Bridging Ral GTPase to Rho Pathways

RLIP76, A Ral EFFECTOR WITH CDC42/Rac GTPase-ACTIVATING PROTEIN ACTIVITY*

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RalA and RalB are GTPases of unknown function and are activated by proteins, RalGDS, that interact with the active form of another GTPase, Ras. To elucidate Ral function, we have searched for proteins interacting with an activated form of RalA using the two-hybrid method and a J urkat cell library. We have identified a partial cDNA encoding a protein, RLIP1, which binds to activated RalA and this binding requires an intact effector domain of RalA. Biochemical data with purified RalA confirm the genetic results. This protein also bears a region of homology with GAP-activating protein (GAP) domains that are involved in the regulation of GTPases of the Rho family and, indeed, RLIP1 displays a GAP activity acting upon Rac1 and CDC42, but not RhoA. This GAP region is not required for RFLIP1 binding to Ral.

The whole cDNA was cloned, and it encodes a 76-kDa polypeptide, RLIP76, which also binds RalA. The Rho pathway is involved in membrane and cytoskeleton modifications after mitogenic stimulation and acts in parallel to and synergistically with the Ras pathway. We propose that these pathways are linked through a cascade composed of Ras → RalGDS → Ral → RLIP76 → CDC42/Rac1/Rho, allowing modulation of the Rho pathway by the Ras pathway.

Ral proteins are biochemically well characterized GTPases whose functions have long remained elusive (1, 2). A potential clue was provided by the finding that RalGDS* and a RalGDS-like protein, which are activators of RalA and RalB (3), interact with the activated form of Ras and that this interaction requires the integrity of the Ras effector domain (4–6). Thus RalGDS, and therefore Ral proteins, might be involved in transducing pathways that signal through Ras.

In order to decipher Ral function, we were searched for proteins that interact with the activated form of RalA. Using a two-hybrid method and a mutant of RalA deficient in its intrinsic GTPase activity (RalAV23), we have isolated a partial cDNA encoding a protein (RLIP1, RalA interacting protein 1) that has characteristics of a Ral effector protein.

The whole cDNA was isolated and sequenced; it contains an ORF encoding a predicted 76-kDa protein (RLIP76) that binds RalA.

Out of the Ral binding region, RLIP76 contains a GAP region related to RhoGAP domains and this structural homology reflects a functional homology with a GAP activity acting upon CDC42HS and Rac1.

EXPERIMENTAL PROCEDURES

Two-hybrid Screen—The two-hybrid system and the J urkat cells library used in this study have already been described (7–9). For LexA fusion protein expression, we have used plasmids pBTM116 or derivatives with modified polylinkers. When necessary, PCR using Pfu polymerase was performed to generate adequate cloning sites. The bait of our screen was a fusion between LexA and a Val-23 mutant of RalA (equivalent to RasV12) deleted of its 27 C-terminal amino acids (RalAV23ΔCT) supposed to be involved in post-translational modifications and membrane localization. This fusion protein was expressed in yeast from plasmid pLRTA. LexA fusion proteins expression was checked on Western blots with anti-LexA antibodies (a gift from P. Moreau, Gif sur Yvette, France).

The two-hybrid procedures were handled according to published methods (9, 10). Library plasmids from transformed yeast colonies were recovered using HB101 as a recipient strain, selected on M9 medium lacking leucine. When mating was used for two-hybrid tests, strain L40 was mated with strain AMR70 (MATa, leu2, trp1, his3, ade2, URA3::lexAop-iao2) (a gift from S. Fields). When two-hybrid results are presented, we are showing the results of β-galactosidase test on filter paper. There was no discrepancy between the His auxotrophy test and the β-galactosidase test.

When required, point mutations were introduced using the Transformer site-directed mutagenesis kit (Clontech). Any DNA fragment submitted to mutagenesis and all PCR products were sequenced.

Gene Expression—Gene expression was analyzed on a multiple tissue Northern blot (Clontech) where mRNAs from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas were represented. A β-actin cDNA was used as a control.

cDNA Cloning—5′-RACE was carried out using 5′-RACE-ready cDNA from Clontech and following manufacturer’s instructions. Isolation of cDNA from phage was performed according to usual techniques (11).

In Vitro Transcription/Translation of RLIP1—A PCR reaction was carried out with pRLIP1 as a template and adequate primers. The 5′ primer contains a T3 RNA polymerase recognition site upstream of an ATG initiation codon in frame with RLIP1 (CGAATAAGCCTACTA- AAGAAGTGGGAATCTAGATCGG) (12). The 3′ primer is downstream of the stop codon of the amplified fragment (GTAAAC-
RLIP76, a Ral Effector Connecting Ral to Rho Pathways

GACGCCGAG.

Transcription and translation in presence of [35S]methionine were performed using 1 μg of PCR product, and, sequentially, a mRNA capping kit and an in vitro translation kit (Stratagene).

Preparation of Proteins from Escherichia coli and in Vitro Binding Experiments—GST and of GST-Ral proteins from E. coli transformed with either plasmid or recombinant plasmid pGEX-4T1 were prepared following classical methods. All buffers contained 5 mM MgCl2. For in vitro binding studies, 5 μg of glutathione-Sepharose 4B-bound proteins were washed twice in ice-cold binding buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM MgCl2, 1 mM Pefabloc, and 0.5% Nonidet P-40) and incubated overnight at 4 °C with 1 μl of in vitro translated [35S]-RLIP1 in 50 μl of binding buffer containing 0.5 mM GTP (experiments with GST and GST-RalAV23), or GDP (in the cases of GST-Ral). After sedimentation of the beads, the supernatant ("the unbound fraction") was removed and the beads were washed three times with binding buffer containing 1 mM dithiothreitol. "Bound proteins" were recovered by boiling the beads in sample buffer. Unbound and bound fractions were subjected to SDS-PAGE on a 10% acrylamide gel. After staining with Coomassie Blue to detect the GST and GST-Ral proteins, gels were treated with Amplify (Amersham Corp.) and dried, and the presence of 35S-RLIP1 was detected by autoradiography.

In vitro binding studies after guanine nucleotide exchange were performed as described above with the following alterations; 10 μg of glutathione-Sepharose 4B-bound proteins were washed twice in ice-cold exchange buffer (20 mM Tris-HCl, pH 7.5, 10 mM EDTA, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM Pefabloc), and incubated for 3 h at 4 °C in 50 μl of exchange buffer containing 90 μM GTP or GDP. The nucleotide exchange reaction was stopped by adding MgCl2 to 20 mM followed by two washes with binding buffer containing 20 mM MgCl2. In all cases, protein concentrations were estimated by Coomassie Blue staining of SDS-PAGE gels and adjustments were made for the same amount to be used in all experiments.

Protein Purification and GTP Hydrolysis Assay—RLIP1 was expressed as a MBP fusion protein from vector pMal-c2 (New England Biolabs). Rac1 and Bcr-GAP proteins were expressed in E. coli and purified as GST fusion proteins, then digested with thrombin. CDC42 was a gift from P. Bouquet. [γ-32P]GTP-bound Rac1, CDC42, and Rap2A were prepared by incubating 200 nM protein with 25 μM Tris, pH 7.5, 5 mM EDTA, 0.2 mM MgCl2, 0.1 mM bovine serum albumin, 1 mM dithiothreitol, 10 mM [γ-32P]GTP (2 μCi to 30 Ci/mmol, DuPont NEN) in a 50-μl volume for 15 min at room temperature. GTP hydrolysis was initiated by raising MgCl2 and GTP to final concentrations of 20 mM and 200 μM, respectively. GTP hydrolysis was stopped at different time points by addition of 2 ml of 50 mM ice-cold Tris, pH 8, 35 mM MgCl2, 1 mM dithiothreitol, 150 mM NaCl, then quantitated by rapid vacuum filtration on BA 85 nitrocellulose filter and radioactivity counting (derived from Ref. 13). The GTP hydrolysis was conducted as described above, but in the presence or absence of Bcr-GAP (400 nM), or MBP-RLIP1 (800 nM), and stopped after a 10-min incubation at room temperature.

FISH Analysis—Fluorescence in situ hybridization (FISH) to metaphase chromosomes prepared from a normal male was carried out according to an usual technique (14). The probe was a 1.6-kilobasepair DNA fragment from one of the inserts isolated during the two-hybrid screen.

Sequence Analysis—We used a Sequenase sequencing kit (Amerham Corp.) or a deaza-T7-sequencing kit (Pharmacia Biotech Inc.) for classical sequencing, or a Dye-deoxy terminator kit (Perkin Elmer) and an Applied Biosystems model 373A automatic sequencer. Sequence analysis was performed using computer facilities provided by the Centre Interuniversitaire de Traitement Informatique (CITI2) (15).

RESULTS

Two-hybrid Screen—Around 1,000,000 colonies were screened with RaIAV23ΔCT as a "bait" and a J urkat cells library. Two library plasmids, pRLIP1 and pRLIP2, were recovered that contained partial cDNAs expressing proteins RLIP1 and RLIP2 (for RRall interacting protein) fused to GAL4 activation domain, respectively. RLIP1 and RLIP2 interact specifically with RaIAV23ΔCT, as opposed to several irrelevant proteins (lamin, statmin (Ref. 16), hSos1 (Ref. 17)) (data not shown). RLIP2 is a C-terminal part of RLIP1.

Ral allele dependence of the interaction was checked using plRLIP1, RaIAV23, RaIAV23A46 (an effector domain mutant), RaIAA26 (which mimics a Ras GDP-blocked mutant), and RaIAV23A26 were cloned in pBTM116. Fig. 1A shows the signals displayed in a β-galactosidase test. First, RLIP1 is able to interact not only with RaIAV23ΔCT (data not shown) but also with RaIAV23 and RaIAwt. Second, RLIP1 interaction with RaIAA26 or with RaIAV23A26 is undetectable. Since a G26A mutation is supposed to block RaA in a GDP-bound state, this result suggests that RLIP1 binds to RaA only when this latter is bound to GTP and not to GDP. Third, RLIP1 is unable to interact with a RaIAA46 mutant. Based on sequence and structural similarities with c-Ha-Ras, a T46A RaA mutant should have an impaired effector domain. This result suggests that RaA requires an intact effector domain to bind to RLIP1. It also suggests that, in yeast, LexA-RalAwt is, at least in part, in the GTP-bound conformation, as is the case for other GTPases expressed as LexA fusions (9).

From these data, it emerges that RLIP1 is a good candidate to be an "effector" of RaA function.

In Vitro Binding—In order to confirm the genetic data, we have tested in vitro RLIP1 binding to GST-RaA and GST-RaIAV23 proteins prepared from E. coli. Around 30% of both RaA proteins bind GDP and GTP (data not shown). RLIP1 cDNA was amplified by PCR. The PCR product was transcribed from a T3 promoter sequence included at the 5' end of the 5' PCR primer, and translated in vitro. A 35S-labeled protein of apparent molecular mass of 66 kDa was produced (Fig. 2A). Fig. 2B shows that RLIP1 did not bind to GST or to GST-RaA but does bind to GST-RaIAV23.

In vitro guanine nucleotide exchange was performed prior to RLIP1 binding. RLIP1 again did not bind to GST. It did...
not bind GST-RalA or GST-RalAV23 loaded with GDP. It bound GST-RalA and, even better, GST-RalAV23 loaded with GTP (Fig. 2C). These data show that RLIP1-RalA interaction is not mediated by a yeast protein and are consistent with the genetic results, i.e. RLIP1 interacts directly with the GTP-bound form of RalA whose effector domain is required.

RLIP1 Binds Ral but No Other GTPase except Rac—We addressed the question whether RLIP1 discriminates among GTPases. Table I summarizes the results obtained with the two-hybrid method, using different GTPases and pRLIP1. It also gives the positive controls used in each case.

In the Ras superfamily, within the Ras branch (to which Ra1A belongs; Ref. 18), RLIP1 was not able to interact with c-Ha-Ras or with Rap1A, Rap2A, or Rap2B. However, it does interact with RalB.

In the Rab branch, RLIP1 was not able to interact with Rab5, Rab6, Rab7, or Rab13.

Finally, in the Rho/Rac branch, we were not able to detect any interaction with RhoA, RhoB or RhoG, but RLIP1 is able to interact with Rac1 (Fig. 1B).

This latter interaction was further investigated using Rac1 alleles. Fig. 1B shows that RLIP1 is able to interact with Rac1V12S189 but not with Rac1V12N17S189, a dominant negative mutant blocked in the GDP-bound form, or Rac1V12A35S189 and Rac1V12A38S189, two effector domain mutants (19). These results suggest that Rac1 bound to GTP is able to interact with RLIP1 through its effector domain.

The GAP-like Region Displays a GAP Activity—Sequence analysis of RLIP1 (see below) has revealed a region highly homologous to GAP regions acting upon GTPases of the Rho family. The question whether the region of RLIP that looks like a GAP region is a GAP was addressed. RLIP1 was expressed fused to MBP from plasmid pMAL-c2. The fused protein was purified and assayed for stimulation of the GTPase activity of purified CDC42 and Rac1, and Rap2A as a control. Fig. 3 shows that there is no effect on Rap2A (specificity control), a significant and reproducible effect on Rac1 and a stronger effect upon CDC42. It also shows that this effect is weaker that the one obtained with Bcr-GAP protein (positive control) (20). RLIP1 exhibited no GAP activity upon RhoA (data not shown).

Molecular Biology of the cDNA—Northern blot analysis revealed that RLIP1 is expressed in all tested tissues as a 4-kytobase mRNA of low abundance (data not shown).

Two consecutive rounds of 5’-RACE were required to obtain the full-length cDNA that was also recovered from a skeletal muscle cDNA library in λgt10 and from a placenta cDNA library in λEXlox. The sequence of the full-length cDNA was

| GTPase | Interaction with pRLIP1 | Protein expression | Control by two-hybrid assay | Reference |
|--------|------------------------|--------------------|-----------------------------|-----------|
| Ra1A   | +                      | +                  | +                           | This work |
| Ra1B   | +                      | +                  | +                           | This work |
| cHRas  | +                      | ND                 | +                           | (9)       |
| cHRas(V12) | -               | ND                 | +                           | (9)       |
| Rap1A  | -                      | ND                 | +                           | J. de Gunzburg, personal communication |
| Rap2A  | -                      | + (ref)            | +                           | J. de Gunzburg, personal communication |
| Rap2A(V12) | -                  | + (ref)            | +                           | J. de Gunzburg, personal communication |
| Rab6   | +                      | +                  | M. Zerial, personal communication |
| Rab5   | -                      | ND                 | +                           | (34)      |
| Rab5L(79) | -                   | + (ref)            | +                           | M. Zerial, personal communication |
| Rab6L(72) | -                   | + (ref)            | +                           | (34)      |
| Rab7L(67) | -                   | + (ref)            | ND                          | P. Chavrier, personal communication |
| Rab13  | -                      | + (ref)            | +                           | A. Zahrour, personal communication |
| RhoA   | -                      | +                  | +                           | B. Olofsson, personal communication |
| RhoB   | -                      | +                  | +                           | B. Olofsson, personal communication |
| RhoB(V14) | -                 | + (ref)            | +                           | B. Olofsson, personal communication |
| Rac1(V12S189) | +              | +                  | +                           | This work; J. Camonis and G. Gacon, unpublished data |
| Rho2(V12A18T) | -            | +                  | +                           | P. Fort, personal communication |
established.

There is one main reading frame (ORF), from base 224 to base 2188, preceded by a correct translation initiation sequence (21). Two short ORFs (13 and 3 codons) are found within the 5' end of this cDNA but none of them is preceded by a correct translation initiation sequence. A 1664-base pair non-coding sequence is found 3' to the ORF.

This ORF encodes a protein made of 655 amino acids and of predicted molecular mass 76 kDa that we named RLIP76 (Fig. 4A). RLIP1 in plasmid pRLIP1 starts at amino acid 185, and RLIP2 starts at amino acid 403.

Data bank comparison revealed that the region extending...
from amino acid 210 to amino acid 353 shares significant homology to regions of proteins bearing a CDC42/Rho/Rac-GAP activity, like Bcr, chimaerins, Drosophila roundabout, and the Ras-GAP-binding protein p190 (22). Fig. 4B shows this striking homology with Bcr and n-chimaerin.

Fig. 4C gives the results obtained with two-hybrid plasmids expressing different parts of RLIP76. These results allow definition of the maximum size of the region required for RalA binding. Together with secondary structure predictions (Fig. 4D), the overall structure of RLIP76 can be depicted schematically as composed of four regions: an N-terminal region where amino acids 65–170 are predicted to be structured in α-helices, the GAP-like region (aa 210–353), the Ral binding region (aa 403–499) predicted to be composed in part of α-helices, and a C-terminal region (aa 499–655). Part of this latter region and part of the Ral binding region (aa 440–610) are predicted to be able to form a coiled-coil structure.

By FISH analysis of 19 R-banded metaphase cells, RLIP1 gene was localized on band 18p11 (25 chromosomes positive on both chromatid out of 38). A minor localization on band 3q26 was also detected (9 out of 38) that might suggest the existence of a related gene (data not shown).

DISCUSSION

We have identified a cDNA encoding a protein, RLIP1, that is able to interact with RalA and Rab8, and which has the characteristics of a Ral effector; biochemical data and genetics suggest that RLIP1 binds better to Ral-GTP than to Ral-GDP and that this interaction requires a functional effector domain. According to Northern blot analysis, RLIP1 is ubiquitously (but at low levels) expressed, as are Ral and RalGDS, a Ral activator (3).

Although able to discriminate Ral from other GTPases, RLIP1 also binds to the active form of Rac1. We suppose that the molecular avatar of this binding is a GAP-like region whose absence impairs interaction with Rac1 but not with RalA; domains involved in Rac binding and in Ral binding are physically distinct. We also show that the structural homology with GAP-like regions reflects a functional homology. RLIP1 is able to activate specifically hydrolysis of GTP bound to Rac1 and to CDC42, but not, as expected, to Rap2A.

The whole cDNA was cloned; it encodes a 76-kDa protein, RLIP76, able to bind to RalA as based on a two-hybrid assay. These findings raise several questions. Our results allow us to conclude that the GAP-like region of RLIP76 displays a bona fide GAP activity acting upon CDC42 and Rac1. However, this GAP activity is rather weak when compared to the GAP activity of Bcr tested in parallel. This could be due either to technical problems (we do not know how much of purified RLIP1 is active), to structural problems (either a larger part or a smaller part of RLIP76 could do better) or to biological constraints (a companion protein might increase this activity). These considerations lead to more questions. What is Ral doing to RLIP76? Is it localizing RLIP76 in the vicinity of its target, as happens to be the case for other GTPases involved in the subcellular localization of certain effectors (23, 24), and/or is it modulating RLIP76-GAP activity?

Ras and Rho pathways are both activated during mitogenic signaling through transmembrane receptors. It is unclear if their activation is sequential or parallel, but they seem to work synergistically (19, 25–29). The Rho pathway, a cascade of GTPases, from CDC42 to Rho passing by Rac, acts upon structures involved in cell shape plasticity. Activation of Ras leads to several cytoplasmic and nuclear phenomena as well as membrane modifications. And Ral proteins are potentially switched to their active form through interaction of activated Ras with Ral activators. We propose that RLIP76 participates in the cross-talk between these GTPase cascades, modulating the state of activity of the CDC42/Rac/Rho pathway in response to Ras activation.

Finally, regions of RLIP76 seem a priori not to participate in the above functions. The α-helix-rich regions, especially the coiled-coil region, might be involved in interactions with other proteins. Alternatively, the coiled-coil region might participate in the homodimerization of RLIP76. After Ral and subsequent Ral activation, Ral binding to RLIP76 could separate the monomers and render the GAP catalytic region accessible to its target.

It will be of great interest in future RLIP studies to analyze the regulation and interplay of the various separate functional domains.

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REFERENCES

1. Chardin, P., and Tavitian, A. (1986) EMBO J. 5, 2203–2208
2. Fred, M., Schlichting, I., Wittinghofer, A., and Chardin, P. (1990) J. Biol. Chem. 265, 6353–6359
3. Albright, C. F., Giddings, B. W., Liu, J., Vito, M., and Weinberg, R. A. (1993) EMBO J. 12, 339–347
4. Ikuchi, A., Demo, S. D., Ye, Z.-H., Chen, Y.-W., and Williams, L. T. (1994) Cell Biol. 14, 7483–7491
5. Hofer, F., Fields, S., Schneider, C., and Martin, G. S. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 11089–11093
6. Spaargaren, M., and Bisschop, J. R. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 12609–12613
7. Chien, C., Bartel, P. L., Sternglanz, R., and Fields, S. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 9578–9582
8. Berchich, S., Bomsel, M., Boggs, M., Durand, H., Dubé, M., Letourneur, F., Camonis, J., and Benarous, R. (1994) J. Biol. Chem. 269, 30073–30076
9. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214
10. Kaiser, C., Michaelis, S., and Mitchell, A. (1994) Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY
11. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Gaspar, M.-L., Meo, T., Bourgard, P., Guenet, J.-L., and Tosi, M. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8606–8610
13. Xu, X., Barry, D. C., Settleman, J., and Weinberg, R. A. (1992) Proc. Natl. Acad. Sci. U.S.A. 91, 348, 125–132
14. Benichou, S., Bomsel, M., Boggs, M., Durand, H., Dubé, M., Letourneur, F., Camonis, J., and Benarous, R. (1994) J. Biol. Chem. 269, 30073–30076
15. Dessen, P., Frédant, C., Valencien, C., and Mignier, C. (1990) Cell 63, 355–356
16. Mauzer, A., Camonis, J. H., and Sobel, A. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 3100–3104
17. Chardin, P., Camonis, J. H., Gale, N. W., Van Aelst, L., Schlessinger, J., Wigger, M., and Bar-Sagi, D. (1993) Science 260, 1338–1343
18. Valandra, A., Chardin, P., Wittinghofer, A., and Sander, C. (1991) Biochemistry 30, 4637–4648
19. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 401–410
20. Diekmann, D., Briss, S., Garrett, M. D., Totty, N., Hsuam, J., Monfrieds, C., Hall, C., Lim, C., and Hall, A. (1991) Nature 351, 400–402
21. Kozak, M. (1987) Nucl. Acids Res. 15, B125–B148
22. Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
23. Leeser, S. J., Paterson, H. F., and Marshall, C. J. (1994) Nature 369, 411–414
24. Stocker, D., MacDonald, S. G., Cadwallader, K., Symons, M., and Hancock, J. F. (1994) Science 264, 1463–1467
25. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
26. Nobes, C. D., and Hall, A. (1993) Cell 71, 53–62
27. Nobes, C. D., Hawkins, P., Stephens, L., and Hall, A. (1995) J. Cell Biol. 108, 225–233
28. Qi, R.-G., Chen, J., Kim, D., McCormick, F., and Symons, M. (1995) Nature 374, 457–459
29. Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) Nature 348, 125–132
30. Settleman, J., Albright, C. F., Foster, L. C., and Weinberg, R. A. (1992) Nature 359, 153–154