Vol. 206, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 5, 1995 Pages 253-259

THE MINIMAL ACTIVE DOMAIN OF THE MOUSE RAS EXCHANGE FACTOR CDC25^{Mm}

P.Coccetti[a,b] *, I. Mauri [a], L. Alberghina [a], E. Martegani [a], and A. Parmeggiani [b]

[a] Dipartimento di Fisiologia e Biochimica Generali, Università di Milano, I-20133 Milano, Italy

[b] SDI n° 61840 du CNRS, Laboratoire de Biochimie, Ecole Polytechnique , F-91128 Palaiseau Cedex, France

Received November 23, 1994

SUMMARY: The minimal active domain of the mouse CDC25^{Mm}, a GDP/GTP exchange factor (GEF) active on H-ras protein, was determined by constructing several deletion mutants of the C-terminal domain of the protein. The functional activity of these fragments was analyzed for the ability to complement the yeast temperature sensitive mutation *cdc25-1* and to catalyze the GDP/GTP exchange on Ras proteins *in vitro*. A C-terminal domain of 256 residues (CDC25$^{Mm}_{1005\text{-}1260}$) was sufficient for full biological activity *in vivo*. Deletion of 27 C-terminal amino acids (CDC25$^{Mm}_{1005\text{-}1233}$) abolished the complementing activity while deletion of 25 N-terminal residues (CDC25$^{Mm}_{1030\text{-}1260}$ corresponding to the most conserved domain) led to a complete loss of expression. The results *in vivo* were supported by experiments *in vitro*. Highly purified CDC25$^{Mm}_{1005\text{-}1260}$, expressed in *E. coli* using the pMAL system, enhanced the GDP release from both H-ras p21 and *S. cerevisiae* Ras2p and its activity was nearly as high as that of CDC25$^{Mm}_{974\text{-}1260}$. Comparison with the Cdc25p protein yielded further evidence that the minimal active domain of CDC25^{Mm} is shorter than the yeast one.

The *ras* gene products belong to the superfamily of the guanine nucleotide binding proteins that are GTPases with a very low intrinsic catalytic activity, sharing common functional and structural properties. The ras proteins are key-elements for the regulation of cell growth and differentiation and act as molecular switches cycling between the active GTP-bound state and the inactive GDP-bound state (1,2). The balance between the "on" and "off" forms is set by the guanine nucleotide exchange proteins (GEF) and the GTPase activating proteins (GAP). In *Saccharomyces cerevisiae* the *CDC25* gene product acts as GEF promoting the formation of the active Ras2p-GTP complex (3-5). A similar action is exerted by the C-domain of the SDC*25* gene product (550 aa), a suppressor of *cdc25* mutations (6,7).Two mouse cDNAs coding for C-terminal fragments of 472 and 287 residues, respectively, were cloned by functional complementation of *S. cerevisiae cdc25* mutants. They showed a 34% identity with the C-terminal domain of Cdc25p (8). The corresponding full-length CDC25^{Mm} cDNA (1260 residues) was cloned by Cen *et al.* (9). The CDC25$^{Mm}_{974\text{-}1260}$ produced in *E. coli* as fusion protein with gluthatione S-transferase (GST) strongly enhanced the GDP release from H-ras p21 and yeast Ras2p (10). Cdc25p-like GDP/GTP exchange factors have also been found in rat and human brain (11-13). This kind of GEFs appear to be tissue-specific and

* Corresponding author . Fax xx39-2-70632811.

Abbreviations: ME, 2βmercaptoethanol; IPTG, isopropylβD-thiogalactopyranoside; GST, glutathione-S-transferase; MBP, maltose binding protein; GEF, guanine nucleotide exchange factor.

0006-291X/95 $5.00

are present only in the central nervous system; no information is yet available about their regulation and upstream pathway. On the contrary, the SOS-like GEFs are ubiquitous and linked to tyrosine-kinase receptors (14).

Genetic evidence (4,15-17) has indicated that *in vivo* the minimum active Cdc25p domain should contain around 300 C-terminal residues. However recent biochemical evidence derived from the work of different authors (4,5,26) has suggested an *in vitro* activity only with fragments of 429 residues, whereas shorter domains are totally inactive. In contrast to this, a fragment of only 287 residues in mouse ($CDC25^{Mm}_{974-1260}$) is active *in vivo* and *in vitro* (5,8). In this work we have defined the smallest catalytic domain of $CDC25^{Mm}$ active *in vivo* and *in vitro*. The activity of the C-terminal region of mouse protein was compared with that of yeast Cdc25p using diverse fragments and chimeric constructions.

MATERIALS AND METHODS

Plasmids constructed for in vivo studies. The yeast strain used was TC7 (*MATα ade lys trp1 ura3 cdc25-1*^{ts}) (8) and the two episomal *URA3* -based expression vectors were pVTU (18) and pEMBLyEX4 carrying the *ADH1* and the *CYC1 /UAS*$_{GAL}$ promoter respectively. In this and the following paragraph, the preparation of the various $CDC25^{Mm}$ and Cdc25p constructions is briefly outlined without explaining all the steps in detail. pVT-$CDC25^{Mm}_{1005-1260}$: the *CDC25*^{Mm} fragment, encoding the 256 C-terminal residues starting from Met 1005, was isolated after *PstI* digestion and cloned in the *PstI* site of pVTU. pVT-$CDC25^{Mm}_{1030-1260}$: the expression of $CDC25^{Mm}$ from the Ala 1030 codon was obtained by introducing a Met codon via PCR amplification. The $CDC25^{Mm}$ fragment , encoding the 231 C-terminal residues, was isolated after *PstI* digestion and cloned in the *PstI* site of pVTU. pVT-$CDC25^{Mm}_{1005-1233}$: the $CDC25^{Mm}$ fragment encoding the 1005-1233 region was isolated after *PstI* and *ScaI* digestion and cloned into pVTU digested with *PstI* and *HindIII*. pVT-$Cdc25p_{1309-1589}$: the 1.3 Kb *NdeI-PvuII* fragment of *CDC25* was cloned in *SalI* site of pUC18 to insert the ATG initiation codon (pUC18-$Cdc25p_{1309-1589}$). The 1.3 Kb *EcoRI -HindIII* fragment of pUC18-$Cdc25p_{1309-1589}$ was cloned in *PvuII* site of pVTU. pEM-$Cdc25p_{1280-1589}$: the 1.5 Kb *BamHI-PvuII* fragment of *CDC25* was cloned in *BamHI* site of pEMBLyEX4, the translation of Cdc25p begins at Met 1280. pVT-$CDC25^{Mm}_{1005-1032}$/$Cdc25p_{1256-1589}$: this fusion allowed the expression of Cdc25p from the residue 1256 by utilising the Met 1005 of the mouse protein. The 1.5 Kb *BamHI-PvuII* fragment of *CDC25*

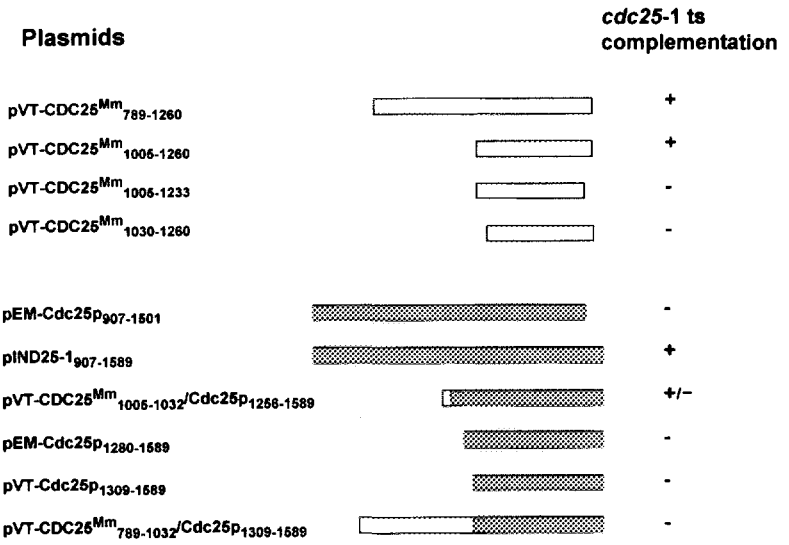

Fig. 1. $CDC25^{Mm}$ and CDC25 fragments used in complementation studies. Complementation of *cdc25-1* mutation by expression of various constructs concerning the C-terminal domains of Cdc25p and $CDC25^{Mm}$. The strain TC7 was transformed with the different recombinant plasmids. The *URA3* transformants were selected at 24°C and tested for growth at 37°C by replica plating. Empty bars: $CDC25^{Mm}$; dashed bars: yeast Cdc25p; shaded area: homology region. (+ /- means that the efficiency of transformation was lower in comparison with the other constructions and the resulting colonies grew slowly at 37°C.)

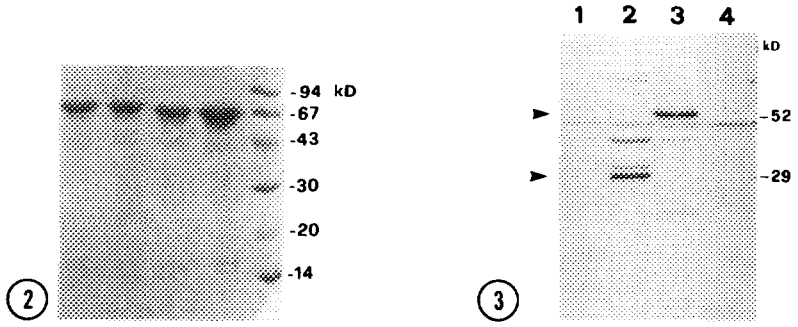

Fig. 2. SDS-PAGE of MBP-fused CDC25$^{Mm}_{1005-1260}$ and CDC25$^{Mm}_{1005-1233}$ after purification. Purified preparation on amylose-resin of MBP-fused CDC25$^{Mm}_{1005-1260}$ (lane 1-2) and of MBP-fused CDC25$^{Mm}_{1005-1233}$ (lane 3-4). Molecular weight markers (lane 5). The gel was stained with Coomassie Brilliant Blue R-250.

Fig. 3. Western blot with antibodies against the various CDC25^{Mm} fragments. Yeast cells carrying pVTU (lane 1), pVT-CDC25$^{Mm}_{1005-1260}$ (lane 2), pVT-CDC25$^{Mm}_{789-1260}$ (lane 3), pVT-CDC25$^{Mm}_{1030-1260}$ (lane 4).

was cloned in the *PvuII* site (nucleotide 3322) of CDC25$^{Mm}_{1005-1260}$ previously cloned in pVTU (pVT-CDC25$^{Mm}_{1005-1260}$). pVT-CDC25$^{Mm}_{789-1032}$/Cdc25p$_{1309-1589}$: this fusion was obtained by inserting the 1.3 Kb *NdeI-PvuII* fragment of *CDC25* in the *PvuII* site (nucleotide 3322) of CDC25$^{Mm}_{789-1260}$ previously cloned in pVTU (pVT-CDC25$^{Mm}_{789-1260}$, see ref. 27). pEM-Cdc25p$_{907-1501}$: the digestion of the *HindIII* site of pInd25-1 (containing Cdc25p$_{907-1589}$) (19) and filling-in led to the expression of Cdc25p from the Met 907 to Ser 1501. **Fig.1** shows the various *CDC25*^{Mm} and *CDC25* fragments.

Plasmids used for in vitro studies. The plasmids utilised for purification from *E. coli* of the various fragments of CDC25^{Mm} as fusion proteins were: pMAL-cRI (20) and pGEX-2TH (10). pMAL-CDC25$^{Mm}_{1005-1260}$: *CDC25*$^{Mm}_{1005-1260}$ was isolated after digestion with *SalI* and *XhoI* and cloned into pMAL-cRI digested with *BamHI*. pMAL-CDC25$^{Mm}_{1005-1233}$: *CDC25*$^{Mm}_{1005-1233}$ was isolated after digestion with *SalI* and *ScaI* and cloned into pMAL-cRI digested with *BamHI*. pGEX-CDC25$^{Mm}_{1030-1260}$: *CDC25*$^{Mm}_{1030-1260}$ was isolated after *PvuII* digestion and cloned into pGEX-2TH digested with *HindIII* .

Protein purifications. The pMAL-CDC25$^{Mm}_{1005-1260}$, pMAL-CDC25$^{Mm}_{1005-1233}$ and pGEX-CDC25$^{Mm}_{1030-1260}$ were expressed in *E.coli* strain DH5α after induction with 0.2 mM IPTG at a cell concentration of 0.6 A600 units and overnight incubation at 27°C. The cell extract and supernatant were prepared essentially as reported (5). The supernatant was mixed with amylose-resin (Biolabs) or glutathione-agarose (Sigma) (2ml per 1-Liter culture cells) and gently shaked for 30 min at 4°C. After washing with 20 mM Na_2HPO4 pH 7.5, and 150 mM NaCl, the bound protein was eluted with 50 mM Tris-HCl, pH 8.0, containing either 10 mM reduced glutathione or 10 mM maltose. The purified proteins were kept at -20°C in 25 mM Tris-HCl pH 7.5, 25 mM NaCl, 14 mM ME and 50% glycerol. **Fig.2** shows a SDS-PAGE of the purified CDC25$^{Mm}_{1005-1260}$ and CDC25$^{Mm}_{1005-1233}$.

Assays. For complementation *in vivo* yeast strain TC7 was transformed with 1-2μg of the various vectors (21). After 36 hours growth on minimal medium at 24°C the transformants were transferred to the restrictive temperature (36°C) by replica plating. To determine the GEF activity in vitro, the dissociation rate of the Ras guanine nucleotide complexes and the GDP to GTP exchange reactions on RAS2p or c-Ha-ras-p21 were measured by the nitrocellulose binding assay at 30°C using [^{3}H]GDP or [^{3}H]GTP (DuPont/NEN) (10). For Western blot assay, total yeast extracts were prepared by cells distruption with glass beads (4 vortex cycles of 30 sec each), denatured with SDS-sample buffer at 100°C and analysed on SDS-PAGE (approximately 30μg of protein for each lane). The blotted SDS-PAGE (8%) was reacted with anti-CDC25^{Mm} (23) or anti-Cdc25p antibodies (kindly provided by Dr. A. Levitzky) (24). Immunoreactive bands, revealed by horseradish peroxidase-conjugated anti-rabbit antibodies, were developed with ECL-chemiluminescent assay system (Amersham). H-ras-p21 and Ras2p were produced and purified as reported in (5) and in (10).

RESULTS AND DISCUSSION

Minimal domain of CDC25^{Mm} active in vivo. All the components of the CDC25-like proteins share a significant homology in the C-terminal domain. This domain interacts directly with the ras proteins and is responsible for the GEF activity. The sequence alignment with the MACAW program (25) has revealed that

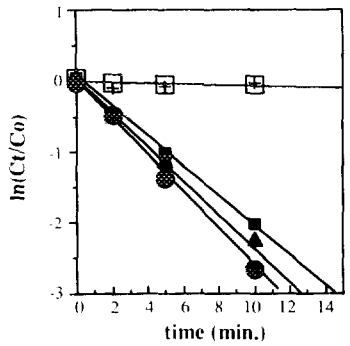

Fig. 4. Stimulation by CDC25$^{Mm}_{1005-1260}$ of the dissociation of the p21·[^{3}H]GDP complex. The dissociation rates of the p21·[^{3}H]GDP complexes (1μM, specific activity: 55 Bq·pmol^{-1}) was measured with 20 nM CDC25$^{Mm}_{1005-1260}$ (●), 400 nM CDC25$^{Mm}_{1005-1233}$ (+), 20 nM CDC25$^{Mm}_{1005-1260}$ plus 400 nM CDC25$^{Mm}_{1005-1233}$ (▲), 20 nM CDC25$^{Mm}_{1005-1260}$ plus 800 nM CDC25$^{Mm}_{1005-1233}$ (■), or in absence of CDC25^{Mm} (□). The reaction was started by the addition of a 500-fold excess of cold GDP.

the most conserved region lies between the amino acids 1309-1542 for the yeast Cdc25p and between the amino acids 1030-1260 for the mouse homologous CDC25^{Mm}. In order to identify the minimal domain of CDC25^{Mm} active *in vivo* yeast strain TC7 was transformed with expression vectors containig *CDC25*^{Mm} fragments of different lenght. As shown in **Fig. 1** the most conserved domain (CDC25$^{Mm}_{1030-1260}$) did not complement the thermosensitive mutation *cdc25-1*. To have activity *in vivo* it was necessary to express the domain comprising 1005-1260 residues (CDC25$^{Mm}_{1005-1260}$), since the deletion of the 27 C-terminal residues (1234-1260) of this fragment abolished the activity of the corresponding product (CDC25$^{Mm}_{1005-1233}$). Using antibodies against the C-terminal region of the mouse protein (22) we observed an efficient production of both the active CDC25$^{Mm}_{1005-1260}$ and the inactive CDC25$^{Mm}_{1005-1233}$ whereas no CDC25$^{Mm}_{1030-1260}$ could be detected (**Fig. 3**).

These results show that for biological activity the most conserved region alone is not sufficient, a fragment encoding additional 25 N-terminal residues (1005-1029) is crucial for the expression and/or the stability of the mouse protein and a fragment encoding the last 27 C-terminal residues is essential for the activity. Having determined the minimal region required for activity *in vivo* in the next section we tested the ability of highly purified CDC25$^{Mm}_{1005-1260}$ and CDC25$^{Mm}_{1005-1233}$ to enhance the dissociation rate of the Ras-guanine-nucleotide complexes in an *in vitro* system.

Minimal domain of CDC25^{Mm} for in vitro activity. We constructed bacterial expression vectors where the two domains CDC25$^{Mm}_{1005-1260}$ and CDC25$^{Mm}_{1005-1233}$ were fused with the Maltose Binding Protein and the domain CDC25$^{Mm}_{1030-1260}$ with the GST. We could only test the activities of CDC25$^{Mm}_{1005-1260}$ and CDC25$^{Mm}_{1005-1233}$ since the domain CDC25$^{Mm}_{1030-1260}$ was completely insoluble. This strongly suggests that the nonconserved residues 1005-1029 are important to assure the correct folding to the C-terminal domain of the mouse protein.

As shown in **Fig. 4** the addition of 20 nM CDC25$^{Mm}_{1005-1260}$ increased strongly the intrinsic dissociation rate of the p21·GDP complex. The rate was enhanced from $1.4 \cdot 10^{-4}$ sec^{-1} to $3 \cdot 10^{-3}$ sec^{-1} thus showing a 21 times stimulation. CDC25$^{Mm}_{1005-1260}$ was also active on Ras2p. The intrinsic dissociation rate of the Ras2p·GDP complex was enhanced by 25 nM CDC25$^{Mm}_{1005-1260}$ sixfold (from $2.3 \cdot 10^{-4}$ to $1.4 \cdot 10^{-3}$ sec^{-1}) while that of the GTP-complex was only increased threefold (from $1.2 \cdot 10^{-4}$ to $3.3 \cdot 10^{-4}$ sec^{-1}) further confirming that the GDP complex of ras proteins is the privileged target for the CDC25-like proteins. Unlike CDC25$^{Mm}_{1005-1260}$ a 20 times higher concentration of CDC25$^{Mm}_{1005-1233}$ was completely inactive on both p21 and Ras2p. Nevertheless competition experiments indicated that the inactive domain CDC25$^{Mm}_{1005-1233}$ could somewhat bind to the p21 protein; in fact, there was a slight inhibition of the

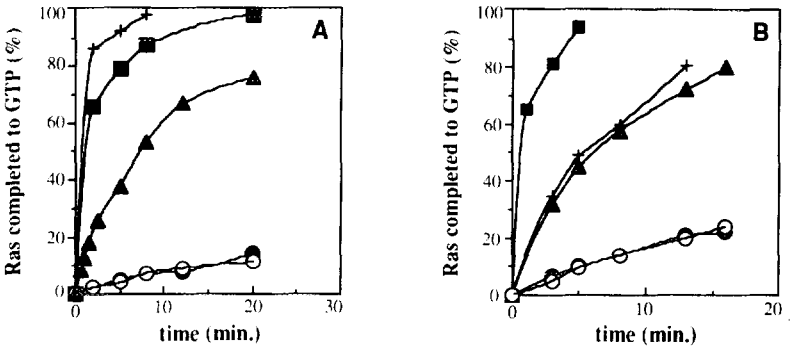

Fig. 5. Stimulation by CDC25$^{Mm}_{1005-1260}$ of the GDP to [^{3}H]GTP exchange reaction of ras p21(A) and Ras2p (B). **A:** The p21·GDP complex (0.5μM) was incubated with 20 nM CDC25$^{Mm}_{1005-1260}$ (▲), 40 nM CDC25$^{Mm}_{1005-1260}$ (■), 40 nM CDC25$^{Mm}_{1005-1233}$ (●), 400 nM CDC25$^{Mm}_{1005-1233}$ (o), or 3 mM EDTA (+). The reaction was started by the addition of a 20-fold molar excess of [^{3}H]GTP (55 Bq·pmol^{-1}) over the p21·GDP complex. **B:** The Ras2p·GDP complex (0.5μM) was incubated with 25 nM CDC25$^{Mm}_{1005-1260}$ (▲), 40 nM MBP (o), 40 nM CDC25$^{Mm}_{1005-1233}$ (●), 20 nM CDC25$^{Mm}_{974-1260}$ (+), or 3 mM EDTA (■). The reaction was started by the addition of a 20-fold molar excess of [^{3}H]GTP (55Bq·pmol^{-1}) over the Ras2p·GDP complex.

activity of CDC25$^{Mm}_{1005-1260}$ on p21 if CDC25$^{Mm}_{1005-1233}$ was added simultaneously in a 20 and 40 molar excess (**Fig. 4**). As expected from the stimulation of the dissociation rate, the GDP to GTP exchange rate of both p21 and Ras2p was also enhanced by CDC25$^{Mm}_{1005-1260}$ (**Fig. 5**) increasing as a function of its concentration (**Fig. 5A**) in a manner similar to that induced by the larger fragment CDC25$^{Mm}_{974-1260}$ (**Fig. 5B**) (10).

It has been proposed that a specific sequence of 48 amino acids, conserved in various GEFs, is responsible for the interaction with the Ras proteins (4). This domain, that in Cdc25p corresponds to the residues 1119-1168 could be important for the activity of this GEF but our results show that it is not necessary for the activity of the CDC25^{Mm} protein since the domains encompassing residues 974-1260 (8,10) and 1005-1260 (this work), lacking the corresponding sequence, are active both *in vivo* and *in vitro* (10). Noteworthy, the deletion of residues 1234-1260 within the most conserved domain inactivates the GEF activity of the mouse protein and of residues 1005-1029 renders CDC25^{Mm} completely insoluble.

Comparison of in vivo properties of CDC25^{Mm} and Cdc25p C-terminal domain. In the following experiments we have compared the various constructions of the 3'-terminal region of *CDC25*^{Mm} with those of the corresponding regions of yeast *CDC25*. As in the case of CDC25^{Mm} the most conserved domain Cdc25p$_{1309-1589}$ was not sufficient to complement the mutation *cdc25-1*^{ts} (**Fig. 1**). This region was also inactive when expressed in a chimaera of 525 residues, of which the N-terminal region is formed by residues 789-1032 of CDC25^{Mm} and the C-terminal one by the last 281 residues (1309-1589) of Cdc25p. In line with this was also the observation that Cdc25p$_{1280-1589}$ did not lead to the recovery of the growth capacity at the restrictive temperature. Only Cdc25p$_{1256-1589}$, corresponding to the active domain of the mouse protein CDC25$^{Mm}_{1005-1260}$, could complement *cdc25-1*^{ts} but with a less efficiency than CDC25$^{Mm}_{1005-1260}$ (**Fig. 1**). Since a reason for the lack of complementation could be a low expression of the deleted Cdc25p domains, antibodies against the catalytic region of Cdc25p were used to analyse the production level of the various Cdc25p constructions. Both the chimaera CDC25$^{Mm}_{789-1032}$/Cdc25p$_{1309-1589}$ and the Cdc25p$_{1256-1589}$ were well produced, whereas no production of Cdc25p$_{1309-1589}$ could be seen (**Fig. 6A**). This fragment of 281 amino acids was not detectable in the yeast cell, whereas, when fused with CDC25$^{Mm}_{789-1032}$ though inactive, it was well produced. Furthermore the gene fragment encoding the inactive domain Cdc25p$_{1280-1589}$, cloned under the control of the inducible promoter *CYC1/UAS*$_{GAL}$ was stable and well expressed only in galactose medium (**Fig. 6B**). Therefore, as found for the mouse GEF, also the most

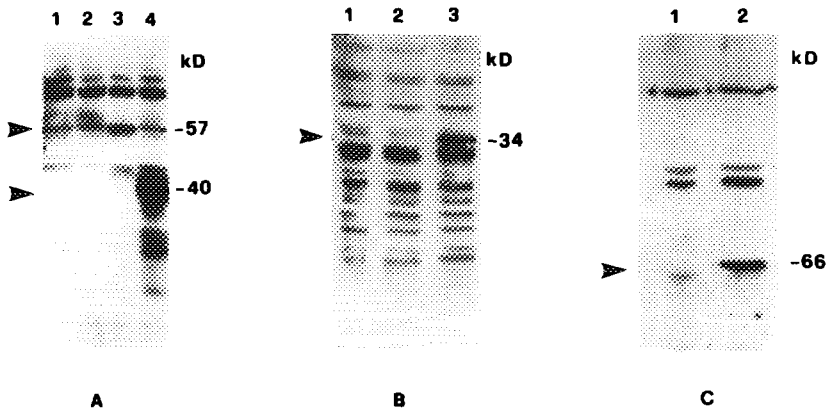

Fig. 6. Western blot with antibodies against the various Cdc25p fragments. A: Yeast cells carrying pVTU (lane1), pVT-Cdc25p$_{1309-1589}$ (lane 2), pVT-CDC25$^{Mm}_{789-1032}$/Cdc25p$_{1309-1589}$ (lane 3), pVT-Cdc25p$_{1256-1589}$ (lane 4). **B:** Yeast cells carrying pEMBLyEX4 (lane 1), pEM-Cdc25p$_{1280-1589}$ in glucose medium (lane 2), pEM-Cdc25p$_{1280-1589}$ in galactose medium (lane 3). **C:** Yeast cells carrying pEM-Cdc25p$_{907-1501}$ in glucose medium (lane 1), pEM-Cdc25p$_{907-1501}$ in galactose medium (lane 2).

conserved domain of Cdc25p needs additional nonconserved residues (1256-1308) for stability and/or for the correct folding of its catalytic domain.

A few conclusions can be drawn from this work. First of all, the short N-terminal amino acid sequence absolutely required for assuring the correct folding and/or stability of the most conserved domain of ras GEFs are specific for each GEF. In the mouse GEF this crucial sequence only comprises 25 nonconserved amino acids (1005 to 1029). In yeast Cdc25p a longer nonconserved amino acid stretch of 53 residues is required. No *in vitro* activity can be obtained with the fragment of 334 residues (Cdc25p$_{1256-1589}$) despite an efficient production and a weak complementation *in vivo*. For a full activity *in vitro* it is required the domain Cdc25p$_{1084-1589}$ (4,5) and *in* vivo the domain Cdc25p$_{1102-1589}$ (4). The expression of smaller regions leads to partial or total inactivation of the protein *in vivo* and *in vitro*, very likely due to uncorrect folding.

The integrity of the most conserved region is crucial for the activity of the catalytic region in both mouse and yeast GEF. The deletion of residues 1234-1260 inside of the conserved region of CDC25^{Mm} was found to inactivate the mouse protein as the deletion of the corresponding region of Cdc25p (1502-1542) (**Fig. 1**). The latter phenomenon was also reported by Lai *et al.* (1993).

The finding that it is possible to isolate in pure form a highly soluble, stable and active CDC25^{Mm} of only 256 residues is of interest for functional studies directed to define the interaction site(s) between ras proteins and GEF and the transmission pathway of signals inducing the release of the bound nucleotide. This minimal catalytic domain can also be an useful tool for structural studies aimed at characterizing the tridimensional structure of an active GEF and the stable complex that the C-terminal domain of a GEF is known to form with ras proteins (4,25,E. Jacquet unpublished results).

ACKNOWLEDGMENTS: P.C. was supported by a short EMBO fellowship and I.M. by a AIRC fellowship. This work was partially supported by a grant CEE BIOTECH BIO2CT-930005 to L.A. and A.P. and by the grant n° 6377 of the ARC to A.P. We are indebted to Drs. E. Jacquet and M.C. Parrini for discussion and criticism, and to Dr. A. Levitzki for yeast Ccd25p antibodies.

REFERENCES

1. Bourne H.R., Sanders D.A., McCormick F. (1991) Nature 349, 117-127.
2. Bollag G., McCormick F. (1991) Annu. Rev. Cell. Biol. 7, 601-632.
3. Jones S., Vignais M., Broach J. R. (1991) Mol . Cell . Biol. 11, 2641-2646.

4. Lai C.C., Boguski M., Broek D., Powers S. (1993) Mol. Cell. Biol. 13, 1345-1352.
5. Jacquet E., Parrini M.C., Bernardi A., Martegani E., Parmeggiani A. (1994) Biochem.Biophys. Res. Commun. 199, 497-503.
6. Boy-Marcotte E., Damak F., Camonis J., Garreau H., Jacquet M. (1989) Gene 77, 297-304.
7. Créchet J.B., Poullet P., Mistou M.Y., Parmeggiani A., Camonis J., Boy-Marcotte E., Damak F., Jacquet M. (1990) Science 248, 866-868.
8. Martegani E., Vanoni E., Zippel R., Coccetti P., Brambilla R., Ferrari C., Sturani E., Alberghina L.(1992) EMBO J. 11, 2151-2157.
9. Cen H., Papageorge A. G., Zippel R., Lowy D.R., Zhang K. (1992) EMBO J. 11, 4007-4015.
10. Jacquet E., Vanoni M., Ferrari C., Alberghina L., Martegani E., Parmeggiani A. (1992) J. Biol. Chem. 267, 24181-24183.
11. Shou C., Farnsworth C.L., Neel B.G., Feig L.A. (1992) Nature 358, 351-354.
12. Schweighoffer F., Faure M., Fath I., Chevallier-Multon M.C., Apiou F., Dutrillaux B., Sturani E., Jacquet M., Toque B. (1993) Oncogene 8, 1477-1485.
13. Wei W., Schreiben S.S., Bandry M., Tocco G., Broek D. (1993) Mol. Brain Res. 19, 339-344.
14. Chardin P., Camonis J.H., Gale N.W., Van Aelst L., Schlessinger J., Wigler M.H., Bar-Sagi D. (1993) Science 260, 1338-1343.
15. Martegani E., Baroni M.D., Frascotti G., Alberghina L. (1986) EMBO J. 5, 2363- 2369.
16. Petitjean A., Higler F., Tatchell K. (1990) Genetics 124, 797-806.
17. Damak F., Boy-Marcotte E., Le-Roscouet D., Guibaud R., Jacquet M. (1991) Mol. Cell. Biol. 11, 202-212.
18. Vernet T., Diguard D., Thomas D.Y. (1987) Gene 52, 225-233.
19. Frascotti G., Coccetti P., Vanoni M. A., Martegani E., Alberghina L. (1991) Biochem. Biophys. Acta 1089, 206-212.
20. Guan C., Li P., Riggs P.D., Inouye H. (1987) Gene 67, 21-30.
21. Ito H., Fukuda Y., Murata K., Kimura A. (1983) J. Bacteriol. 153, 163-168.
22. Ferrari C., Zippel R., Martegani E., Gnesutta N., Carrera V., Sturani E. (1994) Exp. Cell Res. 210, 353-357.
23. Gross E., Marbach I., Engelberg D., Segal M., Simchen G., Levitzki A. (1992) Mol. Cell. Biol. 12, 2653-2661.
24. Schuler G. D., Altschul S. F., Lipman D. J. (1991) Proteins 9, 180-190.
25. Hwang Y., Zhong Y., Poullet P., Parmeggiani A. (1993) J. Biol. Chem. 268, 24692-24698.
26. Porfiri E., Evans T., Chardin P., Hancock J.F. (1994) J. Biol. Chem. 269, 22672-22677.
27. Martegani E., Zippel R., Ferrari C., Coccetti P., Campiglio S., Denis-Donini S., Sturani E., Vanoni M., Alberghina L. (1993) Eur. J. Histochem. 4,73-78.