The Activation of Rac1 by M₃ Muscarinic Acetylcholine Receptors Involves the Translocation of Rac1 and IQGAP1 to Cell Junctions and Changes in the Composition of Protein Complexes Containing Rac1, IQGAP1, and Actin

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The abilities of the M₃ muscarinic acetylcholine receptor (mAChR) and Rac1 to regulate similar cellular responses, including cadherin-mediated adhesion, prompted us to investigate Rac1 regulation by M₃ mAChR. We characterized changes in Rac1 induced by stimulating transfected M₃ mAChR in Chinese hamster ovary cells stably expressing hemagglutinin (HA)-tagged wild-type or mutant Rac1. mAChR activation converts endogenous Rac1 to the GTP-bound form in cells expressing HA-Rac1 but not in cells expressing dominant negative HA-Rac1Asn-17 or constitutively active HA-Rac1Val-12. The competitive binding of endogenous IQGAP1 by HA-Rac1Val-12 may diminish the mACHR-mediated activation of endogenous Rac1. HA-Rac1 and HA-Rac1Val-12, but not HA-Rac1Asn-17, accumulate with IQGAP1 at cell junctions during mAChR-induced cell-cell compaction. Co-localization studies suggest that Rac1 can accumulate at junctions without IQGAP1, but IQGAP1 cannot accumulate at junctions without Rac1. mAChR activation also induces GTP-independent changes in Rac1 because mAChR activation redistributes HA-Rac1Asn-17, which does not bind GTP. Actin associates with complexes containing HA-Rac1 or HA-Rac1Val-12 after prolonged mAChR activation. We also demonstrate that Rac1 participates in mAChR-induced cell-cell compaction and c-Jun phosphorylation. These results indicate that M₃ mAChR activation converts Rac1 to the GTP-bound form, alters interactions between Rac1, IQGAP1, and actin, and causes the junctional accumulation of Rac1 and IQGAP1.

The small GTPase Rac1 is emerging as an important participant in a variety of signaling pathways. Activation of Rac1 contributes to many responses in smooth muscle cells including c-Jun NH₂-terminal kinase (JNK)³ activation (1), reactive oxygen species generation (2), and contraction induced by M₃ mAChR activation significantly alters pulmonary and cardiovascular function (reviewed in Refs. 16 and 17). The induction of E-cadherin-mediated adhesion by M₃ mAChR activation in lung carcinoma cells may diminish metastatic potential (reviewed in Refs. 11 and 15). The M₃ mAChR-mediated activation of JNK (12) may play an important role in AP-1-mediated transcription in a variety of cell types (reviewed in Ref. 18). The probability that these M₃ mAChR-dependent functions involve Rac1 provides a strong rationale for investigating how M₃ mAChR activation alters Rac1.
Potential participants in the M₃ mACHR-mediated activation of Rac1 include IQGAP1 and the Rho guanine nucleotide dissociation inhibitor RhoGDI. IQGAP1, which derives its name from the presence of several IQ domains and some sequence similarity to GTPase-activating proteins, binds multiple proteins in addition to Rac1, including Cdc42, calmodulin, E-cadherin, β-catenin, and actin (reviewed in Ref. 19). IQGAP1 may promote Rac1 activation by diminishing Rac1 GTPase activity and by preventing active Rac1 from interacting with negative regulators (reviewed in Ref. 20). In contrast, RhoGDI may impede Rac1 activation by diminishing the ability of Rac1-GDP to convert to Rac1-GTP (reviewed in Ref. 21).

We are using Chinese hamster ovary (CHO) cells stably transfected with M₃ mACHR (CHO-m3 cells) to help define how M₃ mACHR may activate Rac1. M₃ mACHR-mediated responses in these cells resemble those induced by activating endogenous M₃ mACHR in other cell types. For example, M₃ mACHR activation affects actin/myosin interactions in CHO-m3 cells as it does in smooth muscle cells (reviewed in Ref. 14). Cadherin-mediated adhesion induced by M₃ mACHR activation in CHO-m3 cells (11) mimics almost exactly cadherin-mediated adhesion induced by activating endogenous M₃ mACHR in lung carcinoma cells (15). These similarities indicate that CHO-m3 cells are a reasonable model system to investigate how M₃ mACHR activation affects Rac1. The M₃ mACHR preferentially couples to heterotrimeric G proteins in the Gₛ/₁₁ family (reviewed in Ref. 12). Thus, the activation of Rac1 by other GPCR that preferentially couple to Gₛ/₁₁ proteins, such as receptors for angiotensin-II (1, 2), thrombin, and histamine (reviewed in Ref. 9), may involve changes in Rac1 similar to those that are induced by M₃ mACHR activation.

We found that M₃ mACHR activation profoundly affects both endogenous Rac1 and hemagglutinin (HA)-tagged wild-type and mutant Rac1 proteins that were stably transfected in these CHO-m3 cells. The M₃ mACHR-dependent activation of Rac1 involves the conversion of Rac1 to the GTP-bound form, altered interactions between Rac1, IQGAP1, and actin, and translocation of Rac1 and IQGAP1 to cell junctions. These changes in Rac1 affect specific M₃ mACHR-dependent signaling pathways because expression of dominant negative HA-Rac1 (13,17) inhibits the M₃ mACHR-dependent compaction of the cells and activation of JNK but not the activation of extracellular regulated kinase (ERK)-1 and ERK-2. These results help define the changes in Rac1 that allow it to participate in M₃ mACHR-dependent signaling.

**EXPERIMENTAL PROCEDURES**

**Antibodies—**Antibodies to Rac1, IQGAP1, RhoGDI (Transduction Laboratories, San Diego, CA), HA (Covance, Berkeley, CA), calmodulin (Upstate Biotechnology, Lake Placid, NY), and actin (amino acids 20–33 (Sigma) were used in the assays. The sc-94 antibody (recognizing ERK-1 and ERK-2) and the sc-7383 antibody (recognizing phosphorylated ERK-1 and ERK-2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Cell Lines—**The pEF-BOS-HA-Rac1 and pEF-BOS-HA-Rac1Val-12 plasmids coding for HA-tagged wild-type Rac1 or constitutively active Rac1Val-12, respectively, were a gift from Dr. Andrew Kraft (University of Colorado). Cells were incubated with 10 μM carbachol and lysed (12). Bacterially expressed GST-PBD followed HA precipitation with glutathione-Sepharose 4B beads, as described previously (20). The precipitates were subjected to ECL-Western blotting using antibodies to Rac1 or HA. Densitometry of the samples in the ECL-Western blots was performed to quantify the relative amounts of endogenous Rac1 and HA-tagged Rac1 proteins that co-precipitated with GST-PBD. Optical density values higher than 2.4 may not accurately indicate the relative amounts of proteins in the samples because these values are above the maximum densitometry detection limit.

**Phosphorylation of c-Jun—**Phosphorylation of c-Jun was assayed as described by Wylie et al. (12) using a bacterial expression vector for GST-tagged c-Jun (amino acids 5–89) (24), which was provided by Dr. Andrew Kraft (University of Colorado). Cells were incubated with 10 μM carbachol and lysed (12). Bacterially expressed GST-c-Jun was incubated (1 h, 4°C) with the cleared lysates in the presence of glutathione-Sepharose 4B beads, precipitated, and analyzed by autoradiography (20). In the presence of 10 μM carbachol with kinase buffer (12) containing 100 μCi/ml [³²P]ATP (4.5 Ci/mmol), the cells were boiled in sample buffer for 5 min and subjected to SDS-PAGE followed by autoradiography. Densitometry of the bands was performed to quantify the relative amounts of phospho-c-Jun in the samples. Optical density values higher than 4.4 may not accurately indicate the relative amounts of proteins in the samples because these values are above the maximum densitometry detection limit.

**Phosphorylation of ERK-1 and ERK-2—**Cells were incubated with 10 μM carbachol and lysed (12). Densitometry of the samples in the autoradiographs was performed to quantify the levels of c-Jun phosphorylation.
Activation of Rac1 by M₃ mAChRs

The Wild-type and Mutant Rac1 Proteins Have Different Rates of GTP Binding and Associate with Different Intracellular Proteins—To examine the regulation of Rac1 by M₃ mAChR, CHO-m3 sublines stably expressing HA-tagged wild-type or mutant Rac1 proteins were established. The m3WTRac cell line expresses wild-type HA-Rac1, the m3CARac cell line expresses constitutively active HA-Rac1Val¹¹², and the m3DNRac cell line expresses dominant negative HA-Rac1Arena¹⁻¹⁷.

To test the expectation that these wild-type and mutant HA-tagged Rac1 proteins have different rates of GTP binding and hydrolysis, we measured the binding of [³⁵S]GTPγS by the HA-tagged GTPases expressed in the cells. Although significant levels of [³⁵S]GTPγS are bound by both HA-Rac1 and HA-Rac1Val¹¹², less [³⁵S]GTPγS is bound by HA-Rac1Val¹⁻¹² (than by HA-Rac1) (Fig. 1A). This finding is consistent with constitutively active HA-Rac1Val¹¹² having a slower rate of GTP hydrolysis, which would result in a slower rate of [³⁵S]GTPγS binding. As expected, dominant negative HA-Rac1Arena¹⁻¹⁷ binds minimal levels of [³⁵S]GTPγS (Fig. 1A).

To examine the protein interactions of the HA-tagged wild-type and mutant Rac1 proteins, the HA-tagged GTPases were immunoprecipitated from [³⁵S]GTPγS-labeled cells and examined for co-precipitating proteins (Fig. 1B). For comparison HA-tagged wild-type RhoA, constitutively active RhoAVal¹⁻¹⁴, and dominant negative RhoArena¹⁻¹⁷ were also immunoprecipitated from [³⁵S]GTPγS-labeled stably transfected CHO-m3 sublines that we established previously (14). IQGAP1 was identified as the 195-kDa protein that co-precipitates with HA-Rac¹¹² and to a lesser extent with HA-Rac1 (Fig. 1B). Enzymatic digestion and peptide sequencing of this 195-kDa protein yielded a peptide with the sequence LPYDVTPEQA, which corresponds to residues 1112–1121 of murine IQGAP1 (Swiss-Prot accession number P9JFKP). The identification of this protein as IQGAP1 was further indicated by its reaction with IQGAP1 antibodies in ECL-Western blots of immunoprecipitated HA-Rac1¹¹² (Fig. 1C). The inability of IQGAP1 to co-precipitate with HA-Rac¹Arena¹⁻¹⁷ or the HA-tagged RhoA proteins (Fig. 1B) is consistent with the report that IQGAP1 preferentiallyassociates with the GTP-bound form of Rac1 and does not associate with RhoA (25).

Two proteins that co-precipitate with HA-Rac¹¹² and to a much lesser extent with HA-Rac1 have approximate relative molecular masses of 19 and 16 kDa (Fig. 1B). Several lines of evidence indicate that these two proteins are calmodulin. Calmodulin reportedly migrates on SDS gels with relative molecular masses of 21 and 15 kDa when the protein is Ca²⁺-free and -bound, respectively (reviewed in Ref. 16). Calmodulin also physically associates with IQGAP1, which has three binding sites for Ca²⁺-free calmodulin and one binding site for Ca²⁺-bound calmodulin (reviewed in Ref. 27). The ratio of IQGAP1/19-kDa protein/16-kDa protein is 1.057 ± 0.09:0.23 ± 0.05 in HA-Rac¹¹² immunoprecipitates (n = 5 independent experiments). These proteins are present in a similar ratio in 1.047 ± 0.08:0.24 ± 0.05 in HA-Rac1 immunoprecipitates, even though much less IQGAP1 co-precipitates with HA-Rac1 than with HA-Rac¹¹² (n = 5 independent experiments). These similar ratios support the possibility that the 19- and 16-kDa proteins are Ca²⁺-free and Ca²⁺-bound calmodulin, respectively, associated with IQGAP1. This possibility is further supported by the demonstration that calmodulin antibodies recognize the 19-kDa protein in immunoprecipitates of HA-Rac¹¹² (Fig. 1D, top panel). The lack of detectable reactivity of these antibodies with the 16-kDa protein may be because of an inability of the calmodulin antibodies to recognize Ca²⁺-bound calmodulin.

RhoGDI was identified by ECL-Western blotting (Fig. 1C) as the 28-kDa protein that co-precipitates with HA-Rac1 and to a lesser extent with HA-Rac¹¹² (Fig. 1B). The ratio of HA-Rac¹/RhoGDI is 1:1.14 ± 0.07 in HA-Rac1 immunoprecipitates from m3WTRac cells, whereas the ratio of HA-Rac¹¹²/RhoGDI is 1:0.44 ± 0.06 in HA-Rac¹¹² immunoprecipitates from m3CARac cells (n = 5 independent experiments). Undetectable amounts of RhoGDI co-precipitate with HA-Rac¹Arena¹⁻¹⁷ or HA-RhoArena¹⁻¹⁷ (Fig. 1B).

We hypothesized that Rac1 associates with IQGAP1 and RhoGDI in two mutually exclusive complexes. This hypothesis was tested by comparing immunoprecipitates of IQGAP1, RhoGDI, and HA-Rac¹² from m3CARac cells. Immunoprecipitates of RhoGDI contain neither calmodulin nor proteins that co-migrate with IQGAP1 (Fig. 1E). Conversely, immunoprecipitates of IQGAP1 contain calmodulin but not RhoGDI (Fig. 1E). These results indicate that HA-Rac¹¹² forms a complex with RhoGDI and forms another complex with IQGAP1 and calmodulin in m3CARac cells.

The Wild-type and Mutant HA-Rac1 Proteins Have Different Susceptibilities to Activation by mAChR and Different Effects on the Activity of Endogenous Rac1—The effects of M₃ mAChR activation on the activities of the HA-tagged Rac1 proteins were investigated by precipitating the GTP-bound forms of the proteins using GST-PBD and examining the precipitates by ECL-Western blotting using antibody to HA (Fig. 2A, top panel, and Fig. 2B). We found that the association of HA-Rac1 with GST-PBD is increased by M₃ mAChR activation or by the nonspecific activation of GTPases with GTPγS or guanosine 5′-[(β,g-imido)triphosphate (GppNHp). In contrast, constitutively active HA-Rac¹¹² maximally associates with GST-PBD, and this association is not enhanced by mAChR activation or by exposure to GTPγS or GppNHp. Dominant negative HA-Rac¹Arena¹⁻¹⁷ does not detectably associate with GST-PBD and cannot be induced to associate with GST-PBD by mAChR agonists or by GTPγS or GppNHp (Fig. 2A, top panel, and Fig. 2B).

To determine whether expression of the mutant HA-tagged Rac1 proteins interferes with the activation of endogenous Rac1 by M₃ mAChR, GST-PBD was used to precipitate endogenous Rac1 from the different sublines. The precipitated endogenous Rac1, which migrates with a relative molecular mass of 25 kDa, was detected by ECL-Western blotting using antibodies to Rac1 (Fig. 2A, bottom panel, and Fig. 2C). As expected, the association of endogenous Rac1 with GST-PBD is increased by mAChR activation in m3WTRac cells but not in m3DNRac cells (Fig. 2A, bottom panel, and Fig. 2C). Surprisingly, the association of endogenous Rac1 with GST-PBD is not increased by mAChR activation in m3CARac cells expressing constitutively active HA-Rac¹¹² (Fig. 2A, bottom panel, and Fig. 2C). This response is not due to a general depression of Rac1 activity in these cells because nonspecific activation of GTPases with either GTPγS or GppNHp increases the association of Rac1 with GST-PBD in all of the sublines (Fig. 2A, bottom panel, and Fig. 2C).

Activation of M₃ mAChR Induces the Translocation of IQGAP1 and the Wild-type and Mutant HA-Rac1 Proteins—The effects of M₃ mAChR activation on the intracellular distributions of IQGAP1 and the HA-tagged Rac1 proteins were examined by incubating the cells with carbachol for 0–90 min and immunofluorescently staining the cells using antibodies to IQGAP1 and HA. Translocation of the proteins was consis-
tently detectable within 10 min after exposure to carbachol, resulting in a redistribution that culminated 30 min after exposure. Subsequent translocation resulted in a new distribution of the proteins that was most noticeable 90 min after exposure to carbachol. Because differences in the distributions of the proteins were most obvious at 30 and 90 min after carbachol treatment, images of the intracellular localization of the proteins in control cells and in cells treated with carbachol

FIG. 1. The wild-type and mutant Rac1 proteins have different rates of GTP binding and associate with different intracellular proteins. A, the amount of [35S]GTPγS bound by HA-tagged wild-type or mutant Rac1 proteins was determined by incubating [35S]GTPγS with membranes isolated from equal numbers of m3WTRac, m3DNRac, and m3CARac cells and immunoprecipitating the HA-tagged proteins. Control values were obtained by subjecting an equal number of m3Zeo-2 cells to the identical treatment. Values shown are the means ± 1 S.E. from three independent experiments, each conducted with triplicate samples. B, HA-tagged wild-type or mutant Rac1 proteins (lanes 1–3) or RhoA proteins (lanes 5–7) were immunoprecipitated with HA antibody from lysates of equal numbers of the indicated 35S-labeled cells. As a control, lysates of 35S-labeled m3Zeo-2 cells were subjected to the same procedure (lane 4). The immunoprecipitates were subjected to SDS-PAGE and autoradiography. A representative autoradiograph from five independent experiments is shown. C, HA-Rac1Val-12 was immunoprecipitated with HA antibody from lysates of m3CARac cells and immunoblotted with antibody to RhoGDI (lane 1), HA (lane 2), or IQGAP1 at antibody concentrations of 0.25 μg/ml (lane 3), 0.5 μg/ml (lane 4), and 1 μg/ml (lane 5). A representative immunoblot from three independent experiments is shown. D, lysates were prepared from equal numbers of m3Zeo-2 cells (lanes 1, 2, 9, and 10), m3CARac cells (lanes 3, 4, 11, and 12), m3DNRac cells (lanes 5, 6, 13, and 14), or m3WTRac cells (lanes 7, 8, 15, and 16). The lysates were immunoprecipitated with HA antibody (lanes 1–8) or boiled in sample buffer (lanes 9–16) and immunoblotted with antibody to HA (lanes 1, 3, 5, and 7), calmodulin (even-numbered lanes), or Rac1 (lanes 9, 11, 13, and 15). Representative immunoblots from three independent experiments are shown. E, lysates of 35S-labeled m3CARac cells were immunoprecipitated with antibody to HA (lane 1), RhoGDI (lane 2), or IQGAP1 (lane 3). The immunoprecipitates were subjected to SDS-PAGE and autoradiography. A representative autoradiograph from three independent experiments is shown.
for 30 and 90 min are presented.

In m3WTRac cells, HA-Rac1 is diffusely distributed throughout the cell (Fig. 3A), whereas IQGAP1 accumulates at spherical membrane protrusions at the cell surface (Fig. 3B). Exposure to carbachol for 30 min induces cell-cell compaction and results in the localization of HA-Rac1 at most cell junctions (Fig. 3C) and IQGAP1 at many cell junctions (Fig. 3D). Decom-paction of the cells occurs within 90 min of carbachol exposure, resulting in spread cells that exhibit a diffuse distribution of HA-Rac1 (Fig. 3E) and high concentrations of IQGAP1 at punctate membrane protrusions at the cell surface (Fig. 3F).

To determine whether the mAChR-mediated redistribution of IQGAP1 also occurs in cells expressing normal levels of Rac1, we examined the localization of IQGAP1 in m3Zeo-2 cells, which are CHO-m3 cells stably transfected with the empty pZeoSV2 vector (14). IQGAP1 is present in spherical membrane protrusions on m3Zeo-2 cells (Fig. 4A), and it accumulates at the junctions of these cells that are compacted because of exposure to carbachol for 30 min (Fig. 4B). IQGAP1 subsequently accumulates at punctate membrane protrusions on m3Zeo-2 cells that are decompacting after exposure to carbachol for 90 min (Fig. 4C). These results indicate that mAChR activation induces a similar redistribution of IQGAP1 in m3Zeo-2 cells and m3WTRac cells.

Both dominant negative HA-Rac1\textsuperscript{Asn-17} and IQGAP1 localize in unique sites in m3DNRac cells (Fig. 5), which are more spread than m3WTRac cells or m3Zeo-2 cells. Activation of mAChR does not induce compaction of m3DNRac cells as it does in m3WTRac cells or m3Zeo-2 cells. However, dominant negative HA-Rac1\textsuperscript{Asn-17} translocates from a juxtanuclear distribution (Fig. 5A) to a more diffuse distribution after treatment with carbachol for 30 min (Fig. 5C). Some relocalization of HA-Rac1\textsuperscript{Asn-17} to the juxtanuclear region occurs after treatment with carbachol for 90 min (Fig. 5E). IQGAP1 does not accumulate in spherical membrane protrusions in m3DNRac cells (Fig. 5B) as much as it does in m3WTRac cells or m3Zeo-2 cells. However, these spherical membrane protrusions, exhib
iting high concentrations of IQGAP1, are sometimes visible on m3DNRac cells that are not completely spread (Fig. 5B).

IQGAP1 does not translocate to junctions following M3 mAChR activation in m3DNRac cells (Fig. 5D) but accumulates in punctate membrane protrusions in cells treated with carbachol for 90 min (Fig. 5F).

Both constitutively active HA-Rac1 Val-12 and IQGAP1 accumulate in prominent membrane ruffles on m3CARac cells (Fig. 6, A and B). Cell-cell compaction and translocation of HA-Rac1 Val-12 to cell junctions occurs within 30 min of carbachol exposure (Fig. 6C). In contrast, IQGAP1 is detectable at both cell junctions and membrane ruffles after 30 min of carbachol exposure (Fig. 6D). Neither HA-Rac1 Val-12 nor IQGAP1 are detectable at the junctions of cells that are decompacting after 90 min of carbachol exposure; HA-Rac1 Val-12 is diffusely distributed with some relocalization to membrane ruffles (Fig. 6E), whereas IQGAP1 is present at some membrane ruffles and at punctate membrane protrusions (Fig. 6F).

We consistently found that the junctional localization of HA-Rac1 Val-12 exceeds that of IQGAP1 in m3CARac cells exposed to carbachol for 30 min (Fig. 6, C and D). To test the possibility that HA-Rac1 Val-12 accumulates at some junctions in the absence of IQGAP1, HA-Rac1 Val-12 and IQGAP1 were detected simultaneously in m3CARac cells incubated with or without carbachol for 15 min (Fig. 7). Prior to mAChR activation, HA-Rac1 Val-12 and IQGAP1 co-localize on prominent membrane ruffles (Fig. 7, A and B). After 15 min of mAChR activation, HA-Rac1 Val-12 accumulates at junctions both in the presence and absence of IQGAP1 (Fig. 7, C and D). We did not observe any localization of IQGAP1 to junctions in the absence of HA-Rac1 Val-12. Similar results were obtained when the co-localization of HA-Rac1 and IQGAP1 in m3WTRac cells was examined (data not shown).

Because the activation and intracellular localization of Rac1 is regulated by signaling cascades involving pertussis toxin-sensitive Gi proteins in some systems (28–32), we investigated the effects of pertussis toxin on the carbachol-induced redistribution of the HA-tagged wild-type and mutant Rac1 proteins and IQGAP1 in the CHO-m3 sublines. Cells that were pre-incubated in the absence or presence of pertussis toxin (100 ng/ml, 20 h) exhibited similar carbachol-induced changes in the intracellular localization of the HA-tagged wild-type and mutant Rac1 proteins and IQGAP1 (data not shown). This apparent lack of Gi participation in the mAChR-mediated regulation of Rac1 is perhaps not surprising because M3 mAChR preferentially couple to Gq/11 proteins rather than Gi proteins (reviewed in Ref. 12). Our previous finding that CHO cell compaction is induced by activating transfected Gq/11-coupled M2 or M3 mAChR, but not by activating transfected Goi/o-coupled M3 mAChR (11), is consistent with the lack of Gi participation in the mAChR-mediated regulation of Rac1 in this system.

Activation of M3 mAChR Alters the Association of HA-Rac1 with Intracellular Proteins—The effects of mAChR activation on the protein interactions of Rac1 were tested by immunoprecipitating HA-Rac1 and HA-Rac1 Val-12 from 35S-labeled cells treated with carbachol for different times (Fig. 8). Somewhat
surprisingly, mAChR activation did not induce detectable changes in the association of RhoGDI with HA-Rac1 (Fig. 8A) or HA-Rac1Val-12 (Fig. 8C). However, mAChR activation increased the co-precipitation of HA-Rac1 with proteins that migrate in the region of IQGAP1 (Fig. 8B). Activation of mAChR also caused two proteins with relative molecular masses of 58 and 42 kDa to co-precipitate with HA-Rac1 (Fig. 8A) and to a greater extent with HA-Rac1Val-12 (Fig. 8C). The 42-kDa protein was identified as actin by ECL-Western blotting (Fig. 8D), and the 58-kDa protein has not yet been identified. Actin and the 58-kDa protein co-precipitate with IQGAP1 (Fig. 8A), consistent with previous reports that actin associates with IQGAP1 (33).

Activation of M₃ mAChR causes the Rac1-dependent phosphorylation of c-Jun and the Rac1-dependent phosphorylation of ERK-1 and ERK-2—A previous report that JNK and ERK-1 are activated by M₃ mAChR stimulation in CHO-m3 cells (12) prompted us to examine whether these mAChR-induced events are altered in m3DNRac or m3CARac cells. The mAChR-induced phosphorylation of c-Jun is significantly enhanced in m3DNRac cells and significantly diminished in m3DNRac cells, when compared with the responses induced in m3WTRac and m3Zeo-2 cells (Fig. 9, A and B). In contrast, the mAChR-induced phosphorylation of ERK-1 and ERK-2 is similar in all of the sublines except m3CARac cells, which exhibit a more prolonged elevation of ERK-1 and ERK-2 phosphorylation following mAChR activation (Fig. 9, C–E). These results indicate that functional Rac1 is required for c-Jun phosphorylation, but not for ERK-1 or ERK-2 phosphorylation, induced by M₃ mAChR activation.

DISCUSSION

This study indicates that M₃ mAChR activation has multiple effects on Rac1 and IQGAP1. Activation of mAChR converts Rac1 to the GTP-bound state and induces the translocation of Rac1 and IQGAP1 to cell junctions. Prolonged M₃ mAChR activation causes actin to associate with protein complexes containing Rac1. By comparing these mAChR-induced effects in cells expressing wild-type Rac1, constitutively active Rac1Val-12, and dominant negative Rac1Asp-17, we developed a model depicting the mAChR-dependent changes in Rac1 and IQGAP1, as shown in Fig. 10.

Effects of M₃ mAChR Activation on the Association of Rac1 with GST-PBD—The increased association of HA-Rac1 with GST-PBD in carbachol-treated cells indicates that M₃ mAChR activation increases the GTP-bound state of HA-Rac1. Greater association of HA-Rac1 with GST-PBD is induced by exposing cells to GTPγS or GppNHp rather than to carbachol. Several factors may contribute to this finding. Neither GTPγS nor GppNHp, which are non-hydrolyzable analogs of GTP, would be hydrolyzed readily to GDP during the isolation of HA-Rac1. Thus, the association of HA-Rac1 with GST-PBD may be enhanced or prolonged when the GTPase is bound to GTPγS or GppNHp rather than to GTP.

An additional and complementary explanation for the modest association of GST-PBD with HA-Rac1 induced by mAChR agonists as compared with GTPγS or GppNHp is that only a small proportion of HA-Rac1 in m3WTRac cells is converted to the GTP-bound form following mAChR activation. This is a reasonable possibility because it would be physiologically detrimental for the activity of all Rac1 molecules expressed in a cell to be regulated by only one receptor such as the M₃ mAChR. According to this model some HA-Rac1 molecules, such as those associated with RhoGDI, may be resistant to M₃ mAChR.
that some of the phenotypic alterations induced by dominant negative Rac1<sub>Asn-17</sub> in Dictyostelium are also induced by the expression of constitutively active Rac1<sub>Val-12</sub>. These investigators speculated that constitutively active Rac1<sub>Val-12</sub> may have a negative effect by competitively binding proteins, such as the IQGAP1-related protein DGAP1, which may be required for the normal functioning of endogenous Rac1 in Dictyostelium. Our results are consistent with this possibility. If HA-Rac1<sub>Val-12</sub> competitively binds all of the IQGAP1 that is available to interact with Rac1, then HA-Rac1<sub>Val-12</sub> could inhibit IQGAP1 from participating in the mAChR-mediated activation of Rac1. The potential involvement of IQGAP1 in the GPCR-dependent activation of GTPases is supported by a recent report that expression of mutant IQGAP1 inhibits the bradykinin-dependent activation of Cdc42, whereas expression of wild-type IQGAP1 enhances Cdc42 activity (19). These findings suggest that several of the reported negative effects of constitutively active Rac1<sub>Val-12</sub> (reviewed in Ref. 9) may be caused by the competitive binding of IQGAP1 by Rac1<sub>Val-12</sub>.

**Effects of M<sub>3</sub> mAChR Activation on the Association of Rac1 with RhoGDI**—Our observation that RhoGDI co-precipitates more readily with HA-Rac1 than with HA-Rac1<sub>Val-12</sub> is consistent with reports that RhoGDI preferentially associates with the GDP-bound form of Rho family members (reviewed in Ref. 21). Measurements of GTP<sub>γ</sub>S binding indicated that HA-Rac1 may be in the GDP-bound form more often than HA-Rac1<sub>Val-12</sub>. These different amounts of GDP-bound HA-Rac1 and HA-Rac1<sub>Val-12</sub> may explain the greater association of RhoGDI with HA-Rac1 than with HA-Rac1<sub>Val-12</sub>.

Based on previous reports (21, 35, 36), we expected that complexes of HA-Rac1/RhoGDI would dissociate when HA-Rac1 converts to the GTP-bound form and translocates to cell junctions following mAChR activation. Surprisingly, however, M<sub>3</sub> mAChR activation does not detectably alter the co-precipitation of RhoGDI with HA-Rac1 or HA-Rac1<sub>Val-12</sub>. Complexes of HA-Rac1/RhoGDI may not dissociate in carbachol-treated cells because HA-Rac1 that is complexed with RhoGDI may not readily convert to the GDP-bound form following mAChR activation. This proposition is supported by reports that interactions with RhoGDI inhibit the dissociation of GDP from Rho family members (reviewed in Ref. 21). Overexpression of RhoGDI also inhibits p21-associated kinase activation, consistent with RhoGDI sequestering Rac1 and inhibiting its activity (37). Furthermore, RhoGDI inhibits mAChR-mediated responses involving RhoA, suggesting that the binding of RhoA by RhoGDI blocks the mAChR-mediated activation of RhoA (36). These findings support the possibility that HA-Rac1 and HA-Rac1<sub>Val-12</sub> are most responsive to mAChR-mediated signals when the GTPases are complexed with proteins other than RhoGDI. Effects of M<sub>3</sub> mAChR Activation on Rac1/IQGAP1 Complexes—Activation of mAChR increases the co-precipitation of HA-Rac1 with proteins that migrate in the region of IQGAP1 on SDS-PAGE gels (Fig. 8B), suggesting that mAChR activation increases the association of HA-Rac1 with IQGAP1. Our finding that mAChR activation causes HA-Rac1 to co-localize with IQGAP1 at some cell junctions is also consistent with mAChR activation causing Rac1 to associate with IQGAP1.

The co-precipitation of actin and an unidentified 58-kDa protein with either HA-Rac1<sub>Val-12</sub> or HA-Rac1 is probably caused by the interactions of these proteins with IQGAP1. A previous study found that actin incorporates into complexes of IQGAP1 and Cdc42 when actin polymerization is induced by phalloidin (33). We reported previously that stress fibers form within 90 min of mAChR activation in CHO-m<sub>3</sub> cells (14), which are the parental cells of the lines used in this study. It is
intriguing to speculate that the mAChR-mediated formation of stress fibers stimulates the incorporation of actin into complexes of HA-Rac1/IQGAP1 and HA-Rac1Val-12/IQGAP1. How-

**FIG. 9.** Activation of mAChR causes the Rac1-dependent phosphorylation of c-Jun and the Rac1-independent phosphorylation of ERK-1 and ERK-2. A, phosphorylation of c-Jun was measured after equal numbers of cells from the indicated sublines were incubated with 10 μM carbachol for the specified times. A representative autoradiograph from three independent experiments is shown. Densitometry of the autoradiographs was performed (B) to quantify the level of c-Jun phosphorylation induced by mAChR activation. Values shown are the means ± 1 S.E. from three independent experiments. C, an equal number of cells from the indicated sublines were incubated with 10 μM carbachol for the specified times and lysed. The lysates were immunoblotted with an antibody that recognizes the phosphorylated forms of ERK-1 and ERK-2 (lanes 1–7) and an antibody that recognizes ERK-1 and ERK-2 (lanes 8–14). A representative immunoblot from three independent experiments is shown. Densitometry of the immunoblots was performed to quantify the phosphorylation of ERK-1 (D) and ERK-2 (E) induced by mAChR activation. Values shown are the means ± 1 S.E. from three independent experiments.

**Fig. 10.** A model depicting changes in Rac1 and IQGAP1 induced by M₃ mAChR activation. A, before mAChR activation, the GDP-bound form of Rac1 associates with RhoGDI in the cytoplasm, whereas IQGAP1 accumulates at the plasma membrane. The GTP-bound form of Rac1, which may be present because of stimulatory signals other than mAChR activation, associates with IQGAP1/calmodulin complexes at membrane ruffles. B, within 30 min of stimulating M₃ mAChR, which activates Gₛ/₁₁ proteins, Rac1 is converted to the GTP-bound form and accumulates at the junctions of cells that have undergone cell-cell compaction. Rac1-GTP apparently can accumulate at cell junctions either in the absence or presence of IQGAP1. However, IQGAP1 apparently accumulates at cell junctions only in the presence of Rac1-GTP. Our results indicate that mAChR activation must induce further changes in Rac1, in addition to its conversion to the GTP-bound form and association with IQGAP1, to cause Rac1 to accumulate at cell junctions. Asterisks represent these additional uncharacterized changes in Rac1-GTP induced by mAChR activation. The inability of mAChR activation to alter Rac1/RhoGDI complexes suggests that the majority of these complexes may be relatively insensitive to mAChR-mediated signals. C, prolonged mAChR activation results in the departure of Rac1 and IQGAP1 from cell junctions and the decompaction of the cells. Rac1 associates with actin and an unidentified 58-kDa protein in complexes of IQGAP1 and calmodulin.
ever, we did not observe the immunolocalization of these complexes to stress fibers in cells treated with carbachol for 0–90 min (data not shown). Alternatively, it is more likely that mAChR-mediated changes in cortical actin are associated with the incorporation of actin into IQGAP1 complexes. This possibility is consistent with the localization of IQGAP1 in membrane protrusions, where IQGAP1 might interact with cortical actin.

The mAChR-mediated changes in the shape of membrane protrusions exhibiting high levels of IQGAP1 may involve remodeling of cortical actin. Prior to mAChR activation, IQGAP1 accumulates in spherical membrane protrusions that are prominent on most m3Zeo-2 and m3WT Rac cells. Although these structures may be unique to the CHO-m3 sublines examined in this study, published photomicrographs suggest that other cell types may have similar spherical membrane protrusions exhibiting high levels of IQGAP1 (see Fig. 7D in Ref. 8). The disappearance of these spherical membrane protrusions on cells that are spreading because of migration, HA-Rac1Asn-17 expression, or mAChR activation may result from reorganization of the cortical actin cytoskeleton. Remodeling of cortical actin may also cause these surface structures to change from a spherical form to the more punctate form that is detectable on the cells after prolonged mAChR activation.

Effects of M₃ mAChR Activation on the Translocation of Rac1 and IQGAP1 to Cell Junctions—Several of our findings indicate that conversion to the GTP-bound form and/or association with IQGAP1 may promote the localization of Rac1 at cell junctions after mAChR activation. Activation of mAChR induces greater localization of constitutively active HA-Rac1Val-12 than HA-Rac1 at cell junctions. Constitutively active HA-Rac1Val-12 is in the GTP-bound form more often than HA-Rac1 and exhibits greater association with IQGAP1 than does HA-Rac1. Furthermore, dominant negative HA-Rac1Asn-17, which neither binds GTP nor associates with IQGAP1, does not localize at cell junctions after mAChR activation. Interestingly, IQGAP1 does not co-localize with HA-Rac1 or HA-Rac1Val-12 at all cell junctions. This finding indicates that the mAChR-induced localization of Rac1 at cell junctions may depend more on the conversion of Rac1 to the GTP-bound form rather than on the association of Rac1 with IQGAP1.

Intriguingly, our studies indicate that further mAChR-mediated changes in Rac1, in addition to conversion to the GTP-bound form and potential interactions with IQGAP1, are required for the localization of Rac1 at cell junctions. If the GTP-bound state and association with IQGAP1 are the only requirements for the junctional localization of Rac1, then HA-Rac1Val-12 would be expected to accumulate at cell junctions in the absence of mAChR activation. However, HA-Rac1Val-12 only accumulates at cell junctions after mAChR activation. These findings support our model that mAChR activation promotes the junctional localization of Rac1 by converting Rac1 to the GTP-bound form (and potentially by enhancing IQGAP1 association) and by inducing additional uncharacterized GTP-independent changes in Rac1 (Fig. 10B).

The homophilic engagement of cadherins may provide additional signals that promote the localization of both Rac1 and IQGAP1 to cell junctions following mAChR activation. Rac1 and IQGAP1 are recruited to cell junctions when E-cadherin adhesive activity is experimentally increased in several different cell types (38–41). We reported previously that several forms of cadherins, including E-cadherin, are expressed by CHO-m3 cells (11). Transient cadherin-mediated adhesion induced by mAChR activation in CHO-m3 cells (11) temporally correlates with the transient junctional localization of HA-Rac1, HA-Rac1Val-12, and IQGAP1 induced by mAChR activation in the CHO-m3 sublines used in this study. Thus, signals arising from homophilic interactions between cadherins may promote the junctional localization of HA-Rac1, HA-Rac1Val-12, and IQGAP1 following mAChR activation.

Homophilic interactions of cadherins following mAChR activation may also promote Rac1 activation. The homophilic ligation of transfected C- or E-cadherin activates Rac1 within 30–60 min in CHO cells, as indicated by the increased precipitation of Rac1 with GST-PBD (40, 42). Rac1 activity also increases 30 min after E-cadherin-mediated adhesion of MDCKII cells is induced by the Ca²⁺ switch technique (41). Although these studies suggest differences in the kinetics of Rac1 activation induced by cadherin ligation and by mAChR activation, both mAChR- and cadherin-mediated signals may promote the observed activation and junctional localization of HA-Rac1 and HA-Rac1Val-12 in m3WT Rac and m3CARac cells, respectively.

Although mAChR activation does not cause dominant negative HA-Rac1Asn-17 to accumulate at cell junctions, it causes HA-Rac1Asn-17 to translocate from the juxtanuclear region to a more diffuse distribution in m3DN Rac cells. Because HA-Rac1Asn-17 cannot be converted to the GTP-bound form, mAChR activation must induce a GTP-independent change in HA-Rac1Asn-17, which causes it to adopt a more diffuse distribution. Activation of mAChR may alter a protein that associates with HA-Rac1Asn-17, resulting in translocation of a protein complex containing HA-Rac1Asn-17. Alternatively, mAChR activation may release HA-Rac1Asn-17 from its association with a GEF in the juxtanuclear region, resulting in the diffusion of HA-Rac1Asn-17 from this area. HA-Rac1Asn-17 may not accumulate at cell junctions after mAChR activation because HA-Rac1Asn-17 cannot convert to the GTP-bound form, as discussed above.

The inability of IQGAP1 to accumulate at cell junctions in m3DN Rac cells following mAChR activation suggests that the junctional localization of IQGAP1 requires active Rac1 at these junctions. Our observation that IQGAP1 localizes to junctions only in the presence of HA-Rac1 or HA-Rac1Val-12 (Fig. 7) provides further evidence that active Rac1 must be present at junctions for IQGAP1 to accumulate at these regions.

Consequences of the mAChR-mediated Activation of Rac1—Our results provide evidence that Rac1 activation contributes to cadherin-mediated adhesion induced by M₃ mAChR activation, which was reported previously to occur in lung carcinoma cells (15) and CHO-m3 cells (11). The mAChR-mediated localization of HA-Rac1, HA-Rac1Val-12, and IQGAP1 at the junctions of CHO-m3 cells occurs when cadherin-mediated adhesion is strongest (11), and the disappearance of these proteins from junctions occurs when cadherin-mediated adhesion is diminishing (11). The reduced mAChR-mediated compaction of m3DN Rac1 cells expressing dominant negative HA-Rac1Asn-17 is also consistent with the participation of Rac1 in cadherin-mediated adhesion (reviewed in Refs. 7, 9, and 43).

Our findings are consistent with several aspects of a model recently presented by Fukata and Kaibuchi, which proposes that E-cadherin-mediated adhesion is strengthened by complexes of E-cadherin/β-catenin/α-catenin and diminished by complexes of E-cadherin/β-catenin/IQGAP1 (reviewed in Ref. 43). According to this model, the presence of GTP-bound Rac1 at cell junctions causes IQGAP1 to dissociate from E-cadherin/β-catenin/IQGAP1 complexes and form Rac1-GTP/IQGAP1 complexes instead. These events promote the formation of E-cadherin/β-catenin/α-catenin complexes, resulting in increased E-cadherin-mediated adhesion (reviewed in Ref. 43). In agreement with this model, we found that complexes of HA-
Rac1/IQGAP1 and HA-Rac1Val-12/IQGAP1 accumulate at cell junctions during mAChR-induced cell-cell compaction. The absence of HA-Rac1A61-75 and IQGAP1 from cell junctions in m3DN Rac cells is also consistent with this model.

Our results also indicate that Rac1 activation is required for the mAChR-dependent activation of JNK but not ERK-1 or ERK-2. It was reported previously that JNK is maximally active 40 min after exposing CHO-m3 cells to carbachol (12), similar to our findings. It is interesting that the mAChR-mediated rise in JNK activity in CHO-m3 cells temporally correlates with the mAChR-mediated translocation of Rac1 to cell junctions. Whether this temporal correlation between JNK activation and Rac1 translocation to cell junctions is a causal or coincidental relationship remains to be determined. It is conceivable that Rac1 translocation stimulates JNK activity by affecting JNK signaling complexes located at the cell surface. This possibility is supported by studies indicating that JNK associates at the cell surface with complexes containing scaffolding proteins, cell surface receptors, and regulators of Rho family members (44–46).

Conclusions—Our results indicate that Rac1 participates in M3 mAChR-induced cell-cell compaction and JNK activation in CHO-m3 cells. The activation of Rac1 by M3 mAChR involves the conversion of Rac1 to the GTP-bound form, the translocation of Rac1 and IQGAP1 to cell junctions, and the association of actin with Rac1 protein complexes. Each of these events may be affected by interactions between Rac1 and IQGAP1. The association of Rac1 with IQGAP1 may promote the conversion of Rac1 to the GTP-bound form or help maintain Rac1 in the GTP-bound form. Interactions between activated Rac1 and IQGAP1 may also promote the accumulation of these proteins at cell junctions. The interaction of IQGAP1 with a wide variety of proteins supports the possibility that IQGAP1 complexes bring together Rac1 and other proteins, such as actin, in several M3 mAChR-mediated signaling pathways.

Our results also indicate that M3 mAChR activation induces additional GTP- and IQGAP1-independent changes in Rac1. This conclusion is based on our finding that mAChR activation induces the redistribution of HA-Rac1A61-75, which neither binds GTP nor associates with IQGAP1, as well as the translocation of HA-Rac1Val-12, which is already in the GTP-bound form and associated with IQGAP1 before mAChR activation. These events are probably caused by M3 mAChR-dependent changes in proteins that are associated with HA-Rac1A61-75 or HA-Rac1Val-12. However, the identities of these Rac1-associated proteins and their responses to M3 mAChR activation remain to be determined.

Acknowledgments—We thank Drs. Henry Puhl, Lisa May, Robert Aronstam, and John Noti at the Guthrie Research Institute for assistance in the generation of the CHO-m3 sublines used in this study. We also thank Drs. Shinya Kuroda, Gary Bokoch, and Andrew Kraft for generous gifts of plasmids.

REFERENCES
1. Schmitz, U., Thommes, K., Becker, I., Wagner, W., Sachinidis, A., Dusing, R., and Vetter, H. (2001) J. Biol. Chem. 279, 22003–22010.
2. Wassmann, S., Laufs, U., Baumel, A., Muller, K., Konkol, C., Sauer, H., Bohm, M., and Nickenig, G. (2001) Mol. Pharmacol. 59, 646–654.
3. Van Eck, J., Arrell, D., Foster, D., Strauss, J., Heimosen, T., Furmaniak, J., Kozma, R., Sarner, S., Ahmed, S., and Lim, L. (1996) Nature 379, 837–840.
4. Van Eys, J., Arrell, D., Foster, D., Strauss, J., Heimosen, T., Furmaniak, J., Kozma, R., Sarner, S., Ahmed, S., and Lim, L. (1996) Nature 379, 837–840.
5. Zeng, H., Zhao, D., and Mukhopadhyay, D. (2002) J. Biol. Chem. 277, 628–634.
6. Kozma, R. (1999) J. Biol. Chem. 274, 6346–6349.
7. Kozma, R., Sarner, S., Ahmed, S., and Lim, L. (1999) J. Biol. Chem. 274, 630–634.
8. Wylie, P., Chelli, R., and Blank, J. (1999) Biochem. J. 338, 619–628.
9. Naarala, J., Tervo, P., Loikkanen, S., and Svolainen, K. (1997) Life Sci. 60, 1963–1975.
10. Strassheim, D., Strassheim, D., Varkony, K., Puhl, H., Phelps, S., Porter, R., Aronstam, R., Noti, J., and Williams, C. L. (1999) J. Biol. Chem. 274, 7069–7072.
11. Shafer, S. (1999) Cell Biol. 121, 63–65.
12. Van Zwiert, P., and Doos, H. (1995) Cardiovasc. Drugs Ther. 9, 159–167.
13. White, M. (1995) J. Allergy Clin. Immunol. 95, 1065–1068.
14. Herdegen, T., and Waetzig, V. (2001) Oncogene 20, 2424–2437.
15. Hart, M. J., Callow, M., Souza, B., and Polakis, P. (1996) Biochem. J. 327, 4753–4763.
16. Machesky, L. M. (1998) Curr. Biol. 8, R226–R230.
17. Strassheim, D., Porter, R., Phelp, S., and Williams, C. L. (2000) J. Biol. Chem. 275, 6699–6702.
18. Ren, T. X., Kioos, D. S. W., and Schwartz, M. a. (1999) EMBO J. 18, 578–585.
19. Adler, V., Poholecke, A., Wagner, F., and Kuhl, A. S. (1996) J. Biol. Chem. 271, 17001–17005.
20. Hart, M. J., Callow, M., Souza, B., and Polakis, P. (1996) EMBO J. 15, 2997–3005.
21. Rogers, M., and Strehler, E. E. (1996) in Calmodulin: Guidebook to the Calcium-binding Proteins (Celio, M., Pauls, T., and Schwaller, B., eds) pp. 34–40, Oxford University Press, New York.
22. Ho, Y., Joyal, J., Li, Z., and Sacks, D. B. (1999) J. Biol. Chem. 274, 446–470.
23. Fleming, I. E., Elliot, C. M., and Exton, J. M. (1996) J. Biol. Chem. 271, 33067–33072.
24. Buchanan, F. G., Elliot, C. M., Gibbs, M., and Exton, J. M. (2000) J. Biol. Chem. 275, 9742–9748.
25. Valentin, F., Bueb, J., Capdeville-Atkinson, C., and Tschirhart, E. (2001) Cell Calcium 29, 409–415.
26. Hahn, A., Barth, H., Kress, M., Mertens, P., and Goppelt-Streebe, M. (2002) Biochem. J. 362, 33–40.
27. Zeng, H., Zhao, D., and Mukhopadhyay, D. (2002) J. Biol. Chem. 277, 4003–4009.
28. Ericsson, J., Cerione, R. A., and Hart, M. J. (1997) J. Biol. Chem. 272, 24443–24447.
29. Dumotier, M., Hocht, P., Minter, U., and Faix, J. (2000) J. Cell Sci. 113, 2253–2265.
30. Bokoch, G. M., Bohl, B., and Chuang, T. H. (1994) J. Biol. Chem. 269, 31674–31679.
31. Guo, M., Goreme, I., Read, P., Jia, T., Nakamoto, R., Sonomyo, A. Y., and Sonomyo, A. P. (2001) Am. J. Physiol. Cell Physiol. 281, C257–C269.
32. Lu, W., and Mayer, B. J. (1999) Oncogene 18, 797–806.
33. Kuroda, S., Fukuta, M., Nakagawa, M., Fuji, K., Nakamura, T., Okubo, T., Izawa, I., Nagase, T., Noma, N., Tani, H., Shoji, I., Matusuba, Y., Yonehara, S., and Kabiuchi, K. (1998) Science 281, 832–835.
34. Li, Z., Kim, S., Higgins, J., Brenner, M., and Sacks, D. B. (1999) J. Biol. Chem. 274, 37885–37892.
35. Kovacs, E., Ali, R., McCormack, A., and Yap, A. (2002) J. Biol. Chem. 277, 6708–6718.
36. Nakagawa, M., Fukuta, M., Yamagata, M., Itoh, N., and Kabiuchi, K. (2001) J. Cell Sci. 114, 1829–1838.
37. Aebischer, P., Kuan, C., Kenneson, N., Keljari, N., Haydar, T., Mordes, J., Appel, M., Rossini, A., Jones, S., Flavell, R., Rakic, P., and Davis, R. J. (2001) Genes Dev. 15, 2421–2432.