Effector Recognition by the Small GTP-binding Proteins Ras and Ral*

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The Ral effector protein RLIP76 (also called RIP/RalBP1) binds to Ral-GTP via a region that shares no sequence homology with the Ras-binding domains of the Ser/Thr kinase c-Raf-1 and the Ras-specific guanine nucleotide exchange factors. Whereas the Ras-binding domains have a similar ubiquitin-like structure, the Ral-binding domain of RLIP76 was predicted to comprise a coiled-coil region. In order to obtain more information about the specificity and the structural mode of the interaction between Ral and RLIP, we have performed a sequence space and a mutational analysis. The sequence space analysis of a comprehensive nonredundant assembly of Ras-like proteins strongly indicated that positions 36 and 37 in the core of the effector region are treedeterminant positions for all subfamilies of Ras-like proteins and dictate the specificity of the interaction of these GTPases with their effector proteins. Indeed, we could convert the specific interaction with Ras effectors and RLIP by mutating these residues in Ras and Ral. We therefore conclude that positions 36 and 37 are critical for the discrimination between Ras and Ral effectors and that, despite the absence of sequence homology between the Ras-binding and the Ral-binding domains, their mode of interaction is most probably similar.

Ral is a small GTP-binding protein belonging to the subfamily of Ras proteins (1, 2), which function as molecular switches in signal transduction pathways by alternating between an active GTP-bound and an inactive GDP-bound conformation. The ratio between the active and inactive form of the Ras proteins is regulated by the action of two types of proteins (3): GTPase activating proteins (GAPs), which inactivate GTPases by stimulating the slow GDP dissociation rate, allowing the protein to rapidly come at equilibrium with the cellular pool of guanine nucleotide (4). The guanine nucleotide affinities and the intrinsic GTPase activity of Ral are very similar to that of Ras (5). Ral is ubiquitously expressed, but it is especially abundant in brain, testis, and platelets (6–10). The protein was found both in the plasma membrane and in cytoplasmic vesicles (11, 12).

The cellular function of Ral remained elusive for a long time. Recently, however, progress was made via the discovery of Ral-specific regulatory activities. Multiple Ras-specific guanine nucleotide exchange factors (RalGEFs) have been isolated (13–18). Furthermore, although so far the corresponding genes have not been isolated, Ras-specific GAP activities were identified in brain, testis, and platelets (19–20). Strikingly, in addition to a Ras-specific GEF domain, the RalGEF's contain a C-terminally located Ras-binding domain (RBD), which is able to bind the GDP-bound forms of Ras and Rap proteins, so that the RalGEF's were recognized as one of the new families of Ras protein effectors (Ref. 21 and references therein). The RBDs of Rap1GDS (22, 23) and Rif (24) were shown to be structurally similar to the RBD of the Ser/Thr kinase c-Raf-1 (25–27), a well characterized Ras-effector protein. Interestingly, a Ras-dependent stimulation of Ral was proposed to function parallel to the Ras-Raf-Mek-Erk pathway in several types of cells (28–35), but in platelets, Ral appears to be stimulated in a fashion similar to Rap1A, suggesting that also Rap1A can function upstream of Ral (36). In addition to the regulatory proteins, a putative effector protein was identified: RLIP76, also named RalBP1 or RIP (37–39). Its Ral-binding domain (RalBD) was identified by deletion studies (37) and was used successfully as a probe in a pull-down assay to measure the activation of endogenous Ral upon cellular stimulation (35, 36). Finally, Ral was shown to be involved in the phorbol ester-stimulated phospholipase D activity, apparently through a direct interaction of Ral with phospholipase D 1 (28, 30, 40–42). Additional complexity in the Ral pathway(s) emerged with the isolation of the RLIP76-binding protein Reps1, which can also bind to the adaptor proteins Crk and Grb2 (43), and with the observation that calmodulin binds to RalA (44) and that both Ral and Arf are needed for phospholipase D activation (45).

Even though Ral interacts with RLIP76, it is largely unknown how this interaction takes place. Secondary structure prediction of RLIP76 indicated that the structure of RalBD comprises a coiled-coil region (37) and thus differs from the ubiquitin-like fold of the RBDs. In this work, we have investigated the effects of several mutations on the interaction between Ral and RLIP76 by double hybrid analysis and biochemical methods. Moreover, the specificity of the interaction of Ras and Ral proteins with their effectors was studied by sequence

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1 The abbreviations used are: GAP, GTPase activating protein; GDS, guanine nucleotide dissociation stimulator; GEF, guanine nucleotide exchange factor; Gpp(N)I, 5'-guanylylimidodiphosphate; GST, glutathione S-transferase; RBD, Ras-binding domain; PCR, polymerase chain reaction; wt, wild type; RalBD, Ral-binding domain.

2 The ratio between the active and inactive form of the Ras proteins is regulated by the action of two types of proteins that activate or inactivate GTPases.

3 GTPase activating proteins (GAPs) stimulate the slow GDP dissociation rate, allowing the protein to rapidly come at equilibrium with the cellular pool of guanine nucleotide.
The Ras mutants were made as described above for the Ras mutants using upstream primer 5'-CCGGATCCTCAGAAGATACACACCCGTTTGGTGGTGTTG-3' (the BamHI restriction site is underlined), downstream primer 5'-CCACGTGGGTCGGACAGCTTCAC-3' (Ras residue mutants are designated from Val-121 to Val-125), 10 mM GTPγS·H2O, 25 mM NaCl, and 10% glycerol. Thereafter, 20 mg of Gpp(NH)p-bound sRal were loaded on the column. The GST fusion was cleaved with 40 NIH units of thrombin at 4 °C overnight, after which the complex was eluted. The complex was separated from noncomplexed RalBD by two successive chromatographic steps on a Superdex 75 gel filtration column in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 14 mM 2-mercaptoethanol, 10 mM dithioerythritol, 10 μM Gpp(NH)p, 25 μM Pefabloc SC, 2 μM MgCl2, and 10% glycerol.

Two-hybrid Analysis—The wild type and mutated Ras and Ras genes were subcloned by BamHI/SalI fragments into pVJL10, a derivative of pBTM116 (47). Fragments containing the RalBD or the RBD of c-Raf-1 (amino acids 51–131) were subcloned in the pGAD3S2X vector. The Ras-binding domain of mRalGDS (amino acids 702–852) or mRlf, RBD of mRl, 5 mg of GST-RalBD was bound to 5 mg of GST-Rl fusion protein was eluted with Buffer A containing 10 mM of reduced glutathione and subsequently dialyzed against Buffer B (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10% glycerol). Mixtures were loaded on the column. The affinity chromatography analysis was performed as described (24, 49). Measurements were performed in Buffer B containing 5% glycerol at 37 °C; excitation wavelength was 366 nm, and emission wavelength was 450 nm. The determined dissociation constants were corrected with the percentage of the activity of the effector, which was assessed by an active site titration as described (48).

Fluorescence Measurements—The interaction of hRalB wt and the hRalB/K471A/48E mutant with the Raf-, Rgl-, and RalGDS-RBDs was characterized with a Perkin-Elmer fluorescence spectrometer LS50B as described before (24, 49). Measurements were performed in Buffer B containing 5% glycerol at 37 °C; excitation wavelength was 366 nm, and emission wavelength was 450 nm. The determined dissociation constants were corrected with the percentage of the activity of the effector, which was assessed by an active site titration as described (48).

Sequence and Structural Analysis—An assembly of 476 Ras sequences was collected from different data bases with the help of the Genequix software (50). A comprehensive nonredundant alignment of these sequences was built with CLUSTALW (51) using the BLOSUM62 matrix (52). Only sequences with maximal 80% similarity were accepted, with the exception of Ras: only 8 Ras sequences were found in the data bases, all of which were accepted for the alignment. The final multiple sequence alignment that was obtained after some hand editing included 176 sequences, corresponding to 98 Ras, 36 Rho, 37 Ras, and 8 Ras sequences. This alignment can be found at http://www.cmb.uman. ca/cnbprot/ral.dir.

The detection of those residues that allot specificity to a subfamily, i.e. those residues that are conserved within a given subfamily and
Specificity of the GTPase-Effector Protein Interaction

RESULTS

Isolation of the Complex of sRalA-Gpp(NH)p with RLIP76

The first trials to isolate the complex by incubating the purified proteins sRalA-Gpp(NH)p and RalBD, and subsequent purification over gel filtration column in 50 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 150 mM NaCl, 5 mM dithioerythritol, were unsuccessful. Under these conditions, Ral behaved as a dimer and RalBD as a multimer (not shown). Consequently, a proper separation of the complex from the single proteins was not possible, and we could not be certain that a complex was formed.

Therefore, we modified our strategy. We bound GST-RalBD to a GSH-Sepharose column, loaded sRalA-Gpp(NH)p to the column, and, after washing in order to remove unbound Ral, cleaved with thrombin and eluted the complex from the column. Only with sRalA-Gpp(NH)p could a complex be eluted (Fig. 1); one could not be eluted with sRalA-GDP (not shown), demonstrating that the interaction between Ral and RalBD is GTP-specific, consistent with earlier findings with different techniques (37–39).

The complex-containing fractions of the GSH-Sepharose elution were further purified by gel filtration to remove noncomplexed proteins and to determine the ratio in which the complex is built. A partial separation of noncomplexed RalBD, which here behaved as a dimer, from the complex could be obtained by a first Superdex-75 chromatography step (Fig. 1A). The pooled fractions containing the complex (fractions 18–22) were subjected to a second gel filtration, after which a nearly pure complex (fractions 20–22) could be obtained (Fig. 1B). The complex eluted as a 33-kDa protein, showing it to be a 1:1 complex of the Ral protein (20.4 kDa) and RalBD (14.7 kDa). It was noted that the nonfused form of RalBD runs with a too high apparent molecular mass (Fig. 1), whereas GST-fused RalBD runs normally on SDS-polyacrylamide gel electrophoresis (Fig. 2) (molecular mass, 40.9 kDa).

In Vitro Characterization of the Ral-RLIP76 Interaction

It was shown by fluorescence measurements for several effector molecules that they can inhibit the intrinsic dissociation rate of the GTP-bound complex of Ras-like proteins in a concentration-dependent manner (15, 49, 58). However, addition of up to 10 μM RalBD did not lead to any effect on the intrinsic dissociation rate of sRalA-mGpp(NH)p or hRalB-mGpp(NH)p (not shown), nor to a change in emission spectrum.

Because fluorescence measurements did not allow the characterization of the interaction, we used a pull-down assay to analyze the binding of Ral to RLIP in vitro. We used a GST-fused RalBD and full-length RLIP76 as baits and tested the binding to Ral preloaded with either GDP or Gpp(NH)p. Partially purified GST-coupled full-length RlIP76 was able to specifically bind the Gpp(NH)p-bound forms of sRalA and hRalB (not shown). Also, the truncated form, RalBD, can interact specifically with the Gpp(NH)p-bound forms of sRalA and hRalB (Fig. 2). In comparison, Ras does not bind to RalBD.

What Makes the Specificity of the Interaction with Effector Molecules

A Theoretical Approach

The complete functional interchange between a Ras protein and a Ral protein requires a large number of changes equal to the number of differences between the sequences. In order to select among them those residues that will affect the specificity of the interaction of Ras and Ral with their regulating proteins, we have chosen a general approach described before (53). After building a representative alignment of Ras-like proteins (see under “Experimental Procedures”), we could define those positions that correspond to good tree-determinants, i.e. that are conserved within a subfamily and differ from the other subfamilies. In addition, tree-determinants that are in the protein interior were eliminated as putative participants in the binding site. Finally, because we were mostly interested in the specific interaction with effector proteins, the exposed tree-determi-

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2 P. Bork, C. Sander, and A. Valencia, unpublished results.
3 I. R. Vetter, manuscript in preparation.
Specificity of the GTPase-Effectector Protein Interaction

FIG. 3. Sequence space analysis of the different Ras subfamilies and alignment of the consensus sequences in the switch I region. A, sequence space analysis of the Ras \(^{2}\) (comprising Ras and Rap proteins but not Ral), Ral and Rho subfamilies basically as described (53). The sequence of each subfamily is represented as a vector point in a multidimensional space (sequence space), with residue positions and types as the basic dimensions. Directions in sequence space represent specific sequence patterns and are depicted as planar projections. The tree-determinant residues for each subfamily can be recognized as the extreme positions on the subfamily axis and are here indicated by circle clusters. In addition, the positions of the tree-determinant residues are numbered near these clusters. Positions 53 and 130 are conserved but shared by the Rho and Ras subfamilies and are located between these subfamily axes. Positions shared with Ral were not considered due to the low number of Ral sequences. The positions depicted in boldface are tree-determinant residues for each subfamily or shared by two subfamilies. Position 37 is specific of different subsets of Ras subfamilies. B, sequence space analysis of the Ras, Rho, and Rab subfamilies. Note that whereas position 37 is conserved for all subfamilies and located within the tree-determinant cluster of each subfamily, position 36 is conserved for all subfamilies but shared between the Ras and Rab subfamilies. Consequently, it is located between the clusters of these subfamilies. C, sequence alignment of four Ras subfamilies (Rab, Rho, Ras, and Ral). The number of protein sequences used for the analysis is indicated in parentheses. The numbering of the Ras residues is indicated above the alignment. The mutated positions described in this work are indicated by arrows.

nents positions that do not change their surface exposition between the GTP- and GDP-bound states were eliminated.

Sequence space analysis was performed as described (53). A comparison of the Ras, Rho, and Rab subfamilies and of the Ras subfamily (comprising the Ras and Rap proteins but not Ral), Ral, and Rho subfamilies is depicted in Fig. 3. Only 14 of the completely conserved residues in the eight known Ras sequences are different in the other subfamilies (Ral-tree-determinants), namely positions 7, 24, 25, 33, 36, 43, 46, 53, 67, 70, 92, 93, and 160 (Fig. 3A; note that we used the Ras numbering in these comparisons). When comparing the Ras subfamily, Ral, Rho, and Rab subfamilies, only position 37 is conserved but different in each subfamily (Fig. 3A; note that we used the Ras numbering in these comparisons). When comparing the Ras subfamily, Ral, Rho, and Rab subfamilies, only position 37 is conserved but different in each subfamily (Fig. 3A; note that we used the Ras numbering in these comparisons). When comparing the Ras subfamily, Ral, Rho, and Rab subfamilies, only position 37 is conserved but different in each subfamily (Fig. 3A; note that we used the Ras numbering in these comparisons).
assay, we observed that mutation A48E strongly affects the interaction, whereas the double mutation K47I/A48E practically abrogates the binding of hRalB to RaBD (Fig. 2). As expected, the dominant-negative mutant hRalB(S28N), which is homologous to Ras(S17N), did not show any interaction with the effector molecule RLIP (Fig. 2). We undertook the reciprocal experiment and tried to turn a Ras in a Ral. An identical 2H assay to test interaction between RaBD or Ras effectors on one hand and Ras wt, Ras(I36K), Ras(I36K/E37A), or Ras(E37A), or Ras(E37A) on the other hand showed only weak interaction (not shown). Therefore, we decided to measure the interactions with the more quantitative assay, we observed that mutation A48E strongly affects the interaction, whereas the double mutation K47I/A48E practically abrogates the binding of hRalB to RaBD (Fig. 2). As expected, the dominant-negative mutant hRalB(S28N), which is homologous to Ras(S17N), did not show any interaction with the effector molecule RLIP (Fig. 2). We undertook the reciprocal experiment and tried to turn a Ras in a Ral. An identical 2H assay to test interaction between RaBD or Ras effectors on one hand and Ras wt, Ras(I36K), Ras(I36K/E37A), or Ras(E37A), or Ras(E37A) on the other hand showed only weak interaction (not shown). Therefore, we decided to measure the interactions with the more quantitative assay, we observed that mutation A48E strongly affects the interaction, whereas the double mutation K47I/A48E practically abrogates the binding of hRalB to RaBD (Fig. 2). As expected, the dominant-negative mutant hRalB(S28N), which is homologous to Ras(S17N), did not show any interaction with the effector molecule RLIP (Fig. 2). We undertook the reciprocal experiment and tried to turn a Ras in a Ral. An identical 2H assay to test interaction between RaBD or Ras effectors on one hand and Ras wt, Ras(I36K), Ras(I36K/E37A), or Ras(E37A), or Ras(E37A) on the other hand showed only weak interaction (not shown). Therefore, we decided to measure the interactions with the more quantitative assay, we observed that mutation A48E strongly affects the interaction, whereas the double mutation K47I/A48E practically abrogates the binding of hRalB to RaBD (Fig. 2). As expected, the dominant-negative mutant hRalB(S28N), which is homologous to Ras(S17N), did not show any interaction with the effector molecule RLIP (Fig. 2). We undertook the reciprocal experiment and tried to turn a Ras in a Ral. An identical 2H assay to test interaction between RaBD or Ras effectors on one hand and Ras wt, Ras(I36K), Ras(I36K/E37A), or Ras(E37A), or Ras(E37A) on the other hand showed only weak interaction (not shown). Therefore, we decided to measure the interactions with the more quantitative assay, we observed that mutation A48E strongly affects the interaction, whereas the double mutation K47I/A48E practically abrogates the binding of hRalB to RaBD (Fig. 2). As expected, the dominant-negative mutant hRalB(S28N), which is homologous to Ras(S17N), did not show any interaction with the effector molecule RLIP (Fig. 2). We undertook the reciprocal experiment and tried to turn a Ras in a Ral. An identical 2H assay to test interaction between RaBD or Ras effectors on one hand and Ras wt, Ras(I36K), Ras(I36K/E37A), or Ras(E37A), or Ras(E37A) on the other hand showed only weak interaction (not shown). Therefore, we decided to measure the interactions with the more quantitative
has a high affinity for RalGDS (27, 63). Comparable to the Ras-Raf interaction (58), mutation Y43W (Ras Tyr-32) does not seem to affect the interaction with RLIP. Residue Glu-37 in Ras has been demonstrated to be important for interaction with c-Raf-1, but not with RalGEFs (24, 31–33, 64–66). In Raf, mutation A48E (Ras Glu-37) affects the interaction with RLIP, but not with RalGEFs (24, 31–33, 64–66). In Ral, has been demonstrated to be important for interaction with RalGDS and Rlf (34) but not with c-Raf-1 (64, 65, 68).

Interestingly, mutation K47I (Ras Ile-36) hardly affects the interaction of Ral with RLIP but enables Ral to interact with Ras effector proteins RalGDS and Rlf (Fig. 4). In combination with mutation A48E, which strongly reduces the Ral-RLIP interaction, double mutant hRalB(K47I/A48E) has practically no affinity for RLIP (Figs. 2, 4, 6, and 7). At the same time, this double mutant is able to interact with the Ras-specific effector molecules, of which at least the RalGEFs are bound with affinities that are comparable to those of Ras (Table I). On the other hand, introduction of mutations I36K and E37A abrogates the interaction of Ras with its effector molecules but enables Ras to interact with RLIP, even though the interaction is still weak (Fig. 6 and 7).

Sequence space analysis shows that Ras positions 36 and 37 (Ral residues 47 and 48) are tree-determinant positions for the Ras, Ras, Rho, and Rab subfamilies because they are conserved in each subfamily but differ between the subfamilies (position 36 is shared by the Rab and Ras subfamilies) (Fig. 3). In contrast, the switch I region of Ras and Ral superimpose very well, with a root mean square deviation of 0.3 143 for 11 Cα-atoms. In Fig. 8, we have depicted the electrostatic potentials of the surfaces of Ha-Ras (in the GDP- and GTP-bound conformation) with that of sRalA and the modeled structure of the double mutant RalA(K47I/A48E), with the switch I region toward the reader. When comparing the GDP-bound structures of Ras and Ral, it becomes evident that the positive charge of Lys-47 in Ral and the negative charge of Glu-37 in Ras represents the main difference in the switch I region between these proteins, in accordance with our mutational analysis.

Remarkably, Ral and its partners RalGDS and RLIP do not exist in Saccharomyces cerevisiae or in any other unicellular eukaryotes (as far as genomic sequences are known), an evident discrepancy with Ras and the Ras-MAP kinase signaling modules. The Ras-Ral signaling module is thus of late appearance in evolution, and one is tempted to correlate the appearance of this Ras pathway with the emergence of multicellular-
interaction with RLIP. Mutation of these residues to the Ras-like amino acids enables Ras to interact with the Ras effector proteins Raf and RafGEFs, whereas the opposite mutations enables Ral to interact with RLIP. Our results thus indicate that effects of mutations in the effector region of small GTPases should be interpreted with care, because interactions with other effector proteins may be induced. In this light, it seems possible that the remarkable dominant negative effects of Rac(Q61L/F37A) may not be caused by unproductive interactions with Rac effectors (69) but by productive or unproductive interactions with effectors of other small GTPases. Last but not least, despite a differently predicted secondary structure for RalGDS- and Rlf-RBDs were kindly provided by Vanessa Nancy and Christian Herrmann for supplying RalGDS-RBD. 2H plasmids express

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REFERENCES

1. Chardin, P., and Tavitian, A. (1986) EMBO J. 5, 2203–2208
2. Chardin, P., and Tavitian, A. (1989) J. Biol. Chem. 264, 6353–6359
3. Boguski, M. S., and McCormick, F. (1993) J. Biol. Chem. 268, 12056–12060
4. Lenzen, C., Cool, R. H., Prinz, H., Kuhlmann, J., and Wittinghofer, A. (1998) Biochemistry 37, 7420–7430
5. Frech, M., Schlichting, I., Wittinghofer, A., and Chardin, P. (1990) J. Biol. Chem. 265, 6535–6539
6. Olofsson, B., Chardin, P., Touchet, N., Zahraoui, A., and Tavitian, A. (1988) Oncogene 3, 231–234
7. Polakis, P. G., Weber, R. F., Nevins, R., Didsbury, J. R., Evans, T., and Snyderman, R. (1989) J. Biol. Chem. 264, 16388–16398
8. Bhullar, R. P., Chardin, P., and Haslam, R. J. (1990) FEBS Lett. 260, 48–52
9. Bhullar, R. P. (1992) FEBS Lett. 298, 61–64
10. Wildey, G. M., Vigrassarapu, M., Rim, S., and Denker, J. K. (1993) Biochem. Biophys. Res. Commun. 194, 552–559
11. Biehn, D. P., Pyun, H. Y., Linko-Stentz, K., Macara, I. G., and Fine, R. E. (1993) Biochem. Biophys. Res. Commun. 194, 246–256
12. Volkan, W., Peve, J., Elferink, L. A., and Scheller, R. H. (1993) FEBS Lett. 317, 53–56
13. Albritt, C. F., Giddings, B. W., Liu, J., Vito, M., and Weinberg, R. A. (1993) EMBO J. 12, 339–347
14. Kikuchi, A., Demo, S. D., Ye, Z.-H., Chen, Y.-W., and Williams, L. T. (1994) Mol. Cell. Biol. 14, 7485–7491
15. Wolkhuis, R. M. F., Bauer, B., van’t Veer, L. J., de Vries-Smits, A. M. M., Cool, R. H., Spaargaren, M., Wittinghofer, A., Burgering, B. M. T., and Bos, J. L. (1996) Oncogene 12, 353–362
16. Peterson, S. N., Trabulzini, L., Brava, T. R., Fischer, T., Altshuler, D. L., Martelli, P., Lapetina, E. G., Der, C. J., and White II, G. C. (1996) J. Biol. Chem. 271, 29963–29968
17. D’Adamo, D. R., Novick, S., Kahn, J. M., Leonard, P., and Pellicer, A. (1997) Oncogene 14, 1295–1305
18. Herberg, J. A., Beck, S., and Trowdale, J. (1998) J. Biol. Chem. 273, 839–857
19. Emkey, R., Freedman, S., and Feig, L. (1991) J. Biol. Chem. 266, 9703–9706
20. Bhullar, R. P., and Senervatrine, H. D. (1996) Biochem. Biophys. Acta 1311, 181–188
21. McCormick, F., and Wittinghofer, A. (1996) Curr. Opin. Biotechnol. 7, 449–456
22. Geyer, M., Herrmann, C., Wittinghofer, A., and Kahlbitzer, H. R. (1997) Nature Struct. Biol. 4, 694–699
23. Huang, L., Weng, X., Hofer, F., Martin, G. S., and Kim, S.-H. (1997) Nature Struct. Biol. 4, 69–75
24. Esser, D., Bauer, B., Wolkhuis, R. M. F., Wittinghofer, A., Cool, R. H., and Bayer, P. (1998) Biochemistry 37, 13453–13462
25. Emerson, S. D., Madison, V. S., Palermo, R. E., Waugh, D. S., Scheffler, J. E., Tsao, K.-L., Kiefer, S. C., Liu, S. P., and Fry, D. C. (1998) Biochemistry 37, 6911–6918
26. Nassar, N., Horn, G., Herrmann, C., Block, C., Janknecht, R., and Wittinghofer, A. (1996) Nature Struct. Biol. 3, 723–729
27. Jiang, H., Luo, J.-Q., Urano, T., Frankel, P., Lu, Z., Foster, D. A., and Feig, L. A. (1996) Nature 376, 409–412

FIG. 8. Comparison of the crystal structures of Ha-Ras and Ral. The electrostatic potentials of the crystal structures of Ha-Ras$^{1–176}$Gpp(NH)p (Protein Data Bank code 5p21) Ha-Ras$^{1–176}$GDP (Protein Data Bank code 4q21), sRalA$^{1–178}$GDP (Footnote 3) and the modeled structure of the double mutant sRalA(K47I/A48E)$^{1–178}$GDP were calculated with GRASP (70). Negative potentials are indicated with red and positive potentials with blue; scale is −15 to +15 kT/e. Selected residues in the switch I region are indicated with numbers.
