Raf is a mitogen-stimulated protein kinase that functions as a component of the signaling cascade that leads to the stimulation of mitogen-activated protein kinase. Here we show that the native structure of Raf is a large multi-subunit protein complex with an apparent mass of 300–500 kDa that interacts with Ras and the mitogen-activated protein kinase kinase Mek. Analysis of the structure of the Raf complex demonstrates that it contains a single Raf protein kinase together with the molecular chaperones hsp90 and p50. The Raf-hsp90-p50 complex was observed in starved cells and in cells activated with serum or phorbol ester. Thus, changes in complex formation with hsp90 and p50 are not required for activation of the Raf protein kinase. However, Raf activation caused by Ras was associated with the translocation of the cytoplasmic Raf-hsp90-p50 complex to the cell membrane. Significantly, it is only the membrane-bound complex that exhibits increased protein kinase activity. Thus, the Raf-activated Raf protein kinase functions as a membrane-bound multi-subunit complex.

**EXPERIMENTAL PROCEDURES**

Materials—[32P]Phosphate and [35S]methionine were purchased from Dupont-New England Nuclear. [γ-32P]ATP was prepared using a Gamma-Prep A kit (Promega Biotech) as described by the manufacturer. Polyvinylidene difluoride membranes (Immobilon-P) were obtained from Millipore Corp. PMA and Protein A-Sepharose were from Sigma. Protein G-Sepharose and the Superose 6 gel filtration column HR10/30 were obtained from Pharmacia LKB Biotechnology Inc. Recombinant GST-MAPK and GST-Mek were prepared by bacterial expression and glutathione agarose (Sigma) affinity chromatography using previously described methods (26). The MAPK substrate peptide KRELVEPLT669PSGEAPNQALLR was obtained from the Peptide Synthesis Core Facility (University of Massachusetts Medical School).

**Antibodies**—The rabbit α-Raf antisera 1558 was prepared using the synthetic peptide sequence CTLLTSRPLPVF (Multiple Peptide Systems Inc., San Diego, CA). The peptide was coupled to bovine serum albumin carrier by incubation with glutaraldehyde, and the cross-linked protein complex was used as an immunogen in New Zealand White rabbits. The rabbit α-Mek antisera 2880 was raised against the synthetic peptide CPOCKKTPIQLPNSPEG-NH2 (Multiple Peptide Systems Inc., San Diego, CA). The peptide was coupled to keyhole limpet hemocyanin by incubation with maleimidobenzoyl-N-hydroxysuccinimide ester, and the cross-linked protein complex was used as an immunogen in New Zealand White rabbits. Donkey anti-rabbit IgG antibody (Amersham International PLC), sheep α-mouse IgG antibodies (Amersham International PLC), sheep α-rat IgG antibody (Amersham International PLC), goat α-mouse IgM(μ) antibody (Life Technologies, Inc.; coupled to horseradish peroxidase) were purchased from the suppliers indicated. The monoclonal α-Flag antibody M2 was from IBI (Kodak). The monoclonal α-Ras antibody Y15-250 was obtained from the American Type Culture Collection (Bethesda, MD). The monoclonal α-hsp90 antibody SPA-830 was purchased from StressGen Biotechnologies Corp. (Victoria, British Columbia). The monoclonal α-p50 antibody 3M1/185/50 (27) was provided by Dr. G. Perdue (Perdue University).

**Plasmids**—The plasmids pCMV-Ras61L and pCMV-Ras17N were obtained from Dr. L. Kozma (University of Massachusetts Medical School). The plasmid pCMV-Raf was prepared using the vector pCMV5 (28) and a 2114-base pair EcoRI-XbaI fragment of a human c-ras-1 cDNA (29) that was isolated by polymerase chain reaction from a human fibroblast cDNA library. The plasmid pCMV-Flag-Raf was constructed using insertional overlapping polymerase chain reaction (30) to

**The Native Structure of the Activated Raf Protein Kinase Is a Membrane-bound Multi-subunit Complex**

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introduce the Flag epitope (sequence DYKDDDK, Immunex Corp.) between codons 1 and 2 of the c-raf-1 cDNA. The plasmid pCMV-Raf-Flag was constructed using insertional overlapping polymerase chain reaction to fuse the Flag epitope at the carboxyl terminus of the Raf-1 protein. The GST-Mek expression vector (26) was provided by Dr. R. L. Erikson (Harvard University). The plasmid pCMV-Mek was constructed subcloning a small sheep c-raf-1 cDNA, designated HindIII restriction fragment into the polylinker of the expression vector pCMV5 (BglII and HindIII sites). The GST-MAP kinase expression vector was constructed from pGEX-3X (Pharmacia LKB Biotechnology Inc.) and the human ERK2 cDNA (31). The structure of the recombinant protein is confirmed by dideoxy sequencing.

**Western Blot Analysis**—Proteins resolved by SDS-PAGE (7%) gel were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P) and analyzed by Western blotting. The blots were probed with: (1) α-Raf antisemur 1558 or α-Mek antisemur 2880 and horseradish peroxidase-linked donkey anti-rabbit IgG antibody; (2) α-asparagin monocalonal antibody SPA-830 and horseradish peroxidase-linked goat anti-mouse IgM antibody; and (3) α-Ras monocalonal antibody Y13-259 and horseradish peroxidase-linked sheep α-rat IgG antibody. Immunocomplexes were visualized using the enhanced chemiluminescence procedure (Amersham International PLC).

**In Vivo Protein Kinase Assays**—Raf protein kinase activity was measured in an immune complex kinase assay using immunoprecipitates (M2 antibody) and recombinant Mek as an exogenous substrate. The immunoprecipitates were washed three times with buffer A and twice with buffer C. The washed Flag-Raf immunoprecipitates were incubated with 50 μg [γ-32P]ATP (10 Ci/mmol) and 1 μg of recombinant Mek in a final volume of 100 μl for 30 min at 30 °C. Control experiments demonstrated that the phosphorylation reaction under these conditions was linear for >60 min. The reactions were terminated by boiling with 50 μl of two times sample buffer prior to analysis by SDS-PAGE (7%). The gel was dried, and the phosphorylation of GST-Mek was quantitated using a PhosphorImager and Image-Quant software (Molecular Dynamics, Sunnyvale, CA). Mek activity was measured in an immune complex kinase assay using recombinant Mek as an exogenous substrate. The immunoprecipitates were washed with 5 μl of rabbit α-Mek antisemur 2880 immobilized on 20 μl of Protein A-Sepharose for 1 h at 4 °C. The immunoprecipitate was washed three times with buffer D twice with buffer C and then incubated in buffer C together with 1 μg of GST-MAPK and 50 μg [γ-32P]ATP (10 Ci/mmol) in a final volume of 50 μl at 30 °C. The reaction was terminated by boiling with 50 μl of two times sample buffer prior to analysis by SDS-PAGE (7%). The gel was dried, and the phosphorylation of recombinant MAPK was quantitated using a PhosphorImager and Image-Quant software (Molecular Dynamics, Sunnyvale, CA). The Mek protein kinase activity is presented as the electrophoretic migration of α-Flag-Raf. This protein was stably expressed in CHO cells to fuse the Flag epitope at the carboxyl terminus of c-Raf.

Results

In order to facilitate the analysis of the properties of the Raf protein kinase, we have employed an epitope tag to enable the efficient isolation of highly purified Raf from cells with a specific monoclonal antibody. An epitope-tagged Raf protein was constructed by adding the sequence Asp-Tyr-Lys-Asp-Asp-Asp-Lys (Flag) at the NH2 terminus of c-Raf to create the protein Flag-Raf. This protein was stably expressed in CHO cells by cotransfection of the expression vector pCMV-Flag-Raf together with a plasmid that confers resistance to G418. In initial experiments, we examined the regulation of Raf by phorbol ester. Western blot analysis demonstrated that a marked electrophoretic mobility shift of the Raf protein occurred 15–60 min after the treatment of the CHO cells with phorbol ester (Fig. 1A). Raf kinase activity was measured using an immune complex protein kinase assay with recombinant Mek as an exogenous substrate (Fig. 1B). This analysis demonstrated that the Raf kinase activity was markedly and transiently increased after phorbol ester treatment with the maximal increase after 10 min followed by a lower level of kinase activity that was sustained for at least 60 min (Fig. 1B). Comparison of the time course of the decreased electrophoretic mobility of Raf (Fig. 1A) and the increased Raf kinase activity (Fig. 1B) indicates that these processes are poorly correlated and demonstrate that the marked electrophoretic mobility shift is not required for Raf kinase activation.

Mek is phosphorylated and activated by the Raf protein kinase (21–23). We therefore investigated the time course of the activation of the MAP kinase pathway in CHO cells. Consistent
Structure of the Raf Protein Kinase

![Graph A](image1)

α Raf

![Graph B](image2)

Time course of activation of the MAP kinase signaling cascade. Panel A, CHO cells expressing epitope-tagged Raf (Flag-Raf) were incubated in serum-free medium for 18 h at 37 °C and then treated with 100 nM PMA for 10 min. The cells were lysed and the soluble extract obtained was analyzed by gel filtration chromatography using a Superose 6 HR10/30 column. The gel filtration column was calibrated using protein standards obtained from Pharmacia-LKB Biotechnology Inc. The elution of Raf from the column was examined by Western blot analysis using the α-Raf antiserum 1558 as described under “Experimental Procedures.” Panel B, the Raf protein kinase activity was measured with the exogenous substrate Mek as described under “Experimental Procedures.” The protein kinase activity is presented as arbitrary units obtained by PhosphorImager analysis. The standard error was less than 10% of the mean of each data point.

Identification of the Raf Protein Kinase as a High Molecular Weight Protein Complex—To examine the structure and biochemical properties of the native Raf protein kinase, we examined the behavior of Raf during gel filtration chromatography. CHO cells were activated by treatment with phorbol ester for 10 min, and a cytosolic extract containing Raf was prepared for injection onto a Superose-6 HR10/30 column. Fig. 2A shows that the 74-kDa Raf protein eluted in the early fractions from the column with an apparent mass of 300-500 kDa. A similar elution profile of Raf immunoreactivity was observed for extracts prepared from serum-starved cells (data not shown). To investigate Raf protein kinase activity, we employed an immune complex assay with recombinant Mek as an exogenous substrate. Fig. 2B shows that the Raf protein kinase activity also eluted from the gel filtration column with a high apparent mass (300–500 kDa). Significantly, no Raf protein or Raf kinase activity was found to be eluted from the gel filtration column at the position expected for a Raf monomer (74 kDa). Together, these observations suggest that the native structure of basal and activated Raf is a large protein complex.
of the cells by immunoprecipitation with the M2 monoclonal antibody. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography.

Epitope-tagged Raf (Flag-Raf) were labeled with [\(^{32}\)P]phosphate or [\(^{35}\)S]methionine. Detergent extracts of these cells were prepared, and the Raf proteins were isolated by immunoprecipitation using the monoclonal antibody (M2) that binds to the Flag epitope. The immunoprecipitates were then analyzed by SDS-PAGE. Fig. 3 shows that Raf was specifically immunoprecipitated together with the ~90-kDa p50 and ~50-kDa p90 associated with Raf detected by coimmunoprecipitation (data not shown). This observation indicates that Raf activation is not associated with a change in the amount of p50 and p90 associated with Raf detected by coimmunoprecipitation (data not shown). This observation indicates that activation does not alter the elution profile of Raf during gel filtration chromatography (data not shown).

To identify the Raf-associated p90 and p50 proteins, we performed Western blot analysis of the Raf immunoprecipitates using antibodies directed against specific 90- and 50-kDa proteins. This analysis demonstrated that the Raf-associated 90-kDa protein is hsp90 and that the 50-kDa protein is the hsp90-associated p50 protein (Fig. 3).

**A. [\(^{32}\)P]**  **B. [\(^{35}\)S]**

**C. Western Blot**

**FIG. 3.** Identification of Raf-associated proteins as p50 and hsp90. Panels A and B, parental CHO cells and CHO cells expressing epitope-tagged Raf (Flag-Raf) were labeled with [\(^{32}\)P]phosphate or [\(^{35}\)S]methionine. The Raf proteins were isolated from detergent extracts of the cells by immunoprecipitation with the M2 monoclonal antibody. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The molecular mass standards (116, 97, 66, and 45 kDa) are indicated with arrows at the left of each panel. Panel C, epitope-tagged Raf was immunoprecipitated with the M2 monoclonal antibody from detergent extracts of CHO cells and examined by Western blot analysis using the \(\alpha\)-Raf antiserum 1558, the \(\alpha\)-hsp90 monoclonal antibody SPA-830, and the \(\alpha\)-p50 monoclonal antibody 3M/1B5p50. Open arrows indicate the cross-reactivity of the secondary antibodies employed in the Western blot analysis with the M2 monoclonal antibody used for immunoprecipitation. Filled arrows indicate the bands corresponding to Raf, hsp90, and p50. Molecular mass standards (110, 80, and 49.5 kDa) are indicated at the left of each panel.

**FIG. 4.** Mek binds to the Raf protein complex. Panel A, Raf was immunoprecipitated with the M2 monoclonal antibody from COS cells transfected with expression vectors encoding epitope-tagged Raf (Flag-Raf) and Mek. The level of Raf in the M2 immunoprecipitates was investigated by Western blot analysis with the \(\alpha\)-Raf antiserum 1558. The Raf protein in the Western blot is indicated with an arrow. Molecular mass standards (80 and 49.5 kDa) are indicated on the left. Panel B, the presence of Mek in the M2 immunoprecipitates was examined by Western blotting with the \(\alpha\)-Mek antiserum 28x0. The open arrow (IgG) indicates the cross-reactivity of the secondary antibody employed with the M2 monoclonal antibody used for immunoprecipitation.

**C. Western Blot**

**Fig. 3. Identification of Raf-associated proteins as p50 and hsp90. Panels A and B, parental CHO cells and CHO cells expressing epitope-tagged Raf (Flag-Raf) were labeled with \([^{32}\text{P}]\)phosphate or \([^{35}\text{S}]\)methionine. The Raf proteins were isolated from detergent extracts of the cells by immunoprecipitation with the M2 monoclonal antibody. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The molecular mass standards (116, 97, 66, and 45 kDa) are indicated with arrows at the left of each panel. Panel C, epitope-tagged Raf was immunoprecipitated with the M2 monoclonal antibody from detergent extracts of CHO cells and examined by Western blot analysis using the \(\alpha\)-Raf antiserum 1558, the \(\alpha\)-hsp90 monoclonal antibody SPA-830, and the \(\alpha\)-p50 monoclonal antibody 3M/1B5p50. Open arrows indicate the cross-reactivity of the secondary antibodies employed in the Western blot analysis with the M2 monoclonal antibody used for immunoprecipitation. Filled arrows indicate the bands corresponding to Raf, hsp90, and p50. Molecular mass standards (110, 80, and 49.5 kDa) are indicated at the left of each panel.

**Hsp90 and p50 Are Components of the Raf Protein Kinase Complex**—The high apparent mass of the Raf kinase (300–500 kDa) that is indicated by gel filtration chromatography (Fig. 2) may be caused by the association of Raf with other proteins. To test this hypothesis, we examined Raf-associated proteins using a coimmunoprecipitation assay. CHO cells were metabolically labeled with \([^{32}\text{P}]\)phosphate or \([^{35}\text{S}]\)methionine. Detergent extracts of these cells were prepared, and the Raf proteins were isolated by immunoprecipitation using the monoclonal antibody (M2) that binds to the Flag epitope. The immunoprecipitates were then analyzed by SDS-PAGE. Fig. 3 shows that Raf was specifically immunoprecipitated together with two additional proteins (50 and 90 kDa). Treatment of the CHO cells with serum or phorbol ester caused no significant change in the amount of p50 and p90 associated with Raf detected by coimmunoprecipitation (data not shown). This observation indicates that Raf activation is not associated with a change in the level of Raf complex formation with p90 and p50. This finding is consistent with the observation that activation does not alter the elution profile of Raf during gel filtration chromatography (data not shown).

To identify the Raf-associated p90 and p50 proteins, we performed Western blot analysis of the Raf immunoprecipitates using antibodies directed against specific 90- and 50-kDa proteins. This analysis demonstrated that the Raf-associated 90-kDa protein is hsp90 and that the 50-kDa protein is the hsp90-associated p50 protein (Fig. 3).

**A Single Raf Protein Kinase Is Located within the Native Complex**—The dimerization of protein kinase domains has been proposed to be the mechanism of activation of receptor tyrosine kinases (36). It is therefore possible that the mitogen activation of the Raf protein kinase may be mediated by oligomerization. To test this hypothesis we investigated whether the native Raf complex contains one or more Raf protein molecules. The approach that we employed was to investigate the coimmunoprecipitation of Raf molecules. In order to distinguish between different Raf molecules, we constructed a mutated Raf protein (Raf-Flag) in which the epitope recognized by the \(\alpha\)-Raf antiserum 1558 was changed by the addition of a synthetic epitope (Flag) to the COOH terminus of Raf. Control experiments demonstrated that Raf-Flag was not immunoprecipitated by the 1558 antiserum and that the wild-type Raf was not immunoprecipitated by the M2 (anti-Flag) monoclonal antibody. We then coexpressed Raf and Raf-Flag in COS cells and immunoprecipitated the Raf proteins from serum-starved and phorbol ester-treated cells using isoform-specific antibodies (1558 and M2). Western blot analysis of the immunoprecipitates failed to detect the presence of heterodimeric complexes containing Raf and Raf-Flag (data not shown). Together, these
data indicate that the native Raf complex contains a single Raf molecule.

Mek Binds to the Raf Protein Kinase Complex—The Raf protein kinase substrate Mek has been demonstrated to interact with the carboxyl terminal domain of Raf in the yeast *Saccharomyces cerevisiae* using the two-hybrid method (9). We therefore investigated whether the native Raf protein kinase complex was bound to Mek. Western blot analysis of Raf immunoprecipitates failed to detect the presence of Mek (Fig. 4). This observation indicates that Mek is not a stable component of the Raf protein kinase complex. However, the possibility that Mek binds to the native Raf complex is not excluded by these data because the immunoprecipitation assay employed may be too stringent to detect low affinity interactions. To test this hypothesis, we used an over-expression strategy to investigate whether the interaction of Mek with the Raf complex can be detected. Fig. 4 shows that Mek can be coimmunoprecipitated with the Raf complex when these proteins are over-expressed in COS cells. Together, these data demonstrate that the native form of the Raf protein kinase interacts with Mek.

Ras-dependent Membrane Translocation of the Raf Protein Kinase Complex—The Raf protein is an important intermediate in the signal transduction pathway initiated by growth factor receptors that result in MAP kinase activation. Recently, the NH$_2$-terminal domain of the Raf protein has been demonstrated to bind directly to the effector domain of activated GTP-bound Ras (9–14). This observation suggests that the activation of the Raf protein kinase may require the physical interaction of Raf with activated Ras. A prediction of this hypothesis is that the activated Raf protein kinase in mitogen-stimulated cells should be present on cell membranes where the Ras protein is located. We therefore examined the subcellular distribution of Raf in CHO cells. Fig. 5 shows that the majority of Raf is present as a soluble cytoplasmic protein in starved cells and that only a small amount of Raf is bound to membranes. However, treatment of the cells with serum causes translocation of Raf from the cytosol to the membrane. The kinetics of serum-stimulated membrane translocation (Fig. 5A) and Raf kinase activation (Fig. 5B) were observed to be similar. This observation suggests that the association of Raf with membranes is significant for the process of Raf protein kinase activation.

To examine the role of Ras in the membrane translocation and activation of the Raf protein kinase, we examined the effect of Ras expression on the biochemical properties of Raf. Fig. 6A shows that the expression of activated Ras61L (37) caused a marked increase in the membrane-bound form of the Raf protein kinase and a corresponding decrease in the level of Raf in the cytosol. Furthermore, it was found that only the membrane-bound form of Raf exhibited increased protein kinase activity (Fig. 6B). In contrast, expression of dominant-negative Ras17N (38) caused no significant change in the activity or subcellular distribution of Raf. Together, these observations indicate that Raf protein kinase stimulation requires activated (GTP-bound) Ras and membrane association.

The cytoplasmic Raf protein kinase exists as a high molecular mass complex with hsp90 and p50 (Fig. 3). We therefore investigated whether the membrane translocation of Raf represents the binding of the Raf-hsp90-p50 complex to the membrane or whether the Raf protein is released from the hsp90-p50 complex prior to membrane association. Western blot analysis demonstrated that hsp90 and p50 were coimmunoprecipitated with the membrane-bound Raf protein kinase indicating that it is the Raf-hsp90-p50 complex that associates with the membrane during Raf protein kinase activation (Fig. 6).

**DISCUSSION**

Hsp90 is an essential protein that is required for cell viability (39) and is thought to function as a molecular chaperone (40). Biochemical analysis has demonstrated that hsp90 forms heteromeric complexes with a 50-kDa protein (p50) and other proteins (27). Examples of proteins that have been found in complexes with hsp90 include steroid hormone receptors (41–43), dioxin receptors (44, 45), actin (46), Src tyrosine kinase (47, 48), Elf-2a kinase (49–51), and casein kinase II (52). The results of this study demonstrate that the Raf kinase represents an additional example of a protein that is found in complexes with hsp90 and p50. A similar conclusion has been reached independently by others (53). Both the basal and activated forms of the Raf protein kinase were found in complexes with hsp90 and p50 (Fig. 6). Thus, changes in the level of complex formation between Raf and hsp90-p50 do not account for the mechanism of Raf kinase activation.

The size of the Raf complex (300–500 kDa) observed during gel filtration chromatography could be accounted for by the 74-kDa Raf protein bound to hsp90-p50. In previous studies it has been reported that hsp90 functions as a dimer (54–56). Thus, the Raf complex is likely to contain two molecules of hsp90, one (or more) molecule(s) of p50, and one Raf protein kinase. However, the possibility that additional proteins are also present in the Raf complex cannot be excluded by the data.
obtained in this study. For example, proteins weakly bound to Raf or proteins labeled poorly with [35S]methionine and [32P]phosphate may not have been detected by the coimmunoprecipitation assay (Fig. 2). One example is provided by Mek [32P]phosphate which was observed to associate with the Raf complex only when expressed at high levels in COS cells (Fig. 4). A similar weak interaction of Raf with Ras has been observed in coinmunoprecipitation assays when a large excess of recombinant Ras is added to cell extracts (14).

The finding that Raf is present in cells as a large protein complex provides an explanation for the results of previous studies of the biochemical properties of a MAPKKK activity detected in cell extracts. Chromatographic analysis by Matsuda et al. (57) demonstrated the presence of a single major peak of a MAPKKK activity with an apparent mass of approximately 400 kDa. Similarly, Itoh et al. (17) have described a MAPKKK activity with high apparent mass that was required for Ras-induced Raf activation. As Raf functions as a MAPKKK that interacts directly with Ras, the MAPKKK activities described by Matsuda et al. (57) and Itoh et al. (17) are likely to be accounted for by the Raf-hsp90-p50 complex described in this report (Fig. 4).

In previous studies a broad spectrum of functional roles for the binding of hsp90 to proteins has been described. For example, the hsp90-p50 complex with Src is a transient intermediate that occurs during biosynthesis of the v-Src (but not c-Src) tyrosine kinase (47). Hsp90 is therefore not a significant regulator of the mature Src kinase. However, an important regulatory role for hsp90 has been described for other proteins. For example, the glucocorticoid receptor is complexed with hsp90, and it has been found that the binding of the receptor to hsp90 is required for hormone-stimulated gene expression (58). In addition, it has been demonstrated that hsp90 binding causes stimulation of the protein kinase activity of casein kinase II (52) and eIF-2α kinase (49–51). Together, these data establish that signaling proteins can be regulated by interaction with hsp90. The observation that Raf interacts with hsp90-p50 suggests that the Raf-hsp90-p50 complex is likely to be significant for the physiological function of the Raf protein kinase. However, as both basal and activated Raf are bound to hsp90-p50, it is unlikely that the formation of this complex accounts for Raf kinase activation. Indeed, the level of complex formation by Raf with hsp90-p50 was not found to change during Raf kinase activation.

It is known that Raf can be activated by Ras (59) and that this process is likely to be mediated by the direct interaction of these proteins (9–14). However, the detailed molecular basis for the stimulation of Raf kinase activity has not been established. The presence of Raf in a large protein complex that interacts with Ras and Mek suggests that protein-protein interactions may represent the molecular mechanism of regulation of the Raf protein kinase. The importance of these protein interactions for the normal functioning of Raf is consistent with the observed dominant-negative phenotype of defective Raf proteins (60). We observed that both the cytosolic and membrane-bound forms of the Raf protein kinase are present in a complex with the molecular chaperones hsp90 and p50 (Fig. 6). However, it is only the Ras-induced membrane-bound Raf complex that exhibits increased protein kinase activity (Fig. 6). It is therefore possible that the Ras-mediated translocation of the Raf-hsp90-p50 complex brings the Raf kinase in proximity to an activating molecule located within the membrane. We propose the hypothesis that Raf activation is mediated by a conformational change in the Raf-hsp90-p50 complex after Ras-induced recruitment to the membrane.

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