Cloning and Characterization of cDNA Encoding Cardosin A, an RGD-containing Plant Aspartic Proteinase*

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Carlos Faro‡§, Miguel Ramalho-Santos‡¶, Margarida Vieira‡¶, Alexandra Mendes‡¶, Isaura Simões‡¶, Rita Andrade‡¶, Paula Veríssimo‡¶, Xin-li Lin**, Jordan Tang**‡, and Euclides Pires‡¶

From the ‡Department of Bioquímica, Faculdade de Ciências e Tecnologia and Departamento Biologia Molecular e Biotecnologia, Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3000 Coimbra, Portugal and the ¶**Protein Studies Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

Cardosin A is an abundant aspartic proteinase from pistils of *Cynara cardunculus* L. whose milk-clotting activity has been exploited for the manufacture of cheese. Here we report the cloning and characterization of cardosin A cDNA. The deduced amino acid sequence contains the conserved features of plant aspartic proteinases, including the plant-specific insertion (PSI), and revealed the presence of an Arg-Gly-Asp (RGD) motif, which is known to function in cell surface receptor binding by extracellular proteins. Cardosin A mRNA was detected predominantly in young flower buds but not in mature or senescent pistils, suggesting that its expression is likely to be developmentally regulated. Procardosin A, the single chain precursor, was found associated with microsomal membranes of flower buds, whereas the active two-chain enzyme generated upon removal of PSI is soluble. This result implies a role for PSI in promoting the association of plant aspartic proteinase precursors to cell membranes. To get further insights about cardosin A, the functional relevance of the RGD motif was also investigated. A 100-kDa protein that interacts specifically with the RGD sequence was isolated from octyl glucoside pollen extracts by affinity chromatography on cardosin A-Sepharose. This result suggests that the 100-kDa protein is a cardosin A receptor and indicates that the interaction between these two proteins is apparently mediated through RGD recognition. It is possible therefore that cardosin A may have a role in adhesion-mediated proteolytic mechanisms involved in pollen recognition and growth.

Aspartic proteinases (1) are widely distributed in the plant kingdom, and they have been purified and characterized from several species of monocotyledons, dicotyledons, and gymnosperms (2). In common with most of the aspartic proteinases from retrovirus, bacteria, yeast, fungi, and vertebrates, plant aspartic proteinases are inhibited by pepstatin (a hexapeptide from *Streptomyces*), have an acid pH optimum, and preferentially cleave peptide bonds between hydrophobic residues. The amino acid sequences of some plant aspartic proteinases have recently been deduced (3–7). As compared with those of mammalian or microbial origins, they have an extra segment encoding about 100 amino acids known as the plant-specific insert (PSI).1 This segment is partially or totally removed from the precursors to render active two-chain mature enzymes (8, 9).

Although the molecular and physiological relevance of this domain is not yet known, a significant secondary structural similarity with saposins has been noted (10). Several plant aspartic proteinases have been localized to the vacuoles (11–14), and there is biochemical evidence that some of them are secreted (15, 16). However, the biological functions of these proteinases still remain to be elucidated.

We have previously reported the isolation and characterization of two aspartic proteinases, cardosin A and cardosin B (17, 18), whose milk-clotting activity has traditionally been used in Portugal for cheese making. Both cardosins are two-chain glycosylated enzymes, which are thought to have arisen by gene duplication (17). Although they preferably cleave peptide bonds between residues with bulky hydrophobic side chains, cardosin B displays a broader specificity and a higher proteolytic activity (17, 18). Cardosin A, the more abundant one, is accumulated to a high amount in protein storage vacuoles of the stigmatic papillae and is also found in vacuoles of the epidermic cells of style (11). These observations suggest that cardosin A may be involved in pollen-pistil interaction and possibly in the defense against pathogens or invasion (11). The evidence, however, is lacking to support these hypotheses.

To gain more insights about the possible biological function(s) of cardosin A, we have cloned its cDNA. As will be described herein, a unique feature of cardosin A, among plant aspartic proteinases, is the presence of a functional RGD sequence. The RGD motif is well known as an integrin-binding sequence in mammalian tissues in which it facilitates many cell recognition functions such as adhesion, migration, signaling, differentiation, and growth (19). Although these events have been well studied in animals, little evidence is available for the occurrence of integrin-like proteins and their ligands in plants. Integrin-like proteins of plant origin have recently been identified either by their binding to RGD-containing peptides (20–23) or by their cross-reactivity to antibodies against integrin

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1 The abbreviations used are: PSI, plant-specific inserted sequence; Me₂SO, dimethyl sulfoxide; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RACE, rapid amplification of cDNA ends; RT, reverse transcription; PCR, polymerase chain reaction; Bicine, N,N-bis(2-hydroxyethyl)glycine.
subunits (20, 24–27). Similarly, vitronectin-like proteins, which are supposed to interact with plant integrin-like proteins through their RGD sequence, were detected in the extracellular matrix of several plant tissues (28–31). However, the RGD-containing protein and its interacting receptor have not both being identified in any case.

In the present paper, we described the cloning of cardosin A cDNA and the identification of a 100-kDa receptor from pollen that interacts specifically with the region of the proteinase that contains an RGD cell attachment sequence. The physiological relevance of these findings, in particular the possible involvement of a proteinase in RGD-mediated molecular mechanisms in plants, is also discussed.

**EXPERIMENTAL PROCEDURES**

**Plant Material—**Organs of *Cynara cardunculus* L. were collected from plants previously used for biochemical studies. These plants were grown from seeds supplied by the Botanical Garden of the University of Coimbra. In most cases, organs were collected, frozen immediately in liquid nitrogen, and kept at −80 °C until use.

cDNA Cloning—Total RNA was isolated from flower buds of *C. cardunculus* L. essentially as described in Ref. 32, and the poly(A) mRNA was isolated using a PolyATract mRNA isolation kit (Clontech). The RNA was used to generate double-stranded cDNA, and upon ligation of the Marathon cDNA adaptor, 5′- and 3′-RACE were performed using the Marathon cDNA amplification kit (CLONTECH) according to the manufacturer's instructions. RACE was performed with cardosin A-specific primers (card 1S, GAGTTGTGTGAACACTTATCCA; card 1R, TGATGAGTTGTCTACAACTC) and the adaptor primer AP1 (ACTCTACTATAGGGCTCGAGCGGC). The PCR-amplified cDNA fragments were cloned and sequenced. Based on these sequences, specific primers for the 5′- (primer S, ATGGGTACCTCAATCAGAGCA) and 3′- (primer R, TCAAAGCTGTGCTTCGAAATTC) ends of the open reading frame were synthesized, and full-length cDNAs of cardosin A were PCR-amplified from the cDNA library. The PCR products were cloned, and both strands were sequenced by automated DNA sequencing. Based on these sequences, specific primers for the 5′- (primer S, ATGGGTACCTCAATCAGAGCA) and 3′- (primer R, TCAAAGCTGTGCTTCGAAATTC) ends of the open reading frame were synthesized, and full-length cDNAs of cardosin A were PCR-amplified from the cDNA library. The PCR products were cloned, and both strands were sequenced by automated DNA sequencing.

DNA sequencing was performed in a Vistra DNA Automatic Sequencer (Amersham Pharmacia Biotech), and sequencing reactions were carried out with Texas Red using the 5′-oligonucleotide Texas Red labeling kit (Amersham Pharmacia Biotech). Sequencing reactions were carried out with the Thermo sequenase preemixed cycle sequencing kit (Amersham Pharmacia Biotech) using the dyeexonucleotide chain termination method.

Northern Blotting—Total RNA was isolated from 100 mg of plant tissue using the RNasey Plant Mini kit (Qiagen) according to the manufacturer's instructions. RNA (5 μg) was separated on 1.2% agarose gels containing formaldehyde and transferred to a positively charged nylon membrane (Roche Molecular Biochemicals) by capillarity overnight at 4 °C. RNA was cross-linked to the membrane by baking at 120 °C for 30 min. Prehybridization was performed in standard hybridization buffer with formamide for 1 h at 50 °C. A 1.5-kilobase fragment of cardosin A cDNA corresponding to the coding region was labeled with DIG High Prime (Roche Molecular Biochemicals) by random primer labeling technique and used as a probe. Hybridization was carried out in hybridization buffer overnight at 50 °C, and detection was performed with the DIG luminescent detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions.

RT-PCR Analysis of Cardosin A mRNA Expression—Total RNA was isolated as described above for Northern blotting. RT-PCR was performed with the Superscript RT-PCR kit (Life Technologies, Inc.) using similar amounts of total RNA and the following primers (forward, 5′-CTC GCC CTT TCA TTT CAAC AGG-3′; reverse, 5′-CGG GTT GTA TCT TAG ATC GG-3′). PCR products were visualized on agarose gel as 437-base pair cDNA fragments.

Expression in Escherichia coli and Production of an Antibody Against Recombinant Procardosin A—Two oligonucleotides containing 5′-flanking restriction site NheI (primer 5′-NheI, GCTAGCTCGTCTGAGGATTAGCTCGAGA and primer 3′-NheI, GCTAGCTCCAGCTGCTGCTGGAGAAC-3′) were used to PCR amplify cardosin A. The resulting fragment was subjected to putative signal sequence, was cloned into a TA vector and then subcloned into the NheI cloning site of the pET11a expression vector. The recombinant plasmid (pET11a-PcardA) was transformed into *E. coli* strain BL21 cells, and expression was carried out under the control of the T7 promoter. Induction, isolation of inclusion bodies, refolding, and purification of recombinant procardosin A were carried out essentially as described for canditarpin (33). The proteolytic activity was assayed using the chromogenic synthetic peptide Lys-Pro-Ala-Glu-Phe-(NO2)-Ala-Leu as substrate (17). For antibody production, 100 μg of recombinant procardosin A was emulsified with Freund's complete adjuvant and injected subcutaneously into a New Zealand rabbit from which preimmune blood had been collected. A second immunization was made 6 weeks later using recombinant procardosin A emulsified with Freund's incomplete adjuvant. The antiserum was prepared from blood collected 3 weeks after this last injection.

**Protein Extraction and Western Blotting Analysis—**Organs and pistils at different stages of development were ground in a mortar and pestle under liquid nitrogen and then homogenized at 20% (w/v) in 10 mM Tris, 2% (m/v) SDS, 5% urea. Extracts obtained were centrifuged at 12,000 × g for 15 min at 4 °C, and samples of the supernatants containing about 10 μg of protein were loaded onto 15% polyacrylamide gels for SDS-PAGE. Electrophoresis and transfer onto nitrocellulose membranes were performed as described previously (19). For immunodetection, the membranes were incubated in blocking solution, 0.5% (m/v) skim milk in phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 for 45 min at room temperature and then incubated overnight with a 1:500 dilution of the recombinant cardosin A antibody in blocking solution. The membranes were washed 3 times in blocking solution for 10 min and incubated with a 1:1000 dilution of swine anti-rabbit IgG conjugated to horseradish peroxidase for 1 h. The membranes were again washed 3 times in blocking solution for 10 min, and horseradish peroxidase activity was detected as described previously with luminol chemiluminescence using the ECL method according to the manufacturer's instructions (Amersham Pharmacia Biotech).

**Microsomal Membrane Association Studies—**Young flower bud extracts obtained in 8.0% sucrose, 2 mM EDTA, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 mM Tris, 20 mM HEPES, pH 7.4, were centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was centrifuged at 100,000 × g for 1 h at 4 °C, and the resulting pellet was washed with extraction buffer and resuspended in SDS-PAGE loading buffer. SDS-PAGE, electrotransference, and immunodetection were essentially performed as described above except that a monospecific antibody for PSI (9) was used as primary antibody at a 1:200 dilution.

**Isolation of the Cardosin A-binding Protein—**Pollen (100 mg) was grown under mortar and pestle under liquid nitrogen. Proteinase activity was detected as described above except that a monospecific antibody for PSI (9) was used as primary antibody at a 1:200 dilution.

**RESULTS**

**Molecular Cloning of Cardosin A cDNA and Characterization of the Deduced Amino Acid Sequence—**In the first stage, a 0.8-kilobase internal segment of the cardosin A cDNA was PCR-amplified using degenerated primers encoding two amino acid sequences previously determined by protein sequencing (17). Based upon the sequence of this fragment, the nature and content of the putative amino acid sequence of cardosin A was determined by comparison with the partial amino acid sequence of cardosin A, primers were designed to amplify a full-length cDNA from a young flower bud cDNA Marathon library. Most of the 5′-RACE-PCR fragments contained a putative ATG start codon, whereas all 3′-RACE-PCR fragments contained three stop codons plus the poly(A) sequence. The cardosin A
cDNA was finally obtained from the cDNA library using specific primers for the 5'- and 3'-ends of the open reading frame. The nucleotide and deduced amino acid sequences are shown in Fig. 1A. The 1515-base pair cDNA sequence encodes a signal peptide of about 24 amino acids, a prosegment of 42 residues, and a 438-amino acid-long polypeptide containing the two chains of mature cardosin A. In common with other plant aspartic proteinases, the cDNA-derived amino acid sequence of cardosin A also contains an internal segment of 104 amino acids, which bears no homology with mammalian or microbial aspartic proteinases. This fragment known as PSI separates the two chains that occur in mature form of cardosin A (Fig. 1B). Critical residues for aspartic proteinase activity can be identified in the sequence. They include two catalytic triads (DTG and DSG) both located at the 31-kDa chain and a conserved tyrosine residue (Tyr-75 in pepsin numbering) present in the sequence.

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\begin{align*}
\text{PSI} & \quad \text{underlined}\text{, this PSI is removed during processing of cardosin A}\text{, yielding a two-chain mature enzyme with apparent molecular masses of 31} & \text{and 15 kDa}\text{. Putative N-glycosylation sites are circled and the catalytic aspartates are in bold}\text{. The RGD cell attachment motif is boxed}\text{.}\n\end{align*}
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Fig. 1. A cDNA and deduced amino acid sequence of cardosin A. A, The pre- and pro-sequences are in italic. The PSI is underlined. This sequence is removed during processing of cardosin A, yielding a two-chain mature enzyme with apparent molecular masses of 31 and 15 kDa. Putative N-glycosylation sites are circled and the catalytic aspartates are in bold. The RGD cell attachment motif is boxed. B, Domain organization of cardosin A. The structure of cardosin A includes a signal peptide, a prosegment, the 31-kDa domain containing the two catalytic triads and the RGD sequence, the PSI domain whose predicted secondary structure display similarity to saposins, and the 15-kDa domain, which corresponds to the C-terminal domain of mature cardosin A. The putative N-glycosylation sites, previously shown to be occupied (42), are located in each chain of the active enzyme. The RGD sequence is located at the C-terminal part of 31-kDa chain upstream of the PSI domain.
Cardosin mRNA was consistently not detected in several organs tested other than young flower buds, suggesting that its expression is highly restricted to this organ. Conversely, when RT-PCR was used to study cardosin A mRNA expression in the same organs, besides being detected in flower buds, expression was detected in seeds, pollen, and bracteas but not in roots or leaves (Fig. 3). In this assay, cardosin A mRNA was detected as a PCR-amplified fragment of about 450 base pairs. These results suggest that cardosin A is likely to be expressed in these organs at low levels that can only be detected when a more sensitive technique is used to examine its expression.

**Association of Procardosin A with Microsomal Membranes**—
The predicted secondary structure of PSI suggests that this extra domain of plant aspartic proteinases has an amphipathic character (10). Because proteins with such domains are known to interact with lipid membranes it would be possible that PSI might promote association with cell membranes. To test this hypothesis, fractionation studies were carried out to determine whether the precursor form of cardosin A containing the PSI domain (procardosin A) is soluble or membrane-associated. Protein extracts of young flower buds were subjected to differential centrifugation, and the resulting pellets were analyzed by immunoblotting using an antibody raised against a peptide whose sequence corresponds to a segment of PSI predicted to be exposed. As shown in Fig. 4, procardosin A was detected as a 67-kDa band in the pellet of the 100,000 × g fraction, indicating that the precursor form is associated with microsomal membranes. Conversely, the mature two-chain enzyme was detected in supernatants of 10,000 and 100,000 × g centrifuged samples. Therefore these results suggest that the PSI domain may have a role in the membrane association of the precursor form of cardosin A.

**Identification of a 100-kDa Putative Cardosin A Receptor from Pollen that Interacts Specifically with the RGD Sequence**—
The presence of the RGD cell attachment sequence raised the question of whether it could be functionally active. To determine whether a cardosin A-binding protein occurs in pollen, cardosin A was immobilized in a Sepharose matrix and used for affinity chromatography of detergent-extracted pollen (Fig. 5). Elution was made with an RGD-containing synthetic peptide designed from the amino acid sequence of cardosin A. A pollen cardosin A-binding protein with an apparent molecular mass of 100 kDa was specifically eluted with 1 mM RGD-containing peptide (Fig. 5, lanes 7 and 8). This 100-kDa protein was not eluted when a nonrelated peptide was used (Fig. 5, lanes 5 and 6) or when the chromatography was performed in an albumin-Sepharose column. Other proteins were released throughout the wash fractions, but they were not influenced by the presence of the RGD peptide in the elution buffer. Some of

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**Fig. 2. Developmental expression of cardosin A mRNA and its translational product in the flower of C. cardunculus L.** Northern blot analysis (A) of cardosin A mRNA expression and Western blot analysis (B) of cardosin A using an antirecombinant procardosin A polyclonal antibody are shown. Expression was monitored in pistils throughout floral development at three different stages: closed (lane 1), partially opened (lane 2), and fully opened (lane 3) capitula. RNA and protein were extracted at the same stage of development as described under “Experimental Procedures.”

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**Fig. 3. Organ-specific expression of cardosin A mRNA.** Total RNA was extracted from various organs of C. cardunculus L., and RT-PCR was performed using similar amounts of RNA and primers specific for cardosin A. The source of mRNA is indicated in each lane. bp, base pairs.

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the 100-kDa protein appeared to be gradually released during washing, indicating a low affinity binding to cardosin A. An additional experiment using ligand blotting analysis was performed to confirm that the purified 100-kDa protein interacts with cardosin A. The purified 100-kDa protein was blotted onto polyvinylidene difluoride membrane and incubated with cardosin A either in the absence or presence of an excess of the RGDS peptide. As shown in Fig. 6, the antibody raised against recombinant cardosin A and a more sensitive detection assay allowed the detection of cardosin A in those organs, although at low levels. Cardosin A mRNA was not detected in mature leaves and roots, and thus its expression is apparently and interestingly associated with organs or tissues that undergo substantial morphological changes. In this respect, it is worthwhile to note that expression of cardosin A mRNA in immature pistils also precedes pistil growth and elongation suggesting an involvement in such developmental processes.

Cardosin A is unique among plant aspartic proteinases for having an RGD motif. The crystal structure of cardosin A3 shows that this sequence is located in a loop that connects two β-strands and projects itself above the molecular surface. A similar structure is found in fibroectin (37), suggesting that cardosin A might be functionally active in promoting binding to integrin or integrin-like proteins. This hypothesis is further supported by the identification of the 100-kDa protein from pollen that specifically interacts with the RGD sequence in cardosin A. Similarly to the binding of integrins to their ligands (19), the interaction between cardosin A and its putative receptor seems to be of low affinity. We have at this point no inforn...
mation on the distribution of the putative receptor in the plant, and further studies will be required for substantiating its in vivo role in cardosin A recognition.

The results herein described are the first evidence for the involvement of a proteinase in RGD-dependent recognition in plants. In animals, cell migration both during development and tumor invasion relies on adhesion to extracellular matrix proteins and often requires proteolytic remodeling of the matrix by cell-bound metalloproteinases containing RGD sequences that promote their attachment to integrins (38–40). Plants may also use proteinases in a similar way in molecular events of cell growth and differentiation. Because cardosin A is abundantly expressed in the stigmatic papillae and its putative receptor is present in pollen, it is likely that this RGD-dependent recognition may be active in pollen-pistil interaction. Lord and Sanders (41) have proposed a model for pollen tube extension that predicts the existence and involvement of integrins and extracellular matrix ligands. This process occurs by tip extension in a way analogous to axonal growth and can be viewed as a special case of cell migration (41). It is thus possible that cardosin A participates in adhesion-mediated proteolytic mechanisms associated with pollen tube growth, very much in the way of integrin-bound proteases in cell proliferation and invasion. Further studies are required to elucidate the involvement of this RGD-containing proteinase in pollen-pistil interaction.

REFERENCES

1. James, M. (1998) in Aspartic Proteinases (James, M., ed) Plenum Press, New York
2. Kervinen, J., Tormakangas, K., Runenberg-Ross, P., Guruprasad, K., Blundell, T., and Teeri, T. H. (1995) in Aspartic Proteinases: Structure, Function, Biology and Biomedical Implications (Yakahashi, K., ed) pp. 241–254, Plenum Press, New York
3. Runenberg-Ross, P., Tormakangas, K., and Ostman, A. (1991) Eur. J. Biochem. 202, 1021–1027
4. Cordeiro, M. C., Xue, Z.-T., Pietrzak, M., Pais, M. S., and Brodelius, P. E. (1994) Plant Mol. Biol. 24, 733–741
5. Akasaka, T., Watanabe, H., Abe, K., and Arai, S. (1995) Eur. J. Biochem. 232, 77–83
6. Schaller, A., and Ryan, C. A. (1996) Plant Mol. Biol. 31, 1073–1077
7. D’Hondt, K., Stack, S., Gutteridge, S., Vandenbergh, J., Krebers, E., and Gal, S. (1997) Plant Mol. Biol. 33, 187–192
8. Glathie, S., Kervinen, J., Nimtz, M., Li, G. H., Tobin, G. J., Copeland, T. D., Ashford, D. A., Wlodawer, A., and Costa, J. (1998) J. Biol. Chem. 273, 31230–31236
9. Ramalho-Santos, M., Verissimo, P., Cortes, L., Samyn, B., Van Bree, J., Pires, E., and Faro, C. (1998) Eur. J. Biochem. 255, 133–138
10. Guruprasad, K., Tormakangas, K., Kervinen, J., and Blundell, T. L. (1994) FEBS Lett. 352, 131–136
11. Ramalho-Santos, M., Pissarra, J., Verissimo, P., Pereira, S., Salema, B., Pires, E., and Faro, C. J. (1997) Planta (Berl.) 203, 204–212
12. Math, A., Pleil, J. E., and Gal, S. (1998) Phytochemistry 47, 1453–1459
13. Runenberg-Ross, P., Kervinen, J., Kovalova, V., Raikhel, N. V., and Gal, S. (1994) Plant Physiol. (Bethesda) 105, 321–329
14. Elpidina, E. N., Dunaevsky, Y. E., and Belouzovski, M. A. (1990) J. Exp. Bot. 41, 969–977
15. Rodrigo, I., Vera, P., Van Looi, L. C., and Conjeiro, V. (1991) Plant Physiol. (Bethesda) 95, 616–622
16. Tokes, Z. A., Woon, W. C., and Chambers, S. M. (1974) Planta (Berl.) 119, 39–46
17. Verissimo, P., Faro, C., Moir, A. J. G., Lin, Y., Tang, M., and Pires, E. (1996) Eur. J. Biochem. 235, 762–768
18. Ramalho-Santos, M., Verissimo, P., Faro, C., and Pires, E. (1996) Biochim. Biophys. Acta 1297, 83–89
19. Rozoalhi, E. (1996) Annu. Rev. Cell Dev. Biol. 12, 697–715
20. Schindler, M., Meiners, S., and Cheresh, D. A. (1989) J. Cell Biol. 108, 1955–1964
21. Wayne, R., Staves, M. P., and Leopold, A. C. (1992) J. Cell Sci. 101, 611–623
22. Zhu, J., Shi, J., Singh, U., Wyatt, S. E., Bressan, R. A., Hasegawa, P. M., and Carpen, N. C. (1995) Planta 193, 637–646
23. Canut, H., Carrasco, A., Galau, J.-P., Cassan, B., Boyoussou, H., Vita, N., Ferrara, P., and Pont-Lezica, R. (1998) Plant J. 16, 63–71
24. Quirino, R. S., Brian, L., Aldridge, J., and Schult, T. (1991) Development 1, 11–16
25. Gens, J. S., Reuzeau, C., Doolittle, K., McNally, J. G., and Pickard, B. G. (1996) Proc. Natl. Acad. Sci. U. S. A. 99, 3644–3648
26. Pawlowski, K., Kirenk, R., de Vries, S., and Bisseling, T. (1994) Plant Mol. Biol. 26, 1–13
27. Lin, X., Tang, J., Kielsh, G., Monod, M., and Fouling, S. (1993) J. Biol. Chem. 268, 20143–20147
28. Vacasso, A. M., Ciaffoni, F., Talti, M., Salvengi, R., Bara, A., Tognazzi, D., and Screra, C. (1995) J. Biol. Chem. 270, 30576–30580
29. Liepinsh, E., Anderson, M., Roysschaert, J.-M., and Otting, G. (1997) Nat. Struct. Biol. 4, 793–795
30. Anderson, M., Cursted, T., Jornvall, H., and Johansson, J. (1995) FEBS Lett. 362, 328–332
31. Leahy, D. J., Aukhli, I., and Erickson, H. P. (1996) Cell 84, 155–164
32. Basbaum, C. B., and Verk, Z. (1996) Curr. Opin. Cell Biol. 8, 731–738
33. Brooks, P. C., Stromblad, S., Sanders, L. C., von Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., Quigley, J. P., and Cheresh, D. A. (1994) Cell 83, 683–693
34. Rao, P. M., Pan, X., Wu, X., and Rubin, G. M. (1996) Science 273, 1227–1231
35. Lord, E. M., and Sanders, L. C. (1992) Dev. Biol. 153, 16–28
36. Costa, J., Ashford, D. A., Nimtz, M., Bento, I., Frazio, C., Estevos, C. L., Faro, C. J., Kervinen, J., Pires, E., Verissimo, P., Wlodawer, A., and Carron, M. A. (1997) Eur. J. Biochem. 243, 695–700