on a substrate (Gerald et al., 1998). Similar to wild-type, racE null cells growing on a substrate formed a cleavage furrow and were able to successfully divide into two daughter cells. After division, wild-type cells rapidly crawled away by extending large pseudopods. In contrast, racE null cells did not form these distinct processes, and, instead, moved using a single broad lamellipodium at the leading edge. Thus, while racE null cells on a substrate successfully completed division, the resulting daughter cells displayed abnormal motile behavior.

Since *Dictyostelium* cells on a substrate were reported to produce cleavage furrows independently of myosin II (Neujahr et al., 1997), it was important to determine if myosin II properly localized to the furrow in racE null cells attached to a substrate. To answer this question, we observed the distribution of GFP-tagged myosin II in fixed wild-type and racE null cells. Similar to wild-type cells, racE null cells localized GFP-myosin II to successful cleavage furrows that formed at the appropriate time and place in the cells.

The above observations of racE null cells, up to this point, suggest that cytokinesis proceeds in a normal fashion when these cells are grown as an attached culture. We, therefore, are very interested in trying to determine the cause of cytokinesis failure when these cells are grown in suspension culture. Under these conditions, do these cells never form cleavage furrows, or do furrows form and cytokinesis fails at a different point? In order to address this problem, we had to devise a method of maintaining the cells in suspension culture in such a way that would also allow us to make microscopic observations of the cells. Typically, cells are maintained in suspension culture by shaking them on an orbital shaker. For microscopic observation, we developed a new method of maintaining the cells in suspension by culturing them in media containing a small amount of low-melting temperature agarose. A 0.1% stock solution of low gelling temperature agarose (SeaPlaque, cat no 50101; FMC Bioproducts, Rockland, ME) in HL5 medium was made fresh each time. The stock solution was warmed briefly to dissolve the agarose and subsequently chilled to 19°Celsius. The agarose media was diluted 1:3 with cells in suspension. Cells in this media were then placed in an observation

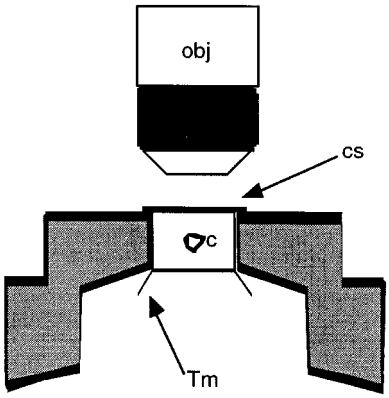


Fig. 4. A schematic cross section of the chamber used to observe cells in agarose culture. A complete diagram of the chamber and the microscopy setup has been published (Kiehart et al., 1994). The top of the chamber consists of a glass coverslip (cs) for use with an upright microscope. For the bottom of the chamber, a clear Teflon membrane (Tm) fitted with a metal gasket allows gas exchange and light transmission. The chamber was viewed on an upright Zeiss (Oberkochen, Germany) axioplan microscope. To maintain maximun range of focus through the chamber, a phase contrast Zeiss LD Achroplan 40× .6NA objective was used in conjunction with a 2× optivar setting. Cells in suspension have also been viewed with a Zeiss Achroplan 20× .45NA objective with a 4× optivar setting.

chamber that allowed oxygenation of the culture with a gas-permeable membrane (see Fig. 4).

We found that the agarose culture mimics conditions of cells maintained in suspension. Sparsely polymerized agarose provides enough resistance to keep cells from settling down to the substrate, but does not provide the tensile strength that would support efficient cell spreading and traction. At low concentrations of agarose, the extent of polymerization is not homogeneous. The agarose media is more accurately described as a "slurry" in which some cells settle out to attach and crawl normally, while others remain suspended above the substrate.

Wild-type cells in agarose culture divided similarly to cells on a substrate. Cells formed an equatorial cleavage furrow that constricted to completion. Because the daughter cells cannot maintain traction, they do not crawl very far and frequently tumbled around one another. Occasionally, wild-type daughter cells in agarose remained attached by a thin cytoplasmic bridge after furrow constriction, similar to attached cells. In the absence of traction or shear forces, these bridges may persist. However, the vast majority of wild-type cells rapidly completed division, and achieved several rounds of division if left undisturbed in agarose culture.

Similar to wild-type cells, racE null cells in agarose culture were able to initiate an equatorial cleavage furrow. However, in 21 examples, we never observed a racE null cell that successfully completed division in agarose. In 6 of these cells, the furrow initiated constriction only to halt midway and slowly revert. In the remaining 15 cells, however, the cells displayed violent shape changes as they failed in cytokinesis. These cells exhibited large blebs accompanied by strong cortical contractions in many different regions of the cells. Ultimately, the cleavage furrow was disrupted in all of the racE null examples, and the cells reverted to a spherical shape (Fig. 5).

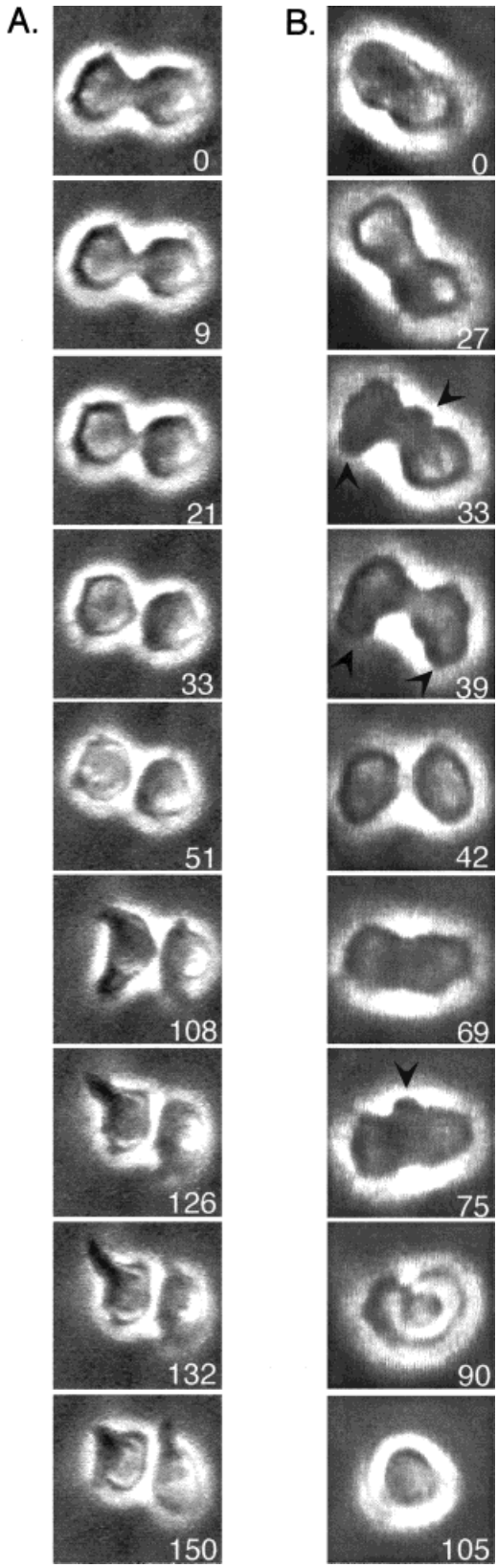
Since racE null cells in agarose were able to initiate a cleavage furrow, but unable to complete it, we wanted to determine the distribution of myosin II in these structures. To answer this question, we fixed wild-type and racE null cells expressing GFP-myosin II while in shaking suspension culture. Similar to wild-type cells, racE null cells in suspension localize GFP-myosin II to the cleavage furrow. However, direct measurement of the equatorial diameter of these fixed cells confirmed that racE null cells do not constrict these furrows to the same extent as wild-type cells.

DETERMINATION OF CORTICAL TENSION AND ACTIN DISTRIBUTION

The observation that racE null cells bleb in suspension culture suggested that the cell cortex of these cells might be particularly weak in the absence of attachment. To investigate this, we used a micropipet aspiration method to measure the cortical tension of cells in suspension (Gerald et al., 1998). Interestingly, the cortical tension of racE null cells was 1/5 the level measured for wild-type cells under similar conditions. Since this drop in tension was measured in interphase cells, this showed that racE null cells also have defects outside of cytokinesis. Importantly, this cortical tension defect was directly linked to the activity of racE protein. Expression of the constitutively active racE in the null background rescued the cortical tension to wild-type levels. In contrast, expression of the constitutively inactive racE did not increase cortical tension in racE null cells. Activated racE is essential for normal cortical tension in *Dictyostelium*.

The above observations (i.e., blebbing during cytokinesis and reduced cortical tension in racE null cells) suggested that the cortical cytoskeleton might be abnormal in these cells. Since other members of the rho family of GTPases are involved in the polymerization of F-actin structures in the cell, it was possible that racE augmented cell stiffness by increasing the amount of F-actin at the cortex. However, we found that the F-actin content of racE null cells is similar to the content seen in wild-type cells. This was true for cells both under attached and suspended conditions.

Alternatively, it was possible that racE might have a role in organizing the existing F-actin cortex. Initial observations showed that under attached conditions, racE null cells have F-actin distributions that are similar to wild-type cells. In these cells, F-actin was prevalent throughout the cortex and enriched in ruffles, crowns, and filopodia. These actin-based structures were also visible in all interphase cells fixed in suspension. Interestingly, however, racE null cells attempting cytokinesis in suspension did display aberrant F-actin distributions. While wild-type cells in suspension resembled attached examples, 40% of racE null cells attempting cytokinesis in suspension displayed abnormal aggregates of F-actin at the cortex. These aggregates were not visible at any other part of the cell cycle, but only appeared as racE null cells attempted division in suspension.



ISOLATION OF racE BINDING-PARTNERS

Being a small GTPase, racE must interact with multiple binding-partners that define a regulatory pathway required for cytokinesis. To define this pathway, we have begun to identify racE binding-partners using an affinity purification approach (Vithalani et al., 1998). In bacteria we have expressed GST-fusion proteins of racE in all its forms (wild-type and constitutively active and inactive). These fusion proteins were then used as affinity ligands to purify binding proteins from *Dictyostelium* cytosolic extracts. Using this approach we isolated a protein of 88 kDa that we cloned and named Darlin (*Dictyostelium* armadillo-like protein). As its name indicates, Darlin contains multiple copies of a peptide repeat related to that found in the armadillo/β-catenin proteins. The protein most closely related to Darlin is the mammalian rho-binding protein smgGDS (Yamamoto et al., 1990). Intriguingly, like smgGDS, Darlin is a broad-spectrum binding protein; it binds not only to racE but also to racC and even to mammalian cdc42 and TC4Ran. Like smgGDS, Darlin also binds preferentially to the GDP-loaded constitutively inactive forms of small GTPases.

It has been suggested that smgGDS is a nucleotide-exchange factor, although its activity is very poor. We have not been able to demonstrate such activity for Darlin. However, given their sequence organization, it is likely that Darlin and smgGDS fold into elongated superhelical structures similar to that of β-catenin. As in β-catenin, this structure probably allows Darlin and smgGDS to bind multiple proteins in a large regulatory complex. Thus, we postulated that Darlin is a scaffold protein that may participate in multiple signaling pathways that involve different small GTPases. Hence, its function would not be restricted to cytokinesis.

To test this hypothesis, we disrupted the gene encoding for Darlin. Remarkably, Darlin mutants did not have any defect in cytokinesis indicating that the interaction with racE is not essential for cell division. Darlin mutants did have a defect during the early aggregation phase of development.

CONCLUSIONS

Our studies of racE in *Dictyostelium* have provided us with a picture of what racE does in the cell that is required for cytokinesis. The mutagenesis experiments indicate that racE does not act as a switch in cell division, but rather that its activation is required to initiate a cascade of downstream events leading to an increase in the global cortical tension of *Dictyostelium* cells. Consistent with these results is the distribution of racE uniformly throughout the plasma membrane, where it is required for the development of this global

Fig. 5. RacE null cells form blebs and fail to divide in agarose culture. Wild-type and racE null cells were placed in 0.03% low melt agarose to mimic growth conditions in suspension. Cells were observed by video microscopy (t = seconds). **A:** Wild-type cells form a cleavage furrow that rapidly divides the cells. After division, the daughter cells extend pseudopods as they attempt to gain traction in the agarose. **B:** RacE null cells form a cleavage furrow, but cannot complete cytokinesis in suspension. As the cells attempt to divide, they form multiple blebs (arrowheads) and their furrows regress.

cortical tension. Thus, racE is not required at a particular time or place of the cell during the cell cycle, but rather it is always required at the cortex for the proper development of tension.

How is racE involved in cortical tension? Our studies indicate that racE is not simply inducing the polymerization of actin filaments at the cortex. Instead, it seems that racE is required for the proper organization of actin at the cortex. This organization may be achieved through the anchoring of filaments to the membrane or their cross-linking into a stronger cortical meshwork. Further studies are aimed at defining in better detail the cause of decreased cortical tension in racE mutants. Coincident with this we would also like to identify proteins, in addition to Darlin, that interact with racE. Our data indicates that the function of racE may not be redundant with that of other related small GTP-binding proteins in *Dictyostelium.* This implies that there must be proteins that interact specifically with racE in allowing it to carry out its unique function. We are in the process of identifying these proteins as a means of identifying the specific signal transduction pathway through which racE functions.

Why is cortical tension required for cytokinesis? Previous studies have shown that cells experience an increase in internal pressure as they enter cytokinesis (Burton and Taylor, 1997; Erickson and Trinkaus, 1976; Laster and Mackenzie, 1996). This is probably induced by the increased activity of myosin II during cytokinesis (Chen et al., 1994; Egelhoff et al., 1993; Larochelle and Epel, 1993; Pollenz et al., 1992; Satterwhite et al., 1992). Thus, cells must have enough tensile strength in their cortices to withstand the changes in internal pressure generated during cell division. This tensile strength may also be important for the successful constriction of the contractile ring.

Using a combination of molecular, genetic, and microscopy techniques we hope to delineate in molecular terms the contribution of racE for cytokinesis.

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