A Similar Pool of Cyclic AMP Phosphodiesterase in Xenopus Oocytes Is Stimulated by Insulin, Insulin-like Growth Factor 1, and [Val^{12}, Thr^{59}]Ha-ras Protein*

(Received for publication, June 7, 1988)

Susan E. Sadler‡ and James L. Maller
From the Department of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262

Pharmacological analysis of in vivo cAMP phosphodiesterase in Xenopus oocytes using the nonselective enzyme inhibitors 3-isobutyl-1-methylxanthine (IBMX), theophylline, and papaverine, demonstrated inhibition of insulin- and insulin-like growth factor-1-induced maturation at concentrations that were 17-60-fold lower than those required to inhibit progesterone-induced germinal vesicle breakdown. The abilities of the phosphodiesterase inhibitors to block the maturation response showed the same rank order of potencies for each hormone: papaverine > IBMX > theophylline. Insulin-induced oocyte maturation that was accelerated by 0.01 μM progesterone was also inhibited by low micromolar concentrations of IBMX, demonstrating that the accelerated time course was due to a synergistic potentiation of insulin action by progesterone. Both insulin-induced maturation and insulin-stimulated phosphodiesterase activity displayed similar sensitivities to inhibition by IBMX, suggesting that hormone-stimulated phosphodiesterase activity is required for the peptide hormone action. Furthermore, microinjection of the transforming ras gene product [Val^{12}, Thr^{59}]Ha induced oocyte maturation and stimulated oocyte phosphodiesterase activity by approximately 50%, and both of these actions were inhibited by IBMX. These results suggest that oocyte maturation induced by insulin, insulin-like growth factor 1, and transforming ras protein involves stimulation of a similar phosphodiesterase.

Hormone-induced maturation in Xenopus laevis oocytes is clearly a complex process that involves many cellular events which finally culminate in germinal vesicle breakdown (GVBD). One early event in progesterone-induced maturation is a decrease in cAMP levels that is at least partially due to inhibition of oocyte adenylate cyclase, as demonstrated by numerous investigators (1-7). Inhibition of oocyte membrane adenylate cyclase has been correlated with binding of steroid to a plasma membrane receptor (4) and slowing of guanine nucleotide exchange (5). Furthermore, in contrast to other cell systems in which the actions of inhibitory hormones on the adenylate cyclase system are mediated by a well characterized guanine nucleotide-binding protein, termed G₁ (or N₁), the inhibitory action of progesterone in the oocyte system involves a novel mechanism of action that is independent of the classic inhibitory protein (6-9). The induction of oocyte maturation by insulin and insulin-like growth factor 1 (IGF-1) has also been shown to involve inhibition of oocyte adenylate cyclase (10).

Recently, use of an in vivo assay system demonstrated that both insulin and IGF-1 stimulate cAMP phosphodiesterase activity in intact Xenopus oocytes (10). While both peptide hormones were shown to stimulate enzyme activity measured after microinjection of 200 μM [³H]cAMP, progesterone had no significant effect on enzyme activity. The dose-response curves for stimulation of phosphodiesterase by insulin and IGF-1 correlated with the ability of each peptide to inhibit oocyte adenylate cyclase activity, demonstrating a dual regulation of oocyte adenylate cyclase and phosphodiesterase by insulin and IGF-1. Furthermore, basai oocyte phosphodiesterase activity was shown to be inhibited by extracellular application of methylxanthines, and the IC_{50} values for IBMX and theophylline correlated with the ability of each drug to inhibit progesterone-induced oocyte maturation.

Microinjection of the ras oncogene product (p21) has been shown to induce oocyte maturation, and [Val^{12}]Ha is nearly 100-fold more potent than the protein encoded by the wild-type gene (11). In this initial report, p21 had no effect on oocyte adenylate cyclase activity and did not elicit measurable changes in oocyte cyclic AMP levels, although p21-induced germinal vesicle breakdown was inhibited by cholera toxin. These results suggested that the effects of p21 in the oocyte system are not strictly mediated by the adenylate cyclase system, an unexpected observation in view of previous studies using the budding yeast Saccharomyces cerevisiae, in which both yeast and mammalian ras proteins were shown to activate adenylate cyclase (12, 13).

The ras gene product has been implicated as a mediator of the actions of growth-promoting hormones. Induction of oocyte maturation by transforming p21 ras protein and insulin has been reported to be blocked by injection of monoclonal antibody Y13-259, suggesting that the action of insulin is ras-dependent (14). Similarly, microinjection of monoclonal antibody 6B7, an antibody directed against a synthetic peptide that corresponds to a highly conserved region of p21 required for oncogene function, was reported to inhibit insulin-induced maturation in Xenopus oocytes (15). These results suggest that p21 proteins take part in a cascade of events which...
mediate insulin-induced oocyte maturation and that the insulin receptor tyrosine kinase somehow interacts with this family of proteins to initiate the maturation process.

In view of the evidence that insulin and IGF-1 activate a cyclic AMP phosphodiesterase in Xenopus oocytes, the present studies were undertaken to characterize more fully the actions of these hormones. The nonselective phosphodiesterase inhibitors IBMX, theophylline, and papaverine were used as pharmacological tools to investigate the nature of the hormone-sensitive enzyme activity. In addition, a recombinant ras construct, [Val'2, Thr'59]Ha (16), was used to compare insulin- and IGF-1-induced oocyte maturation to that elicited by microinjection of the p21 protein. The results suggest that a distinctive pool of oocyte phosphodiesterase is stimulated by insulin and IGF-1, that hormone-stimulated activity is required for the maturation response, and that the growth-promoting action of the transforming ras protein [Val'2, Thr'59] Ha involves stimulation of the same enzyme.

MATERIALS AND METHODS

In Vivo Phosphodiesterase Assay—The assay for in vivo measurement of phosphodiesterase activity is similar to the method of Allende et al. (17) and uses a modification of the Thompson and Appleman procedure (18). Animals were primed, oocytes were isolated, and the assay was performed as described previously (10), with the single exception that the content of Buffer A was modified to contain the following: 70 mM NaCl, 10 mM NaHCO3, 1 mM MgCl2, 0.5 mM CaCl2, 1 mM KCl, 25 mM Hepes, pH 7.8. Enzyme activity was measured by injecting [2,8-3H]cAMP (30–38 Ci/mmol; ICN Biochemicals) into each oocyte to a final concentration of 200 μM (final specific activity of 500–700 cpm/pmol of injected label) either alone or in combination with the indicated agents. When [Val'2, Thr'59] Ha was injected, the ras protein was suspended in p21 buffer containing 25 mM NaHepes, pH 7.5, 0.5 mM EDTA, 0.5 mM dithiothreitol, 0.005% 1-Octyl-d-glucopyranoside. An assay time of 5 min was used, and the results in each figure are presented as the mean ± S.E. of triplicate measurements using oocytes from a single frog. Each figure contains representative data selected from at least three reproducible experiments. Statistical significance was evaluated using the Student’s t test.

Oocyte Maturation (GVBD)—Groups of 15–20 oocytes were incubated at room temperature in 2-ml volumes of Buffer A containing the indicated additions. During time course studies, each treated group was scored for GVBD, as evidenced by the appearance of a white spot on the pigmented animal pole at successive times after addition of inducing agents. Responses were verified by manual dissection of oocytes after fixation in 2% trichloroacetic acid. In dose-response studies, oocytes were fixed after the indicated time period and scored to verify the maturation response. The results of these experiments are expressed as the percentage of the population responding (% GVBD) as a function of incubation time or drug concentration at the designated time.

Hormones and Reagents—Monocomponent porcine zinc insulin was a gift from Eli Lilly & Co. IGF-1 was purchased from Angen Biologicals. Bovine serum albumin free of all insulin-like growth factor activity was purchased from Reheis Chemicals. Recombinant [Val'2, Thr'59] Ha was generously supplied by Dr. J. B. Gibbs of Merck, Sharp, and Dohme Research Laboratories. All other reagents were purchased from Sigma.

RESULTS

When theophylline and IBMX were used to characterize hormone-induced oocyte maturation, the actions of insulin and IGF-1 could be clearly distinguished from that of progesterone on the basis of the observed sensitivities to inhibition. As shown in Figs. 1 and 2, theophylline inhibits insulin-induced oocyte maturation with an IC50 of approximately 30 μM, but progesterone-induced GVBD is less sensitive to inhibition, requiring almost millimolar concentrations of inhibitor (Fig. 1A). Similarly, IBMX is a more potent inhibitor of insulin-induced oocyte maturation than it is of progesterone-induced maturation (Fig. 1B). The same relative comparison can be made of the abilities of theophylline and IBMX to inhibit oocyte maturation induced by IGF-1 and progesterone, as demonstrated in Fig. 2. Both theophylline and IBMX inhibit peptide hormone-induced oocyte maturation at concentrations 10–25-fold lower than those required to inhibit the progesterone-induced response.

Using oocytes taken from different frogs, numerous dose-response experiments were performed to evaluate the potencies of IGF-1, theophylline, and papaverine, and the results are summarized in Table I. Of the three phosphodiesterase inhibitors tested, papaverine was the most potent inhibitor of oocyte maturation induced by all three hormones. Insulin-induced GVBD was the most sensitive to inhibition. The IC50 values for inhibition of IGF-1- and insulin-induced GVBD by each drug were similar, with the exception that the concentration of theophylline required to inhibit IGF-1-induced maturation was approximately 2-fold that required to inhibit the insulin response. In all animals tested, the concentrations of inhibitor required to prevent progesterone-induced maturation were significantly greater than those needed to inhibit the peptide hormone actions. For example, the IC50 for inhibition of progesterone-induced GVBD by papaverine (18.4 μM) was 60 times greater than the concentration of papaverine required to inhibit insulin- or IGF-1-induced GVBD (0.286 and 0.356 μM, respectively). For all inducing hormones, the rank order of potencies was: papaverine > IGF-1 > theophylline. Two other nonselective phosphodiesterase inhibitors, 8-chlorotheophylline and 8-chlorotheophylline were also tested for their abilities to inhibit hormone-induced oocyte maturation,

FIG. 1. Dose-dependent inhibition of insulin- and progesterone-induced GVBD by theophylline and IBMX. Groups of 20 oocytes were added to 2-ml volumes of Buffer A containing 1 mg/ml bovine serum albumin, either 1 μM insulin (C) or 1 μM progesterone (○), and the indicated molar concentrations of theophylline (△) or IBMX (□), and the time course of oocyte maturation was monitored, as described under "Materials and Methods." The results shown are data recorded at the time that control groups (in the absence of added inhibitor) achieved 100% GVBD: 4 h 55 min after addition of insulin; 3 h 55 min after addition of progesterone.

### Table I

<table>
<thead>
<tr>
<th>Hormone</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>1.75</td>
</tr>
<tr>
<td>IGF-1</td>
<td>2.00</td>
</tr>
<tr>
<td>Progesterone</td>
<td>63.0</td>
</tr>
</tbody>
</table>

Hormone- and p21-stimulated Cyclic AMP Phosphodiesterase
Hormone- and p21-stimulated Cyclic AMP Phosphodiesterase

FIG. 2. Dose-dependent inhibition of IGF-1- and progesterone-induced GVBD by theophylline and IBMX. Groups of 20 oocytes were added to 2-ml volumes of Buffer A containing 1 mg/ml bovine serum albumin, either 0.01 μM IGF-1 (O) or 1 μM progesterone (●), and the indicated molar concentrations of theophylline (A) or IBMX (B), and the time course of oocyte maturation was monitored, as described under "Materials and Methods." The results shown are those recorded at the time that control groups (in the absence of added inhibitor) achieved 100% GVBD: 6 h 30 min after addition of IGF-1; 3 h 30 min after addition of progesterone.

TABLE I
Relative abilities of papaverine, IBMX, and theophylline to inhibit hormone-induced oocyte maturation

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Insulin IC_{50} (μM)</th>
<th>IGF-1 IC_{50} (μM)</th>
<th>Progesterone IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaverine</td>
<td>0.286±0.148(3)*</td>
<td>0.356±0.075(3)</td>
<td>18.4±5.2 (4)</td>
</tr>
<tr>
<td>IBMX</td>
<td>5.4±1.4 (5)</td>
<td>7.4±2.5 (4)</td>
<td>137±31 (7)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>31.3±0.7 (3)</td>
<td>61±6.5 (5)</td>
<td>845±171 (4)</td>
</tr>
</tbody>
</table>

*Mean ± S.E.

and the IC_{50} values for these drugs were >5 mM (data not shown).

IBMX was used to evaluate more fully the accelerating action of low doses of progesterone on the insulin- and IGF-1-induced responses. As reported previously (10, 15, 19–21), the addition of a low dose of progesterone to oocytes treated

FIG. 3. Time course and dose-response analysis of acceleration of insulin-induced GVBD by low dose progesterone. Groups of 20 oocytes were placed in 2-ml volumes of Buffer A containing 1 mg/ml bovine serum albumin and 1 μM insulin (C), 10 μM progesterone (C), or 1 μM insulin + 0.01 μM progesterone (●) in the absence or presence of the indicated molar concentrations of IBMX. A, time courses of GVBD. During the first 6 h, 0.01 μM progesterone had no effect (data not shown). B, dose-dependent inhibition of hormone-induced GVBD by IBMX. Each curve was generated at the time that control groups of oocytes (in the absence of added inhibitor) reached 100% GVBD: 3 h 15 min after addition of progesterone; 4 h 30 min after addition of insulin + progesterone; 22 h after addition of insulin.

FIG. 4. Time course and dose-response analysis of acceleration of IGF-1-induced GVBD by low dose progesterone. Groups of 20 oocytes were placed in 2-ml volumes of Buffer A containing 1 mg/ml bovine serum albumin and 10 nM IGF-1 (●), 10 μM progesterone (C), or 10 nM IGF-1 + 0.01 μM progesterone (●) in the absence or presence of indicated molar concentrations of IBMX. A, time courses of GVBD. During the first 5 h, there was no response to 0.01 μM progesterone alone (data not shown). B, dose-dependent inhibition of hormone-induced GVBD by IBMX. Each curve was generated at the time that control groups of oocytes (in the absence of added inhibitor) reached 100% GVBD: 3 h 45 min after addition of progesterone; 4 h after addition of IGF-1 + progesterone; 5 h 15 min after addition of IGF-1.
with approximately 20 nl of [Val', Thr'] Ha p21 (2.5 mg/ml stock trichloroacetic acid and dissected to verify the maturation response, and placed in 2-ml volumes of Buffer A containing the indicated solution in 25 mM Tris, pH 7.5, 2 mM MgCl₂, 2 mM dithiothreitol) for 90 min in Buffer A containing 1 mg/ml bovine serum albumin and 1 mM insulin or 10 μM progesterone in the presence of indicated molar concentrations of IBMX. Each curve was generated at the time that control groups of oocytes (in the absence of inhibitor) reached 100% GVBD: 3 h 15 min in progesterone, 22 h in insulin. B, inhibition of in vivo oocyte phosphodiesterase activity by IBMX. Oocytes were preincubated for 90 min in Buffer A containing 1 mg/ml bovine serum albumin in the absence or presence of insulin or 10 μM IBMX and the indicated molar concentrations of IBMX. Oocyte phosphodiesterase (PDE) activity was then measured in triplicate groups of oocytes after microinjection of 200 μM [³H]cAMP, as described under “Materials and Methods.” *, greater than untreated controls, p < 0.05.

with insulin significantly accelerated the time course of oocyte maturation (Fig. 3A). This set of oocytes responded slowly to insulin alone (achieving only 5% GVBD after 6 h of incubation), and addition of a low dose of progesterone (0.01 μM) to the insulin incubation resulted in a dramatic acceleration of the time course of maturation (100% maturation in only 4 h 20 min, Fig. 3A). The combined hormone action (insulin plus a low dose of progesterone) displayed a sensitivity to inhibition by IBMX that approximated the sensitivity of insulin alone (Fig. 3B). A low dose of progesterone (0.01 μM) also accelerated oocyte maturation induced by IGF-1 (Fig. 4A), and the combined hormone effect was inhibited by IBMX with a potency that also approximated the sensitivity of IGF-1 alone (Fig. 4B).

As demonstrated previously, insulin and IGF-1 stimulate cyclic AMP phosphodiesterase activity measured after microinjection of 200 μM [³H]cAMP, while progesterone has no effect on measured enzyme activity (10). The correlation between inhibition of hormone-induced oocyte maturation and inhibition of oocyte phosphodiesterase activity is shown in Fig. 5. Insulin-induced GVBD was completely inhibited by 20 μM IBMX, but this same concentration of inhibitor had no effect on progesterone-induced maturation (Fig. 5A). When oocytes were treated with 1 μM insulin for 90 min, phosphodiesterase activity was stimulated by 33%, and co-treatment with companion oocytes with 20 μM IBMX completely blocked the insulin stimulation but had no effect upon basal phosphodiesterase activity measured in control oocytes that were not treated with insulin (Fig. 5B). When the IBMX concentration was increased to 1 mM, progesterone-induced GVBD was completely inhibited (Fig. 5A), and this inhibition of the steroid effect was accompanied by a further reduction of oocyte phosphodiesterase activity, measured both in the presence and absence of insulin (Fig. 5B).

IBMX was also used to investigate the possible role for phosphodiesterase in the maturation-inducing action of the ras protein, [Val', Thr'] Ha. As shown in Fig. 6, oocyte maturation elicited by microinjection of p21 was inhibited in a dose-dependent manner by IBMX with an IC₅₀ of approximately 10 μM. These results raised the possibility that transforming p21 might also stimulate oocyte phosphodiesterase. To test this possibility, the effect of microinjected p21 on oocyte phosphodiesterase was measured. After coinjection of [Val', Thr'] Ha (0.12 mg/ml final intracellular concentration) with substrate at the initiation of the reaction sequence,
phosphodiesterase activity was stimulated approximately 50% above buffer injected controls (Fig. 7). The level of stimulation measured after p21 injection varied from 30 to 100% in four separate experiments using oocytes from different frogs (data not shown). When the final concentration of 50 μM IBMX was included in the substrate mixture, the stimulatory effect of [Val'2, Thr'59]Ha was blocked (Fig. 7). Similarly, preincubation of oocytes for 90 min in Buffer A containing 300 μM IBMX also blocked stimulation of oocyte phosphodiesterase by subsequently injected [Val'2, Thr'59]Ha (data not shown).

**DISCUSSION**

Within the last few years, an increased appreciation of the involvement of phosphodiesterase in hormonal regulation of cyclic AMP levels has begun to emerge, and multiple molecular forms of the enzyme have been described in numerous cell types (see Ref. 22 for review). As with other tissues, the existence of multiple forms of *Xenopus* oocyte phosphodiesterase has also been described, but with some discrepancies in the results. Various methods of preparation and analysis have yielded evidence suggesting the existence of a calmodulin-stimulated enzyme that hydrolyzes both cAMP and cGMP, and another that is calmodulin-insensitive and specifically hydrolyzes cAMP (23, 24). These *in vitro* data gave no indication of hormone sensitivity, and it has been suggested that calmodulin activation of oocyte phosphodiesterase activity is inhibited in living oocytes (25).

Even though it has long been known that theophylline and related xanthines inhibit progesterone-induced oocyte maturation (29), the present study is the first to compare the abilities of IBMX, theophylline, and papaverine to inhibit oocyte maturation stimulated by progesterone, insulin, and IGF-1. In a previous study, inhibition of basal phosphodiesterase activity by IBMX and theophylline was shown to be dose-dependent and to correlate with the ability of each drug to inhibit progesterone-induced GVBD (10). Close examination of inhibitor effects clearly distinguishes peptide action from steroid action (Figs. 1 and 2). Oocyte maturation induced by the peptide hormones is more sensitive to inhibition by all three drugs than is progesterone-induced GVBD, and the order of potencies for inhibition of hormone-induced GVBD is the same for each inducing agent: papaverine > IBMX > theophylline (Table I). This rank order of potencies is identical to that described for other tissue preparations. For example, the following IC50 values have been reported for soluble phosphodiesterase from calf liver: 1.0 μM papaverine, 2.6 μM IBMX, 120 μM theophylline (29).

Acceleration of insulin-induced oocyte maturation by low doses of steroids has been reported previously (15, 19–21). In these studies, low concentrations of various steroids including progesterone, testosterone, and ethinyl estradiol were shown to accelerate the time course of insulin-induced oocyte maturation. It could not be established from these studies, however, which hormonal pathway was amplified when oocytes were treated with a combination of steroid and insulin. The acceleration of insulin-induced maturation by progesterone was recently correlated with synergistic stimulation of oocyte phosphodiesterase activity (10). Although progesterone alone has no effect on phosphodiesterase activity in oocytes, when a low dose of progesterone is combined with insulin, oocyte phosphodiesterase activity is stimulated to levels greater than those measured in response to insulin alone (10). The pharmacological evidence presented here also supports the hypothesis that hormone-stimulated phosphodiesterase activity is involved in the accelerating action of low doses of steroid. Oocyte maturation induced by IGF-1 was also accelerated by a low dose of progesterone in a fashion similar to that described for insulin (Figs. 3 and 4). Furthermore, the maturation responses elicited by both insulin plus progesterone and IGF-1 plus progesterone were inhibited by low micromolar concentrations of IBMX, the same concentrations that specifically inhibited the actions of the growth peptides alone (Figs. 3 and 4). These results suggest that the hormone-sensitive phosphodiesterase that is involved in insulin-induced GVBD is also targeted by combined peptide and steroid and that the accelerating action of the combined hormones is due to potentiation of peptide action by progesterone.

Previously, it was shown that dose-dependent stimulation of oocyte phosphodiesterase activity by low nanomolar concentrations of IGF-1 directly correlated with the ability of the peptide hormone to both inhibit oocyte adenylate cyclase in membrane preparations and stimulate oocyte maturation (10). Dose-dependent stimulation of oocyte phosphodiesterase by insulin was directly correlated with its ability to inhibit oocyte adenylate cyclase activity (effective concentrations approximating 10 nM). These results demonstrated dual regulation of phosphodiesterase and adenylate cyclase activity by insulin and IGF-1, and suggested involvement of phosphodiesterase in the induction of oocyte maturation by peptide hormones. The correlation between dose-dependent inhibition of insulin-induced GVBD and phosphodiesterase by IBMX (Fig. 5) further supports the hypothesis that hormone-stimulated phosphodiesterase plays an integral role in the action of insulin and IGF-1. The ability of 20 μM IBMX to inhibit both insulin-stimulated phosphodiesterase activity and insulin-induced maturation (Fig. 5) suggests that stimulation of a specific pool of oocyte phosphodiesterase is required for the insulin-induced maturation response. It is also interesting to note that higher concentrations of IBMX both inhibit progesterone-induced GVBD and elicit a further decrease in phosphodiesterase activity measured in both the absence and presence of added insulin. High doses of IBMX apparently affect a second pool of oocyte phosphodiesterase that is hormone-insensitive. Even though the ability of progesterone to stimulate oocyte maturation does not correlate with an effect of steroid on oocyte phosphodiesterase activity (10), a requisite level of phosphodiesterase activity is apparently essential for progesterone activity, since inhibition of the basal level of enzyme activity with high concentrations of IBMX correlates with concomitant inhibition of progesterone-induced oocyte maturation (Fig. 5). These results are consistent with previous studies in which theophylline treatment was shown to increase basal levels of oocyte cAMP, limit progesterone-induced decreases in intracellular cAMP, and inhibit progesterone-stimulated oocyte maturation (30).

The ras family of cellular proto-oncogenes, the so-called p21 proteins, have been implicated as potential mediators of insulin action in *Xenopus* oocytes, and it has been suggested that p21 proteins affect enzymes involved in regulatory pathways that interconnect with the insulin pathway (31). This hypothesis is supported by the results presented in Figs. 6 and 7. The ability of IBMX to inhibit oocyte maturation stimulated by microinjection of [Val'2, Thr'59]Ha closely paralleled the ability of IBMX to inhibit oocyte maturation stimulated by insulin and IGF-1 (IC50 values approximating 5–10 μM). In addition, microinjected p21 was shown to stimulate oocyte phosphodiesterase, and this stimulatory action was inhibited by co-injection of IBMX (50 μM final intracellular concentration) with substrate at the initiation of the reaction sequence (Fig. 7). Since peptide hormone-induced GVBD was specifically inhibited by IBMX in this lower concentration range (Figs. 1–3, Table I), these results suggest...
that the actions of p21 are dependent upon the activity of the same oocyte phosphodiesterase that is involved in the actions of insulin and IGF-1. Further work is needed to more fully characterize the effect of p21 on oocyte phosphodiesterase and to determine which form of the enzyme is involved in the growth-promoting actions of insulin, IGF-1, and the transforming ras gene product.

Acknowledgments—We wish to thank Janet Lee Kyes and Barbara Quarantillo for their skilled technical assistance and Karen Kail Eckart for expert secretarial help.

REFERENCES