The N-terminal Moiety of CDC25\textsuperscript{Mm}, a GDP/GTP Exchange Factor of Ras Proteins, Controls the Activity of the Catalytic Domain

MODULATION BY CALMODULIN AND CALPAIN\textsuperscript{*} 

Soria Baouz\textsuperscript{‡}, Eric Jacquet, Alberto Bernardi\textsuperscript{§}, and Andrea Parmeggiani\textsuperscript{¶}

From the Groupe de Biophysique-Equipe 2, Ecole Polytechnique, F-91128 Palaiseau Cedex, France and \Laboratoire d'Enzymologie du Centre National de la Recherche Scientifique, F-91198 Gif-sur-Yvette and Institut Jacques Monod, Université Paris 7, F-75251 Paris 05, France

This work describes the \textit{in vitro} properties of full-length CDC25\textsuperscript{Mm} (1262 amino acid residues), a GDP/GTP exchange factor (GEF) of H-ras p21. CDC25\textsuperscript{Mm}, isolated as a recombinant protein in \textit{Escherichia coli} and purified by various chromatographic methods, could stimulate the H-ras p21 GDP dissociation rate; however, its specific activity was 25 times lower than that of the isolated catalytic domain comprising the last C-terminal 285 residues (C-CDC25\textsuperscript{Mm285}) and 5 times lower than the activity of the C-terminal half-molecule (631 residues). This reveals a negative regulation of the catalytic domain by other domains of the molecule. Accordingly, the GEF activity of CDC25\textsuperscript{Mm} was increased severalfold by the Ca\textsuperscript{2+}-dependent protease calpain that cleaves around a PEST-like region (residues 798–853), producing C-terminal fragments of 43–56 kDa. In agreement with the presence of an IQ motif on CDC25\textsuperscript{Mm} (residues 202–229), calmodulin interacted functionally with the exchange factor. Depending on the calmodulin concentration an inhibition up to 50\% of the CDC25\textsuperscript{Mm}-induced nucleotide exchange activity on H-ras p21 was observed, an effect requiring Ca\textsuperscript{2+} ions. Calmodulin also inhibited C-CDC25\textsuperscript{Mm285} but with a ~100 times higher IC\textsubscript{50} than in the case of CDC25\textsuperscript{Mm} (~10 \textmu M \textit{versus} 0.1 \textmu M, respectively). Together, these results emphasize the role of the other domains of CDC25\textsuperscript{Mm} in controlling the activity of the catalytic domain and support the involvement of calmodulin and calpain in the \textit{in vivo} regulation of the CDC25\textsuperscript{Mm} activity.

The mouse CDC25\textsuperscript{Mm} protein is a guanine nucleotide exchange factor (GEF) regenerating the active form of H-ras p21, the complex with GTP (1–3). Homologous products were found in rat (p140-rasGRF) (4) and human (H-GRF) (5, 6). These rasGEFs have been described to be specific for the central nervous system (4–9). Some evidence has also been reported for the existence of full-length and truncated forms of these exchange factors in other tissues (10, 11). Experiments \textit{in vivo} suggest that the upstream connection of this GEF involves G-protein-coupled receptors (9, 12, 13) and not hormone-receptor-bound tyrosine kinases via the adaptor protein GRB2, as has been found for SOS, a ubiquitous rasGEF (14–16). CDC25\textsuperscript{Mm} contains in the N-terminal moiety two domains of pleckstrin homology (PH1 and PH2), one of DBL homology (DH) and a coiled-coil region that follows the PH1 domain (cf. Ref. 17). PH domains are frequently present in signaling proteins and represent regions of interactions with specific ligands such as the \beta\gamma-subunits of heterotrimeric G-proteins (18, 19) and phospholipids (20). Coiled-coils are specific tertiary structures involved in protein interactions (21) and the DH is a domain sharing similarity with a GDP/GTP exchange factor of members of the Rho family (2, 4, 22, 23). Farnsworth et al. (24) reported that \textit{in vivo} the activity of the homologous p140-rasGRF from rat brain is enhanced by raising the calcium concentration, an effect associated with the binding of calmodulin, and that p140-rasGRF and calmodulin form a stable complex. A direct action of calmodulin was supported by the presence in the N-terminal region of CDC25\textsuperscript{Mm} of an IQ domain, a sequence frequently found in proteins interacting with calmodulin (25, 26). Very recent experiments in \textit{vivo} have indicated that PH1, coiled-coil and IQ domains act cooperatively to facilitate the activation of p140-rasGRF by calcium (17). Moreover, the presence in p140-rasGRF of two adjacent PEST sequences has suggested potential cleavage by the Ca\textsuperscript{2+}-dependent protease calpain (26). Concerning \textit{in vitro} properties, whereas C-terminal catalytic fragments spanning 256 to 488 amino acid residues have been biochemically characterized (3, 5, 27, 28), little is known about the functional properties \textit{in vitro} of the full-length molecule comprising 1262 (CDC25\textsuperscript{Mm}) or 1244 (p140-rasGRF) amino acid residues, of which the purification has yet to be reported.

Therefore, with the aim at deepening our knowledge of the mechanisms controlling the activation of H-ras p21, we have produced and purified the full-length CDC25\textsuperscript{Mm} as recombinant protein in \textit{Escherichia coli} and characterized its properties under well defined conditions \textit{in vitro}. The purified GEF shows a specific activity much lower than its isolated catalytic domain or the C-terminal half-molecule; it is inhibited by calmodulin and is specifically cleaved by calpain.

\textbf{MATERIALS AND METHODS}

Production and Purification of CDC25\textsuperscript{Mm}—The entire open reading frame of murine CDC25\textsuperscript{Mm} gene from a BamHI-EcoRI fragment of pHc28 (22) was cloned in a pGEX2TH in which the HindIII site had
been replaced by a SalI site. A BamHI-SalI fragment, containing the total full-length CDC25Mm gene, was cloned in BamHI-SalI of pMAL-c2 (New England Biolabs) and expressed in E. coli strain SCS1 as N-terminal fusion with the maltose-binding-protein (MBP), comprising a Xa-specific cleavage site. Cell cultures (1.5 liters) were grown at 30°C in LB medium containing 50 μg/ml ampicillin. The induction was started with 0.1 mM isopropyl-β-D-thiogalactopyranoside at a cell density of 0.2 A500 units and continued at 24°C to a density of 1.5 A260 units. After centrifugation (7,000 × g for 10 min), the resuspended pellet was sonicated for 5 min in buffer (25 mM Tris-HCl, pH 7.5, 50 mM NaCl, 7 mM ME) containing 10% glycerol, 1 mM EDTA, and 1 mM Pefablock-SC and centrifuged for 20 min at 25,000 × g at 4°C, a temperature at which all the subsequent purification steps were carried out. The supernatant was loaded on a 6-ml ResourceQ column (fast protein liquid chromatography system, Pharmacia Biotech Inc.) equilibrated with buffer A and eluted with the same buffer at a flow rate of 3 ml/min. Unlike the bulk of E. coli proteins, the largest portion of MBP-CDC25Mm was not retained on the resin. The nonretained active fractions were mixed with 10 ml of amylase-resin (New England Biolabs) equilibrated in buffer A and gently shaken for 30 min. After centrifugation at low speed (2,500 × g for 2 min), the supernatant was discarded, and the resin mixed again with 50 ml of buffer A was centrifuged. This step was repeated four times. Finally, MBP-CDC25Mm was removed from the amylase resin by two washes with 10 ml of buffer A containing 10 mM maltose. After centrifugation, the combined supernatant was passed on a HiTrap heparin column of 5 ml (Pharmacia) that was step-eluted with 250, 400, 600, and 1000 mM NaCl solutions in 25 mM Tris-HCl, pH 7.5, and 7 mM ME (flow rate: 5 ml/min) under the control of the fast protein liquid chromatography system. MBP-CDC25Mm emerged at 600 mM NaCl. After dialysis against buffer A plus 50% glycerol, the purest fractions on SDS-PAGE, were stored at −20°C. Their activity was stable for at least 6 months.

Assay for GEF Activity—The dissociation rates of the p21-[3H]GDP complexes were measured kinetically at 30°C after addition of a 500-fold excess of unlabeled nucleotide using the nitrocellulose binding assay. The labeled p21-[3H]GDP was prepared by incubation for 5 min at 30°C in 100 μl of buffer B (50 mM Tris-HCl, pH 7.5, 1 mM MgCl2, 100 mM NaCl, 0.1 mM CaCl2, 0.5 mg/ml Bovine serum albumin, containing 2 μM p21, 3 mM EDTA, and 6 μM [3H]GDP (350 Bq/mmol). Du Pont NEN). Then, 3 mM MgCl2 was added. The reaction mixture for the dissociation experiments contained in buffer B, 0.1–0.2 μM p21-[3H]GDP and either MBP-CDC25Mm, GST-CDC25Mm631, or C-CDC25Mm285, calmodulin, calpain, CaCl2, or EGTA as indicated in the legends to figures. The concentration of glycerol carried over from the CDC25Mm storage buffer was only usually 10%. An equal amount of storage buffer was added to the control. For calpain treatment see legend to Fig. 4 or the text in the relative section of “Results.” At the given times, the samples (5–10%) were filtered through nitrocellulose discs (Sartorius 11306, 0.45 μm), washed twice with 3 ml of ice-cold 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, and 100 mM NaCl, and the retained p21-bound radioactivity was measured in a liquid scintillation counter LKB/Pharmacia, model Wallac 1410.

Other Materials and Methods—C-CDC25Mm631 and human C-H-ras p21 were isolated and purified as described (27, 29). C-CDC25Mm631 was produced in E. coli, as GST fusion and purified by affinity chromatography on glutathione-Sepharose, under the same conditions as reported for C-CDC25Mm631 by Jacquet et al. (27). This was followed by chromatography on HiTrap Heparin column (see above). Stepwise elution of C-CDC25Mm631 took place at 400 mM NaCl. Calmodulin and calpain were purchased from Sigma. SDS-PAGE was carried out using a 10% 0.25% acrylamide/bisacrylamide gel and stained with Coomassie Blue. The anti-C-CDC25Mm631 antibodies were produced in rabbit, and those anti-MBP were purchased from New England Biolabs. Protein concentration was determined by the Bio-Rad assay, using bovine serum albumin as a standard. The concentration of p21 was checked by [3H]GDP binding. To determine the percentage of protein components, Coomassie Blue-stained gels were analyzed with the Apple scanner system, and the results were evaluated with the Scan Analysis software.

RESULTS

Purification of CDC25Mm and C-CDC25Mm631—Cloning of the full-length CDC25Mm gene in pGEX resulted in low expression and little soluble product, whereas the use of a pMAL vector allowed the production of 1 mg of CDC25Mm/1 liter of cell culture at an 0.1% cell density of 1.5 units. More than 60% of the produced CDC25Mm remained soluble after a 20-min centrifugation at 25,000 × g. The temperature of induction was important; incubation at 24°C gave higher concentrations of soluble CDC25Mm than at 37°C. On SDS-PAGE the MBP-fused product showed the expected apparent molecular mass of 188 kDa and corresponded, after purification, to the slowest migrating band (~50% of the total protein) (Fig. 1A, lane 4). In the purified preparations, Western blot with anti-C-CDC25Mm631 antibodies revealed only one band corresponding to the molecular mass of the MBP-fused full-length protein (188 kDa) (Fig. 1B, lane 2) while most other SDS-PAGE bands were responsive to anti-MBP antibodies (Fig. 1B, lane 1). Since no proteolysis was detectable in the cell extract, these bands consist of N-terminal incomplete translational products of MBP-CDC25Mm. Accordingly, a second passage on amylase resin gave the same gel pattern without any further purification. Also the use of other chromatographic techniques such as ion exchange (ResourceQ, Pharmacia, at pH from 7.0 to 9.5) and hydrophobic resins (Pharmacia kit), or of hydroxylapatite and filtration methods (Superdex, Pharmacia and Microcon-100, Amicon) did not improve purification. Together, these properties show that our CDC25Mm preparations were not contaminated by C-terminal fragments and contained little, if any, E. coli proteins. Protein concentration by means of Aquacide II or Amicon ultrafiltration was avoided, since it caused loss of activity, likely due to aggregation. Cleavage of the fused MBP by factor Xa led to degradation of CDC25Mm; therefore, we have used the fused protein. C-CDC25Mm631 was obtained >90% pure as GST-fused protein. C-CDC25Mm631 was homogeneous on SDS-PAGE, as reported (27).

Comparison of the Activities of Full-length CDC25Mm, C-CDC25Mm631, and C-CDC25Mm285—Experiments in vivo suggest that CDC25Mm has a constitutive GEF activity (9). In line with this, the [3H]GDP/GDP exchange rate of p21-[3H]GDP in vitro was enhanced by the purified full-length CDC25Mm (Fig. 2A). The stimulation of the p21-GDP dissociation rate constant increased linearly with increasing the CDC25Mm concentration (Fig. 2B). Fig. 2C shows that the C-terminal half-molecule C-CDC25Mm631 displayed a constitutive GEF activity several times higher than the full-length molecule. Also in this case, increasing concentrations of C-CDC25Mm631 enhanced linearly the GDP/GDP exchange activity of H-ras p21 (Fig. 2D).

FIG. 1. SDS-PAGE of MBP-CDC25Mm prior to and after purification (A) and Western blot with anti-MBP or anti-C-CDC25Mm631 antibodies (B). A, SDS-PAGE of molecular weight markers (lane 1), total cell extract prior to induction of MBP-CDC25Mm (lane 2), total cell extract after induction of MBP-CDC25Mm (lane 3), purified preparation of MBP-CDC25Mm (lane 4). B, Western blots of purified preparation of MBP-CDC25Mm revealed with anti-MBP (lane 1) and with anti-C-CDC25Mm631 antibodies (lane 2).
Similar results were obtained by determining the GDP/GTP exchange rate (not shown).

The activity of CDC25Mnm and C-CDC25Mnm631 was then compared with that of the C-terminal fragment C-CDC25Mnm285, the best studied catalytic fragment of CDC25Mnm (3, 27), slightly longer than the shortest catalytic domain isolated so far (last 256 C-terminal residues, ref 28). C-CDC25Mnm285 was by far the most active of the three rasGEF forms. At a concentration of 0.2 μM, the activities of CDC25Mnm and C-CDC25Mnm631 were 4.4 and 23%, respectively, that of C-CDC25Mnm285 (Fig. 3). This reveals a negative influence on the activity of the C-terminal domain by other domains of CDC25Mnm.

CDC25Mnm is specifically cleaved by calpain around a PEST region—Cheney and Mooseker (26) identified in the homologous p140-rasGRF (residues 198–225T) (26) suggests a C-terminal CDC25Mnm fragments with constitutive activities higher than the activity of the full-length molecule, further confirms the negative influence of the N-terminal moiety on the activity of the C-terminal catalytic domain and suggests a regulatory role by proteases on the activity of this rasGEF.

Calmodulin Action on CDC25Mnm Activity—Farnsworth et al. (24) observed that H-ras p21 activation by p140-rasGRF in vivo was enhanced by raising the concentration of Ca2⁺, a mechanism mediated by calmodulin, since this ubiquitous protein, known to be involved in cellular processes controlled by Ca2⁺-dependent signaling (cf. Refs. 32 and 33), was coprecipitated with p140-rasGRF. The fact that CDC25Mnm has the same recognition sequence for calmodulin (IQ motif, residues 202–229), as found in p140-rasGRF (residues 198–225T) (26) suggests a productive interaction also between CDC25Mnm and calmodulin. As shown in Fig. 3, in our in vitro system Ca2⁺-calmodulin was found to inhibit the rasGEF activity of CDC25Mnm. With increasing concentrations of calmodulin, the inhibition leveled at ~50% of the rasGEF activity, the concentration of calmodulin inducing half maximum inhibition (IC₅₀) being ~0.1 μM (Fig. 5, inset).

In experiments not shown, we have observed that the treatment of CDC25Mnm by calpain leading to N-terminal and C-terminal-truncated fragments abolished the inhibition of the GEK activity induced by 1 μM calmodulin. Thus, one can conclude that the inhibitory effect of calmodulin on the catalytic activity of the C-terminal domain is due to an intramolecular mechanism originating from the N-terminal moiety.

As shown in Fig. 6, the omission of Ca2⁺ and the addition of 0.1 mM EGTA led to a progressive slight decrease of the calmodulin inhibition on the CDC25Mnm activity, the obtained values displaying a marked variability. Only at 1 mM EGTA, the calmodulin effect was completely abolished.

Ca2⁺-calmodulin also inhibited the GEK activity of C-CDC25Mnm285, but at a concentration about 100 times higher than in the case of the full-length molecule (IC₅₀ = ~10 versus 0.1 μM, respectively) (Fig. 7). Calmodulin had no effect on the intrinsic GDP exchange activity of H-ras p21.

![Figure 2](image-url)  
**Fig. 2.**[1H]GDP/GDP exchange rate of H-ras p21 and stimulation of the dissociation rate constants of H-ras p21-GDP as a function of CDC25Mnm or C-CDC25Mnm631 concentration. The reaction mixture contained 0.2 μM H-ras p21-[1H]GDP minus (□) and plus (■) 0.140 μM CDC25Mnm (A) or 0.040 μM C-CDC25Mnm631 (C). The same conditions were used to determine kinetically the dissociation rate constants and the stimulation factor as a function of the concentration of CDC25Mnm (0.025 to 0.200 μM, panel B) and C-CDC25Mnm631 (0.010 to 0.120 μM, panel D).

![Figure 3](image-url)  
**Fig. 3.** Comparison of the GEK activity of CDC25Mnm, C-CDC25Mnm631, and C-CDC25Mnm285 as determined from the stimulation of the dissociation rate constants of H-ras p21-[1H]GDP. The concentration of the GEK forms was 0.2 μM. Open bar, C-CDC25Mnm285; shaded bar, C-CDC25Mnm631; solid bar, CDC25Mnm.
The biochemical characterization of purified full-length CDC25Mm shows that its noncatalytic moiety, comprising two PH, one DH, one coiled-coil and one IQ domain, has a regulatory function on the GEF activity of CDC25Mm. In fact, the specific activity of the full-length protein is 4% that of the isolated catalytic C-terminal fragment of 285 residues. The observation that the C-terminal half-molecule shows an activity, whose extent lies between the activity of the full-length CDC25Mm and that of C-CDC25Mm<sub>285</sub>, further confirms a regulatory function of the noncatalytic moiety of the molecule and supports the existence in the cell of mechanisms activating this GEF. This is in agreement with experiments in vivo showing that serum stimulation of NIH3T3 transformants leads to an increase in Ras<sub>z</sub>GTP only in cells expressing the entire CDC25Mm but not in cells expressing smaller C-terminal forms of CDC25Mm (9, 22). Thus, the N-terminal region is essential for the response to serum. In line with this, is also the observation that the C-terminal truncated CDC25Mm lacking the last 600 amino acids behaves as dominant negative to the response to serum of the full-length molecule (9).

The specific cleavage by calpain around the PEST region increases the GEF activity due to the production of C-terminal fragments. The observation that the induced activity is of the same range as the activities of C-CDC25Mm<sub>631</sub> and the p140-rasGRF C-terminal fragment of 456 residues (4) suggests that CDC25Mm and that of C-CDC25Mm<sub>285</sub>, further confirms a regulatory function of the noncatalytic moiety of the molecule and supports the existence in the cell of mechanisms activating this GEF. This is in agreement with experiments in vivo showing that serum stimulation of NIH3T3 transformants leads to an increase in Ras<sub>z</sub>GTP only in cells expressing the entire CDC25Mm but not in cells expressing smaller C-terminal forms of CDC25Mm (9, 22). Thus, the N-terminal region is essential for the response to serum. In line with this, is also the observation that the C-terminal truncated CDC25Mm lacking the last 600 amino acids behaves as dominant negative to the response to serum of the full-length molecule (9).
most noncatalytic domains participate in regulating the activity of the catalytic C-terminal conserved region. This indicates that the signal activating the productive interaction between the CDC25Mm C-terminal region and H-ras p21 evokes a global effect on the CDC25Mm molecule.

It is known that the activity of calpain can be coordinated with that of calmodulin, as shown by the frequent presence on calmodulin-binding proteins of a PEST motif and the functional relationship between calpain action and calmodulin binding site (cf. Ref. 34). In a large number of proteins (cf. Refs. 30 and 31), PEST sequences appear to represent a signal for rapid degradation by non-ubiquitin-mediated proteolysis that can involve calpain. Interestingly, in the mouse embryo brain a 58-kDa form of CDC25Mm was described, whereas in the more slowly metabolizing adult brain only the full-length 140-kDa protein could be detected (7). In the human brain, besides the full-length 140-kDa H-GRF, shorter forms of 43–50 kDa were reported (5) that might also be present in tissues other than brain (11). The results of our work show that C-terminal CDC25Mm fragments of 43–56 kDa can originate from the action of calpain. Therefore, as reported in this work, activation of the GEF activity by calpain appears to be mediated by C-terminal fragments that are more active than the full-length molecule. In vivo, an eventual action of calpain could contribute to modulate the activity of CDC25Mm. In this context, it is worth mentioning that the expression of C-CDC25Mm285 in fibroblasts was found to enhance the GDP/GTP exchange activity on H-ras p21 and the tumor formation in the nude mice (35). Moreover, the homologous Saccharomyces cerevisiae ras-GEF Cdc25p, containing a cyclin destruction box, has been described to display a rapid metabolism (half-life time, ~20 min) due to proteolytic degradation (36). Whether proteases also act on the mammalian ras-GEF in vivo remains to be determined, since the half-life of CDC25Mm in neuronal cells is still unknown.

From our results, Ca²⁺-calmodulin interacts efficiently with CDC25Mm inhibiting the ras-GEF activity with a IC₅₀ of 0.1 μM, an effect dependent on Ca²⁺, corresponding approximately to an equimolar ratio between calmodulin and CDC25Mm. The course of inhibition with increasing amounts of calmodulin suggests a noncompetitive effect on the GEF activity of CDC25Mm. Hence, binding of calmodulin and p21 to CDC25Mm should concern distinct sites. The weak interaction of calmodulin with the catalytic domain of CDC25Mm suggests the possibility that in the three-dimensional conformation the IQ sequence at the N-terminal region (residues 202–229) and the C-terminal catalytic domain are vicinal.

Calmodulin is in general known to activate calmodulin-binding proteins. However, in some cases such as calmodulin-kinase II, calmodulin-kinase IV, myosin light chain kinase and calcineurin, incubation with Ca²⁺/calmodulin has been shown to induce inactivation (cf. Refs. 37 and 38). It is interesting to mention that calmodulin-kinase IV interacts with calmodulin with high affinity, comparable to that of CDC25Mm (37), differently from calmodulin-kinase II which is inhibited only by high concentration of calmodulin (38).

Noteworthy is the relatively high concentration of EGTA required for abolishing the Ca²⁺-calmodulin-dependent effect. Several recent studies have indicated that the conformation of the N-terminal and C-terminal calcium binding helix-loop-helix motifs of calmodulin depends on whether calcium is bound or not (cf. Ref. 39). Since the affinity of EGTA for calcium is several orders of magnitude higher than that of calmodulin, this finding suggests that, when occupied, the Ca²⁺-binding site of calmodulin is little accessible to the chelating agent.

From experiments in vivo (24), one would rather expect that Ca²⁺-calmodulin activates CDC25Mm. This difference with our results in vitro suggests the existence of other components affecting the action of calmodulin on CDC25Mm in the cell implying a more complex calmodulin-regulated mechanism. The same authors (24) report that calmodulin-bound p140-rasGRF isolated from stimulated cells and calmodulin-free p140-rasGRF isolated from unstimulated cells display the same GEF activity. The different experimental conditions may be the reason for the discrepancy between these and our results.

In conclusion, our work supports a regulatory role of the N-terminal moiety of CDC25Mm on its catalytic domain and suggests that calmodulin and calpain act as modulators of the CDC25Mm activity in the cell. Very recently, Mattingly and Macara (13) have reported on the basis of experiments in vivo a phosphorylation-dependent activation of CDC25Mm exchange factor by muscarinic receptors and G-protein βγ-subunits. The relationship between this effect and the calmodulin-dependent regulation remains an open question.

Acknowledgments—We are indebted to Dr. D. R. Lowy for sending the CDC25Mm gene cloned in pH28 and Drs. J. B. Créchet, M.C. Parrini, C. Giglione, and I. M. Krab for fruitful discussion and advice.

REFERENCES
1. Martegani, E., Vanoni, M., Zippel, R., Cocetti, P., Brambilla, R., Ferrari, C., Sturani, E., and Alberghina, L. (1992) EMBO J. 11, 2151–2157
2. Cen, H., Papageorge, A. G., Zippel, R., Lowy, D. R., and Zhang, K. (1992) EMBO J. 11, 4007–4015
3. Jacquet, E., Vanoni, M., Ferrari, C., Alberghina, L., Martegani, E., and Farmegianni, A. (1992) J. Biol. Chem. 267, 24181–24185
4. Shou, C., Farnsworth, C. L., Neel, B. G., and Feig, L. A. (1992) Nature 358, 351–354
5. Schwighofer, F., Faure, M., Fath, I., Chevallier-Multan, M.-C., Apis, F., Dutrillaux, B., Sturani, E., Jacquet, M., and Tocque, B. (1993) Oncogene 8, 1477–1485
6. Wei, W., Das, B., Park W., and Broek, D. (1994) Gene (Amst.) 151, 279–284
7. Martegani, E., Zippel, R., Ferrari, C., Cocetti, P., Campiglio, S., Denis-Domini, S., Sturani, E., Vanoni, M., and Alberghina, L. (1993) Eur. J. Histochem. 37, 73–78
8. Ferrari, C., Zippel, R., Martegani, E., Gnasutta, N., Carrera, V., and Sturani, E. (1994) Exp. Cell Res. 210, 353–357
9. Zippel, R., Orecchia, S., Sturani, E., and Martegani, E. (1996) Oncogene 12, 2607–2609
10. Chén, L., Zhang, L.-J., Greer, P., Tung, P. S., and Moran, M. F. (1993) Dev. Genet. 14, 339–346
11. Guerrero, C., Rojas, J. M., Chedid, M., Esteban, L. M., Zimonjic, D. B., Popescu, N. C., Font de Mora, J., and Santos, E. (1996) Oncogene 12, 1097–1107
12. Shou, C., Wurmsker, A., Ling, K., Barbaric, M., and Feig, L. A. (1995) Oncogene 10, 1887–1895

Fig. 7. Calmodulin can interact with the catalytic domain of CDC25Mm. The p21-[3H]GDP dissociation kinetics were determined in buffer B containing 2 mM CaCl₂ in the presence of 0.01 μM C-CDC25Mm285 and increasing concentrations of calmodulin as indicated. The reaction was started by the addition of p21-[3H]GDP complex to a final concentration of 0.2 μM.
Properties of Full-length rasGEF CDC25Mm

13. Mattingly, R. R., and Macara, I. G. (1996) Nature 382, 268–272
14. Chardin, P., Camonis, J. H., Gale, N. W., Van Aelst, L., Schlessinger, J., Wigler, M. H., and Bar-Sagi, D. (1993) Science 260, 1338–1343
15. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M., and Weinberg, R. A. (1993) Nature 363, 45–51
16. Gale, N. W., Kaplan, S., Levenstein, E. J., Schlessinger, J., and Bar-Sagi, D. (1993) Nature 363, 88–92
17. Buchsbaum, R., Telliez, J.-B., Goonesekera, S., and Feig, L. A. (1996) Mol. Cell. Biol. 16, 4888–4896
18. Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993) Trends Biochem. Sci. 18, 343–348
19. Inglese, J., Koch, W. J., Touhara, K., and Lefkowitz, R. J. (1995) Trends Biochem. Sci. 20, 151–155
20. Fergusson, K. M., Lemmon, M. A., Sigler, P. B., and Schlessinger, J. (1995) Structure 3, 715–718
21. Lupas, A. (1996) Trends Biochem. Sci. 21, 375–382
22. Cen, H., Papapostolou, G. L., Vass, W. C., Zhang, K., and Lowy, D. R. (1993) Mol. Cell. Biol. 13, 7718–7724
23. Hart, M. J., Eva, A., Evans, T., Aaronson, S. A., Cerione, R. A. (1991) Nature 354, 311–314
24. Farnsworth, C. L., Freshney, N. W., Rosen, L. B., Ghosh, A., Greenberg, M. E., and Feig, L. A. (1995) Nature 376, 524–527
25. Cheney, R. E., and Mooseker, M. S. (1992) Curr. Opin. Cell Biol. 4, 27–35
26. Cheney, R. E., and Mooseker, M. S. (1994) Mol. Biol. Cell Supp. 5, 21A
27. Jacquet, E., Barouz, S., and Parmeggiani, A. (1995) Biochemistry 34, 12947–12954
28. Cocetti, P., Mauri, I., Alberghina, L., Martegani, E., and Parmeggiani, A. (1995) Biochem. Biophys. Res. Commun. 206, 253–259
29. Parrini, M. C., Jacquet, E., Bernardi, A., Jacquet, M., and Parmeggiani, A. (1995) Biochemistry 34, 13776–13783
30. Reichsteiner, M. (1990) Cell Biol. 1, 433–440
31. Reichsteiner, M., and Rogers, S. W. (1996) Trends Biochem. Sci. 21, 267–271
32. Finn, B. E., and Forsén, S. (1995) Structure 3, 7–11
33. James, P., Vorherr, T., and Carafoli, E. (1995) Trends Biochem. Sci. 20, 38–42
34. Molinari, M., Maki, M., and Carafoli, E. (1995) J. Biol. Chem. 270, 14576–14581
35. Zippel, R., De Maddalena, C., Porro, G., Modena, D., Sturani, E., and Vanoni, M. (1994) Int. J. Oncol. 4, 175–179
36. Kaplon, T., and Jacquet, M. (1995) J. Biol. Chem. 270, 20742–20747
37. Kitani, T., Okuno, S., and Fujisawa, H. (1995) J. Biochem. 117, 1070–1075
38. Ishida, A., Kitani, T., Okuno, S., and Fujisawa, H. (1994) J. Biochem. 115, 1075–1082
39. Swindells, M. B., and Ikura, M. (1996) Nat. Struct. Biol. 3, 501–504