Biophysical Analysis of the Interaction of Rab6a GTPase with Its Effector Domains

Received for publication, August 5, 2008, and in revised form, November 14, 2008. Published, JBC Papers in Press, November 19, 2008, DOI 10.1074/jbc.M806003200

Tim Bergbrede, Nam Chu Ky, Stefan Schoebel, Wulf Blankenfeldt, Matthias Geyer, Evelyn Fuchs, Roger S. Goody, Francis Barr, and Kirill Alexandrov

From the §Department of Physical Biochemistry, Max Planck Institute for Molecular Physiology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany, the ‡Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland 4072, Australia, and the †Cancer Research Centre, University of Liverpool, Liverpool L3 9TA, United Kingdom

Rab GTPases are key regulators of intracellular vesicular transport that control vesicle budding, cargo sorting, transport, tethering, and fusion. In the active (GDP-bound) conformation, Rab GTPases are targeted to intracellular compartments where they are converted into the active GTP-bound form and recruit effector domain containing proteins. Rab6a has been implicated in dynein-mediated vesicle movement at the Golgi apparatus and shown to interact with a plethora of effector proteins. In this study, we identify minimal Rab6a binding domains of three Rab6a effector proteins: PIST, BicaudalD2, and p150glued. All three domains are >15-kDa fragments predicted to form coiled-coil structures that display no sequence homology to each other. Complex formation with BicaudalD2 and p150 has a moderate inhibitory effect on the intrinsic GTPase activity of Rab6a, while interaction with PIST has no influence on the hydrolysis rate. The effectors bind activated Rab6a with comparable affinities with $K_d$ values ranging from high nanomolar to low micromolar. Transient kinetic analysis revealed that effectors bind to Rab6a in an apparent single-step reaction characterized by relatively rapid on- and off-rates. We propose that the high off-rates of Rab6-effector complexes enable GTPase-activating protein-mediated net dissociation, which would not be possible if the off-rate were significantly slower.

Vesicle-mediated transport between intracellular membrane-bound compartments of eukaryotic cells is essential for biosynthesis, secretion, endocytosis, cell differentiation, and growth. Transport involves sequestering of the cargo molecules into vesicles, vesicular budding, transport processes, and finally docking and fusion of vesicles at the target membrane (1). GTPases of the Rab family play a central role in the regulation of these processes by recruiting specific partner (effector) proteins (2). Like all other GTPases involved in regulatory processes, Rab proteins interconvert between active, GTP-bound forms that are capable of effector interactions, and inactive, GDP-bound forms. Rab GTPases, such as Rab1, 6, 8, 12, and 24, play a decisive role in the maintenance and function of the Golgi apparatus, which represents the most active site of transport of vesicles in the cell. The proteins BicaudalD2, p150glued, and PIST, R6IP1, mint3, Rab6IP2A, and B and Rabkinesin-6 had been identified as putative effectors of Rab6a. These proposed effector interactions suggest a role for Rab6a in COPI-independent Golgi to endoplasmic reticulum (ER) or trans-Golgi network to ER retrograde transport, endosome to Golgi transport, and transition from metaphase to anaphase during mitosis (3–9). BicaudalD2, p150glued, and PIST belong to the class of proteins known as Golgins. Golgins associate with Golgi membranes either via transmembrane domains (Giantin), a myristoylated Golgi reassembly stacking protein (GRASP)-adapter domain (golgin-45, GM130) or through recruitment by small GTPases (Rab, Arf, Arl) (for a review see Ref. 10). Golgins bind RabGTPases via their coiled-coil domains; however, a particular Golgin may have multiple Rab binding partners. It was previously found that Rab6a indeed binds to the Golgins BicaudalD1 and D2 as well as to p150glued, a subunit of dynactin, dynamin, and PIST (11). Rab6a laden membranes were shown to be sufficient to recruit the dynein motor complex in vitro and in vivo. This recruitment is a critical step in microtubule-dependent retrograde trafficking. Furthermore, the positioning of the Golgi in the pericentriolar region of the cell is known to be dynein-dependent. These observations point to a central role of Rab6a in dynein-mediated vesicle movement at the Golgi.

The plethora of potential Rab6a effector domains present in structurally and functionally diverse proteins raises the question as to the mechanistic and thermodynamic basis of their function and the order of their recruitment by Rab6a. Because Rab6a has the lowest GTPase activity among RabGTPases (12), it is expected to generate long-lived Rab6-effector complexes. This creates a potential problem for GAP-mediated regulation of Rab6 GTPase activity, because effectors and GAP molecules are predicted to recognize overlapping regions of the GTPase. In this study we identify the minimal Rab6a binding domains of PIST, BicaudalD2, and p150glued and characterize their association with Rab6a using a combination of spectroscopic and biochemical methods. We demonstrate that in all cases the Rab6a binding domains are encoded by small, contiguous domains that selectively bind the GTP-loaded GTPase. The

$^a$ This work was supported in part by DFG Grant AL 484/7-2 (to K. A.) and Grant SFB 642 (to K. A. and R. S. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$^b$ The on-line version of this article (available at http://www.jbc.org) contains supplemental materials, Table S1, and Figs. S1–S5.

$^c$ Supported by a Heisenberg fellowship from the DFG. To whom correspondence should be addressed. E-mail: k.alexandrov@imb.uq.edu.au.

$^d$ F. Barr, unpublished data.
affinity of the domains for Rab6a-GTP is in the low micromolar range, and the interactions are characterized by relatively fast off-rates. The latter feature probably allows regulation of complex stability by Rab6aGAP activity.

**MATERIALS AND METHODS**

**Protein Expression and Purification**—All proteins used in this study were expressed in the *Escherichia coli* BL21 (DE3) RIL strain using the T7-polymersase-based inducible expression vector systems of PET or pTriex origin (13).

**Rab6a**—The expression and purification of Rab6a was performed as described earlier (12). Briefly, a shake flask culture with LB-medium containing 125 mg/liter ampicillin was inoculated with an aliquot of an overnight culture to an A_{600} of ~0.05. The culture was incubated at 37 °C and 100 rpm until the A_{600} reached ~0.6 and induced with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside. The culture was further incubated at 30 °C for 5–6 h, and cells were harvested by centrifugation. The bacterial pellet was washed once with ice-cold phosphate-buffered saline buffer and stored as frozen pellets at −80 °C.

**Effectors**—For expression of Rab6a effectors, 5-liter cultures were grown as described above. After addition of isopropyl-1-thio-β-D-galactopyranoside, the culture was further incubated overnight at 18 °C with 130 rpm agitation, before harvesting the cells 10–15 h postinduction.

**Isolation of Rab6a Effectors**—Effectors containing N-terminal His_{6} or His_{6}-GST\(^3\) fusion tags were purified using a standard combination of affinity and size-exclusion chromatography. Cleared bacterial lysate supplemented with 5 mM imidazole was loaded onto 5 ml of Ni- HisTrap FF crude affinity chromatography columns equilibrated with buffer A. Proteins bound nonspecifically to the column matrix were eluted with a buffer containing 25 mM imidazole. An additional washing step with 6 column volumes of wash buffer containing 0.5% (w/v) CHAPS was applied in some cases. His_{6}-tagged protein was eluted in 50-column volumes of buffer containing 30 mM Tris/HCl, pH 7.5, 50 mM LiCl, 2% (v/v) glycerol, 5 mM DTT. Complex-containing fractions were concentrated by membrane filtration, and aliquots were shock-frozen in liquid nitrogen before storing at −80 °C.

**Preparation of Rab6a-Effector Complexes**—For the preparation of Rab6a-effector complexes with p150 or PIST, 400 μM of the GTPase were mixed with 200 μM of the effector protein, 10 mM MgCl₂, and 1 mM GTP. The samples were incubated for 1 h on ice before resolving them on a Superdex 200 gel filtration column equilibrated with 30 mM Tris/HCl, pH 7.5, 80 mM LiCl, 2% (v/v) glycerol, 5 mM DTT. Complex-containing fractions were identified by SDS-PAGE, the pooled sample was concentrated by membrane filtration, and aliquots were shock-frozen in liquid nitrogen before storing at −80 °C.

**Preparation of the Rab6a-BicaudalD2 Complexes**—For preparation of the Rab6a-bicaudalD2 complexes, the GST-tagged minimal Rab6a binding domain of bicaudalD2 was incubated with GSH-Sepharose for 1 h at 4 °C with shaking. 10 μM GTP was added prior to the addition of Rab6a, and the slurry was further incubated for 1 h. The slurry was then poured into a column and unbound protein removed by washing with 3-column volumes of buffer containing 30 mM Tris/HCl, pH 7.5, 50 mM LiCl, 5 mM DTT, 10 μM GTP. Complex-containing fractions were concentrated by membrane-filtration followed by addition of GTP to a final concentration of 1 mM and shock-frozen in liquid nitrogen before storing at −80 °C.

**Analytical Gel Filtration**—Analytical gel filtration chromatography was carried out using a HPLC 600S controller with 626 pump system and autosampler (717plus). Protein was detected using absorbance (2487 Dual λ Absorbance) and fluorescence (2475 Multi λ Fluorescence) detector (Waters). Depending on the calculated molecular weight of the analyzed protein, Superdex 75 HR10/30 and 200 HR10/30 gel filtration columns were used. The molecular weight proteins were calculated by interpolation on a calibration curve constructed using a molecular weight standard mixture (Bio-Rad).

**Isothermal Titration Calorimetry (ITC)**—All measurements were carried out at 25 °C in reaction buffer containing 30 mM HEPES pH 7.5, 80 mM LiCl, 5 mM DTT, 1 mM MgCl₂, and 100 μM nucleotide (GTP or GDP), using a VP-ITC and Thermovac system (MicroCal). Protein samples were dialysed extensively in the same batch of reaction buffer. All samples were degassed for at least 10 min at 25 °C prior to the experiment. The acquired titration data were corrected for the increase in volume and for the heat of dilution of the titrant. To correct for heat of dilution of the titrant, the heats evolved from injection of titrant into reaction buffer were subtracted from the heat of each injection. The data were analyzed using the manufacturer’s software yielding the stoichiometry (N), the binary equilibrium association constant (K\(0\)), and the enthalpy of binding (ΔH\(f\)). ΔH\(f\) was assumed to be independent of the primary ligand concentration. From the relationship ΔG\(0\) = −RTlnK\(0\) and the Gibbs-Helmholtz equation, the standard free energy (ΔG\(0\)) and the standard entropy of association (ΔS\(0\)) were calculated.

**Stopped-flow Measurements**—To determine the rates of association (k\textsuperscript{on}) and of dissociation (k\textsuperscript{off}) of Rab6a with its
Interaction of Rab6a with Effectors

effectors a SF-61MX spectrofluorimeter with IS-2 Rapid Kinetics (High Tech Scientific) was used. Measurements were carried out at 25 °C in degassed buffer containing 50 mM Tris/Cl, pH 7.5, 80 mM LiCl, 2 mM MgCl₂, and 5 mM DTT. The fluorescence signal of the mant-group at 448 nm, induced by fluorescence resonance energy transfer (FRET) from tryptophan excited at 295 nm, was monitored. In practice, a 420-nm cut-off filter was used in front of the emission monochromator. To improve the signal to noise ratio, data from eight to twelve measurements were averaged and used for further analysis.

For the chosen experimental set-up, where the concentration of effectors was much greater than that of the Rab6a-mantGppNHp, association can be considered a pseudo-first order reaction and the fluorescence signal can be analyzed by fitting to a single exponential equation. Plotting the observed rate constants of the single exponential fit against the corresponding concentrations of the effector yielded a linear relationship over the usable concentration range in all cases. The association rate (k_on) was derived from the slope of the linear fit, while the dissociation rate (k_off) was calculated from the intercept with the ordinate. Based on k_on and k_off, the dissociation constants (K_d) for the interaction of Rab6a with its effectors were calculated.

GTPase Activity Assay—GTP hydrolysis was assayed as a function of time by HPLC as described previously (12). Excess

FIGURE 1. Schematic overview of protein constructs tested for Rab6a-effector interactions. A, expression constructs of bicaudalD2 and their biochemical properties for Rab6a binding. Construct 1 was used, in most cases, as a fusion to a GST tag, to enhance its solubility. The uppercase numbers denote the method used for confirmation of the Rab6a effector interactions: 1, affinity matrix binding assay; 2, analytical size-exclusion chromatography; 3, fluorescence-based interaction analysis; 4, calorimetric interaction analysis; 5, yeast two-hybrid assay. CC denotes the putative coil-coil domains. For further work, the fragments 1, 9, 14 of bicaudalD2 were chosen. B, schematic overview of truncation constructs for expression of the Rab6a binding domain of PIST and the summary of their biochemical properties. Constructs 2, 3, 4, 5, 14, and PIST wt were chosen for further analysis. Construct 14 was assessed by heteronuclear NMR spectroscopy and shown to represent a random coil structure (supplemental information). Constructs are labeled as in A. C, schematic overview of truncation constructs for expression of the Rab6a binding domain of p150glued and the summary of their biochemical properties. Constructs 2 and 11 were chosen for further studies. Constructs are labeled as in A.
GTP from the storage buffer of the protein sample was removed by passing it over a NAP-5 column (Amersham Biosciences) equilibrated with a buffer containing 50 mM Tris/HCl, pH 7.5, 100 mM LiCl, and 5 mM DTT. The sample concentration was adjusted to 150 μM, and the hydrolysis reaction started by addition of 10 mM MgCl₂ at 25 °C. 20-μl samples were withdrawn at appropriate time intervals and analyzed by HPLC.

RESULTS AND DISCUSSION

Identification and Recombinant Production of the Minimal Rab6a Binding Domains of PIST, BicaudalD2, and p150glued

To thermodynamically analyze the interactions of Rab6a with its effector proteins we first sought to identify the minimal Rab6a binding domains of PIST, bicaudalD2, and p150glued. Because no structural information on these Rab6a effectors is available, the expression constructs were designed either by a trial and error approach or were guided by secondary structure predictions and yeast two-hybrid analysis of PIST, p150glued, and bicaudalD2 interactions with Rab6a. Several Rab6a binding fragments of bicaudalD2 and PIST were designed based on limited proteolytic analyses of the Rab6a-effector complexes (supplemental materials). Fig. 1 provides a summary of the generated fragments, their solubility in E. coli and their interaction with GTP-loaded Rab6a. The Rab6a binding domains used in the described studies are named by the sequence source gene followed by the number of the construct. Construct 1 of bicaudalD2 was prepared as an N-terminal GST fusion protein to make the effector domain more amenable to concentration. The protein resulting from construct 14 of PIST was also subjected to heteronuclear NMR spectroscopy to assess its folding state (supplemental materials and Figs. S4A and S5).

Thermodynamics of the Rab6a-Effector Interactions—We initially attempted to study Rab6a interac-
tions with its effector domains by a solid state precipitation assay using GST-tagged recombinant Rab6a as bait (Fig. 2). Although nucleotide-bound state-dependent interactions with some effector domains were observed, the efficiency of effector recovery was low (data not shown). We propose that the effector domains dissociated during the washing procedure due to binding of Rab6a to effectors with an affinity in the micromolar range. As an alternative method that allows detection of complex formation when the interaction affinities are in the micromolar range, we chose size exclusion chromatography. Injection of a mixture of effectors with GTP-bound Rab6aCΔ27 resulted in elution of the Rab6a-effector complexes at positions approximately corresponding to their calculated molecular weights when a 1:1 binding stoichiometry is observed (Fig. 3). The only exception was the full-length construct of the PIST-Rab6a complex, which displayed an apparent stoichiometry of 1:2. This may be due to the extended shape of the PIST molecule imparted by the coiled-coil segments resulting in lower retention times on a gel filtration column.

To characterize the binding affinities and thermodynamics of the interactions of Rab6a with its effectors we used isothermal titration calorimetry (ITC) (14, 15). The advantage of this method is that it does not require modification of the protein molecules for measurements, as it measures the heat that is absorbed or generated by the system due to binding events. The heat generated or absorbed is proportional to the fraction of bound molecules.

In these experiments, 100 μM solutions of PIST wt and p150-2 or a 50 μM solution of GST-bicaudalD2-1 were titrated with a 10-fold molar excess of Rab6a bound to either GDP or GTP (Fig. 4). As the injections proceeded, the size of the injection peaks decreased gradually, indicating saturation of binding sites. The reactions with the GTP-bound form of Rab6a were exothermic and the obtained titration data could be fitted to $K_d$ values of 2.8, 2.2, and 1 μM, respectively. The binding stoichiometry for interaction of the effectors with Rab6a was found to be 1:1. No heat generation or absorption was detected when the GDP form of Rab6a was used confirming that the three proteins were specific for GTP-bound Rab6a, as observed for other GTPase effector molecules (Fig. 4). In the case of PIST and GST-bicaudalD2-1, binding of the effector protein to Rab6a-GTP is an enthalpy-driven process, with a negligible entropic contribution (supplemental Table S1).

Because GST-bicaudalD2-1, PIST-14, and p150-12 remained in solution at higher concentrations, the reverse titrations could be performed. In these experiments a 10-fold molar excess of the effector domains (1 mM) were titrated into 100 μM Rab6a-GTP. The results are summarized in supplemental Table S1. The data indicate that all effector domains interact with the...
active form of Rab6a with comparable micromolar affinities, indicating they have the potential to compete with each other provided that they bind to the same or at least overlapping sites.

Kinetic Analysis of Rab6a Interaction with Its Effectors — The ITC data provides an insight into the thermodynamics of protein-protein interactions, but does not yield information on the kinetics of such processes. To obtain the kinetic parameters of Rab6a-effector interactions, as well as to independently confirm the obtained affinity values, we employed fluorescent stopped-flow methodology. As a fluorescent reporter we chose the well characterized mant group conjugated to either GDP or GppNHp (a non-hydrolyzable GTP analogue). The signal from the well characterized mant group conjugated to either GDP or GppNHp resulted in a dissociation rate of 6.2 s⁻¹, in agreement with the initial estimate.

Thus, the affinity determined using transient kinetic analysis is congruent with the estimated values obtained by ITC. These findings are in keeping with the idea that binding of Rab6a with the bicaudalD2 binding domain occurs in a simple, single-step, reversible binding mode. Of course, it cannot be excluded that an initial very weak binding interaction is followed by a rapid first order process, as seen for many protein-ligand and protein-protein interactions, but the evidence suggests that the interaction is probably not accompanied by large conformational changes in either of the components. The off-rate of the interaction is relatively high, possibly indicating that although the Rab6a-effector complex is long lived due to the very slow intrinsic hydrolysis rate, its dynamic nature enables stimulation of the GTPase activity of Rab6a by a specific GAP protein.

The described approach was also used to characterize the interaction of Rab6a with PIST (Fig. 5C and Table 1). Confirming observations made in ITC experiments, a $K_a$ value of 2.7 μM was derived from the transient kinetic experiments. Similar to the bicaudalD2 binding domain, the Rab6a-PIST interaction appears to be a single-step process. Interestingly, in contrast to other effectors, binding of p150-2 to Rab6a-mantGppNHp resulted in a concentration-dependent fluorescence quenching rather than enhancement (Fig. 5D). The affinity calculated from the measured rates was 2.4 μM.

Influence of Effector Domain Binding on the Hydrolysis Rate of Rab6a — Several Rab GTPases were reported to possess an altered hydrolytic activity when complexed to their effector...
proteins. Interaction of Rabphilin-3a with Rab3a was shown to have a weak stimulating effect on the intrinsic GTPase activity, while concurrently inhibiting GAP-stimulated hydrolysis (16). In a mechanism counteracting inactivation, other Rab-effector interactions reduce the intrinsic GTPase activity of the complex. This has been observed for Rab5a and Rabaptin-5 (17) as well as for Rab4a binding to Rabaptin-4 and -5 (18).

To assess whether similar effects can also be exerted on Rab6a by its effector domains we compared the rate of hydrolysis of Rab6a with that of preformed gel filtration chromatography-purified Rab6a-bicaudalD2-1, Rab6a-PIST, and Rab6a-p150-2 complexes. The samples were incubated at 25 °C over the course of 12 days and continuously analyzed by HPLC (Fig. 6).

The decrease in protein-bound GTP follows a single-exponential time course that can be analyzed to yield the rate constant for the assumed first order reaction. Table 2 summarizes the rates for GTP hydrolysis of the different Rab6a-effector complexes.

Comparison of the observed hydrolysis rates of the GTPase complexes shows that the Rab6a effectors under investigation have different effects on Rab6 GTP hydrolysis. While binding of PIST appears to have only a limited influence, interaction with GST-bicaudalD2-1 or p150-2 leads to a decrease in the already low intrinsic hydrolysis activity of Rab6a by a factor of \( \approx 2-3 \) (12). Because no protein degradation was observed for any of the proteins used in the assay, changes in the hydrolysis rates appear to be the result of changes in protein activity rather than altered protein levels.

**Conclusions**—In this study, we have characterized three Rab6a effector domains derived from bicaudalD2, p150\(_{\text{Glued}}\), and PIST. Similar to previously described effector proteins of other Rab GTPases, minimal Rab6a binding domains are composed of short contiguous coiled-coil segments (19, 20). Besides this proposed domain fold, the Rab6a binding domains do not share sequence similarity, indicating that they probably have different evolutionary origins. This is in contrast to the effectors of the endocytic RabGTPases: Rab5 and Rab22, where the effector domains have emerged via gene duplication. All three Rab6a effector domains bind to the active form of the GTPase with low micromolar affinity. This is similar to the affinity previously determined for the FIP3-Rab11 interaction (19). The binding mechanism

![Figure 5](image_url)

**TABLE 1**

| Effector construct     | \( k_1 \) | \( k_{-1} \) | \( K_d \) |
|------------------------|----------|----------|---------|
| GST-bicaudalD2-1       | 4.3 \( \times 10^6 \) M\(^{-1}\) s\(^{-1}\) | 6.4 s\(^{-1}\) | 1.5 \( \times 10^{-6} \) M |
| PIST wt                | 1.0 s\(^{-1}\) | 2.6 s\(^{-1}\) | 2.7 \( \times 10^{-6} \) M |
| p150-2                 | 7.8 s\(^{-1}\) | 18.5 s\(^{-1}\) | 2.4 \( \times 10^{-6} \) M |

![Graphical abstract](image_url)


**Interaction of Rab6a with Effectors**

The rates for hydrolysis of GTP at 25 °C for Rab6a alone and in complex with bicaudalD2, p150, and PIST.

**TABLE 2**

| Protein complex | Hydrolysis rate $\times 10^{-4}$s$^{-1}$ |
|-----------------|----------------------------------------|
| Rab6a/p150-2    | 2.1                                    |
| Rab6a/bicaudalD2-1 | 2.5                                    |
| Rab6a/PISTwt    | 5.4                                    |
| Rab6a          | 5.8                                    |

The rates for hydrolysis of GTP at 25 °C for Rab6a alone and in complex with bicaudalD2, p150, and PIST.

**Hydrolysis rates**

The transient kinetic analysis, the interactions are best described by a single-step mechanism and do not appear to involve large structural rearrangements in any of the interacting partners. This observation is in line with the rather rigid structure of a coiled-coil domain, where helices are wrapped around each other and do not allow large structural changes besides unwinding (21). The complexes formed are relatively dynamic and display high off-rates, suggesting that electrostatic interactions rather than hydrophobic interactions compose the interaction interface. Again, this observation is in agreement with the secondary structure prediction since coiled-coil structures are typically known to be highly polar at surfaces distinct from their hydrophobic dimer interface (21). The dynamic nature of the interactions presumably enables regulation of the complex stability by a Rab6a GAP that could induce a decay in the complex population by stimulating the otherwise slow GTP hydrolysis of Rab6a.

**Acknowledgments**—We thank M. Terbeck, A. Sander, T. Rogowsky, S. Thuns, and N. Lypilova for excellent technical assistance. A. Rak is acknowledged for advice in the initial stages of the project. We are very grateful to the Dortmund Protein Facility (DPF) for protein expression and purification. Daniel Shaw is acknowledged for critically reading the manuscript.

**REFERENCES**

1. Pfeffer, S. R. (1999) Nat. Cell Biol. 1, E17–E22
2. Grosshans, B. L., Ortiz, D., and Novick, P. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 11821–11827
3. Monier, S., Jollivet, F., Janoueix-Lerosey, I., Johannes, L., and Goud, B. (2002) Traffic 3, 289–297
4. Miserey-Lenkei, S., Waharte, F., Boulet, A., Cuif, M. H., Tenza, D., El M. A., Raposo, G., Salamero, J., Heliot, L., Goud, B., and Monier, S. (2007) Traffic 8, 1385–1403
5. Miserey-Lenkei, S., Couedel-Court, Del, N. E., Bardin, S., Piel, M., Racine, V., Sibarita, J. B., Perez, F., Bornens, M., and Goud, B. (2006) EMBO J. 25, 278–289
6. Teber, I., Nagano, F., Kremerksothen, I., Bilbils, K., Goud, B., and Barnekow, A. (2005) Biol. Chem. 386, 671–677
7. Barr, F. A., and Short, B. (2003) Curr. Opin. Cell Biol. 15, 405–413
8. Barr, F. A. (1999) Curr. Biol. 9, 381–384
9. Bailly, E., McCaffrey, M., Touchot, N., Zahraoui, A., Goud, B., and Bornens, M. (1991) Nature 350, 715–718
10. Short, B., Haas, A., and Barr, F. A. (2005) Biochim. Biophys. Acta 1744, 383–395
11. Short, B., Preisinger, C., Schaletzky, J., Kopajtich, R., and Barr, F. A. (2002) Curr. Biol. 12, 1792–1795
12. Bergbrede, T., Pylpenko, O., Rak, A., and Alexandrow, K. (2005) J. Struct. Biol. 152, 235–238
13. Berrow, N. S., Alderton, D., Sainsbury, S., Nettleship, J., Assenberg, R., Rahman, N., Stuart, D. I., and Owens, R. J. (2007) Nucleic Acids Res. 35, e45
14. Wiseman, T., Willson, S., Brandsd, T. J., and Lin, L. N. (1989) Anal. Biochem. 179, 131–137
15. Indyk, L., and Fisher, H. F. (1998) Methods Enzymol. 295, 350–364
16. Kishida, S., Shirakata, H., Sasaki, T., Kato, M., Kaibuchi, K., and Takai, Y. (1993) J. Biol. Chem. 268, 22259–22261
17. Rybin, V., Ullrich, O., Rubino, M., Alexandrow, K., Simon, I., Seabra, M. C., Goody, R., and Zerial, M. (1996) Nature 383, 266–269
18. Nagelkerken, B., Van, A. E., Van, R. M., Gerez, L., Mohrmann, K., Van, U. N., Hothuizien, J., Pelkmans, L., and Van Der, S. P. (2000) Biochem. J. 346, 593–601
19. Eathiraj, S., Mishra, A., Prekeris, R., and Lambright, D. G. (2006) J. Mol. Biol. 364, 121–135
20. Eathiraj, S., Pan, X., Ritacco, C., and Lambright, D. G. (2005) Nature 436, 415–419
21. Lupas, A. N., and Gruber, M. (2005) Adv. Protein Chem. 70, 37–78