FEBS Letters 453 (1999) 20–24 FEBS 22142

The small GTPases Rab5a, Rab5b and Rab5c are differentially phosphorylated in vitro

Mario Chiariello, Carmelo B. Bruni*, Cecilia Bucci

Dipartimento di Biologia e Patologia Cellulare e Molecolare 'L. Califano' and Centro di Endocrinologia ed Oncologia Sperimentale 'G. Salvatore' del Consiglio Nazionale delle Ricerche, Via S. Pansini 5, 80131 Napoli, Italy

Received 13 April 1999; received in revised form 11 May 1999

Abstract Rab GTPases play a fundamental role in the regulation of membrane traffic. Three different Rab5 isoforms have been reported but no differences in their function in endocytosis have been discovered. As the Rab5 isoforms show a conserved consensus site for Ser/Thr phosphorylation, we investigated whether this site was phosphorylated. Here, we report that the three Rab5 proteins are differentially recognized by different kinases. Rab5a is efficiently phosphorylated by extracellular-regulated kinase 1 but not by extracellular-regulated kinase 2, while cdc2 kinase preferentially phosphorylates Ser-123 of Rab5b. These findings strongly suggest that phosphorylation could be important to differentially regulate the function of the Rab5 isoforms.

Key words: Rab5; Endocytosis; Phosphorylation; Eukaryotic cell

1. Introduction

Rab proteins regulate membrane traffic in the endocytic and biosynthetic pathway [1,2]. Among them, Rab5 is localized to the early endosomal compartment and has been implicated in the regulation of the plasma membrane to early endosomes transport [3,4]. A common feature of the Rab family of small GTPases is the existence of subgroups of structurally related isoforms sharing high a sequence identity (85–95%) [5]. The Rab5 subgroup comprises three isoforms (Rab5a, Rab5b and Rab5c) that are localized to the plasma membrane and early endosomes and are ubiquitously expressed [6,7]. These proteins are essential for in vitro homotypic fusion of early endosomes and in vivo regulation of endocytosis. Overexpression of wild-type or mutant proteins impaired in their ability to hydrolyze GTP causes an increase of the internalization rate of endocytosis, while expression of dominant negative mutants, impaired in their ability to bind GTP, inhibits the kinetics of endocytosis [6]. Interestingly, three highly related Rab5 proteins have been isolated in yeast and have been named Ypt51p, Ypt52p, Ypt53p [8]. These proteins share between 52 and 54% identity with Rab5a and seem to have different but overlapping functions in the endocytic pathway, suggesting a similarity with the situation in mammalian cells [8,9]. It is not yet clear whether the conserved isoforms in the Rab protein family have distinct roles and are responsible for a more fine regulation of membrane traffic or if they are just redundant. Up to now, no differences in the way the three Rab5 proteins control the early steps of endocytosis in mammalian cells have been found [6].

The progressive activation of several cyclin-dependent kinases (CDKs) tightly regulates the progression through the eukaryotic cell cycle. Passage through the different phases greatly affects the intracellular membrane trafficking and cdc2 has been suggested to directly regulate some of these processes by phosphorylating Rab1 and Rab4 proteins, during mitosis [10,11].

Both activation of Ras and of one of its downstream effectors, phosphatidylinositol 3-kinase, stimulate endocytosis [12,13]. Increasing evidences point to Rab proteins (and more specifically to Rab5) as possible mediators of at least some of these effects [14]. Conversely, several reports indicate that direct phosphorylation of Rab proteins may regulate their activity and/or cellular localization. Rab3b, Rab6 and Rab8 are phosphorylated upon thrombin stimulation of platelets [15] and Rab8 itself, in its active form, may interact with a stress-activated GC kinase [16]. Interestingly, upon phosphorylation, Rab1 and Rab4 changed their distribution becoming mostly cytosolic [10]. Rab4 is also phosphorylated by the insulin-activated extracellular signal-regulated kinase (ERK) ERK1, suggesting that the insulin-induced movement of Rab4 from the Glut-4-containing vesicles to the cytosol could result from phosphorylation of Rab4 by ERK [17]. Altogether, these data suggest that a network of kinases may be involved in the regulation of endocytosis, eventually phosphorylating Rab proteins and modifying their activity and/or subcellular localization.

Rab5a, Rab5b and Rab5c have one conserved phosphorylation consensus motif in position 123 that may be recognized by a proline-directed Ser/Thr kinase. In this paper, we show that these proteins can be phosphorylated in vitro by specific kinases. Furthermore, our data indicate that the three Rab isoforms can be differentially phosphorylated by these kinases, suggesting that phosphorylation could specifically modulate the function of the different Rab5 isoforms in vivo.

2. Materials and methods

2.1. Plasmid construction

The previously described cDNAs for Rab5a, Rab5b and Rab5c [6,18] were cloned in pET-16b as *Nde*I-*Bam*HI (Rab5a and Rab5b) and *Nde*I-*Hin*dIII (Rab5c) fragments. The point mutant Rab5bS123Q was created by site-directed mutagenesis using the two oligonucleotides 5′-GCTAGGCTGGGCTTGCCGCTGCAGTTCC-3′ and 5′-AGCCCAGCCTAGCATTGTTATTGCCT-3′. The PCR product was gel-purified and cloned into the pET-16b-rab5b as an *Nde*I fragment. Cloning and purification of GST-c-jun79 substrate have been previously described [19].

2.2. Quantification of Rab5a, Rab5b and Rab5c

Rab5a, Rab5b and Rab5c protein were purified from *Escherichia*

*Corresponding author. Fax: (39) (81) 7703285.
E-mail: brucar@unina.it

PII: S0014-5793(99)00686-9

coli containing the PET-16b expression plasmid as described [20]. Confluent HeLa cells were lysed in Laemmli buffer and loaded on a 12% SDS-PAGE. Proteins were then transferred on a nitrocellulose filter, the filter was then incubated with monoclonal anti-Rab5, polyclonal anti-Rab5b or anti-Rab5c antibody, followed by a HRP-conjugated anti-mouse or anti-rabbit antibody. Bands were then detected by chemiluminescence (ECL, Amersham). Quantification was performed with a phosphorimager using recombinant Rab5a, Rab5b and Rab5c as standards.

2.3. Western blot and GTP overlay

Cells were transfected for 48 h with pCDNA3 plasmids encoding Rab5b wild-type, Rab5bQ79L, Rab5bS123Q and Rab5bN133I. Cells were then lysed in standard SDS sample buffer and extracts were electrophoresed on 12% SDS-PAGE. For immunoblotting, separated proteins were transferred to a nitrocellulose membrane. The filter was then blocked with 5% milk in PBS for 40 min at room temperature. Then, primary mouse monoclonal 9E10 anti-myc antibody was added at the appropriate dilution and incubated for 2 h at room temperature. The filters were washed, incubated with a secondary anti-mouse HRP-conjugated antibody for 1 h at room temperature and the bands were visualized using the enhanced chemiluminescence system (ECL, Amersham). For GTP overlay, after electrophoresis, the gel was treated, transferred to a nitrocellulose filter and incubated with [α-^{32}P]GTP as described.

2.4. cdc2 kinase phosphorylation assay

Confluent plates of HeLa cells were synchronized in the interphase by treatment with 2 mM thymidine for 16 h and in mitosis by a 16 h treatment with 1 mM nocodazole. Interphasic cells were collected by scraping in cold PBS and mitotic cells by pipetting. Cells were then lysed at 4°C in a buffer containing 50 mM Tris pH 7.4, 250 mM NaCl, 0.1% Triton X-100, 5 mM EDTA, 50 mM sodium fluoride, 0.1 mM vanadate, 0.1 mM PMSF. Cleared lysates were immunoprecipitated for 6 h at 4°C with 2 μg of p34cdc2-specific antibody (Gibco). Immunocomplexes were recovered with the aid of protein A-Sepharose. Beads were washed three times with PBS containing 1% Nonidet P-40 and 2 mM vanadate, once with 100 mM Tris, pH 7.5, 0.5 M LiCl and once in kinase reaction buffer (25 mM HEPES, pH 7.6, 20 mM $MgCl_2$, 20 mM β-glycerophosphate, 0.1 mM sodium vanadate and 2 mM DTT). Samples were resuspended in 30 μl reaction buffer containing 1 μCi γ-ATP, 130 μM unlabelled ATP, 3 mM of DTT and 2 μg of different purified substrates. After 15 min at 30°C, reactions were terminated by the addition of 10 μl 5×Laemmli buffer. Samples were heated at 95°C for 5 min and analyzed by 12% SDS-PAGE.

2.5. ERK1 and ERK2 phosphorylation assay

L6 cells, stably expressing insulin receptors, were starved for 16 h in DMEM containing 0.5% BSA. Cells were eventually stimulated for 6 min with 1 μM insulin and then lysed at 4°C in 10 mM Tris pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.4 mM EDTA, 10 mM NaF, 2 mM vanadate, 0.1 mg/ml PMSF. Cleared lysates were then rocked for 2 h at 4°C in the presence of 500 ng α-ERK1- or α-ERK2-specific antibodies (Santa Cruz). Immunocomplexes were recovered with the aid of protein A-Sepharose. Beads were then washed three times with PBS containing 1% Nonidet P-40 and 2 mM vanadate, once with 100 mM Tris, pH 7.5, 0.5 M LiCl and once in kinase reaction buffer (20 mM HEPES pH 7.5, 10 mM MgAc, 0.1 mg/ml PMSF, 2 mM vanadate, 100 μM ATP).

Samples were resuspended in 30 μl reaction buffer containing 1 μCi γ-ATP, 50 μM unlabelled ATP, 3 mM DTT and 8 μg of different purified substrates. After 30 min at 30°C, reactions were terminated by adding 10 μl of 5×Laemmli buffer. Samples were then heated at 95°C for 5 min and analyzed by 12% SDS-PAGE. In some experiments, COS1 cells were used and were transfected by electroporation with 10 μg of pCDNA3-MEK or pCDNA3-MEKEE (the activated form of MEK1) [21] before the starvation of 16 h in DMEM containing 0.5% BSA.

2.6. HA-ERK2 and HA-JNK1 phosphorylation assay

Kinase assays on cells transfected with the epitope-tagged ERK2 or JNK1 were performed as follows. Subconfluent COS1 cells were transfected with 3 μg pCDNA3-HA-ERK2 or pCDNA3-HA-JNK1 and with 3 μg of pCDNA3-EGFR by electroporation. After 24 h, the medium was changed and the cells were then cultured overnight in serum-free medium. Cells were eventually stimulated with insulin for 6 min or with EGF for 15 min and processed as above. The epitope-tagged ERK2 or JNK1 were immunoprecipitated from the cleared lysates by incubation with the specific antibody 12CA5 (Boehringer Mannheim) for 1 h at 4°C. Beads were then washed three times with PBS containing 1% Nonidet P-40 and 2 mM vanadate, once with 100 mM Tris, pH 7.5, 0.5 M LiCl and once in kinase reaction buffer (20 mM HEPES pH 7.5, 10 mM MgAc, 0.1 mg/ml PMSF, 2 mM vanadate, 100 μM ATP for ERK2 or 25 mM HEPES, pH 7.6, 20 mM $MgCl_2$, 20 mM β-glycerophosphate, 0.1 mM sodium vanadate and 2 mM DTT for JNK1).

Samples were resuspended in 30 μl of reaction buffer containing 1 μCi γ-ATP, 50 μM unlabelled ATP, 3 mM DTT and 2 μg of different purified substrates. After 15 min at 30°C, reactions were terminated by adding 10 μl 5×Laemmli buffer. Samples were then heated at 95°C for 5 min and analyzed by 12% SDS-PAGE.

3. Results and discussion

The proper timing and coordination of cell cycle events is ensured, in higher eukaryotes, by a family of closely related enzymes, namely CDKs [22]. The activity of these Ser/Thr protein kinases are, in turn, tightly regulated by several mechanisms, such as binding of regulatory subunits and phosphorylation (or dephosphorylation) of conserved threonine and tyrosine residues [23,24]. The transition from the G2 to M phase is controlled by Cdc2 (CDK1), in association with A- and B-type cyclins. Through the phosphorylation of different substrates, Cdc2 is responsible for many of the cellular events that take place in mitotic cells, chromosome condensation, disassembly of the nuclear lamina, formation of the mitotic spindle and Golgi fragmentation [22,25,26]. Noteworthy, cdc2 kinase is also responsible for Rab1 and Rab4 phosphorylation during mitosis [10,11]. We thereby decided to investigate whether the three Rab5 isoforms were also recognized and phosphorylated by this kinase. We immunoprecipitated inactive or active p34cdc2 kinase from HeLa cells in the interphase (I) or mitosis (M), respectively. We then tested their ability to phosphorylate purified His-Rab5a, His-Rab5b, His-Rab5c proteins and histone H1. Histone H1 is a specific substrate for p34cdc2 and was used as a positive control. As expected, no phosphorylating activity was detected in interphase cell extracts while histone H1 was strongly phosphorylated in extracts derived from mitotic cells (Fig. 1). His-Rab5b was also efficiently phosphorylated by cdc2 kinase in mitotic cells but not (as expected) in interphase cells (Fig. 1). A weak band corresponding to the His-Rab5a protein was visible indicating that Rab5a was phosphorylated by cdc2 kinase

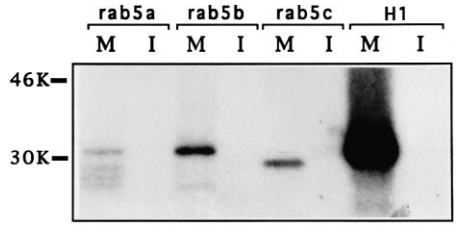

Fig. 1. Rab5 phosphorylation in vitro by the p34cdc2 kinase. p34cdc2-specific antibody (Gibco) was used to immunoprecipitate cdc2 kinase from quiescent and mitotic cells. Immunocomplexes were recovered with the aid of protein A-Sepharose as described in Section 2 and incubated with 2 μg of the different purified isoforms. After 15 min at 30°C, reactions were terminated by the addition of 10 μl 5×Laemmli buffer, heated at 95°C for 5 min and analyzed by 12% SDS-PAGE. I = interphase, M = mitosis, H1 = histone H1.

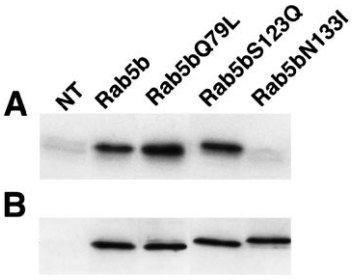

Fig. 2. Western blot and GTP-binding blot of Rab5b wild-type and mutant proteins. Cells were transfected with plasmids encoding the different proteins. The lysates were then run on SDS-PAGE and subjected to GTP-binding (A) or Western blot (B) analysis as described in Section 2. NT = non-transfected.

although to a lesser extent when compared to Rab5b. When Rab5c was used as a substrate, a faster migrating band was detected (Fig. 1). Western blot analysis indicated that this band was not recognized by anti-Rab5c antibodies and that Rab5c had the same migration rate as Rab5a and Rab5b (data not shown). These data thereby show that cdc2 kinase can specifically discriminate between the different Rab5 isoforms, suggesting a mechanism in vivo by which Rab5b, among the three Rab5 isoforms, may selectively mediate the effect of the cell cycle on the early endocytic processes. This is of particular interest considering the widespread presence of the three Rab5 isoforms in cellular types investigated up to date (unpublished data).

To establish whether the three proteins were all expressed at the same time, also in the system used, HeLa cells, and whether there were considerable differences in their concentration, we decided to quantify the amount of Rab5a, Rab5b and Rab5c protein in these cells using recombinant Rab5 proteins as standards (data not shown). The concentration of Rab5a protein was previously found to be ~0.07 pg/cell in MDCKI cells [27]. In HeLa cells, we have found that the concentration was about the same (~0.08 pg/cell). The concentration of Rab5b and Rab5c was about 0.1 pg/cell and 0.04 pg/cell, respectively (data not shown). These data show that the concentration of the three Rab5 isoforms in HeLa cells is very similar, Rab5b being the most abundant one.

To identify the precise amino acid phosphorylated, we then mutated the Ser in the consensus phosphorylation motif that we have identified in the Rab5b protein. Using PCR-mediated site-directed mutagenesis, we substituted the Ser in position 123 with a glutamine, creating the Rab5bS123Q mutated cDNA. The mutated cDNA was cloned in pCDNA3 for the expression in mammalian cells and into PET-16b for the expression and purification in bacteria. We checked if the mutated protein was able to bind GTP to test if the mutant protein was still functional. Total cell extracts from cells expressing Rab5b wild-type, Rab5bQ79L, Rab5bS123Q and Rab5bN133I were run on SDS-PAGE. As shown in Fig. 2, a GTP-binding blot (A) and Western blot (B) analysis were performed. The four proteins were expressed at comparable levels in HeLa cells (Fig. 2B). Moreover, Rab5b wild-type, Rab5bQ79L and Rab5bS123Q bind GTP while the dominant negative mutant Rab5bN133I did not. This shows that the Rab5bS123Q protein is able to bind GTP efficiently, thereby demonstrating that the mutation does not affect the structure of the protein. We then repeated the cdc2 kinase assay using histone H1, Rab5b wild-type and Rab5bS123Q. As shown in Fig. 3, phosphorylation of the Rab5b protein was completely abolished by the mutation demonstrating that the Ser in position 123 is the phosphorylation site recognized by the cdc2 kinase.

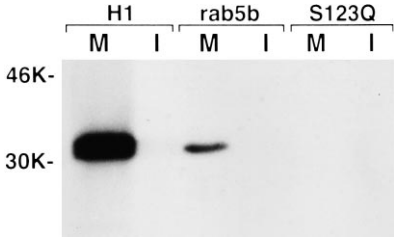

Fig. 3. Identification of the Rab5b phosphorylation site. Immunoprecipitated cdc2 kinase was obtained and incubated with the different substrates as described in Section 2. The reactions were then analyzed by 12% SDS-PAGE. I = interphase, M = mitosis, H1 = histone H1.

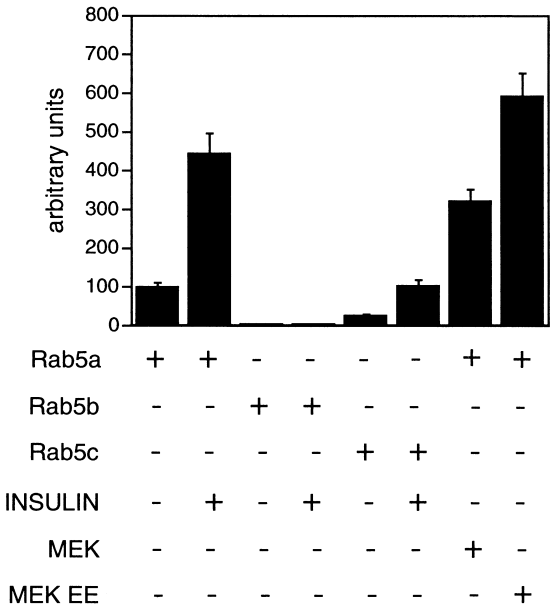

Fig. 4. Phosphorylation in vitro by ERK1. ERK1 was immunoprecipitated from unstimulated cells or from cells stimulated for 6 min with insulin as described in Section 2. In some samples, the cells were previously transfected with a plasmid encoding MEK or MEK EE. The immunoprecipitated kinases were then used to test their ability to phosphorylate Rab5a, Rab5b and Rab5c in vitro as described in Section 2.

Most of the responses of a cell to extracellular stimuli result from the sequential activation of signal transduction cascades, usually consisting of kinase modules that include a MAPK, a MAPK kinase (MEK) and a MEK kinase (MEKK) [28]. The best characterized MAPK cascades culminate with the activation of the extracellular-regulated kinases 1 and 2 (ERKs) and of the c-Jun N-terminal kinases. These, in turn, phosphorylate several specific substrates controlling very different cellular functions such as proliferation, differentiation, apoptosis and cellular metabolism [28–30]. The molecular switch that starts these kinase cascades is usually a small GTP-binding protein, Ras for MEK1 and MEK2 and Rac/Cdc42 for JNK-1 [29]. Due to the complexity of each single transduction pathway and to the number of new small GTP-binding proteins and kinases cloned, there is now a great interest in the study of the

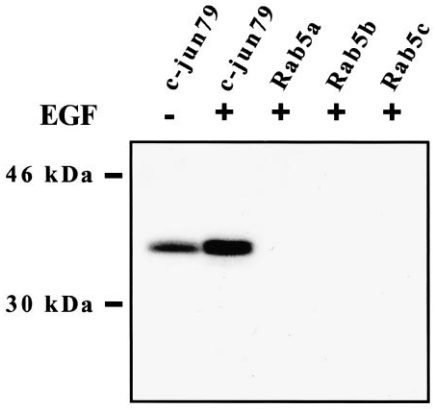

Fig. 5. Phosphorylation in vitro by Jun kinases. Immunoprecipitated Jun kinase was recovered from cells stimulated with EGF and then, an in vitro phosphorylation assay was performed on c-jun79, Rab5a, Rab5b and Rab5c proteins as described in Section 2.

integration of the transduced signals, both at the level of the small GTPases and at the level of the kinases and substrates. MAPKs and CDKs, even if controlled by very different mechanisms, share an important common feature: a substrate specificity markedly dependent on the presence of a proline residue immediately following the site of Ser/Thr phosphorylation, being usually classified in a unique family as 'proline-directed Ser/Thr kinases'. It is thereby possible that these enzymes may share common substrates, that, in turn, may participate to the control of intracellular events, such as the vesicular traffic, whose tight regulation needs to be ensured in situations so different as extracellular stimulation or progression through the different phases of the cell cycle. We then tested the ability of MAP kinase to phosphorylate the three Rab5 isoforms. In particular, we checked whether immunoprecipitated active ERK1 or ERK2 were able to phosphorylate the three Rab5 isoforms. As a control for both the ERK1 and ERK2 kinase assay, myelin basic protein (MBP) was used as it is a specific substrate for these kinases. MBP was highly phosphorylated by both kinases as expected and phosphorylation was strongly increased after insulin stimuli (data not shown). No phosphorylation on Rab5 proteins was detected in our conditions when the ERK2 kinase was immunoprecipitated from insulin-stimulated HeLa, COS1 and L6 cells or when epitope-tagged HA-ERK2 was transfected and then immunoprecipitated (data not shown). Conversely, Rab5a was efficiently phosphorylated by immunoprecipitated ERK1 after insulin stimulation. In Fig. 4, quantitative data are plotted from experiments performed on L6 cells with and without 6 min of insulin stimulation using immunoprecipitated ERK1. The phosphorylation of Rab5a was increased about four times upon insulin induction. Moreover, transfection of a plasmid containing MEK EE (the activated form of MEK1) increased about two times the Rab5a phosphorylation by MEK1, therefore confirming the specificity of the phosphorylation reaction. A weak phosphorylation of Rab5c by ERK1 was detected and insulin stimulation increased it about four times. However, no phosphorylated band was detected in the case of Rab5b. This result is of particular interest since only the Rab5a protein is efficiently recognized by ERK1, thereby again strongly suggesting that the function of the three Rab5 isoforms could be differentially regulated by phosphorylation. Moreover, the fact that no phosphorylation was detected when using the ERK2 kinase (even if we transfected the epitope-tagged construct) indicates that ERK1 and ERK2 may have a different substrate specificity.

We decided to test if jun kinases were also able to phosphorylate any of the three Rab5 isoform. c-jun79 is a natural substrate for JNK1 substrate and its phosphorylation is induced upon stimulation by EGF as shown in Fig. 5. However, when we used EGF stimulation and His-Rab5a, His-Rab5b and His-Rab5c as substrates, no phosphorylation was detected (Fig. 5).

Rab5a, Rab5b and Rab5c are three isoforms sharing more than 80% sequence identity. The three proteins regulate the initial steps of endocytosis, being able to change the kinetics of uptake of different endocytic markers [6]. The presence of isoforms that derive from different genes is a common feature of the Rab protein family. It is still under debate whether these isoforms represent just a safety mechanism of the cell to ensure, for instance, the Rab5 function in the presence of lethal mutations in one of the genes or if their existence could be important to perform specific functions through differential regulation. Our data indicate that the three Rab5 isoforms have a common phosphorylation site for Ser/Thr kinases that is differentially recognized in vitro by specific kinases. Indeed, ERK1 efficiently phosphorylated the Rab5a protein after insulin stimulation but was not able to phosphorylate Rab5b, while p34cdc2 kinase preferentially phosphorylated Rab5b. Conversely, no phosphorylation was detected by Jun kinases.

In conclusion, our data, together with previously published evidence that other Rab proteins are phosphorylated, strongly indicate that phosphorylation plays a fundamental role in controlling the Rab function. In particular, our data suggest that the three Rab5 isoforms are differentially regulated in response to different stimuli and that phosphorylation could be responsible for a fine regulation of endocytosis in vivo. More work will be required to establish how phosphorylation of the three Rab5 isoforms influences their function in the endocytic pathway.

Acknowledgements: This project was supported in part by Grants form the European Community (number ERBFMRKCT960020 to C.B.) and from the Italian Consiglio Nazionale per le Ricerche (Progetto Finalizzato Biotecnologie to C.B.).

References

[1] Olkkonen, V. and Stenmark, H. (1997) Int. Rev. Cytol. 176, 1–85.
[2] Novick, P. and Zerial, M. (1997) Curr. Opin. Cell Biol. 9, 496–504.
[3] Bucci, C., Parton, R.G., Mather, I.H., Stunnenberg, H., Simons, K., Hoflack, B. and Zerial, M. (1992) Cell 70, 715–728.
[4] Li, G. and Stahl, P.D. (1993) J. Biol. Chem. 268, 24475–24480.
[5] Valencia, A., Chardin, P., Wittinghofer, A. and Sander, C. (1991) Biochemistry 30, 4637–4648.
[6] Bucci, C., Lütcke, A., Steele-Mortimer, O., Chiariello, M., Olkkonen, V.M., Dupree, P., Bruni, C.B., Simons, K. and Zerial, M. (1995) FEBS Lett. 366, 65–71.
[7] Wilson, D.B. and Wilson, M.P. (1992) J. Clin. Invest. 89, 996–1005.
[8] Singer-Krüger, B., Stenmark, H., Düsterhöft, A., Philippsen, P., Yoo, J.-S., Gallwitz, D. and Zerial, M. (1994) J. Cell Biol. 125, 283–298.
[9] Singer-Krüger, B., Stenmark, H. and Zerial, M. (1995) J. Cell Sci. 108, 3509–3521.

[10] Bailly, E., McCaffrey, M., Touchot, N., Zahraoui, A., Goud, B. and Bornens, M. (1991) Nature 350, 715–718.
[11] van der Sluijs, P., Hull, M., Huber, L.A., Mâle, P., Goud, B. and Mellman, I. (1992) EMBO J. 11, 4379–4389.
[12] Li, G., D'Souza-Shorey, C., Barbieri, M.A., Cooper, J.A. and Stahl, P. (1997) J. Biol. Chem. 272, 10337–10340.
[13] Li, G., D'Souza-Shorey, C., Barbieri, M.A., Roberts, R.L., Klippel, A., Williams, L.T. and Stahl, P.D. (1995) Proc. Natl. Acad. Sci. USA 92, 10207–10211.
[14] Barbieri, M.A., Kohn, A.D., Roth, R.A. and Stahl, P.D. (1998) J. Biol. Chem. 273, 19367–19370.
[15] Karniguian, A., Zahraoui, A. and Tavitian, A. (1993) Proc. Natl. Acad. Sci. USA 90, 7647–7651.
[16] Ren, M., Zeng, J., de Lemos-Chiarandini, C., Rosenfeld, M., Adesnik, M. and Sabatini, D.D. (1996) Proc. Natl. Acad. Sci. USA 93, 5151–5155.
[17] Cormont, M., Tanti, J.-F., Zahraoui, A., van Obberghen, E. and Le Marchand- Brustel, Y. (1994) Eur. J. Biochem. 219, 1081–1085.
[18] Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K. and Zerial, M. (1990) Cell 62, 317–329.
[19] Coso, O.A., Chiariello, M., Kalinec, G., Kyriakis, J.M., Woodgett, J. and Gutkind, S. (1995) J. Biol. Chem. 270, 5620–5624.
[20] Gorvel, J.-P., Chavrier, P., Zerial, M. and Gruenberg, J. (1991) Cell 64, 915–925.
[21] Coso, O.A., Chiariello, M., Yu, J., Teramoto, H., Crespo, P., Xu, N., Miki, T. and Gutkind, S. (1995) Cell 81, 1137–1146.
[22] Nigg, E. (1995) Bioessays 17, 471–480.
[23] Morgan, D. (1995) Nature 374, 131–134.
[24] Less, E. (1995) Curr. Opin. Cell Biol. 7, 773–780.
[25] Misteli, T. and Warren, G. (1994) J. Cell Biol. 125, 269–282.
[26] Lowe, M., Rabouille, C., Nakamura, N., Watson, R., Jackman, M., Jamsa, E., Rahman, D., Pappin, D.J. and Warren, G. (1998) Cell 94, 783–793.
[27] D'Arrigo, A., Bucci, C., Toh, B. and Stenmark, H. (1997) Eur. J. Cell Biol. 72, 95–103.
[28] Robinson, M. and Cobb, M. (1997) Curr. Opin. Cell Biol. 9, 180–186.
[29] Fanger, G., Gerwins, P., Widman, C., Jarpe, M. and Johnson, G. (1997) Curr. Opin. Genet. Dev. 7, 67–74.
[30] Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215.