G-protein-mediated Epidermal Growth Factor Signal Transduction in a Human Breast Cancer Cell Line

EVIDENCE FOR TWO INTRACELLULAR PATHWAYS DISTINGUISHABLE BY PERTUSSIS TOXIN

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The MDA-468 human breast cancer cell line has an amplified epidermal growth factor (EGF) receptor gene (20 X) and correspondingly overexpresses the EGF receptor. Since this cell line is growth inhibited by supra-physiological levels of EGF in tissue culture, it has been possible to select variant cells which have lost the chromosome bearing the amplified EGF receptor domain and which are capable of growing in high levels of EGF. One such cell line (MDA-468-S4) shows an absolute requirement for EGF for growth in anchorage-independent tissue culture conditions. We have utilized MDA-468 and MDA-468-S4 to examine the intracellular transduction of EGF signals leading to growth inhibition and proliferation, respectively. We report that in anchorage-independent conditions, pertussis toxin can abrogate both the EGF-dependent growth inhibition in MDA-468 cells and the EGF-dependent cell proliferation in MDA-468-S4 cells. This inhibition is paralleled by the ADP-riboseylation of an endogenous 41,000-dalton membrane protein in both MDA-468 and MDA-468-S4 cells. In contrast, the toxin does not prevent the transient, augmented expression of c-myc and c-fos mRNA seen in response to EGF in both cell types. These data suggest 1) the notion of more than one simultaneous, parallel, intracellular EGF-dependent signal transduction pathway and 2) G-protein involvement in at least one pathway mandatory for the growth modulating responses to EGF in anchorage-independent conditions, but distinct from that inducing c-myc and c-fos mRNA expression.

Epidermal growth factor (EGF) is a low molecular weight polypeptide which stimulates the proliferation of a wide variety of cell types (1-3). EGF binds to a specific cell surface receptor which has been shown to possess an intrinsic tyrosine kinase activity (1-3). In addition to phosphorylating a number of endogenous and exogenous proteins (1-3), the receptor kinase is subject to autophosphorylation (4). Other early events that follow EGF-receptor complex formation include increasing levels of intracellular Ca2+ (5, 6), enhanced Na+/H+ antipporter activity (7), and transient expression of several genes including the nuclear-located oncogene products c-myc and c-fos (8-11). In general, it seems that these genes can be induced by membrane-originating stimuli such as receptor-ligand interaction (including EGF) and it is inferred that they are involved in the transduction of such signals (12, 13).

While it is understood that these and possibly other events resulting from EGF binding either facilitate or are required for cell proliferation, the intracellular transduction mechanism(s) of the cell growth signal is unresolved. One possible mechanism that has not been widely associated with the action of EGF involves the guanine nucleotide-binding proteins (G-proteins). These membrane-associated proteins are signal coupling components in the response to many neurotransmitters and hormones (14) and have been well characterized in the adenylate cyclase system (15). An important aspect of one category of G-proteins is sensitivity to the inhibitory effects of Bordetella pertussis toxin which acts by the covalent binding of ADP-ribose to defined sites on the protein (16, 17). Thus, the sensitivity to pertussis toxin has been used to indicate whether the action of a G-protein is involved in a number of hormone-stimulated responses.

This laboratory has recently reported that MDA-468, a human breast cancer cell line, has an amplified EGF receptor gene and consequently a very high number of EGF receptors (approximately 1.5 x 10⁶ receptors cell⁻¹) (18). Although the receptor number is increased, no abnormalities have been found in the properties of the individual receptor molecules (19, 20). As has been demonstrated for A431 cells (21), in supra-physiological EGF concentrations, growth inhibition is induced in MDA-468 cells (18, 19). We have taken advantage of that fact to generate MDA-468 variants which survive in high levels of EGF and were subsequently found to have lost the EGF receptor gene amplification (19). One variant cell line (MDA-468-S4) showed interesting properties with respect to regulation of growth in culture by EGF. This cell line showed an absolute dependence on the addition of exogenous EGF for growth in anchorage-independent conditions but was only moderately stimulated by EGF in anchorage-dependent tissue culture. MDA-468 and MDA-468-S4, therefore, are closely related, differing primarily in the level of EGF-receptor expression, but respond in an opposite fashion to EGF in anchorage-independent tissue culture.

In this study we have used these cell lines to examine the possible role of G-proteins in the specific growth responses of cells to exogenous EGF. Our results suggest that the activated EGF-receptor complex is coupled to a pertussis toxin-sensitive G-protein and that this step is mandatory in producing EGF-mediated responses, which may be either inhibitory (in MDA-468) or proliferative (in MDA-468-S4).
**EXPERIMENTAL PROCEDURES**

Cell Cultures—Cell line MDA-468 was derived from a human breast tumor (22, 23). The variant MDA-468-S4 was generated by this laboratory as reported in Ref. 19. Both cell lines were routinely cultured in L-15 medium, supplemented with 10% fetal calf serum (FCS). Effect of EGF and Pertussis Toxin on Anchorage-independent Growth—A total of 10^4 cells were plated in triplicate in 35-mm plates in a two-layer system of agar (0.5% bottom, 0.3% top) in L-15 medium supplemented with 10% FCS plus EGF and/or pertussis toxin at the concentrations noted. Plates were then incubated for 18 days at 37°C and then analyzed for colony formation. In experiments examining ADP ribosylation, both cell types were grown in methylcellulose. MDA-468 and MDA-468-S4 were plated at a concentration of 10^4 cells in duplicate on 100-mm plates in a two-layer system of 0.5% agar (bottom) and 0.8% methylcellulose (top) in L-15 medium supplemented with 10% FCS plus EGF and/or pertussis toxin at final concentrations shown in Table I. Plates were then incubated for 14 days at 37°C. Cells were harvested from the methylcellulose by centrifugation and a portion of the resulting suspension counted by means of a hemocytometer.

Preparation of Crude Membrane Fractions—MDA-468 and MDA-468-S4 cells were harvested from methylcellulose, washed, and resuspended in 50 mM Tris/HCl, pH 7.5, 5 mM MgCl2. Cells were disrupted by sonication (10 s at 5–7 DC amperes) and the homogenates were centrifuged (50,000 × g, 30 min) and the resulting pellets resuspended, washed twice, and stored at −70°C in 50 mM potassium phosphate, pH 7.5.

**EGF-binding Studies**—Binding studies were carried out in duplicate on subconfluent cells grown on 35-mm dishes. Cells were washed with binding buffer (serum-free L-15 medium with 1 mg/ml bovine serum albumin and 5 mM Hepes, pH 6.8) and then incubated for 4 h at 4°C in binding buffer containing 0.1 to 10 nM EGF and 2.5 × 10^10 cpm [3H]-EGF (100 μCi/μl) for each nanomolar of unlabeled EGF. Cells were then washed six times with binding buffer, solubilized in 0.5 N NaOH, and radioactivity determined. Specific binding was estimated by subtracting counts bound in the presence of excess unlabeled EGF (5 × 10^−10 M) from total counts bound, and Scatchard plots (24) were derived.

ADP Ribosylation of Crude Membrane Fractions—In vitro labeling was performed using a modification of the procedure of Moss et al. (25). MDA-468 or MDA-468-S4 membranes (20–50 μg) (protein measured as in Lowry et al. (26)) were incubated with 30 μM [α-32P]NAD, 20 mM thymidine, 0.5 mM ATP, 0.5 mM GTP, 6.5 mM dithiothreitol, 50 mM potassium phosphate, pH 7.5, and 1 μg/ml pertussis toxin (activated by incubation with 50 mM glycine, pH 8.0, and 20 mM dithiothreitol for 10 min at 37°C) for 30 min at 37°C. The reaction was stopped by adding ice-cold 100% trichloroacetic acid to give a final concentration of 10%. After centrifugation, the pellets were dissolved in sodium dodecyl sulfate sample buffer, fractioned on a 5–20% linear acrylamide gradient gel (as described by Laemmli (27)) and autoradiographed.

Isolation of RNA and RNA Blotting—Total RNA was isolated by guanidine isothiocyanate solubilization and centrifugation over a CsCl cushion (28). Five μg of poly(A)^+ RNA, purified by passage over oligo(dT)-cellulose, was denatured with glyoxal and dimethyl sulfoxide and electrophoresis was performed in a 1.1% agarose gel. The RNA was then transferred to a Zetabind filter and hybridized to a radiolabeled probe using high stringency conditions. Human 28S and 18S rRNAs were used as markers.

**RESULTS AND DISCUSSION**

MDA-468 and MDA-468-S4 respond differently to the addition of EGF in anchorage-independent tissue culture conditions. When plated in agar supplemented with 10% FCS (approximately 10^{-11}–10^{-12} M EGF), MDA-468 demonstrated a high plating efficiency (Fig. 1A), while MDA-468-S4 was unable to proliferate (Fig. 1B). The addition of EGF (10^{-8} M) to the medium resulted in an inhibition of growth of MDA-468 (to 25% of control values) and a marked stimulation of growth of MDA-468-S4 to approximately 5% plating efficiency.

These responses to EGF have allowed us to investigate the possibility of G-protein involvement in signal transduction. We cultured both MDA-468 and MDA-468-S4 in the presence of both 10^{-8} M EGF and increasing concentrations of pertussis toxin (0.1–100 ng/ml). This caused a concentration-dependent reversal of the EGF-mediated growth inhibition of MDA-468 (Fig. 1A). In the presence of 10 ng/ml pertussis toxin and 10^{-8} M EGF, the plating efficiency approximated that of cells not exposed to growth-inhibiting concentrations of EGF. Similarly, pertussis toxin inhibited the EGF-dependent growth of the variant MDA-468-S4 (Fig. 1B). In the absence of EGF, pertussis toxin at all concentrations had no effect on the plating efficiency of MDA-468 (data not shown).

It is well known that the ADP ribosylation of the inhibitory G-protein of adenylate cyclase, catalyzed by pertussis toxin, leads to a stimulation of the enzyme (15). Cholera toxin is also known to cause a chronic stimulation of adenylate cyclase by ADP-ribosylating the stimulatory G-protein in the enzyme-receptor complex (15). However, cholera toxin, included in the anchorage-independent cell culture assay in concentrations from 0.01 to 1 μg/ml, did not alter the EGF-mediated responses of either MDA-468 or MDA-468-S4 (data not shown). The cells did, however, respond to cholera toxin by increased acid-soluble cyclic AMP content. Results from two separate experiments showed that in MDA-468 cells, cholera toxin (2 h; 1 μg/ml) raised cyclic AMP levels (as measured by high pressure liquid chromatography) from 21.3 to 100.8 pmol (10^6 cells)^{-1}, while the same treatment in MDA-468-S4 cells increased cyclic AMP content from 19.3 to 63.4 pmol (10^6 cells)^{-1}. It is therefore unlikely that the pertussis toxin effects seen on the MDA-468/MDA-468-S4 system can be accounted for by stimulation of adenylate cyclase.

We further investigated the effects of pertussis toxin treatment by measuring the binding of EGF to its receptors on MDA-468 and MDA-468-S4 cells (Table I). In duplicate studies, pertussis toxin, at 10 ng/ml, had no significant effect on the affinity or number of binding sites of 125I-EGF as measured by high pressure liquid chromatography. Data represents plating efficiency (number of colonies/10^4 cells × 100 ± S.E.) where a colony is defined as ≥60 cells. A, MDA-468; B, MDA-468-S4.

**FIG. 1. Effect of EGF and/or pertussis toxin on anchorage-independent growth.** Growth studies were carried out by incubating 10^6 cells in agar containing the various concentrations of pertussis toxin (P.T.) and/or EGF as outlined under "Experimental Procedures." Data represents plating efficiency (number of colonies/10^4 cells × 100 ± S.E.) where a colony is defined as ≥60 cells. A, MDA-468; B, MDA-468-S4.
Pertussis Toxin-sensitive EGF Signal Transduction

**Table I**

<table>
<thead>
<tr>
<th></th>
<th>MDA-468 (10^6 cells)</th>
<th>MDA-468-S4 (10^6 cells)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Kd</td>
<td>B_max</td>
</tr>
<tr>
<td>Control</td>
<td>0.41</td>
<td>905.60</td>
</tr>
<tr>
<td>+ 10 ng·ml⁻¹ pertussis toxin</td>
<td>0.55</td>
<td>718.33</td>
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**Table II**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cells harvested (×10⁶)</th>
<th>Cells harvested (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA-468</td>
<td>MDA-468-S4</td>
</tr>
<tr>
<td>None</td>
<td>21 ± 6</td>
<td>2 ± 0.3</td>
</tr>
<tr>
<td>10⁻⁸ M EGF</td>
<td>6 ± 0.5</td>
<td>16 ± 2.2</td>
</tr>
<tr>
<td>10 ng·ml⁻¹ pertussis toxin</td>
<td>14 ± 5</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>10⁻⁸ M EGF + 10 ng·ml⁻¹ pertussis toxin</td>
<td>22 ± 4</td>
<td>2 ± 0.7</td>
</tr>
</tbody>
</table>

measured by Scatchard analysis (24). Thus the effects of the toxin on EGF-mediated growth responses are not simply secondary to changes in the ability of EGF receptors to bind EGF.

To further analyze the biochemical correlates associated with pertussis toxin actions in these cells, it was necessary to perform the tissue culture in a manner which would allow cell harvest. Thus, we tested the effect of EGF and/or pertussis toxin on cells growing in anchorage-independent conditions in viscous medium (methylcellulose) and in anchorage-dependent conditions. The data are shown as cell counts in Table II. The responses of MDA-468 and MDA-468-S4 in methylcellulose culture were qualitatively similar to those seen in agar culture (Fig. 1). In anchorage-dependent conditions, however, the pertussis toxin effect on the two cell lines could be distinguished. MDA-468 showed growth inhibition by EGF which could be blocked by pertussis toxin but MDA-468-S4, no pertussis toxin added to in vitro assay; lane 3, grown in absence of both EGF and pertussis toxin; no pertussis toxin added to in vitro assay; lane 3, grown in absence of both EGF and pertussis toxin; lane 4, grown in presence of 10⁻⁸ M EGF; lane 5, grown in presence of 10⁻⁸ M EGF and pertussis toxin. Pertussis toxin contains a mono-ADP-ribosyltransferase activity that is thought to be responsible for its effects on signal transduction (25). To identify substrates of the toxin in intact MDA-468 and MDA-468-S4 membranes, we grew the cells for 14 days in methylcellulose in the presence and absence of EGF (10⁻⁸ M) and/or pertussis toxin (10 ng·ml⁻¹) as outlined. The cells were then harvested, disrupted, and a crude membrane fraction prepared. Membranes from both cell lines under the various growth conditions were then subjected to ADP ribosylation in vitro by [α-³²P]NAD in the presence of pertussis toxin. Under these conditions, membranes from both cell lines grown in the presence or absence of EGF could incorporate ³²P resulting in the labeling of a 41,000-dalton polypeptide, in addition to high molecular weight substrates (Fig. 2A, lanes 3 and 4; Fig. 2B, lanes 2 and 4). When pertussis toxin was omitted from the in vitro reaction, the [α-³²P]ADP-ribose incorporation in the 41,000-dalton protein was abolished (Fig. 2A, lane 2; Fig. 2B, lane 5). The ³²P-labeling of the high molecular weight substrates was independent of pertussis toxin but was dependent on the presence of the membrane preparation (Fig. 2A, lane 1). In contrast, when membranes were prepared from cells which had been exposed to pertussis toxin during tissue culture, no ADP ribosylation of the 41,000-dalton protein could be induced by in vitro incubation in the presence of pertussis toxin (Fig. 2A, lanes 5 and 6; Fig. 2B, lanes 1 and 3). Our data suggest that the ADP ribosylation of a 41,000-dalton membrane protein is likely involved in the inhibitory effects of pertussis toxin on EGF-mediated events in MDA-468 and variant MDA-468-S4. Certainly this protein is the only pertussis toxin-dependent substrate in the membrane fractions and furthermore, as evidenced by the lack of availability of ³²P-labeling sites in vitro, it becomes ADP-ribosylated in...

**Fig. 2. Autoradiographic analysis of pertussis toxin substrates in crude membrane fractions.** Membranes were ADP-ribosylated in vitro in response to pertussis toxin with [α-³²P]NAD as substrate as outlined under "Experimental Procedures." A, MDA-468: lane 1, no membranes; lane 2, grown in the absence of both EGF and pertussis toxin; no pertussis toxin added to in vitro assay; lane 3, grown in absence of both EGF and pertussis toxin; lane 4, grown in presence of 10⁻⁸ M EGF; lane 5, grown in presence of 10⁻⁸ M EGF and pertussis toxin. Pertussis toxin added to in vitro assay; lane 6, grown in presence of both 10⁻⁸ M EGF and 10 ng·ml⁻¹ pertussis toxin; lane 7, grown in presence of both 10⁻⁸ M EGF and 10 ng·ml⁻¹ pertussis toxin; lane 8, grown in presence of both 10⁻⁸ M EGF and 10 ng·ml⁻¹ pertussis toxin; lane 9, grown in presence of both 10⁻⁸ M EGF and 10 ng·ml⁻¹ pertussis toxin; lane 10, grown in the absence of both EGF and pertussis toxin; lane 11, identical to lane 4 but pertussis toxin not added to in vitro assay. Arrowheads point to pertussis toxin substrates.
intact cells under conditions that lead to suppression of EGF-dependent growth inhibition and proliferation.

The ADP-ribose substrate in these membranes has the same electrophoretic mobility as the α-subunit of the inhibitory G-protein associated with adenylate cyclase (N\textsubscript{a}). This cannot, however, be taken as evidence of identity; work from a number of laboratories has provided evidence that the G-proteins involved in non-adenylate cyclase systems are discrete from that of N\textsubscript{a} but highly homologous in both structure and function (32-36).

Growth inhibition by supra-physiological levels of EGF is a common phenotype of cell lines overexpressing the EGF receptor gene (18, 21). Previous reports have indicated that a reduction in the number of functional EGF receptor-kinase complexes in such cell lines could reduce EGF-mediated growth inhibition (18, 37, 38). The phenomenon has therefore been associated principally with the degree of tyrosine phosphorylation of substrates and/or the resultant ATP depletion (37-39). In this regard, the pertussis toxin-mediated blocking of EGF-dependent growth inhibition in MDA-468 is a significant observation since it provides a direct indication that growth inhibition requires a biochemical step subsequent to receptor activation.

In preliminary experiments designed to examine the possible biochemical pathway(s) through which these pertussis toxin effects are mediated, the effect of the toxin on the transcription of the oncogenes c-myc and c-fos was studied. MDA-468 and MDA-468-S4 growing in anchorage-dependent conditions were treated for 48 h in the presence or absence of pertussis toxin (10 ng.ml\textsuperscript{-1}) prior to treatment (30 min) with EGF (10\textsuperscript{-8} M). This 48-h treatment was sufficient to ribosylate all pertussis toxin substrates; ADP-riboseylation in vitro by [α-\textsuperscript{32}P]NAD, led to an identical pattern of \textsuperscript{32}P-labeling of membranes as that shown in Fig. 2, A and B (data not shown).

The level of c-myc and c-fos transcripts were then assessed by Northern blotting (Fig. 3). Under these conditions c-myc mRNA levels can be increased in MDA-468 cells by EGF exposure (Fig. 3A, lanes 1 and 2). Pertussis toxin alone had little effect on basal transcription (Fig. 3A, lane 4), but additionally, did not block the stimulating effects of EGF on c-myc transcription (Fig. 3A, lane 3). Similarly, pertussis toxin was unable to block the EGF-mediated increase in c-fos mRNA levels (Fig. 3B). Thus, although pertussis toxin could abrogate EGF growth inhibition in MDA-468 cells (Table II), stimulation of c-myc and c-fos mRNA production was unaltered. These data confirm the finding that transient activation of c-myc and c-fos expression can still take place even when there is ultimately no mitogenic response (10, 12, 40, 41).

Similar EGF-mediated increases in c-myc and c-fos mRNA levels were also demonstrable in MDA-468-S4 and the effect was not blocked by pertussis toxin (data not shown). However, since exogenous EGF was not mandatory for MDA-468-S4 growth in anchorage-dependent conditions, nor does pertussis toxin block proliferation under these conditions (Table II), the significance of this result remains unclear.

A number of signal transduction pathways have been implicated in cellular growth control. It is possible therefore to speculate on both the nature of the biochemical transduction pathways activated by EGF and those upon which the pertussis toxin effects are mediated. Recent studies have suggested that phosphatidylinositol metabolism is associated with mitogen binding in many cell lines (42). In addition, the phosphoinositide and diacylglycerol accumulation induced by a number of pharmacological agents has been shown to be dependent on G-protein activation (32-36). However, the role of phosphatidylinositol turnover in the signal transduction of EGF in various epidermoid and breast cancer cell lines is controversial. It does not appear to be a universal phenomenon associated with EGF-mediated mitogenesis in either EGF-receptor gene amplified or nonamplified cell lines (43-47), nor has it been widely correlated with changes in cell proliferation (43-47).

Work published by Pouyssegur and co-workers (48-50) has suggested that activation of phospholipase C in Chinese hamster lung fibroblasts has a crucial determinant function in growth control and that inhibition of the enzyme (for example, with pertussis toxin) can block all aspects of cell proliferation, from activation of c-myc transcription, through change in intracellular pH to DNA replication. They also showed (48-50) that mitogens that act independently of phosphoinositide and diacylglycerol accumulation in fibroblasts (for example, EGF) are insensitive to agents such as pertussis toxin that alter G-protein function. Our results suggest that the intracellular EGF signal transduction pathway(s) in MDA-468 and

**Fig. 3. Effect of pertussis toxin on the stimulation of c-myc (A) and c-fos (B) gene expression in MDA-468 cells.** A, cells were incubated in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 10 ng.ml\textsuperscript{-1} pertussis toxin for 48 h. Following 24-h serum starvation c-myc expression was induced by the addition of 10\textsuperscript{-8} M EGF (lanes 1 and 3) 30 min prior to cell harvest. B, cells were incubated in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 10 ng.ml\textsuperscript{-1} pertussis toxin for 48 h. c-fos expression was induced by the addition of 10\textsuperscript{-8} M EGF (lanes 3 and 4) 30 min prior to cell harvest, following a prior 24-h period of serum starvation. In both A and B, poly(A)\textsuperscript{+} RNA was then examined by Northern blot analysis with \textsuperscript{32}P-labeled nick-translated probes specific for c-myc and c-fos as outlined under "Experimental Procedures." Human 28 S and 18 S rRNA was used as standard (right). Arrows denote c-myc (A) and c-fos (B) mRNA band.
MDA-468-S4 cells is more complex than that proposed in fibroblasts. Whereas the work of Pouyssegur (48–50) could distinguish two separate mitogenic signal pathways, one represented by α-thrombin (i.e. dependent on phosphoinositide metabolism and inhibited by pertussis toxin) and a second represented by EGF (i.e. independent of phosphatidylinositol turnover and thus insensitive to pertussis toxin), we have evidence to suggest at least two separate transduction pathways utilized by the same mitogen, EGF, and distinguishable by pertussis toxin sensitivity. In MDA-468, and variant MDA-468-S4 cells, we have demonstrated that pertussis toxin can block EGF-dependent growth inhibition and proliferation, respectively, and that this blockade takes place without interrupting the transient activation of c-myc and c-fos mRNA synthesis. The precise biochemical pathway which serves as target for the pertussis toxin-mediated inhibition in these cells remains to be elucidated.

In light of the broad definition of regulatory signals and obligatory events provided by Rozengurt (51), it is possible to hypothesize that in normal cells responding to a mitogen there are very likely a number of intracellular pathways leading to protein phosphorylation, cytoplasmic alkalinization, increased gene expression etc., the sum of which leads to cell division and proliferation. Depending upon mitogen and cell type, some postreceptor binding steps would be regulatory and others obligatory. Similarly, these transduction pathways might also be both G-protein dependent and independent. Since MDA-468 cells cannot be growth inhibited by pertussis toxin alone in the presence of low concentrations of EGF, this cell line clearly utilizes mitogenic signals which are independent of G-protein mediation (or mediated by a G-protein which is insensitive to pertussis toxin). However, in the presence of high levels of EGF, where the growth factor is inhibitory, the obligatory step post-EGF binding is mediated by a G-protein and is blocked when the G-protein is ADP-ribosylated by pertussis toxin. In a similar manner we must conclude that the MDA-468 variant MDA-468-S4, where exogenous EGF must be present for proliferation in anchorage-independent conditions that the obligatory intracellular signal mediated by EGF is also both transduced via a G-protein and is pertussis toxin-sensitive.

These studies have been made possible by the unique EGF-dependent growth characteristics of the MDA-468 and MDA-468-S4 cell lines. Further experiments are being directed toward both the identification of the specific response pathways involved and the generality of G-protein participation in EGF-mediated signal transduction.

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REFERENCES