Compartment-Specific Feedback Loop and Regulated Trafficking Can Result in Sustained Activation of Ras at the Golgi

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ABSTRACT Imaging experiments have shown that cell signaling components such as Ras can be activated by growth factors at distinct subcellular locations. Trafficking between these subcellular locations is a regulated dynamic process. The effects of trafficking and the molecular mechanisms underlying compartment-specific Ras activation were studied using numerical simulations of an ordinary differential equation-based multi-compartment model. The simulations show that interplay between two distinct mechanisms, a palmitoylation cycle that controls Ras trafficking and a phospholipase C-ε (PLC-ε) driven feedback loop, can convert a transient calcium signal into prolonged Ras activation at the Golgi. Detailed analysis of the network identified PLC-ε as a key determinant of "compartment switching". Modulation of PLC-ε activity switches the location of activated Ras between the plasma membrane and Golgi through a new mechanism termed "kinetic scaffolding". These simulations indicate that multiple biochemical mechanisms, when appropriately coupled, can give rise to an intracellular compartment-specific sustained Ras activation in response to stimulation of growth factor receptors at the plasma membrane.

INTRODUCTION

Ras, a small GTPase, is a well-studied cellular signal transducer. The canonical pathway for Ras activation begins with dimerization and autophosphorylation of the receptor tyrosine kinases that bind growth factors. The receptor dimers phosphorylate the adaptor protein Shc, which then recruits the adaptor protein Grb2 and the guanine exchange factor (GEF) Sos to the plasma membrane. Sos then catalyzes the exchange of GTP for GDP on Ras (1). Recent development of novel fluorescence reporters of Ras activation has made it possible to monitor both the temporal and spatial dynamics of Ras activation (2,3). Using a green fluorescent protein (GFP)-based probe that binds specifically to GTP-bound Ras, it was shown that Ras is targeted to both the endoplasmic reticulum (ER)/Golgi and the plasma membrane and become activated in both compartments in response to receptor tyrosine kinase signaling (2,4). Furthermore, the activation pathway in each compartment exhibits distinct temporal dynamics. The activation of Ras at the plasma membrane is immediate and transient, whereas the activation at the ER/Golgi appears to be more delayed and persistent.

The mechanisms underlying the spatiotemporal dynamics of Ras activation are not yet known in complete details, but recent works have illuminated the importance of calcium in Ras signaling. In COS-1 cells, binding of epidermal growth factor (EGF) to EGF receptor (EGF-R) activates Src, which then phosphorylates phospholipase C-γ (PLC-γ). PLC-γ stimulates the production of diacylglycerol (DAG) and inositol triphosphate (IP_3), ultimately resulting in an elevated level of intracellular calcium (4,5). Increased intracellular calcium has both positive and negative regulatory functions. Binding of calcium to the Ras GEF RasGRP1 targets it to the ER/Golgi, where it can activate Ras (4,5). Furthermore, calcium binding to the Ras GTPase activating protein (GAP) calcium-promoted Ras inactivator (CAPRI) induces its translocation to the plasma membrane (4,6). This simultaneous activation of positive and negative regulators combined with the distinct spatial localization of the regulators lead to a complex situation in which the location and duration of Ras activation are regulated in a context-dependent manner. How can a transient signal such as calcium be converted into a prolonged signal such as activated Ras at the Golgi? What roles do compartment-specific biochemical reactions play in signal processing at different locations within the cell? What are the biochemical requirements of a network that would yield the observed experimental behavior? To address some of these questions, we have developed and analyzed a multicompartmental model of Ras activation.

MATERIALS AND METHODS

Model formulation

A schematic model of EGF-induced Ras activation in multiple cellular compartments is depicted in Fig. 1 *A*. The activation of Ras on the plasma membrane takes place via the canonical EGFR-Sos-Ras pathway, and was adapted from the model of Bhalla and Iyengar (7). The activation of PLC-γ and the subsequent calcium response were adapted from the work of Fink and colleagues (8–10).

Although the central role of calcium in Ras activation has been demonstrated, how a transient signal such as calcium can generate a sustained response such as Ras activation on the Golgi has not yet been elucidated. Experimental studies showed that EGF-stimulated calcium signaling occurs on the seconds to minutes timescale, whereas Golgi Ras signaling occurs on the minutes to hours timescale (2,11). We therefore hypothesized that the network structure contains both a time delay and a signal amplification

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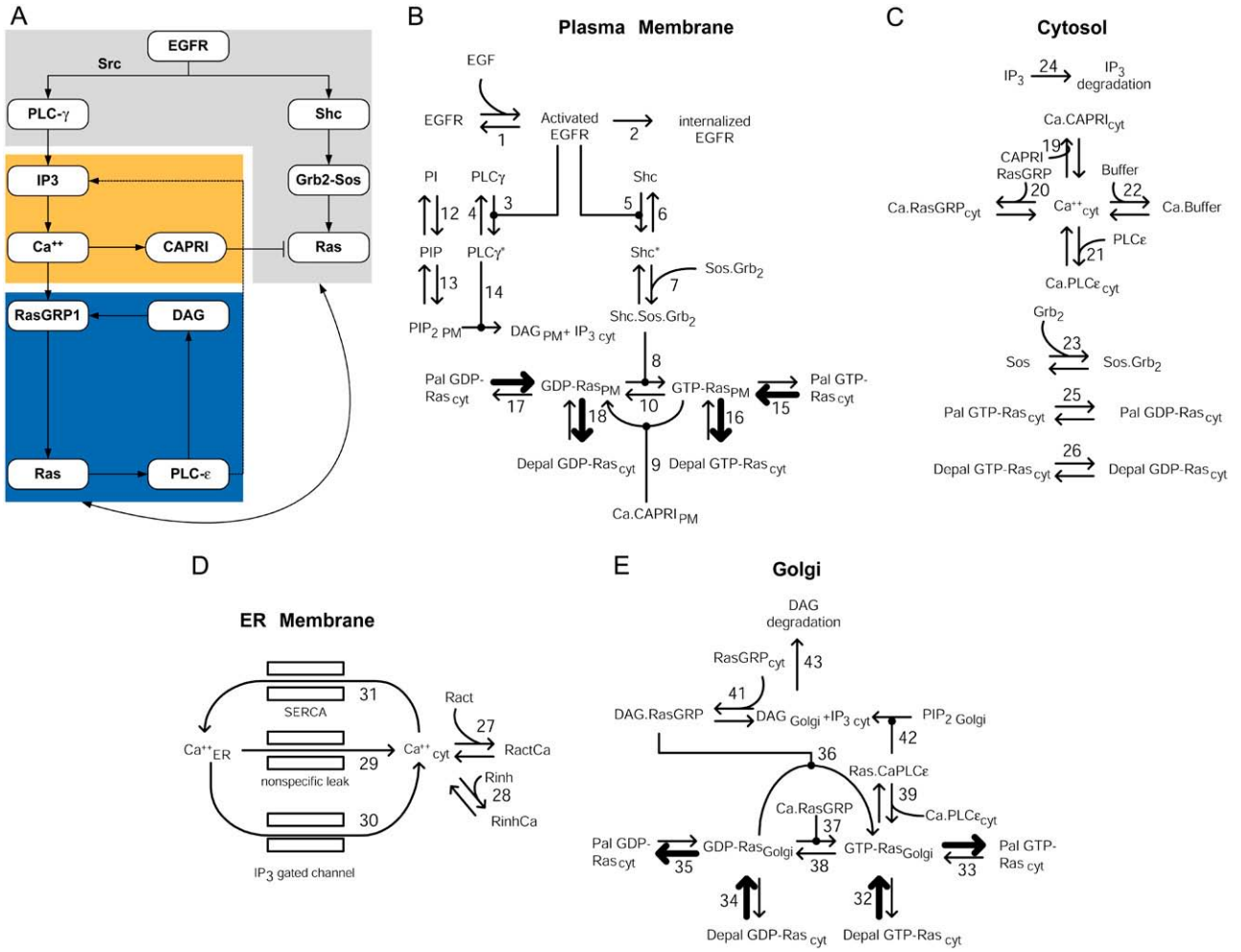

FIGURE 1 Mathematical model for compartment-specific activation of Ras. (*A*) A schematic model of Ras activation. The activation of Ras on the PM takes place via the canonical pathway. In this scheme, phosphorylated EGF receptor (EGFR) activates the adaptor protein Shc. Phosphorylated Shc recruits the adaptor protein Grb2 and the Ras GEF Sos to the plasma membrane. The activation of Ras on the Golgi requires PLC-γ-mediated PIP_2 hydrolysis. The hydrolysis reaction generates the second messenger DAG and IP_3. Binding of IP_3 to IP_3-gated calcium channel results in a transient rise in intracellular calcium. Calcium binding allows a phospholipase enzyme called PLC-ε to translocate to the Golgi. At the Golgi, Ras GTP activates PLC-ε, resulting in a local accumulation of DAG, which can then activate more RasGRP1. In addition to these biochemical reactions, GTP- and GDP-bound Ras molecules are trafficked between the Golgi and the plasma membrane according to their palmitoylation state. Depalmitoylated Ras is trapped on the Golgi, whereas palmitoylated Ras is exported to the plasma membrane. The components are color coded as followed: gray (PM), orange (cytoplasm), and blue (Golgi). (*B–D*) Biochemical reaction networks in various cellular compartments. Enzymatic species are denoted by a solid line with circular termination. Heavy arrows indicated the predominant direction of Ras transport. Each number corresponds to the appropriate reaction numbers given in Supplementary Table 2 (see Supplementary Material).

module. Several lines of experimental evidence suggest that DAG production on the Golgi may fulfill both functions. First, bryostatin, a DAG analog, is sufficient for activation of Ras on the Golgi but not the plasma membrane in Jurkat cells (4). Second, the putative GEF of Ras on the Golgi, RasGRP, is strongly activated by DAG (12,13). Third, overexpression of the protein diacylglycerol kinase zeta (DGK-ξ), which converts DAG to phosphatidic acid, blocks RasGRP1-dependent Ras activation, and expression of dominant negative DGK-ξ prolongs Ras signaling (14). A molecule that may potentially link Ras signaling to Golgi DAG production is the enzyme PLC-ε, which has been experimentally localized to intracellular membranes such as the Golgi and is activated by small G-proteins such as Ras and Rap1 (15,16). Integrating these results together, we developed a model where the initial activation of Ras at the Golgi occurs via calcium-bound RasGRP1. Once Ras is activated, PLC-ε binds Ras-GTP via its Ras-associating domain in a GTP-dependent manner (15,17). This binding stimulates the phospholipids hydrolysis activity of PLC-ε (15). The product of the reaction, DAG and IP_3 act as nested amplification loops. DAG can bind to and activate more RasGRP1, whereas IP_3 may prolong Ras activation by promoting further increase in intracellular calcium (18).

In addition to DAG-dependent Ras activation, two groups have shown that a palmitoylation-depalmitoylation cycle regulates Ras activation on the Golgi (3,19). Using GFP-fused Ras isoforms, it was shown that palmitoylated Ras is trafficked to the plasma membrane, where it can be activated by Sos. Conversely, depalmitoylated Ras ''falls off'' the plasma membrane, and is retrogradely trafficked to the Golgi via a nonendosomal pathway (Fig. 1 *A*). In this way, compartmentalization of Ras signaling may depend on the relative rates of palmitoylation and depalmitoylation. Activation in one compartment may be coupled to activation in the other compartment. Incorporating these features, we have developed the model shown in Fig. 1 *A*. The reactions within the model are shown in Fig. 1, *B–E*.

There are several additional assumptions that we have incorporated into the model, and these are provided in Supplementary Table 1. The rate constants and initial concentrations are listed in Tables 2 and 3 in the Supplementary Material.

Implementation in virtual cell

We developed a compartmental ordinary differential equation model using the Virtual Cell program (http://www.nrcam.uchc.edu/). The model consisted of three compartments: the plasma membrane (PM), the ER, and the Golgi. Detailed biochemical reaction networks in these compartments are shown in Fig. 1, *B–E*, and the parameters used for simulations are given in Tables 1 and 2 in the Appendix. When possible, we used signaling modules that have been described previously in literature (8–10, 20–22). The trafficking of Ras between the Golgi and the PM was modeled as two sequential reversible mass action reactions. For instance, the movement of Ras from the Golgi to the PM was modeled as a reaction that described the palmitoylation reaction as a first-order reaction, and another first-order reaction that described the binding of a transient cytoplasmic pool of Ras to the plasma membrane. The kinetics for Ras palmitoylation and depalmitoylation have been described elsewhere and was qualitatively constrained using the time courses from the work of Bivona and colleagues (2,23,24). The simulation results, as well as the MATLAB (The MathWorks, Natick, MA) m-file, SBML file, and ordinary differential equations used in the model, are available for download from the Virtual Cell website (file name: ''Eungdamrong and Iyengar BiophysJ'').

RESULTS

Input-output characteristics of Ras activation

The signal processing characteristics of the Ras signaling network are summarized in Figs. 2 and 3. At an EGF concentration of 40 ng/mL, the model predicted that intracellular calcium will increase immediately after stimulation and return to baseline within ~1 min (Fig. 2, *top panel*). This finding is qualitatively consistent with experimentally observed time course (11). In contrast to the rapid timescale of calcium signaling, Ras activation on the plasma membrane reached the maximum value in ~15 min and returned to the baseline gradually due to a combination of EGF receptor internalization and CAPRI-mediated inactivation. Ras activation on the Golgi occurred even more slowly. It began only after a delay of ~10 min and reached the maximum ~30–40 min poststimulation (Fig. 2, *lower panel*). These temporal profiles are in agreement with experimentally observed time courses (2,3). In our model, the delay in Ras activation at the Golgi was necessary for accumulation of DAG, which was required for calcium-independent activation of RasGRP. The increase in Golgi Ras GTP activity was concomitant to the increase in Golgi DAG level (data not shown).

It is well known that the amplitude and the duration of the signal may trigger different downstream responses (25). To understand the potential consequences of the balance between the positive and negative regulators, the dose-response curve for Ras activation at the plasma membrane was analyzed in two ways. First, the concentration of Ras-GTP was plotted as a function of EGF concentration at a given point in time (Fig. 3 *B*). We called this the instantaneous dose-response curve, since only the immediate downstream

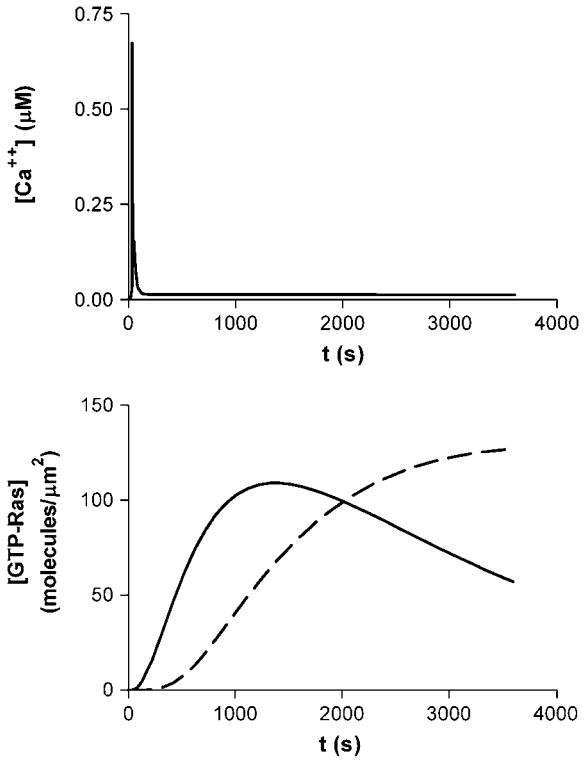

FIGURE 2 Amplification and prolongation of Ras signaling via localized feedback loops. EGF stimulation results in a transient rise in intracellular calcium (*top panel*). However, the increase in Golgi Ras-GTP is more gradual and sustained (*dashed line*, *bottom panel*). This persistent signaling is driven by both the retrograde trafficking of RasGTP from the plasma membrane, and the production and accumulation of diacylglycerol on the Golgi (*solid line*, PM Ras-GTP; *dashed line*, Golgi Ras-GTP).

components at the plasma membrane can sense and respond to transient changes in Ras-GTP concentration. Here, we found that the nature of the dose–response curve depended on the time at which the response was measured. At a relatively early time point (1000 s), the dose-response curve was hyperbolic (*open circles*, Fig. 3 *B*). At 1 h poststimulation, the curve was bell-shaped, indicating that high concentrations of EGF-inhibited Ras activation (*open squares*, Fig. 3 *B*). The inhibition of Ras activation by high EGF concentration has been observed in physiological cases, for example, in EGF-mediated gastric ulcer healing (26). Such ''band-pass'' behavior occurred because EGF activates both the positive (Sos) and negative (CAPRI) regulators of plasma membrane-localized Ras. Intermediate concentration of EGF resulted in the most sustained response. Higher concentration of EGF targeted more CAPRI to the plasma membrane, and thus resulted in a shorter duration of Ras activation. However, the highest EGF concentration also activated more Sos, resulting in the greatest peak response (Fig. 3 *A*).

A second way to describe the input-output relationship is to integrate under the concentration versus time curve. This time-integrated output, which depends on both the duration and the amplitude, reflects the effect of Ras activation on

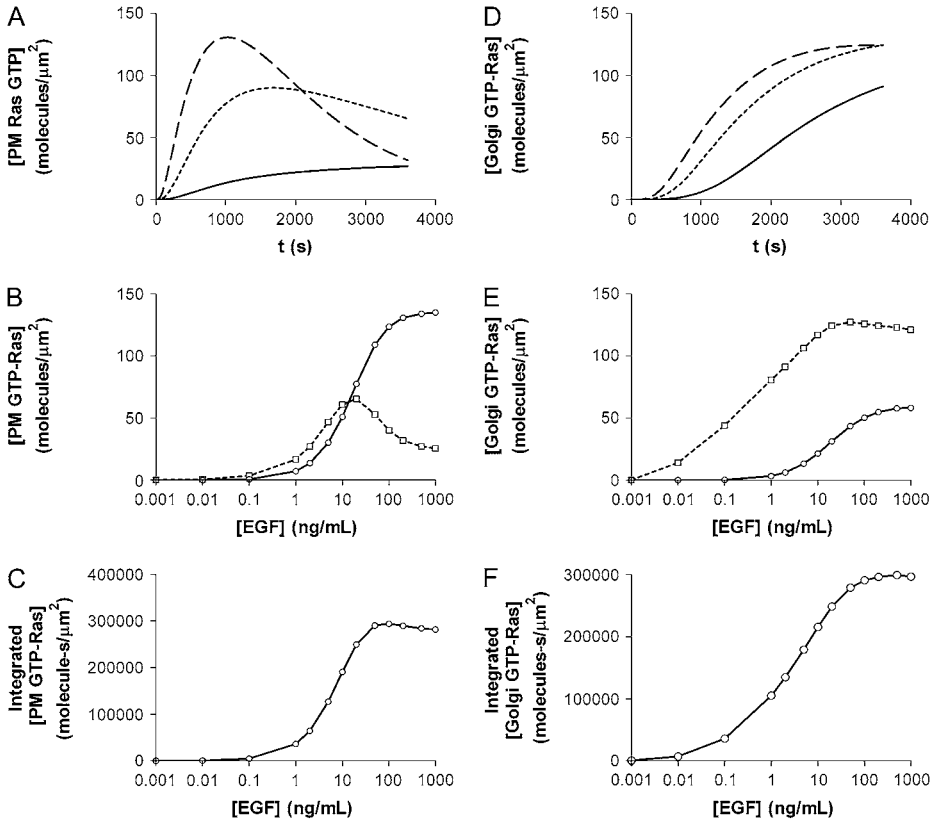

FIGURE 3 Instantaneous and time-integrated dose-response curves for Ras activation. (*A*) The temporal evolution of EGF-induced Ras signaling on the plasma membrane. EGF concentration was varied from 1 ng/mL to 500 ng/mL, and the concentration of Ras was plotted as a function of time. Only three time courses were plotted to maintain clarity. Intermediate concentrations of EGF resulted in a more sustained activation of PM Ras, but the highest concentrations of EGF resulted in a more transient response with a higher maximal amplitude (*solid line*, 2 ng/mL EGF; *dotted line*, 20 ng/mL EGF; *dashed line*, 200 ng/mL). (*B*) Instantaneous dose-response curve of PM Ras. The concentrations of PM Ras-GTP at the specified time points were plotted as a function of EGF concentration. At 1000 s post stimulation (~17 min), the dose-response is hyperbolic (Michaeles-Menten-like) (*solid line*). At 1 h poststimulation, the response is bell-shaped (*dotted line*). (*C*) Time integrated dose-response curve for PM Ras. The time integrated dose-response curve represents an integration of the area under the concentration versus time curves in Fig. 2 *A*. It is therefore a function of both the amplitude and the duration of signaling. The output was integrated over a period of 1 h and plotted as a function of EGF concentration. (*D*) The temporal evolution of EGF-induced Ras signaling on the Golgi. Increasing EGF concentration decreased the time delay and increased the rate at which Golgi-bound Ras is activated. (*solid line*, 2 ng/mL EGF; *dotted line*, 20 ng/mL EGF; *dashed line*, 200 ng/mL). (*E*) Instantaneous dose-response curve of Golgi Ras at 1000 s (*solid line*) and 1 h poststimulation (*dotted line*). (*F*) Time integrated dose-response curve of Golgi Ras.

more distal downstream components such as MAPK 1,2. Downstream components that are located in a different compartment may only respond to this integrated signal since biological processes with slower timescale (e.g., diffusion and trafficking) prevent the effectors from immediately sensing transient changes in Ras-GTP concentration. Fig. 3 *C* showed that this time-integrated dose-response curve was hyperbolic, as expected for most biological systems.

The activation of Ras at the Golgi differed from that on the plasma membrane in three ways. First, a similar maximal response was observed at multiple concentrations of EGF (Fig. 3 *D*). However, higher EGF concentration accelerated the rate at which this plateau is reached. Second, the instantaneous and time-integrated dose-response curves were hyperbolic at all time. Because the GAP activity at the Golgi is constitutive, not regulated by EGF, the band-pass behavior was absent and Ras activation was affected only by the relative ratio of the GEF and the constitutive Golgi GAP activity (Fig. 3, *E* and *F*). Finally, Ras activation at the Golgi was less sensitive to changes in EGF concentration, as evident by the slope of the linear part of the dose-response curve. This robustness emerged because Golgi Ras-GTP can be generated by two different mechanisms: retrograde trafficking of Ras-GTP from the plasma membrane and engagement of the DAG-dependent amplification loop. At early time points when activation of Ras on the plasma membrane is maximal, retrograde trafficking of Ras from the plasma membrane to the Golgi may be more important. It can ''jump start'' the amplification loop. At later time points, when plasma Ras-GTP is low and sufficient Ras-GTP has accumulated on the Golgi for PLC-ε recruitment, DAG-dependent mechanisms become more important.

Effect of constitutive GAP activity on compartment-specific Ras activation

The amplitude and duration of Ras signaling are ultimately determined by a balance between the activating GEF activity and the inactivating GAP activity. Typically, most cells contain multiple GAP proteins, only some of which are regulated by extracellular signals such as EGF (27,28). To examine how the constitutive GAP activity influences Ras signaling, we performed a sensitivity analysis of constitutive GAP activity at both the plasma membrane and the Golgi. Fig. 4 *A* illustrates how variations in calcium-independent GAP activity at the plasma affected Ras signaling. The constitutive GAP activity was varied from 1×10^{-5} to

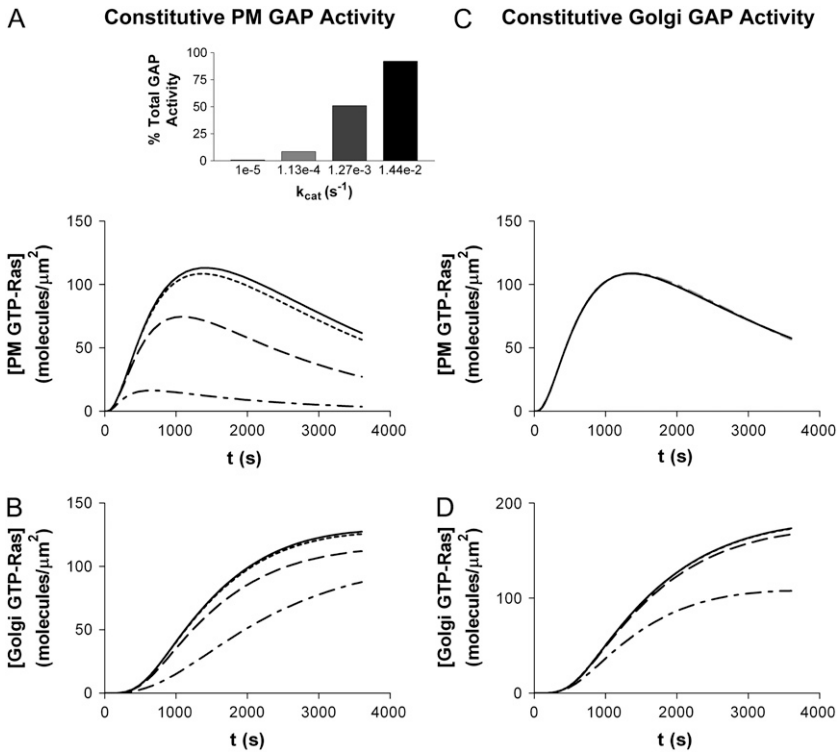

FIGURE 4 Coupling of Ras activation at the plasma membrane and the Golgi. (*A* and *B*) Sensitivity of Ras signaling to variations in noncalcium-regulated (constitutive) plasma membrane GAP activity. The activity of calcium-independent GAP activity at the plasma membrane was varied (baseline constitutive GAP activity = 1×10^{-4} s^{-1}). The concentrations of Ras GTP at the plasma membrane (*A*) and the Golgi (*B*) were then plotted as a function of time. The inset in *A* showed the activity of noncalcium-regulated GAP as a fraction of total GAP activity, both ligand-induced and constitutive, at the plasma membrane (*solid line*, GAP activity = 1×10^{-5} s^{-1}; *dotted line*, 1.13×10^{-4} s^{-1}; *dashed line*, 1.27×10^{-3} s^{-1}; *dash-dotted line*, $1.44 \times 1\ 0^{-2}$ s^{-1}). (*C* and *D*) Sensitivity of Ras signaling to variations in Golgi GAP activity. The activity of constitutive GAP on the Golgi membrane was varied over three orders of magnitude around the baseline case (baseline constitutive GAP activity V_{max} = 1 molecule μm^{-2} s^{-1}). Concentration of Ras GTP at the plasma membrane (*C*) and the Golgi (*D*) were plotted as a function of time (*solid line*, V_{max} = 1×10^{-3} molecules μm^{-2} s^{-1}; *dotted line*, 1.13×10^{-2} molecules μm^{-2} s^{-1}; *dashed line*, 0.127 molecules μm^{-2} s^{-1}; *dash-dotted line*, 1.44 molecules μm^{-2} s^{-1}).

1.44×10^{-2} s^{-1} (baseline rate = 1×10^{-4} s^{-1}). As expected, the maximal concentration of plasma membrane Ras-GTP decreased as the GAP activity increased (Fig. 4 *A*). However, the rate of the signal attenuation appeared unaffected. Furthermore, the activity of Golgi Ras-GTP was also affected since it is coupled to plasma membrane Ras-GTP through retrograde trafficking. However, the changes in Golgi Ras-GTP concentration were not as dramatic as those of plasma membrane-bound Ras-GTP (Fig. 4 *B*). This was because alternative mechanisms to activate Ras, such as RasGRP activation by DAG, existed. Unlike plasma membrane-localized Ras, Golgi-bound Ras is inactivated only by a constitutive GAP. When the Golgi GAP activity was varied (baseline V_{max} = 1 molecule/μm^2-s), the change in Ras-GTP level was not as extensive either at the Golgi or at the plasma membrane (Fig. 4, *C* and *D*). This modest effect at the Golgi was due to the predominant role of the GEF activity and the presence of the nested feedback loops that regulate the level of Ras-GTP at the Golgi.

A dynamic mechanism for compartment switching: ''kinetic scaffolding''

Since the palmitoylation-depalmitoylation cycle plays an important role in regulating the subcellular context of signaling, we investigated how the palmitoylation kinetics affected Ras activation at the plasma membrane and the Golgi. Experimental data showed that Ras is palmitoylated at the Golgi and depalmitoylated at the plasma membrane, and that this cycle results in trafficking of Ras between these two subcellular compartments (3,19). We hypothesized that the competition between anterograde trafficking of Ras from the Golgi to the plasma membrane and the sequestration of Ras on the Golgi due to PLC-ε binding can result in novel switching behaviors. When the palmitoylation rate of Ras GDP (baseline rate = 1.5×10^{-2} s^{-1}) was decreased by fivefold, from 5.07×10^{-3} s^{-1} to 1×10^{-3} s^{-1}, more Ras-GTP accumulated on the Golgi. This relatively modest effect ($\sim$19%) was expected, since decreasing the palmitoylation rate would slow down the rate at which Ras molecules are exported to the plasma membrane (Fig. 5 *A*). In contrast, the effect on Ras signaling at the plasma membrane was far more dramatic. Between a palmitoylation rate of 3.71×10^{-3} s^{-1} and 2.36×10^{-3} s^{-1}, a value about an order of magnitude below that used in the baseline simulation, the concentration of Ras-GTP the plasma membrane decreased rapidly and significantly $\sim$1300 s after EGF stimulation (Fig. 5 *B*). We called this phenomenon, whereby output in one compartment is switched off in favor of increasing output in a different compartment, ''compartment switching''.

The molecular mechanism underlying compartment switching was analyzed by examining the reactions that Ras participated in both at the Golgi and at the plasma membrane. At the Golgi, Ras-GTP binds to PLC-ε, which functions a scaffold to hold Ras at the Golgi. This interaction closes a feedback loop that allows the Golgi to sequester more Ras molecules and maintain them in the activated state. This positive feedback loop results from Ras activation of

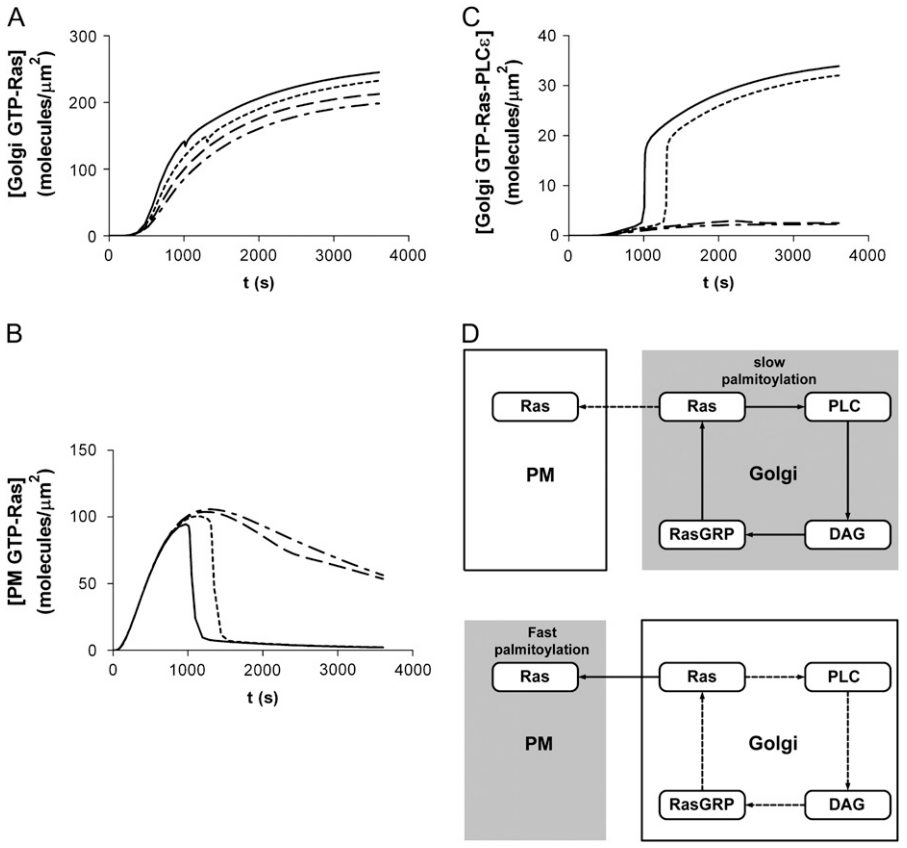

FIGURE 5 Switching of Ras signaling on the plasma membrane by a kinetic scaffolding mechanism. (*A*) Response of Golgi Ras-GTP to variations in the palmitoylation rate of Golgi Ras-GDP. The palmitoylation rate of Golgi Ras-GDP (baseline value = 1.5×10^{-2} s^{-1}) was varied, and the concentration of Golgi Ras-GTP was then plotted as a function of time (*solid line*, 1×10^{-3} s^{-1}; *dotted line*, 2.36×10^{-3} s^{-1}; *dashed line*, 3.71×10^{-3} s^{-1}; *dash-dotted line*, 5.07×10^{-3} s^{-1}). (*B*) Response of PM Ras-GTP to variations in palmitoylation rate of Golgi Ras-GDP. The time course of PM Ras-GTP was plotted for different Golgi Ras-GDP palmitoylation rate. At a palmitoylation rate between 2.36×10^{-3} s^{-1} and 3.71×10^{-3} s^{-1}, switching occurred. Note that these rates are an order of magnitude slower than the rate used for baseline simulation (rate = 0.015 s^{-1}). (*C*) Engagement of the DAG-dependent feedback loop at low palmitoylation rates. The activity of the PLCε-dependent feedback loop, as measured by the formation of the PLCε-Ras-GTP complex, was examined at different palmitoylation rates. The switching of plasma membrane Ras signaling occurred concomitantly with the formation of PLCε-Ras-GTP complex on the Golgi membrane. (*D*) A schematic model for location switching. When the palmitoylation rate is slow (*dotted line*) or PLCε activity is high, Ras accumulates preferentially on the Golgi, and the positive feedback loop involving DAG production is switched on (*shaded background*). When palmitoylation rate is fast (*solid line*), RasGTP is preferentially trafficked to the plasma membrane. However, signaling on the Golgi is not significantly affected since Ras GTP and GDP are depalmitoylated and eventually returned to the Golgi.

PLC-ε, which stimulates DAG production. The accumulation of DAG on the Golgi recruits and activates RasGRP, which in turn activates Ras. It should be noted that the large surface area of the Golgi in relative to that of the plasma membrane (6.67-fold greater) indicates that a small increase in Golgi Ras can correspond to a large decrease in plasma membrane Ras concentration. To determine whether this kinetic scaffolding is the mechanism for the compartment switching, the activity of the feedback loops, as measured by the level of Ras-GTP-PLC-ε complex, was plotted at various rates of palmitoylation. At the lowest rate of palmitoylation, a large amount of Ras-PLC-ε complex accumulates on the Golgi membrane, suggesting that the DAG feedback loop was active and Ras was sequestered by PLC-ε. As the rate of palmitoylation increased, transport to the plasma membrane was favored, and the dynamic trapping of Ras on the Golgi decreased. This was evident by the lower concentrations of Ras-PLC-ε complex on the Golgi (*dashed* and *dash-dotted lines* in Fig. 5 *C*). Under such conditions, Ras-GTP accumulated on the Golgi only as a result of retrograde transport of plasma membrane localized Ras-GTP. Fig. 5 *D* schematically summarizes how changes in the rate of palmitoylation affected the extent of Ras activation at the plasma membrane and the Golgi. When the palmitoylation is relatively slow, Ras is trapped on the Golgi and DAG increases (Fig. 5 *D*, *top panel*). When palmitoylation is fast, plasma membrane is the favored site of Ras activation and the presence of activated Ras at the Golgi occurs mostly by retrograde trafficking (Fig. 5 *D*, *bottom panel*).

Another potential mechanism by which compartment switching could occur is through modulation of the PLC-ε level. Increasing PLC-ε concentration by only threefold led to a switch-like decrease in Ras-GTP levels at the plasma membrane (Fig. 6 *A*). This sharp decrease resulted from a scaffolding effect that trapped Ras at the Golgi as the concentration of PLC-ε increased. As expected, these simulations also showed that there is a substantial increase in the levels of GTP-Ras at the Golgi with increasing levels of PLC-ε (Fig. 6 *B*). Taken together, these results supported the hypothesis that PLC-ε functions as a key determinant of the subcellular location of Ras signaling.

DISCUSSION

The focus of this study was to develop computational approaches to understand how a signal that originated at the

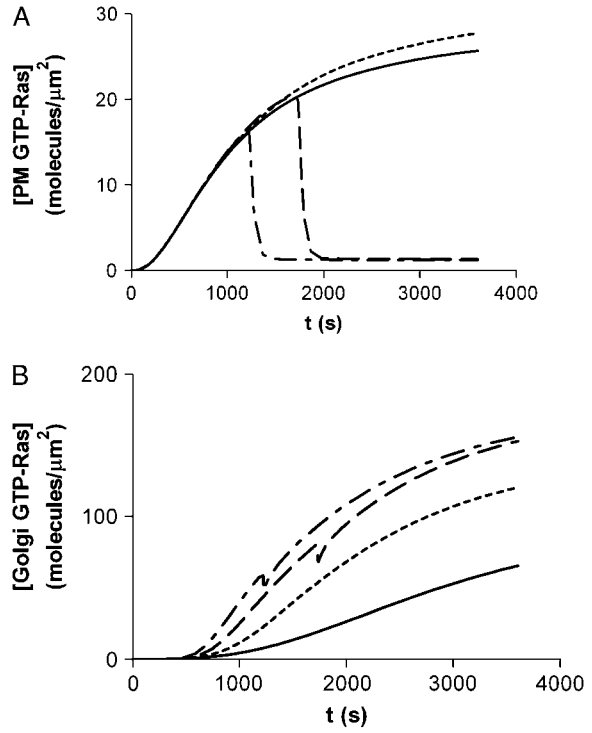

FIGURE 6 Kinetic scaffolding by PLC-ε leads to compartment-switching of Ras signaling. The kinetic scaffolding effect was investigated by varying both PLC-ε (15.1–43.4 nM) and EGF concentrations (0.1–100 ng/mL) simultaneously. At a representative EGF concentration (2 ng/mL), the concentration of Ras GTP at the plasma membrane (*A*) and the Golgi (*B*) were plotted at various initial PLC-ε concentration (*solid line*, [PLC-ε] = 15.1 nM; *dotted line*, 29.3 nM; *dashed line*, 43.4 nM; *dash-dotted line*, 57.6 nM). A similar behavior was observed for each EGF concentration tested.

plasma membrane propagated through different subcellular regions. This analysis showed that the presence of a compartment-specific feedback loop coupled with regulation of cellular transport by mechanisms such as a palmitoylation-depalmitoylation cycle can result in persistent activation of Ras at the Golgi. The numerical simulations showed several interesting systems-level properties. The configuration of the network as deduced from the experimental data indicated that calcium can deactivate Ras at the plasma membrane while simultaneously activating Ras at the Golgi. This concurrent dual regulation results in distinct instantaneous and time-integrated dose-response curves to varying concentrations of EGF. The instantaneous response of plasma membrane-bound Ras changed from a hyperbolic response to a bell-shaped ''band-pass'' response, whereas the responses of Golgi signaling were always hyperbolic. The switch in response from hyperbolic to bell-shaped reflects the role of the calcium-dependent activation of the CAPRI, the Ras-GAP that deactivates Ras at the plasma membrane. This dynamic switching of the dose-response via dual regulation of both the positive and negative regulators may represent a common biological mechanism that explains the behavior of other signaling networks (29,30).

The mechanisms underlying Ras activation at the Golgi are complex and poorly understood. Previous studies indicated that the level of Ras at the Golgi and the plasma membrane are coupled through intracellular trafficking. Our simulations indicate that the experimentally observed persistent activation of Ras at the Golgi is likely to occur due to multiple mechanisms that operate at different timescales. At early times after EGF stimulation, the activated Ras at the Golgi may result from retrograde trafficking of Ras that had been activated at the plasma membrane. At later times, stimulation of PLC-ε by Golgi localized Ras-GTP resulted in de novo production of DAG that activated the GEF RasGRP1. This feedback loop can maintain Ras in its activated state and modulate switching of Ras signaling from the plasma membrane to the Golgi. The nested feedback loops of trafficking, reinforced by local feedback loops, result in relative robust response at the Golgi where Ras activity is not significantly affected by perturbations in EGF concentration, constitutive GAP activity, palmitoylation kinetics, or PLC-ε concentration. In contrast, Ras activation at the plasma membrane is sensitive to these same perturbations, since there is no operational feedback loop at the plasma membrane in this network. Thus, the results of this study illustrate the capability of nested feedback loops not only in maintaining persistent signal output, but also in specifying the subcellular location of such persistent signals.

The role of activation of the various Ras isoforms in cell proliferation and transformation is well-established. Recent experimental data indicate that both the subcellular location and the extent of Ras activation can play a role in signal routing to the various protein kinases and consequently elicit differential biological responses. For instance, H-Ras activation at the Golgi has different activation profiles of the downstream effectors MAPK 1,2, Akt, and Jnk (2). In NIH-3T3 fibroblasts, constitutively activated Ras that has been targeted to the ER can stimulate Ras-dependent transcriptional activity and cause transformation (2). In PC12 cells, activation of Ras on the Golgi appears to play a role in neurite outgrowth. Overexpression of RasGRP1, which selectively activates Ras at the Golgi, promotes neurite outgrowth. Silencing the same gene blocks PC12 cell differentiation (4). Recent studies also indicate that local-activation Ras may play a role in determining directionality during cell migration (31). All of these observations suggest that that not only is the extent of Ras activation important in determining cellular responses, but also where Ras is activated. Our simulations here provide an initial insight into how the interplay of biochemical reactions can lead to changes in levels of active Ras at different subcellular locations in response to receptor stimulation at the plasma membrane.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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