CELL STRUCTURE AND FUNCTION 26: 571–575 (2001)

REVIEW

Identification of Proteins Involved in Cytokinesis of *Dictyostelium*

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ABSTRACT. ***Dictyostelium*** **is one of the model systems of choice for studying the cytokinesis of animal-type cells. Two types of cytokinesis mutants have been used to identify proteins involved in the cytokinesis of *Dictyostelium*: (1) type I, the mutant cells grow on substrates to produce giant multinucleate cells; (2) type II, the mutant cells divide nearly normally on substrates, but are unable to divide at all and get highly multinucleate in suspension culture. These two mutant types might correspond to the myosin II-independent and myosin II-including cytokinesis mechanisms, respectively.**

Key words: cytokinesis/*Dictyostelium*/REMI/gene targeting/IQGAP/GAPA

The cellular slime mold *Dictyostelium discoideum* is a good model system for studying the molecular mechanism of cytokinesis in animal-type cells, because the morphological changes that occur during cytokinesis of *Dictyostelium* ameba on plastic plates are very similar to those of cultured animal cells. In addition, cytokinesis mutants that produce multinucleate giant cells can be isolated due to the haploid genome of this organism. Actually, since the first cytokinesis mutant of *Dictyostelium*, in which the gene encoding a unique myosin II heavy chain was disrupted, was reported in 1987 (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987), a number of proteins have turned out to be involved in cytokinesis by targeted gene disruption (knockout) using efficient homologous recombination of this organism (Table I). These include several actin-binding proteins as well as conventional myosin II and small GTPases of Ras and Rho families. In contrast, identification of genes involved in cytokinesis by classical forward genetics was not so successful at the beginning. This is due to incomplete plasmid libraries constructed on shuttle vectors, since the AT-rich genomic DNA of *Dictyostelium discoideum* is not so stable in *E. coli* cells. Fortunately, in 1992, an efficient tagging method, restriction enzyme-mediated integration (REMI), was successfully used to isolate mutants and clone genes involved in *Dictyostelium* development (Kuspa and Loomis, 1992). Then, using improved REMI (Bsr-REMI), we showed for the first time that it was possible to isolate *Dictyostelium* cytokinesis mutants that grow on plate culture and produce highly multinucleate cells (Adachi *et al.*, 1994). In this short review, we would like to summarize briefly the nature of cytokinesis mutants of *Dictyostelium* and identification of novel proteins involved in cytokinesis by forward genetics.

Two types of cytokinesis mutants in Dictyostelium

The first cytokinesis mutant in *Dictyostelium*, the myosin II heavy chain-null mutant, cannot divide at all in suspension culture; the cells keep getting larger and more multinucleate without cytokinesis until they burst (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). This clearly indicates that cytokinesis without the support of solid surface requires myosin II. However, this lethality is conditional. The myosin II-null cells can grow on plastic plates, and, surprisingly, most of the myosin II-null cells grown continuously on plates are mononucleate. Detailed analyses revealed that myosin II-null cells divide on plates nearly normally (Neujahr *et al.*, 1997; Zang *et al.*, 1997); that is, the cells constrict to form cleavage furrow at the equator region soon after the nuclear division. This myosin II-independent process observed under the support of solid surface is called attachment-assisted mitotic cleavage (Neujahr *et al.*, 1997) or cytokinesis B (Zang *et al.*, 1997). If the cells would be defective in this process, the cells could be highly multinucleate even when grown on plates. Conversely,

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Abbreviations: CHSp, Chediak-Higashi syndrome protein; GAP, GTPase-activating protein; GRD, GAP-related domain; REMI, restriction enzyme-mediated integration.

Table I. PROTEINS INVOLVED in *Dictyostelium* CYTOKINESIS

Protein	Mutant type	Reference
Identified by reverse genetics (knockout, antisense RNA, overexpression of WT, CA or DN forms)		
Myosin II (unique conventional myosin)		
heavy chain	II	(De Lozanne and Spudich, 1987; Knecht and Loomis, 1987)
essential light chain	II	(Pollenz *et al.*, 1992)
regulatory light chain	II	(Chen *et al.*, 1994)
Actin-binding proteins		
Profilin I and II (G actin sequestering)	I	(Haugwitz *et al.*, 1994)
Cortexillin I and II (bundling)	I	(Faix *et al.*, 1996)
Coronin	I	(de Hostos *et al.*, 1993)
DAip1	I	(Konzok *et al.*, 1999)
Small GTPases and their regulators and effectors		
RasG (H-Ras-like)	II	(Tuxworth *et al.*, 1997)
Rac1A, 1B, 1C (Rac1-like)	?*	(Dumontier *et al.*, 2000; Palmieri *et al.*, 2000)
GAPC (RasGAP-like)	II	(Inoue *et al.*, unpublished work)
DGAP1/DdRasGAP1 (IQGAP-like)	?**	(Faix and Dittrich, 1996; Lee *et al.*, 1997)
Protein Kinases		
PAKa (PAK-like)	II	(Chung and Firtel, 1999)
Others		
Clathrin	II	(Niswonger and O'Halloran, 1997)
Calmodulin	I	(Liu *et al.*, 1992)
TalA (Talin-like)	II	(Niewohner *et al.*, 1997)
CluA (novel)	I	(Zhu *et al.*, 1997)
AmiA/PiaA (novel)	I	(Nagasaki *et al.*, 1998)
DtrA (novel)	II	(Ginger *et al.*, 1998)
Identified by forward genetics (tagging = restriction enzyme-mediated integration, REMI)		
Small GTPases and their effectors		
RacE (Rho family GTPase)	II	(Larochelle *et al.*, 1996)
GAPA (IQGAP-like)	I	(Adachi *et al.*, 1997)
Others		
LvsA (beige/CHSp-like/FAN-like)	II	(Kwak *et al.*, 1999)
CykA (novel)	I	(Adachi *et al.*, unpublished work)
CykB (novel)	I	(Yamamoto *et al.*, unpublished work)

The type II mutants include ones that produced multinucleate cells but were not lethal in suspension. *It is not clear if the mutant cells were multinucleate or not on substrate. **The phenotype of the DGAP1-null mutants seemed dependent on the genetic background. The GAPA/DGAP1 double null mutants showed much more severe defect than the GAPA single null mutants on substrate, suggesting that DGAP1 could be involved at least in the myosin II-independent system.

mutant cells showing such a phenotype could be defective in this process. Mutants of this type were actually isolated by Bsr-REMI (Adachi *et al.*, 1994) as well as chemical mutagenesis (Adachi *et al.*, unpublished work).

Therefore, cytokinesis mutants of *Dictyostelium* could be roughly classified into two types: (1) type I, the mutant cells grow on substrates producing giant multinucleate cells; in this case, a gene involved in a myosin II-independent system could be disrupted; (2) type II, like myosin II-null cells, the mutant cells divide nearly normally and are mononucleate on the substrate, but are unable to divide at all getting highly multinucleate until the cells burst and die in suspension culture; in this case, a gene involved in a myosin II-including system could be disrupted.

Proteins identified from type I mutants

Seeking novel genes involved in the myosin II-independent mechanism of cytokinesis, we have isolated more than 20 type I mutants by Bsr-REMI. The method is essentially

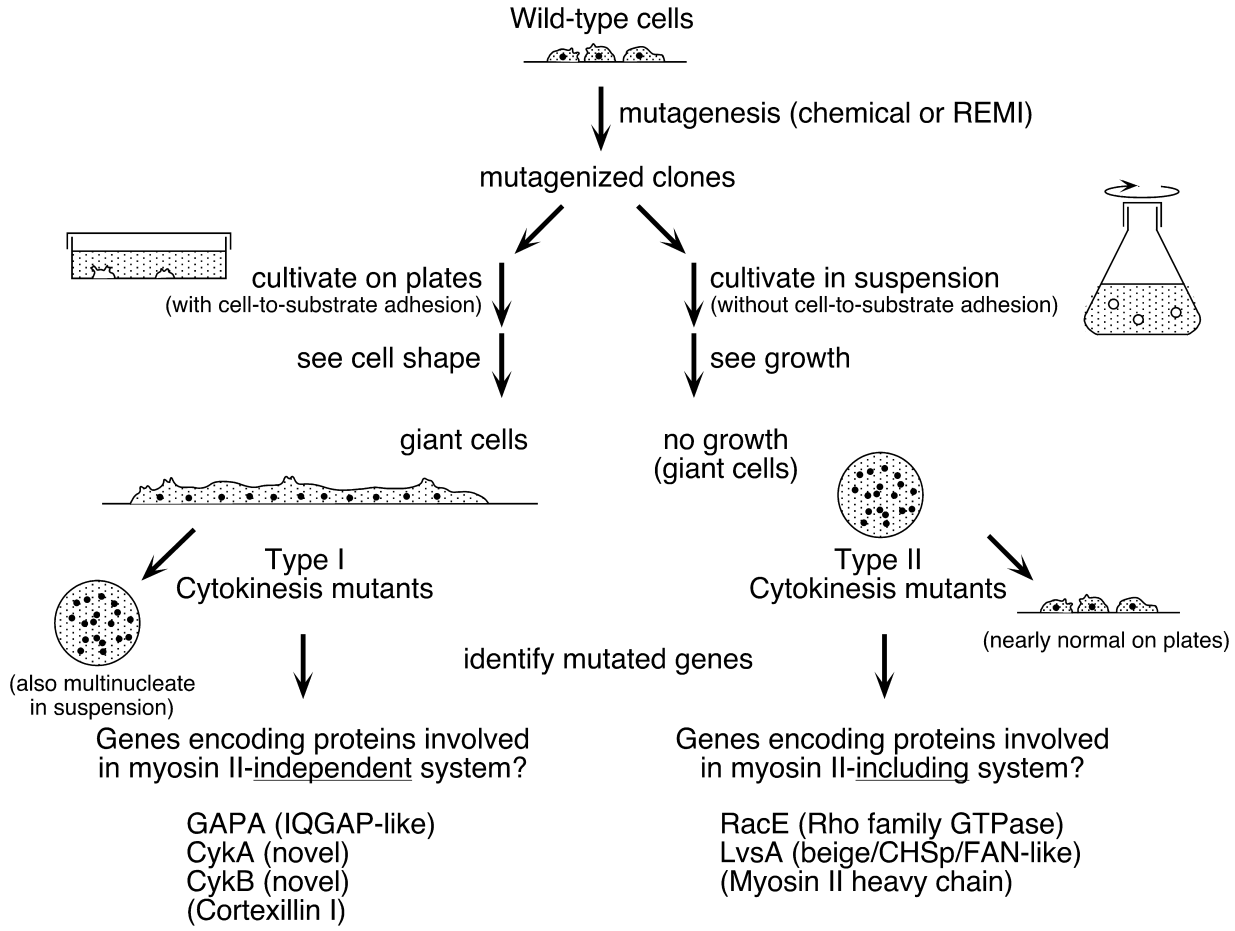

Fig. 1. Two methods of isolating cytokinesis mutants in *Dictyostelium*.

summarized on the left side of Figure 1. Briefly, colonies of REMI transformants were formed on plastic plates and were observed under microscope; the colonies including giant cells were picked up, and the mutant cells were then cloned and stored. The fragments of the disrupted genes were cloned from the genomic DNA of the mutants by the plasmid rescue method. Of ~1,000 independent REMI transformants, one type I cytokinesis mutant was obtained (Adachi *et al.*, 1994). So far, three novel genes, gapA (Adachi *et al.*, 1997), cykA (Adachi *et al.*, unpublished work) and cykB (Yamamoto *et al.*, unpublished work) have been identified. In the 34 Mbp genome of *Dictyostelium*, there are ~10,000 genes. Half the length of the entire genome is occupied by the non-coding regions, thus more type I genes should be identified. Sequencing of the disrupted genes from our library of type I cytokinesis mutants is currently in progress.

The gapA gene codes for an IQGAP-like protein GAPA. GAPA protein is entirely quite similar to the C-terminal half of mammalian IQGAP protein, an effector for Rho family small GTPases Rac1/Cdc42. In the middle of this homologous region, there lies a GAP-related domain (GRD) originally identified as the catalytic domain of RasGAP and thought to be a putative GTPase-binding domain. This is the first example showing that the elimination of an IQGAP gene causes cytokinesis defect. During cytokinesis of the GAPA-null cells, both the mitotic cell rounding and furrow formation normally occur, and only the cleavage of the cellular bridge connecting the daughter cells fails causing the furrow regression, suggesting that GAPA could contribute to this process. As expected, myosin II localized to the cleavage furrow in the GAPA-null cells, supporting the idea that myosin II is independent of the system including GAPA (Adachi *et al.*, 1997). Interestingly, GAPA-null cells were also defective in cytokinesis and produced multinucleate cells in suspension. In contrast to the myosin II-null cells, however, the GAPA-null cells grow and produce giant cells in suspension. All the type I mutants examined also grew and produced multinucleate cells in suspension, although the extent of the defect of each mutant was different (Adachi *et al.*, unpublished work). These findings suggest that the myosin-independent system including GAPA also contribute to cytokinesis in suspension. Thus, at least in

Dictyostelium cytokinesis, the system including IQGAP-like proteins could be a general system, whereas the myosin-including system could be a supporting one for the cell without the support of adhesion to a substrate. Recently, it has been shown that another *Dictyostelium* IQGAP-like protein DGAP1 has in part a function overlapping with that of GAPA (Faix *et al.*, 2001; Sakurai *et al.*, unpublished work). The GAPA/DGAP1 double null mutant was a type I mutant, and its cytokinesis defect was much more severe than that of GAPA.

From reverse genetic approaches including targeted gene disruption, anti-sense RNA expression and expression of dominant negative forms, several type I cytokinesis mutants have been reported (Table I). These include cortexillin-null mutants (Faix *et al.*, 1996). A cortexillin mutant was also isolated by chemical mutagenesis of *Dictyostelium* cells, followed by cDNA library complementation (Robinson and Spudich, 2000). Cortexillin is a homo- or heterodimer of cortexillin I and II (CI and CII, respectively) and an actin bundling protein. The reported phenotype of CI/CII double null mutant is very similar to that of GAPA/DGAP1 double null cells, suggesting some relationship between these proteins. Interestingly, recent works suggested an interaction between IQGAP-like proteins and cortexillin (Faix *et al.*, 1996; Imai *et al*., unpublished work). It is important to identify such interactions between proteins identified from type I mutants, if we are to understand the mechanism of myosin II-independent cytokinesis at a molecular level.

In contrast to the successful identification of interaction between IQGAP-like proteins and cortexillin, putative small GTPases of *Dictyostelium* that bind to GAPA (Sakurai *et al.*, 2001) have yet to be identified in spite of our efforts. It is already reported that DGAP1 binds to *Dictyostelium* homologues of mammalian Rac1, Rac1A, Rac1B and Rac1C (Faix *et al*., 1998). However, *Dictyostelium* Rac1s did not bind to GAPA (Imai *et al*., unpublished work), suggesting that the two IQGAP-like proteins have distinct roles in cytokinesis. Putative small GTPases that bind to GAPA or to both GAPA and DGAP1 have to be identifed in order to extend the signaling pathway including the IQGAP-cortexillin complex.

Proteins identified from type II mutants

Expecting to find novel genes involved in the myosin II-including mechanism of cytokinesis, De Lozanne's group has isolated cytokinesis mutants that cannot grow in suspension culture as candidates for type II mutants. The method is essentially summarized on the right side of Figure 1. Briefly, after REMI mutagenesis, transformants obtained on plates were clonally transferred to suspension culture. They selected mutant cell lines that did not grow to produce multinucleate cells in suspension culture. So far, two novel genes encoding Rho family GTPase RacE (Larochelle *et al.*, 1996) and beige/Chediak-Higashi syndrome protein (CHSp)-related protein LvsA (Kwak *et al.*, 1999) were reported to be identified as well as myosin II heavy chain gene. Interestingly, both RacE-null and LvsA-null mutants scarcely produce multinucleate cells when grown on plates (type II mutant) like the myosin II null mutant. Thus, it is likely that both RacE and LvsA proteins work in the myosin II-including system, although no interactions between these three proteins have not been reported. Detailed analysis revealed that RacE protein localizes to the cell cortex but is not accumulated at the furrow during cytokinesis (Larochelle *et al.*, 1997). RacE is thought to be involved in the generation of cortical tension required for cytokinesis (Gerald *et al.*, 1998). LvsA protein is homologous to the beige/CHS proteins, suggesting that LvsA is involved in membrane traffic (Kwak *et al.*, 1999). It is known that clathrin-null mutant has a phenotype similar to that of LvsA-null cells (Niswonger and O'Halloran, 1997). Thus, both LvsA and clathrin could be involved in membrane traffic required for cytokinesis, although the relationship between these and myosin II are not clear.

From reverse genetic approaches, several type II cytokinesis mutants have been reported (Table I). These include the null mutants of PAK-like protein kinase PAKa (Chung and Firtel, 1999), and *Dictyostelium* homologue of H-Ras, RasG (Tuxworth *et al.*, 1997), as well as essential (Pollenz *et al.*, 1992) and regulatory (Chen *et al.*, 1994) light chains of myosin II. PAKa protein localized to cleavage furrow in cytokinesis and is involved in the assembly of myosin II, probably regulating myosin II heavy chain kinase (Chung and Firtel, 1999). It is very interesting that in *Dictyostelium*, a Ras is involved in cytokinesis. This is supported by our recent result that overexpression of GAPC, a putative GTPase-activating protein for Ras, induced multinucleate cells only in suspension culture (Inoue *et al*., unpublished work).

Conclusion

There seem to be two types of cytokinesis mutants in *Dictyostelium*, each representing two distinct mechanisms of cytokinesis, myosin II-independent and myosin II-including ones. Several proteins likely to be involved in each system have been identified from these two types of cytokinesis mutants. However, in both systems, further identification of their protein components is required for making complete catalogs. Furthermore, only a few interactions between the components have been identified at this stage. In order to understand the molecular mechanism of cytokinesis, protein-protein interactions within the systems as well as those between the systems need to be clarified.

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(*Received for publication, November 29, 2001*
and accepted, November 29, 2001)