Lovastatin Inhibits Prolactin-Induced Nb2 Cell Mitogenesis and Milk Product Synthesis in Mouse Mammary Gland Explants (44162)

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Abstract. **The p21^{ras} protein has been shown to be active in many growth factor signaling systems, including that of prolactin (PRL). In our studies, the main objective was to examine further the involvement of *ras* in prolactin-stimulated mitogenic and metabolic processes. We used the farnesylation inhibitor lovastatin to block effectively *ras*-dependent signaling in Nb2 cells, a pre-T lymphoma cell line, and mammary gland explants derived from 12- to 14-day pregnant mice. Lovastatin completely inhibited PRL-induced Nb2 cell mitogenesis at 1 μm. In the mammary gland, lovastatin at 0.1 μm inhibited the PRL stimulation of lipid and lactose synthesis; at 2 μm, lovastatin abolished the PRL stimulation of casein production. When p21^{ras} was immunoprecipitated from lovastatin (25 μm)-treated Nb2 cells and mammary gland explants, the unfarnesylated (inactive) form of *ras* was shown to be redistributed from the cell membrane to the cytosolic compartment of the cell. This suggests that the anchoring of *ras* to the cell membrane is essential for its action in prolactin signal transduction. Thus, in addition to the well-known active participation of *ras* in mitogenic growth factor signaling, the presence of functional *ras* also appears necessary for the PRL stimulation of specific metabolic processes involved in lactation.** [P.S.E.B.M. 1997,Vol 216]

In recent years, it has become apparent that many growth factors share similar signal transduction pathways. The molecules involved in prolactin signaling are rapidly being identified and have been reported to have some similarities to other growth factor–mediated signaling systems. The prolactin receptor (PRLR) is a member of the superfamily of growth factor receptors known as cytokine receptors (1, 2). Three PRLR isoforms (short, intermediate, and long) have been identified and differ only in the length of their cytoplasmic domains (2, 3). Two primary models employed in the study of prolactin (PRL) signaling mechanisms are Nb2 cells, a rate pre-T lymphoma cell line, and the mouse mammary gland explant system. The Nb2 cell line, the only cell line known to express the intermediate form of PRLR (3), is dependent on lactogenic hormone stimulation for continuous progression through the cell cycle (4).

Most of the research studies focusing on the mitogenic signaling for PRL have been accomplished employing the Nb2 cell line. A host of kinases that are activated in response to PRL have been identified; these include protein kinase C (5), RAF-1 (6), members of the s6 kinase family (7), MAP kinases (8), JAK kinases (9–11), and *src* kinases (12). Also in Nb2 cells, PRL stimulates the activation of various other signaling molecules, which include SHC, GRB, and SOS (13), as well as vav (14) and pim (15). The principal PRL signaling studies accomplished with the mammary gland have been those that identified the activation of the receptor-associated tyrosine kinase JAK 2 (9).

Additional signaling molecules mutual to many mitogenic growth factor signaling pathways are the GTPase p21^{ras} family of proteins (16, 17). The *ras* proto-oncogene product has been shown to be an integral part of the MAP kinase signaling pathway (8, 18, 19). Activated *ras* can alter MAP kinase activity by directly binding to and activating

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the serine/threonine kinase RAF-1 (8). The activity of *ras* is dependent upon its GTP/GDP-bound state. Previous studies with Nb2 cells reported that PRL stimulates *ras* by inducing its shift from the inactive GDP-bound to the active GTP-bound state (8, 13). Until now there has been little emphasis on the possible role of *ras* in PRL-mediated signaling of differentiative processes in the mammary gland. Furthermore, there is a lack of pharmacological evidence that suggests a physiological function for *ras* activation by PRL.

ras proteins undergo the posttranslational modification of farnesylation (20), a crucial process for anchoring *ras* in the cell membrane. Ultimately, only the farnesylated form of *ras* appears to be involved in growth factor signal propagation (21). In the present study, we tested the hypothesis that the mitogenic and metabolic effects of PRL involve the "*ras* signaling pathway." This was accomplished by employing lovastatin, a specific inhibitor of farnesylation (18, 19), to block *ras*-mediated signaling. Accordingly, the effect of lovastatin on PRL-stimulated lipid, lactose, and casein synthesis in mouse mammary gland explants, and mitogenesis in Nb2 cells was tested. Finally, $p21^{ras}$ in Nb2 cells and mammary gland explants was isolated to explore possible changes in both its farnesylation state and cellular distribution. The data presented here suggest the need for functional *ras* in both mitogenic and metabolic PRL signaling pathways.

Materials and Methods

Materials. Stocks of Nb2 cells were provided by C. T. Beer (Cancer Control Agency, Vancouver, British Columbia, Canada). Midpregnant (10–14 days of pregnancy) Swiss-Webster mice were purchased from Harlan Laboratories Inc. (Indianapolis, IN). Ovine PRL (NIH-P-S-17) was a gift from NIDDK. Other materials were purchased from the following sources: cortisol from Charles Pfizer and Co. (New York, NY); Medium 199 Earle's salts, Hanks' balanced salt solution, Fischer's medium, fetal calf serum, and horse serum from GIBCO Laboratories (Grand Island, NY); porcine insulin, penicillin, and streptomycin from Eli Lilly Co. (Indianapolis, IN); [4,5-^{3}H]leucine (53 Ci/m*M*), [1-^{14}C]acetate (59 m*M*/mmol), and [5,6-^{3}H]glucose (66.6 Ci/mmol) from New England Nuclear Corp. (Boston, MA); cellulose TLC plates and 2-propanol from Fisher Chemical Co. (Waltham, MA); rat monoclonal anti-*ras* antibody (clone Y13-259, Cat. #OP01) from Oncogene Science (Cambridge, MA); anti-rat immunoglobulin G agarose (Cat. #A-6524) from Sigma Chemical Co. (St. Louis, MO); anti-rat IgG HRP conjugate (Cat. #NA 932) and enhanced chemiluminescence reagents from Amersham (Arlington Heights, IL); PVDF membranes from Scheicher & Schuell (Keene, NH); and lovastatin from Merck (Rahway, NJ).

Nb2 Cell Culture. A PRL-dependent Nb2 cell line was grown in Fischer's medium supplemented with 10% fetal calf serum and 10% horse serum, 1.0×10^{-4} *M* 2-mercaptoethanol, 100 IU/L penicillin, and 100 μg/ml streptomycin, as previously described (4). Incubation for 24 hr in serum-free Fischer's media effectively arrests growth at the G0/G1 phase of the cell cycle (22). Once serum depletion is complete, cells are harvested and treated with oPRL.

Nb2 Mitogenesis Assay. Nb2 cells arrested at G0/G1 in the cell cycle were resuspended in serum-free Fischer's media (stationary media) at 1×10^5 cells/ml. Cell aliquots of 800 μl were then distributed into 24-well plates, and increasing concentrations of lovastatin in 100 μl quantities were added. Next, either 5 ng/ml oPRL or "stationary media" were added in 100 μl aliquots to bring the total volume of each sample to 1 ml. Incubation of cells was carried out for 72 hr, after which cell number was determined using a Coulter counter.

Mouse Mammary Gland Explant Preparation. Mammary gland explants were prepared as previously described from mice that were pregnant 12–14 days (23). Mice were sacrificed by cervical dislocation; mammary glands were removed and placed in Hanks' balanced salt solution. Explants (3–6 mg each) from each of 8–10 animals were prepared and placed on siliconized lens paper in 60-mm petri dishes containing 6 ml of Medium 199 Earle's salts with 1 μg/ml insulin and 10^{-7} *M* cortisol. Next, tissues were incubated for 24 hr at 37°C under humidified 95% air/5% CO_2 atmosphere prior to experimentation. All studies involving the preparation of mouse mammary gland explants were performed in compliance with the regulations of the Animal Care and Use Committee of Wayne State University.

Mouse Mammary Gland Bioassays. After an initial 24-hr incubation, M199 media was aspirated from tissues and replaced with 4 ml of fresh M199 containing increasing concentrations of lovastatin and/or 1 μg/ml PRL. Incubations were then continued for an additional 24 hr, after which the incorporation of radioactive precursors into macromolecules was determined. In experiments involving the rate of lactose synthesis, mammary gland tissues were exposed to 0.5 μCi/ml [5,6-^{3}H]glucose for the last 2 hr of incubation in order to pulse label lactose. Labeled lactose was separated from labeled glucose on TLC plates and the radioactivity quantitated (24). For experiments in which the rate of casein synthesis was determined, [^{3}H]leucine (0.5 μCi/ml) was added to culture medium for the final 2 hr of incubation. Quantities of [^{3}H]leucine incorporated into the casein-rich phosphoprotein fraction were determined in a protein fraction isoelectrically precipitated at pH 4.6 (25). Finally, in experiments determining the rate of lipid synthesis, tissues were exposed to [^{14}C]acetate (0.5 μCi/ml) for the last 2 hr of incubation. Tissues were then weighed, homogenized in 400 μl of distilled water, and the lipids extracted by the method of Bligh and Dyer (26). Radioactivity in the organic layer was then quantitated. Results are expressed as disintegrations per minute per milligram wet tissue weight.

Nb2 Cell and Mammary Gland Explant Lysate Preparations. Both G1-arrested Nb2 cells (1×10^6/ml) and preincubated mouse mammary gland explants were

treated with or without 25 μ*M* lovastatin and incubated for 24 hr as described above. Samples were then washed twice with ice-cold phosphate-buffered saline–orthovanadate (1 m*M*). Nb2 cells were resuspended, at 4°C, in lysis buffer "A" (2.5×10^7 cells/ml) containing 2% NP4O, 10 m*M* Tris, 50 m*M* NaCl, 30 m*M* sodium pyrophosphate, 2.5 m*M* EDTA, 1 m*M* orthovanadate, 1 m*M* phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, pH 7.6. Mouse mammary gland explants were weighed, added to lysis buffer "A" (1:2, w/v) and disrupted with a ground-glass homogenizer. After 30 min on a rocking platform, lysates were centrifuged (100,000*g*) for 30 min at 4°C. The resulting supernatants were used for immunoprecipitation and subsequently quantitated for total levels of $p21^{ras}$ in the prepared samples.

Nb2 Cell and Mammary Gland Explant Cellular Fractionation. Nb2 cells and mouse mammary gland explants were treated with lovastatin and washed using methods identical to those described in Lysate preparations above. However, both Nb2 cells (2.5×10^7) and mouse mammary gland explants (1:2, w/v) were resuspended in buffer "B" containing 20 m*M* Hepes, 40 m*M* β-glycerophosphate, 2 m*M* $MgCl_2$, 1 m*M* orthovanadate, 1 m*M* phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 2.5 m*M* EDTA, pH 7.6. Nb2 cells were lysed for 5 min at 4°C, while mammary gland explants were disrupted using a polytron homogenizer and incubated at 4°C for 1 hr. Next, both sample preparations were centrifuged (100,000*g*) at 4°C for 30 min. The supernatant (cytosolic fraction) was transferred to a separate tube and the pellet (cell membrane fraction) was resuspended in buffer "B" at a volume equal to that of the supernatant. Finally, 2% NP40 was added to each sample and incubations were performed for 10 min at 4°C for Nb2 cells and 1.5 hr for explant samples. Subsequently, all samples were centrifuged (100,000*g*) for 30 min at 4°C. The resulting supernatants were also used for immunoprecipitation and represent membrane associated and cytosolic $p21^{ras}$ in prepared samples.

Immunoprecipitation and Immunoblot Conditions. After preparation of lovastatin- or control-treated Nb2 and mammary gland total lysate, cell membrane suspensions, and cytosolic samples, 1-ml aliquots of each preparation were precleared with 80 μl of anti-rat immunoglobulin G agarose. Samples were centrifuged at 200*g* and supernatants were immunoprecipitated with a monoclonal rat anti-*ras* antibody (3 μg/ml) overnight at 4°C. After antigen-antibody complexes were isolated by addition of 100 μl of anti-rat protein A agarose, proteins were washed twice with buffer "B" and solubilized by heating at 95°C in 70 μl of SDS-Laemmli sample buffer (27). Samples were separated by SDS-PAGE (15%–25% linear gradient) under reducing conditions and transferred to PVDF membranes. Membranes were probed with anti-*ras* antibody (10 ml at 1.5 μg/ml) for 1 hr followed by treatment with anti-rat IgG HRP conjugate (20 ml at 1:1000 dilution for 30 min). Detection was accomplished by incubation with enhanced chemiluminescence reagents and exposure to x-ray film.

Statistical comparisons were made with Student's *t* test for comparing two means, or with an analysis of variance followed by Scheffe's test for multiple comparisons. Significant differences ($P < 0.05$) are indicated by asterisks in the figures.

Results

In initial experiments, the effect of increasing concentrations of lovastatin on PRL-induced mitogenesis was determined in G1-arrested Nb2 cells. Figure 1 shows that, after a 72-hr culture, PRL-stimulated proliferation is reduced by about 90% when lovastatin levels exceed 1 μ*M*. Control cell numbers are significantly but modestly decreased (less than approximately 40%) by increasing lovastatin concentrations. This effect likely reflects an interference with the doubling of cell number which normally occurs in "control" cells cultured for 72 hr in the absence of serum. Also, DMSO, the solvent for lovastatin, was maintained below 0.05% and had no effect on cell number (data not presented). Thus, these studies support a physiological role for $p21^{ras}$ in the PRL stimulation of mitogenesis in Nb2 cells, as suggested from the results of earlier reports (8, 13).

In further experiments, the effect of lovastatin on the PRL stimulation of milk product synthesis in cultured mouse mammary tissues was determined. As seen in Figure 2, lovastatin at concentrations of 2 μ*M* or higher abolished PRL-induced casein synthesis without affecting the baseline

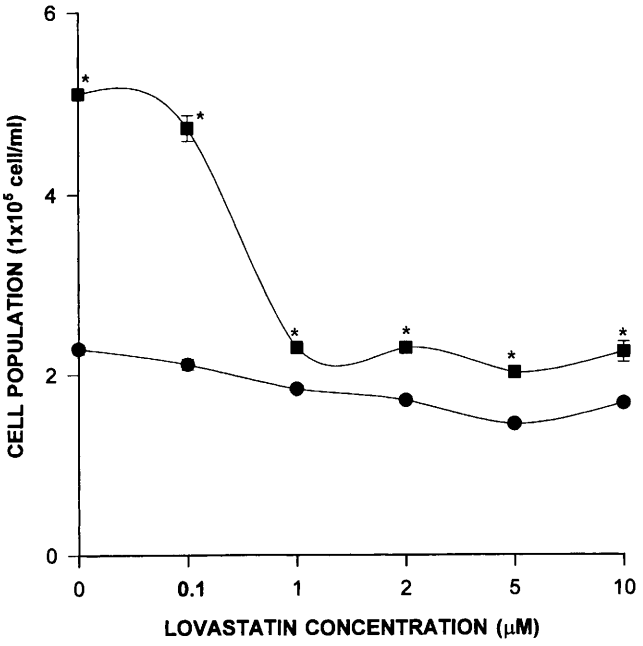

Figure 1. The effects of lovastatin on PRL-stimulated Nb2 mitogenesis. Nb2 cells (1×10^5/ml) arrested in G1 phase were treated with increasing levels of lovastatin in the presence or absence of 5 ng/ml oPRL. After incubation for 72 hr, cell population was determined. ●, control; ■, PRL-treated cells. Values represent the mean ± SEM for six observations.

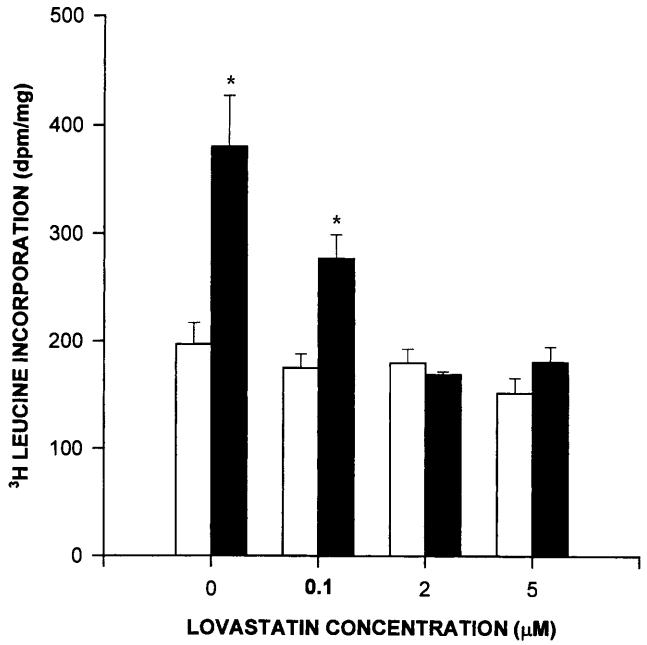

Figure 2. The effect of lovastatin on PRL-stimulated casein synthesis. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} cortisol. After preincubation, increasing concentrations of lovastatin were added in the absence or presence of 1 μg/ml oPRL followed by a second 24-hr incubation. Casein synthesis was then determined as described in Materials and Methods. Values represent the mean ± SEM for six observations. □, control; ■, PRL-treated.

production of these milk proteins. Figure 3 shows the effect of lovastatin on the PRL stimulation of lactose synthesis. At 0.1 μ*M*, lovastatin attenuated the PRL effect on lactose synthesis, while higher lovastatin concentrations abolished the PRL response. All lovastatin concentrations (0.1–5 μ*M*) significantly reduced, by about 30%, the basal rate of [^{3}H]glucose incorporation into lactose. When testing the effect of lovastatin on the PRL stimulation of milk lipid synthesis, all lovastatin concentrations between 0.1 and 5 μ*M* abolished the PRL response and significantly reduced (by approximately 50%) the extent of [^{14}C]acetate incorporation into lipids (Fig. 4). These studies clearly show that lovastatin effectively abolishes the stimulatory effect of PRL on the synthesis of multiple milk products in cultured mammary tissues.

In a subsequent series of experiments, we investigated the mechanisms by which lovastatin inhibits p21^{ras}-mediated PRL signaling in both Nb2 cells and mouse mammary gland explants. Briefly, Nb2 cells and explants were treated with or without lovastatin; then total lysate, cytosolic, and cell membrane fractions were prepared as described in Materials and Methods. Proteins in the various cell fractions were immunoprecipitated with anti-*ras* antibody, separated by PAGE, transferred to PDVF membranes, and blotted with monoclonal anti-*ras* antibody (Figs. 5 and 6). Figures 5 and 6 both show a shift in migration of *ras* in total lysates of Nb2 and mammary cells treated with lovastatin. Apparently, the lovastatin inhibition of farnesylation of *ras* causes a mobility shift to a slower migration in PAGE gels. Another feature in Figs. 5 and 6 is the redistribution of *ras* from the cell membrane to the cytoslic fraction of the cells in response to lovastatin in both Nb2 cells and mammary gland explants. Most apparent is the increased accumulation of unfarnesylated *ras* in the cytosolic fraction of lovastatin-treated cells. Using the NIH imaging program, increases of *ras* in the cytosolic fractions were 5.3-fold for the Nb2 cells and 4.2-fold for the mammary tissues. Also visible is the decrease in amounts of farnesylated *ras* associated with the cell membrane in lovastatin-treated cells. The specificity of the functioning of lovastatin in both the Nb2 cells and mammary tissues is thus established by these experiments.

Discussion

The results of these studies provide substantive evidence supporting the premise that p21^{ras} is an essential component of PRL signaling cascade in both Nb2 cells and mammary tissues. In all experiments, the farnesylation inhibitor lovastatin (18, 19) was employed to block *ras*-dependent signal propagation. The fact that lovastatin abolished PRL-stimulated Nb2 mitogenesis is compatible with earlier studies done by Rao *et al.* (8) and Erwin *et al.* (13), in which PRL was shown to stimulate GTP binding of p21^{ras} in Nb2 cells. Thus, our studies in concert with those from other laboratories clearly suggest a physiological role for *ras* in the signaling pathway for the PRL stimulation of mitogenesis.

Further studies suggest that *ras* is also involved in the

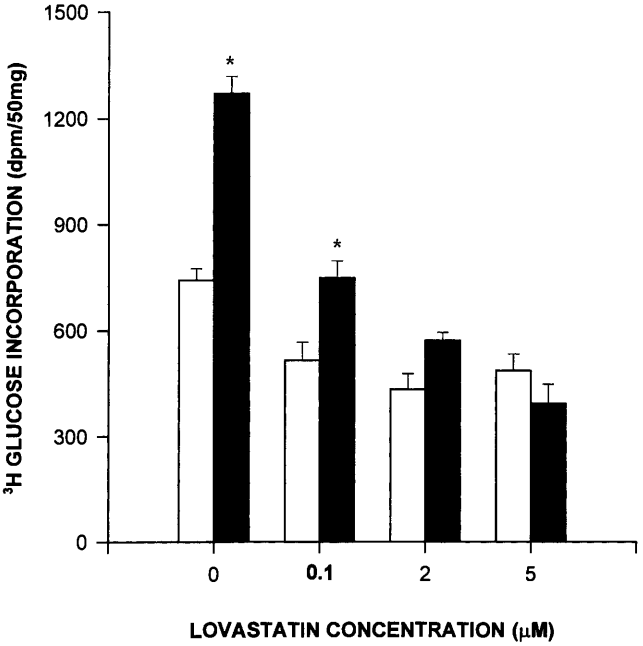

Figure 3. The effect of lovastatin on PRL-stimulated lactose synthesis. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} cortisol. After preincubation, increasing concentrations of lovastatin were added in the absence or presence of 1 μg/ml oPRL followed by a second 24-hr incubation. Lactose synthesis was then determined as described in Materials and Methods. Values represent the mean ± SEM for six observations. □, control; ■, PRL-treated.

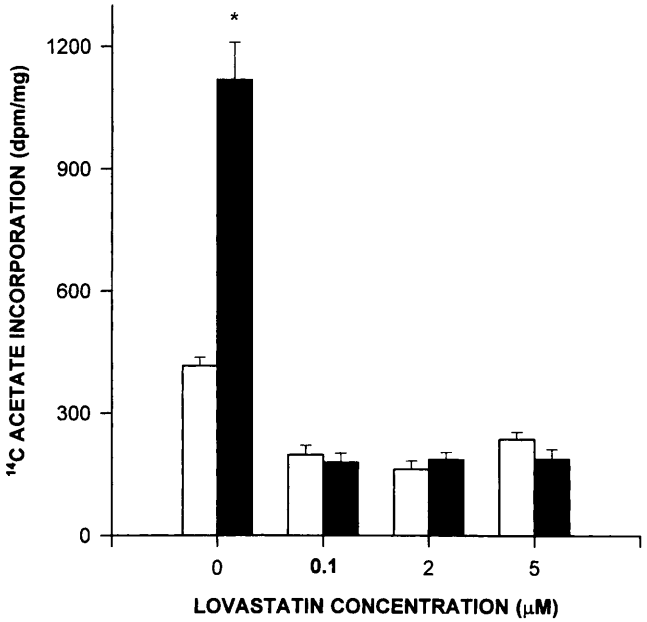

Figure 4. The effect of lovastatin on PRL-stimulated lipid synthesis. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} cortisol. After preincubation increasing concentrations of lovastatin were added in the absence or presence of 1 μg/ml oPRL followed by a second 24-hr incubation. Lipid synthesis was then determined as described in Materials and Methods. Values represent the mean ± SEM for six observations. □, control; ■, PRL-treated.

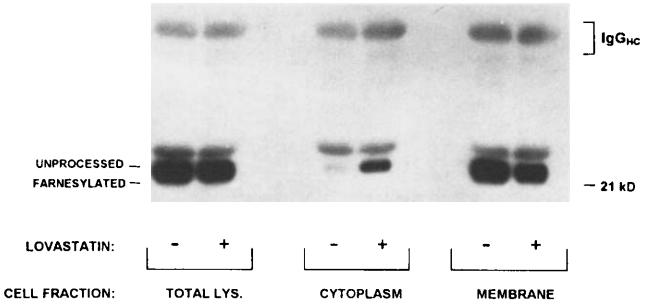

Figure 5. The effect of lovastatin on the membrane association of *ras* in Nb2 cells. Nb2 cells arrested in G1 phase were incubated for 24 hr in the presence of (25 μ*M*) lovastatin. *ras* was immunoprecipitated from total lysate, cytosolic, and cell membrane fractions using a monoclonal anti-*ras* antibody (Y13-259) and then immunoblotted with Y13-259. The two forms of *ras* are indicated by markers at the left. This blot is representative of three separate experiments.

signaling pathway for the PRL regulation of differentiative processes in the mammary gland. Lovastatin clearly abolishes all the stimulatory effects of PRL on the synthesis of milk products including caseins, lactose, and triglycerides. The fact that lovastatin decreases the basal rates of lactose and lipid synthesis suggests a requirement for farnesylated *ras* in maintaining basal rates of synthesis of these milk products; this is clearly not the case regarding the maintenance of the basal rate of casein synthesis. In any event, *ras* appears to be involved in the regulation of milk product synthesis in the mammary gland. It is also possible that *ras* may be involved in scores of other biological processes regulated by PRL.

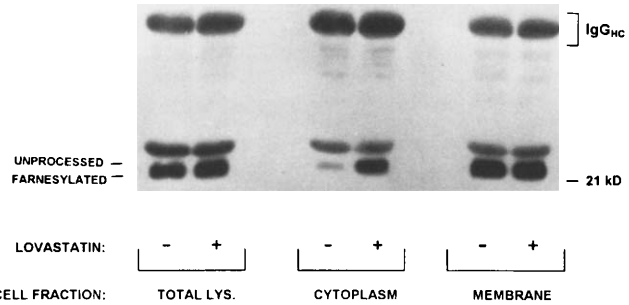

Figure 6. The effect of lovastatin on the membrane association of *ras* in mouse mammary gland explants. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} cortisol. After preincubation, lovastatin (25 μ*M*) was added followed by a second 24-hr incubation. *ras* was immunoprecipitated from total lysate, cytosolic, and cell membrane fractions using a monoclonal anti-*ras* antibody (Y13-259) and then immunoblotted with Y13-259. The two forms of *ras* are indicated by markers at the left. This blot is representative of three separate experiments.

The final series of experiments were designed to establish the specificity for the lovastatin interference with various PRL functions. When *ras* from lovastatin-treated Nb2 cells and mammary gland explants was examined, a number of changes were observed. First, the data suggest that a significant fraction of total cellular *ras* remained in the unfarnesylated form when Nb2 cells and mammary tissues were exposed to lovastatin. This is indicated by the shift of total *ras* to a slightly lower mobility. These results correlate with results from previous lovastatin studies (28, 29). Also observable in each blot was the appearance of a significant amount of unfarnesylated *ras* in the cytosolic fraction of cells and tissues treated with lovastatin. Unfarnesylated *ras* levels are extremely low under normal conditions in the cell (28, 29). From these observations the following conclusions can be made. First, lovastatin impairs the farnesylation of *ras,* thus causing its shifted mobility. Second, the unfarnesylated form of *ras* does not interact with the cell membrane and thus appears in the cytosolic fraction. Lastly, when *ras* is prevented from associating with the cell membrane, PRL effects on target cells are abolished. These studies suggest that p21^{ras} plays a physiological role in both the mitogenic and metabolic signaling pathways employed by PRL. We cannot, however, completely exclude other possible effects of lovastatin which may be responsible for the effects observed in these studies.

1. Morrison DK, Kaplan DR, Escobedo IA, Rapp UR, Roberts TM, Williams LT. Direct activation of the serine/threonine kinase Raf-1 through tyrosine phosphorylation by the PDGF B-receptor. Cell **58:**649–657, 1989.
2. Kelly PA, Boutin J, Jolicoeur C, Okamura H, Gagnon J, Edery M, Sirota M, Banville D, Dusanter-Fourt I, Djiane J. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell **53:**69–77, 1988.
3. Ali S, Pellegrini I, Kelly PA. A prolactin-dependent immune cell line (Nb2) expresses a mutant form of the prolactin receptor. J Biol Chem **266:**20110–20117, 1991.
4. Gout PW, Noble RL, Beer CT. Cultured Nb rat lymphoma cells in endocrine and cancer research. Biochem Cell Biol **64:**659–666, 1985.

5. Buckley AR, Montgomery DW, Kibler R, Putnam CW, Zukoski CF, Gout PW, Beer CT, Russell DH. Prolactin stimulation of ornithine decarboxylase and mitogenesis in Nb2 node lymphoma cells: The role of protein kinase C and calcium mobilization. Immunopharmacology **12:**37–51, 1986.
6. Clevenger CV, Torigoe T, Reed JC. Prolactin induces rapid phosphorylation and activation of prolactin receptor-associated Raf-1 kinase in a T-cell line. J Biol Chem **269:**5559–5565, 1994.
7. Carey GB, Liberti JP. Stimulation of receptor-associated kinase, tyrosine kinase, and map kinase is required for prolactin-mediated macromolecular biosynthesis and mitogenesis in Nb2 lymphoma. Arch Biochem Biophys **316:**179–189, 1995.
8. Rao Y, Buckley DJ, Buckley AR. Rapid activation of mitogen-activated protein Kinase and p21ras by prolactin and interleukin 2 in rat Nb2 node lymphoma cells. Cell Growth Differ **6:**1235–1244, 1995.
9. Campbell GS, Argetsinger LS, Ihle JN, Kelly PA, Rillema JA, Carter-Su C. Activation of JAK tyrosine kinase by prolactin receptors in Nb2 cells and mouse mammary gland explants. Proc Natl Acad Sci USA **91:**5232–5236, 1994.
10. Lebrun JJ, Ali S, Sofer L, Ulrich A, Kelly PA. Prolactin-induced proliferation of Nb2 cells induces tyrosine phosphorylation of the prolactin receptor and its associated tyrosine kinase JAK 2. J Biol Chem **269:**14021–14026, 1994.
11. Rui H, Kirken RA, Farrar WL. Activation of receptor associated tyrosine kinase JAK 2 by prolactin. J Biol Chem **269:**5364–5367, 1994.
12. Clevenger CV, Medaglia MV. The protein tyrosine kinase p59fyn is associated with the prolactin (PRL) receptor and is activated by PRL stimulation of T-lymphocytes. Mol Endocrinol **8:**674–681, 1994.
13. Erwin RA, Kirken RA, Malabarba MG, Farrar WL, Rui H. Prolactin activates RAS via signaling proteins Shc, growth factor receptor bound 2, and son of sevenless. Endocrinology **136:**3512–3518, 1995.
14. Clevenger CV, Ngo W, Sokol DL, Luger SM, Gerwirtz AM. VAV is necessary for prolactin-stimulated proliferation and is translocated into the nucleus of a T-cell line. J Biol Chem **27:**13264–13253, 1995.
15. Buckley AR, Buckley DJ, Leff MA, Hoover DS, Magnuson NS. Rapid induction of pim-1 expression by prolactin and interleukin-2 in rat Nb2 lymphoma cells. Endocrinology **136:**5252–5259, 1995.
16. Bogulski MS, McCormick F. Proteins regulating Ras and its relatives. Nature **366:**643–654, 1993.
17. Ymauchi K, Holt K, Pessin JE. Phosphatidylinositol 3-kinase functions upstream of Ras and Raf in mediating insulin stimulation of c-Fos transcription. J Biol Chem **268:**14597–14600, 1993.
18. Casey PJ, Moomaw JF, Zhang FL, Higgins JB, Thissen JA. Prenylation and G protein signaling. Recent Prog Horm Res **49:**215–238, 1994.
19. Cox AD, Graham SM, Solski PA, Buss JE, Der CJ. The carboxy-terminal CXXX sequence of G_i alpha, but not Rab5 or Rab11, supports Ras processing and transforming activity. J Biol Chem **268:**11548–11552, 1993.
20. Volker C, Miller RA, McCleary WR, Rao A, Poenie M, Backer JM, Stock JB. Effects of farnesylcysteine analogs on protein carboxyl methylation and signal transduction. J Biol Chem **266:**21515–21522, 1991.
21. Philips MR, Pillinger MH, Staud R, Volker C, Rosenfeld MG, Weissmann G, Stock JB. Carboxyl methylation of Ras-related proteins during signal transduction in neutrophils. Science **259:**977–980, 1993.
22. Rillema JA, Tarrant TM, Linebaugh BE. Studies on the mechanism by which prolactin regulates protein, RNA, DNA synthesis in Nb2 node lymphome cells. Biochim Biophys Acta **1014:**78–82, 1989.
23. Rillema JA. Early actions of prolactin on uridine metabolism in mammary gland explants. Endocrinology **92:**1673–1679, 1973.
24. Oppat CA, Rillema JA. Characteristic of the early effect of prolactin on lactose synthesis in mouse mammary gland explants. Proc Soc Exp Biol Med **188:**342–345, 1988.
25. Ashworth US. Rapid method for the determination of casein in milk by the dye binding method and for the detection of mastitis. J Dairy Sci **48:**537–543, 1965.
26. Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can J Biochem Physiol **37:**911–917, 1959.
27. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature **227:**680–685, 1970.
28. Hohl RJ, Lewis K. Differential effects of monoterpenes and lovastatin on ras processing. J Biol Chem **270:**17508–17512, 1995.
29. Xu X-Q, McGuire TF, Blaskovich MA, Sebti SM, Romero G. Lovastatin inhibits the stimulation of mitogen-activated protein kinase by insulin HIRcB fibroblasts. Arch Biochem Biophys **326:**233–237, 1996.