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Stimulus-induced dissociation of α subunits of heterotrimeric GTP-binding proteins from the cytoskeleton of human neutrophils

(signal transduction)

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ABSTRACT **Previous studies on the mechanism responsible for terminating the generation of second messengers induced by chemotactic factor–receptor complexes have, on one hand, suggested a direct role of a GTP-binding protein(s) (G protein), and, on the other hand, proposed that there is a lateral segregation of the ligand–receptor complexes into G protein-depleted domains of the plasma membrane. In the present investigation, which addresses these apparently contradictory findings, we found that a substantial part of the α subunits of the G_n protein ($G_{n\alpha}$) in unstimulated neutrophils were associated with a cytoskeletal fraction and that release of these subunits occurred upon stimulation with the chemotactic factor fMet-Leu-Phe. An identical $G_{n\alpha}$ release could also be induced by direct activation of G proteins with guanosine 5′-[γ-thio]triphosphate or AlF_4^-. In contrast, the α subunits of the stimulatory G protein ($G_{s\alpha}$) also found associated with the cytoskeletal fraction of unstimulated cells were not released by fMet-Leu-Phe stimulation. However, they were effectively released by direct G-protein activation with guanosine 5′-[γ-thio]triphosphate. In addition, inhibition of the fMet-Leu-Phe-stimulated modulation of the actin network by pertussis toxin did not affect the fMet-Leu-Phe-induced release of $G_{n\alpha}$ from the cytoskeletal fraction. These observations indicate that fMet-Leu-Phe-induced activation of neutrophils involves a specific dissociation of $G_{n\alpha}$ from the cytoskeleton and that this release is not a consequence of the well-known effect of fMet-Leu-Phe on the cytoskeleton of neutrophils. The present data contribute ideas concerning the transducing properties of G proteins in cellular signaling and seem to reconcile the apparently contradictory concepts of how the cytoskeleton participates in the termination of the chemotactic-factor-induced generation of second messengers in human neutrophils.**

Human neutrophils play a major role in the body's defense against invading microorganisms. In performance of this function, these cells are able to adhere, migrate, phagocytose, and produce microbicidal substances. These cellular responses occur under the influence of specific ligands and depend on a series of consecutive events: (*i*) the specific binding of a ligand to its receptor (*ii*) the transduction and generation of intracellular signals, and (*iii*) the activation of appropriate biochemical pathways that trigger the cellular responses (1).

The signal derived from the ligand–receptor complex is generally transduced further downstream by GTP-binding proteins (G proteins). The "traditional" G proteins consist of three distinct subunits, α (39–52 kDa), β (35–36 kDa), and γ (8–10 kDa). The GTP-induced activation of the heterotrimeric G proteins results in a subsequent dissociation of the subunits. The βγ subunits are structurally very similar and are, to some degree, functionally interchangeable because they appear to interact identically with different α subunits (2). In contrast, the α subunit is specific for each G protein and is capable of binding and hydrolyzing GTP. The GTP-bound form of the α subunit is believed responsible for the transduction of the ligand-induced signal by coupling to intracellular effector systems (2). Neutrophils have been reported to contain several distinct heterotrimeric G proteins, of which G_n [neutrophil GTP-binding protein (G_{i-2})], with a 40-kDa α subunit, has been proposed to be responsible for transducing the signal between the chemotactic receptor and its effector systems (3).

At present G proteins are not only discussed in the context of cell activation but also in aspects of cell deactivation. It has been suggested that in the plasma membrane of stimulated neutrophils chemotactic factor–receptor complexes undergo lateral segregation from their corresponding heterotrimeric G proteins and might thereby lose their capacity to generate additional second messengers (4–6). Furthermore, the termination of the signaling properties of the chemotactic factor–receptor complex is also believed to be modulated by the cytoskeleton because, within a few seconds after its formation, the complex is known to become associated with the cytoskeleton (7–9). We have previously shown that the association between the chemotactic factor–receptor complex and the cytoskeleton could be inhibited by pretreating electropermeabilized cells with guanosine 5′-[β-thio]diphosphate (GDP[βS]) (10), suggesting a permissive role of G protein activation. Considered together, these data suggest that there is an intimate interaction between ligand–receptor complexes, G proteins, and the cytoskeleton of neutrophils as part of the initiation and termination of second-messenger generation.

The aim of the present study was to gain further knowledge about the basic properties and role of G proteins in cellular activation/deactivation by elucidating whether G proteins do interact with the cytoskeleton of human neutrophils and under what circumstances such an interaction might occur.

MATERIALS AND METHODS

Materials. All reagents used were of analytical grade. Dextran and Ficoll–Paque were obtained from Pharmacia, fMet-Leu-Phe was from Sigma, and guanosine 5′-[γ-thio]triphosphate (GTP[γS]) was purchased from Boehringer Mannheim. The peroxidase-conjugated goat anti-rabbit antiserum was obtained from Dako (Glostrup, Denmark). The enhanced chemiluminescence detection system was from

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Abbreviations: G protein, GTP-binding protein; G_α, α subunit(s) of the heterotrimeric G protein; G_β, β subunit(s) of the heterotrimeric G protein; GDP[βS], guanosine-5′-[β-thio]diphosphate; GTP[γS], guanosine 5′-[γ-thio]triphosphate; G_i, inhibitory G protein; G_n, neutrophil GTP-binding protein (G_{i-2}); $G_{n\alpha}$, α subunit(s) of G_n protein; G_s, stimulatory G protein; $G_{s\alpha}$, α subunit(s) of G_s.

Amersham. The antisera used were as follows: (*i*) the rabbit antiserum R16,17, raised against a peptide corresponding to the 9-amino acid C-terminal sequence of G_i and shown to react specifically with $G_{n\alpha}$ ($G_{i\text{-}2\alpha}$) > $G_{i\text{-}1\alpha} \gg G_{i\text{-}3\alpha}$ or $G_{o\alpha}$ (= anti-$G_{n\alpha}$ antibody) (11); (*ii*) the rabbit antiserum RM/1, raised against a synthetic decapeptide corresponding to the C terminus of stimulatory G protein (G_s). This antiserum is specific for the α subunit of G_s ($G_{s\alpha}$) with crossreactivity to the α subunit of olfactory G protein and was purchased from NEN (= anti-$G_{s\alpha}$ antibody) (12); (*iii*) the rabbit antiserum R3,4, raised against bovine brain 35/36-kDa β subunit of the heterotrimeric G protein (G_β) was shown to react specifically with β subunits from all G proteins (= anti-G_β antibody) (11).

Isolation of Human Neutrophils. Peripheral human blood was obtained from healthy volunteers and collected in heparin-containing vacutainer tubes. After sedimentation on dextran, the neutrophils were isolated according to the method described by Böyum (13). In short, contaminating erythrocytes were removed by a brief hypotonic lysis in distilled water, after which the cell suspension was centrifuged on a Ficoll–Paque gradient to separate the polymorphonuclear leukocytes from lymphocytes, monocytes, and platelets. The neutrophils were then washed twice before resuspension in a calcium-containing medium with the following composition: 136 mM NaCl/4.7 mM KCl/1.2 mM $MgSO_4$/1.1 mM $CaCl_2$/0.1 mM EGTA/1.2 mM KH_2PO_4/5.0 mM $NaHCO_3$/5.5 mM glucose/20 mM Hepes, pH 7.4. The cells were kept on ice pending further processing. Because G_β was extremely sensitive to proteolytic degradation, cells were routinely pretreated with 5 mM diisopropyl fluorophosphate for 10 min.

Permeabilization of Neutrophils. The cells were electrically permeabilized according to a described method (10, 14). Briefly, in this study the cells (10^7 cells per ml) were suspended in 120 mM KCl/10 mM NaCl/2 mM $MgCl_2$/10 mM Pipes/3 mM EGTA/0.2% (wt/vol) human albumin/ ≈250 nM $CaCl_2$, pH 6.8 (15). The cells were kept on ice and rendered permeable by repeated exposures (150 μs each) to an electric field of 1.7 kV/cm. After permeabilization, the cells were immediately used in the different experiments, as described in the figure legends.

Preparation of the Cytoskeletal Fraction. Cytoskeletal fractions were prepared essentially as described (10), using Triton X-100-containing medium with the following composition: 25 mM Hepes (pH 7.4), 2 mM $MnCl_2$, 4 mM iodoacetic acid, 10 μM Na_3VO_4, pepstatin at 5 μg/ml, leupeptin at 5 μg/ml, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 1.0% (vol/vol) Triton X-100. In short, neutrophils (stimulated or unstimulated) were incubated with ice-cold Triton X-100-containing medium for 10 min on ice and then centrifuged for 10 s at ≈9000 × *g*. The obtained cytoskeletal preparations were washed once, pelleted, and prepared for electrophoresis by resuspending the pellets in a sample buffer with the following composition: 62.5 mM Tris·Cl (pH 6.8), 2% (wt/vol) SDS, 5% (vol/vol) mercaptoethanol, and 11.5% (vol/vol) glycerol.

Electrophoresis and Immunoblotting. Electrophoresis was done according to the method described by Laemmli (16). In short, the pellets from intact cells and cytoskeletal preparations were boiled in the sample buffer for 5 min. These samples (1×10^6 cell equivalents per lane) were then applied to a 10–20% polyacrylamide gradient gel containing 10% SDS (wt/vol). The resolved proteins were electrophoretically transferred to a poly(vinylidene difluoride) membrane. After transfer, the membranes were blocked with 5% (wt/vol) bovine serum albumin in phosphate-buffered saline (PBS), pH 7.3, overnight at 4°C, and for an additional 30 min at 37°C in the same medium supplemented with 1% (wt/vol) dried milk. The membranes used for immunoblotting with anti-G_β antibody were blocked solely with 1% (wt/vol) dried milk in PBS for 30 min at 37°C. The immunoblotting was done by exposing the poly(vinylidene difluoride) membranes to the different primary antibodies, after which the immune reaction was detected with a peroxidase-conjugated secondary antibody, followed by enhanced chemiluminescence detection. Densitometer analyses were done with an UltroScan XL enhanced laser densitometer (LKB).

RESULTS

Effects of fMet-Leu-Phe-Stimulation on Localization of $G_{n\alpha}$ and G_β. The immunoblot in Fig. 1*A* shows the presence of $G_{n\alpha}$ in intact cells and cytoskeletal preparations. As expected, the 40-kDa G_α was found in equal amounts in the whole-cell preparations of both unstimulated (lane 1) and fMet-Leu-Phe-stimulated (lane 2) cells. The 40-kDa subunit was also found in the cytoskeletal fractions of unstimulated neutrophils (lane 3), whereas the presence of the protein in the cytoskeletal fraction of fMet-Leu-Phe-stimulated cells was considerably reduced and sometimes totally abolished (lane 4). Densitometer analysis of these blots revealed that only 17 ± 5% (mean ± SEM, $n = 6$) of the protein remained associated with the cytoskeletal fraction after fMet-Leu-Phe stimulation. In contrast, the common 35/36-kDa G_β (Fig. 1*B*) was detected in both unstimulated and fMet-Leu-Phe-stimulated intact cells (lanes 1 and 2), whereas only trace amounts were found in the cytoskeletal fractions (lanes 3 and 4). The $G_{n\alpha}$ and G_β absent in the cytoskeletal fractions (Fig. 1*A*, lane 4 and Fig. 1*B*, lanes 3 and 4) were fully recovered in the supernatants obtained when the cytoskeletal fractions were pelleted (data not shown). On the basis of these findings, we have in the present study concentrated our interest on the interaction between G_α and the cytoskeleton.

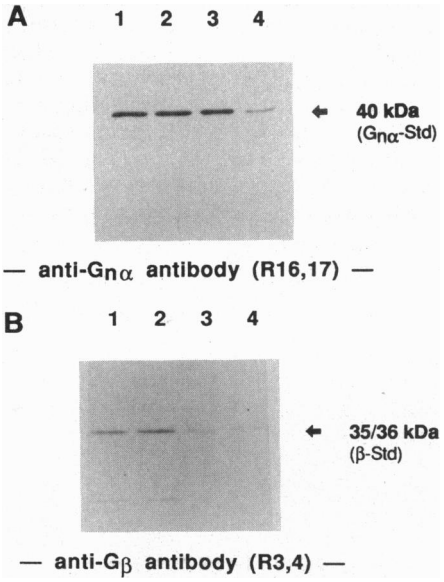

FIG. 1. Immunoblot analysis of the distribution of $G_{n\alpha}$ and G_β after stimulation with fMet-Leu-Phe. Neutrophils were equilibrated for 5 min at 37°C and then stimulated with 20 nM fMet-Leu-Phe for 30 s. The stimulation was stopped by putting the cells on ice and simultaneously adding ice-cold medium. For comparison, other neutrophils were subjected to the 5-min equilibration period at 37°C but were not stimulated with fMet-Leu-Phe. Lanes: 1–4, respectively, unstimulated and fMet-Leu-Phe-stimulated intact cells and cytoskeletal fractions of unstimulated and fMet-Leu-Phe-stimulated cells. The proteins were detected by using a 1:5000 dilution of the anti-$G_{n\alpha}$ antibody (R16,17) (*A*) or a 1:5000 dilution of the anti-G_β antibody (R3,4) (*B*). At this dilution the anti-G_β antibody also weakly detected an unknown protein of a lower molecular mass in intact cells.

Effects of Fluoride- or GTP[γS] Stimulation on Localization of $G_{n\alpha}$. To further examine the mechanism(s) behind the release of $G_{n\alpha}$ from the cytoskeleton, ligand–receptor complex formation was bypassed by directly activating cellular G proteins with either 100 μM GTP[γS] or 10 μM $AlCl_3$ and 20 mM NaF (hereafter referred to as AlF_4^-). To enable these experiments, the cells were first electropermeabilized to allow rapid introduction of the stimuli. The permeabilization had no obvious effect on the cellular content of 40-kDa $G_{n\alpha}$ when comparing intact and permeabilized cells from the same donor (data not shown). $G_{n\alpha}$ was found in roughly equal amounts in the whole-cell preparations of both unstimulated and stimulated permeabilized cells (Fig. 2 *A* and *B*, lanes 1 and 2). As in Fig. 1, a significant amount of $G_{n\alpha}$ was present in the cytoskeletal fraction of unstimulated cells (Fig. 2 *A* and *B*, lanes 3). After stimulating neutrophils with AlF_4^- or GTP[γS], however, the presence of 40-kDa $G_{n\alpha}$ was almost abolished in the cytoskeletal fraction (Fig. 2 *A* and *B*, lane 4). The dissociated $G_{n\alpha}$ was instead fully recovered in the supernatants obtained when the cytoskeletal fractions were pelleted (data not shown). These data support the results obtained with fMet-Leu-Phe (Fig. 1), suggesting that the $G_{n\alpha}$ found associated with the cytoskeletal fraction in unstimulated cells dissociates from this fraction as a result of their activation.

Effects of fMet-Leu-Phe- or GTP[γS] Stimulation on Localization of $G_{s\alpha}$. To further examine the specificity of the mechanism(s) behind the dissociation of $G_{n\alpha}$, we tested the possible effects of fMet-Leu-Phe and GTP[γS] on the interaction of $G_{s\alpha}$ with the cytoskeleton (Fig. 3 *A* and *B*). The $G_{s\alpha}$ were found in roughly equal amounts in the whole-cell preparations of unstimulated and stimulated intact cells (Fig. 3*A*, lanes 1 and 2) as well as that of permeabilized cells (Fig. 3*B*, lanes 1 and 2). As with $G_{n\alpha}$ (Fig. 1), a significant amount of $G_{s\alpha}$ was present in the cytoskeletal fraction of unstimulated cells (Fig. 3 *A* and *B*, lanes 3). However, after stimulating intact neutrophils with fMet-Leu-Phe, no reduction of the presence of $G_{s\alpha}$ was detected in the cytoskeletal fraction (Fig. 3*A*, lane 4). In contrast, after stimulating permeabilized neutrophils with 100 μM GTP[γS], the presence of $G_{s\alpha}$ was abolished in the cytoskeletal fraction (Fig. 3*B*, lane 4). The dissociated $G_{s\alpha}$ were instead fully recovered in the supernatants obtained when the cytoskeletal fractions were pelleted

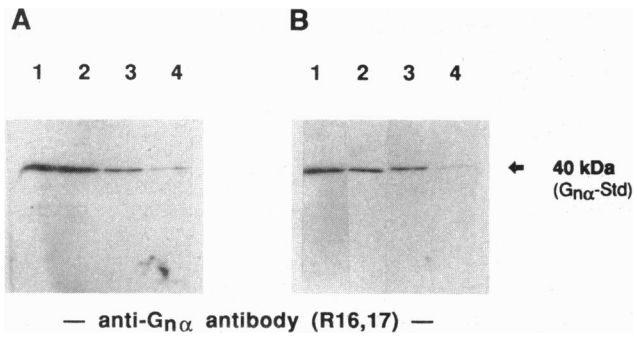

FIG. 2. Immunoblot analysis of the distribution of $G_{n\alpha}$ after stimulation with AlF_4^- or GTP[γS]. Neutrophils were permeabilized, after which 20 mM NaF and 10 μM $AlCl_3$ or 100 μM GTP[γS] was immediately added. Preparations stood at 4°C for 5 min and were then transferred to a 37°C waterbath for an additional 10-min incubation. The stimulation was stopped by putting the cells on ice and simultaneously adding ice-cold medium. For comparison, other neutrophils were permeabilized and subjected to the incubation periods at 4°C and 37°C but were not stimulated with AlF_4^- or GTP[γS]. Lanes 1 and 2, respectively, show unstimulated and AlF_4^-- (*A*) or GTP[γS]- (*B*) stimulated cells; lanes 3 and 4 show the cytoskeletal fractions of unstimulated and AlF_4^-- (*A*) or GTP[γS]- (*B*) stimulated cells. Proteins were detected with a 1:5000 dilution of the anti-$G_{n\alpha}$ antibody (R16,17).

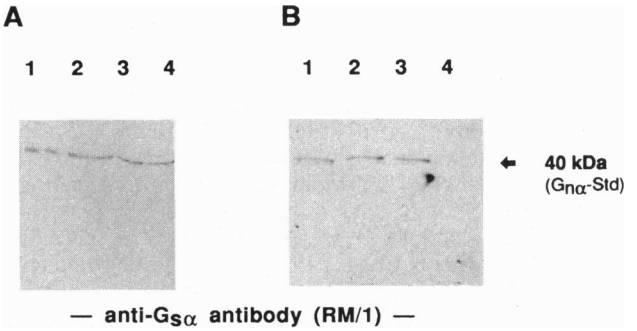

FIG. 3. Immunoblot analysis of the distribution of $G_{s\alpha}$ after stimulation with fMet-Leu-Phe or GTP[γS]. (*A*) Neutrophils were equilibrated for 5 min at 37°C and then stimulated with 20 nM fMet-Leu-Phe for 30 s. For comparison, other neutrophils were subjected to the 5 min equilibration period at 37°C but were not stimulated with fMet-Leu-Phe. (*B*) Neutrophils were permeabilized, after which 100 μM GTP[γS] was immediately added. Preparations stood at 4°C for 5 min and were thereafter transferred to a 37°C waterbath for an additional 10-min incubation. For comparison, other neutrophils were permeabilized and subjected to the incubation periods at 4°C and 37°C but were not stimulated with GTP[γS]. In both *A* and *B* the stimulation was stopped by putting the cells on ice and simultaneously adding ice-cold medium. Lanes 1 and 2, respectively, show unstimulated and fMet-Leu-Phe- (*A*) or GTP[γS]- (*B*) stimulated cells; lanes 3 and 4, respectively, show the cytoskeletal fractions of unstimulated and fMet-Leu-Phe- (*A*) or GTP[γS]- (*B*) stimulated cells. Proteins were detected with a 1:2500 dilution of the anti-$G_{s\alpha}$ antibody (RM/1). The indicated position of the $G_{n\alpha}$ standard in both *A* and *B* was detected by reblotting with the anti-$G_{n\alpha}$ antibody (R16,17).

(data not shown). Reblotting of the immunoblots shown in Fig. 3 *A* and *B* with the anti-$G_{n\alpha}$ antibody yielded the same results as those shown for fMet-Leu-Phe in Fig. 1 and for GTP[γS] in Fig. 2*B* (data not shown). These data point to a specific effect of fMet-Leu-Phe on the release of $G_{n\alpha}$ and, therefore, support the previous results suggesting that G_α associated with the cytoskeletal fraction in unstimulated cells dissociate from this fraction only when activated.

Effect of Pertussis Toxin on the fMet-Leu-Phe-Induced Dissociation of $G_{n\alpha}$. Pertussis toxin was used to investigate whether inhibition of the fMet-Leu-Phe-induced modulation of the actin network could prevent the fMet-Leu-Phe-stimulated release of $G_{n\alpha}$. The experiments presented in Fig. 4*A* show that both the primary association of $G_{n\alpha}$ to the cytoskeleton and the fMet-Leu-Phe-induced release are only marginally effected by incubation at 37°C for 2 hr (lanes 1–4), the time period required for the toxin-induced ADP-ribosylation. The batch of pertussis toxin used in these experiments effectively abolished the fMet-Leu-Phe-induced polymerization of actin (data not shown). Despite this total inhibition, pretreatment with pertussis toxin (1000 ng/ml) did not prevent the fMet-Leu-Phe-induced release of $G_{n\alpha}$ from the cytoskeletal fraction (Fig. 4*B*, lanes 1 and 2). Densitometer analysis of these blots revealed that only 19 ± 3% (mean ± SEM, $n = 3$) of the protein band remained associated with the cytoskeletal fraction after fMet-Leu-Phe stimulation.

DISCUSSION

Immunoblot analysis using antibodies directed against different subunits of heterotrimeric G proteins not only confirmed the appearance of G_α and G_β in whole lysates of human neutrophils but, more interestingly, for G_α also confirmed its appearance in a cytoskeletal fraction of these cells. We were unable, however, to detect a significant amount of G_β in the cytoskeletal fraction of neutrophils. This result agrees with previous data on subcellular fractionation of neutrophils, demonstrating that both G_α and G_β are mainly

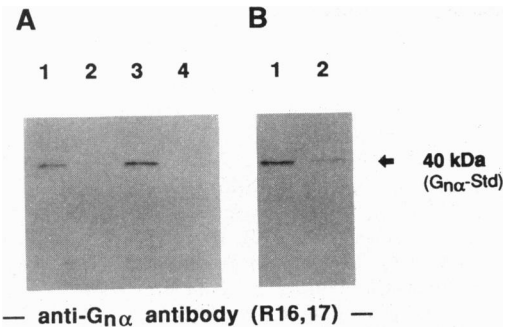

FIG. 4. Immunoblot analysis of the distribution of $G_{n\alpha}$ in neutrophils treated with pertussis toxin. Neutrophils (1×10^7 cells per ml) were incubated for 2 hr at 37°C with pertussis toxin (1000 ng/ml). The cells were then washed, resuspended (5.5×10^6 cells per ml), preincubated for 5 min at 37°C, and then stimulated with fMet-Leu-Phe (20 nM) for 30 s. The stimulation was stopped by putting the cells on ice and simultaneously adding ice-cold medium. (*A*) Control samples—i.e., cells not treated with pertussis toxin but subjected to 2-hr incubations at 37°C (lanes 1 and 2) or 4°C (lanes 3 and 4): analysis of cytoskeletal fractions of unstimulated neutrophils (lanes 1 and 3) and cytoskeletal fractions of fMet-Leu-Phe-stimulated neutrophils (lanes 2 and 4). (*B*) Cytoskeletal fractions of pertussis toxin-treated unstimulated (lane 1) or fMet-Leu-Phe-stimulated (lane 2) neutrophils. Proteins were detected with a 1:5000 dilution of the anti-$G_{n\alpha}$ antibody (R16,17).

recovered from the plasma-membrane fraction, but the former is also found in the cytosolic fraction (11).

The present experiments reveal an association between the cytoskeleton and key elements of the signal-transduction system in nonstimulated cells, which suggests that, in addition to its vital role in cell motility, the cytoskeleton also functions as an organizer and "matrix," optimizing and perhaps regulating the generation of second messengers. This concept has previously been discussed in regard to the regulation of the phosphatidylinositol cycle, as the cytoskeleton can interact and regulate phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (17, 18). Furthermore, other enzymes involved in inositol phospholipid metabolism have also been found associated with the cytoskeleton (19, 20). Of greater interest concerning the present results is a recently proposed hypothetical model of the cellular organization of signal-transduction systems in which G proteins in nonstimulated cells are organized in a multimeric structure, presumably via cytoskeletal interactions (21). In this model, receptor-induced GTP activation of the G protein, as well as any type of GTP activation, would not only cause the structure of the G_α to change to its active form but would also cause the release of G_α, enabling them to interact and activate their target enzymes.

The G_n protein is generally considered to be the one activated by fMet-Leu-Phe and responsible for transducing the signal between the chemotactic receptor and its effector systems (3). In accordance with the model discussed above, the present study shows that fMet-Leu-Phe stimulation selectively triggers dissociation of α subunits of the G_n protein from the cytoskeleton without affecting the cytoskeletal association of $G_{s\alpha}$. This effect of fMet-Leu-Phe on the $G_{n\alpha}$ release from the cytoskeleton can be mimicked by GTP[γS] or AlF_4^-, both of which bypass the ligand–receptor complex formation and directly activate cellular G proteins. In addition, direct activation of cellular G proteins with GTP[γS] also caused an almost total release of $G_{s\alpha}$ from the cytoskeleton. Finally, inhibition of the fMet-Leu-Phe-stimulated modulation of the actin network by pertussis toxin (22, 23) did not affect the fMet-Leu-Phe-induced release of $G_{n\alpha}$ from the cytoskeletal fraction. These data suggest that the release of G_α from the cytoskeleton of human neutrophils is due to an effect on the G protein rather than to an indirect effect on the actin network.

As previously mentioned, both $G_{n\alpha}$ and G_β are mainly recovered from the plasma-membrane fraction, but the former is also found in the cytosolic fraction (11). Taken together with the finding that in human neutrophils the $G_{n\alpha}$ are in large excess of the $G_{\beta\gamma}$ (11), this result suggests the existence of at least two different pools of $G_{n\alpha}$. In relation to the present data and the previous findings discussed above, it seems reasonable to suggest that the excess of G_α is organized in a cytoskeletal-associated G protein pool. Interesting in this context was the recent finding that pertussis toxin catalyzed ADP-ribosylation of G_i protein from bovine brain inhibits the binding of this G_α to solubilized photoaffinity-labeled N-formyl chemotactic peptide receptors (24). The present observation, however, that pertussis toxin did not prevent the fMet-Leu-Phe-induced release of $G_{n\alpha}$ from the cytoskeletal fraction suggests that the fMet-Leu-Phe–receptor complex can still interact with $G_{n\alpha}$ associated with the cytoskeleton. These somewhat contradictory findings may be explained if $G_{n\alpha}$ is divided into at least two different populations, one that is associated with and can be released from the cytoskeleton upon receptor interaction, even if it is ADP-ribosylated, and a second that is not associated with the cytoskeleton, which cannot interact with the fMet-Leu-Phe–receptor complex after ADP-ribosylation.

Our previous results (10) demonstrating that GDP[βS] can inhibit the association between fMet-Leu-Phe–receptor complexes and the cytoskeleton of neutrophils, suggested that a G protein was involved in the regulation of the interaction between the ligand–receptor complexes and the cytoskeleton and the resulting termination of second-messenger generation. In contrast, the simultaneously published data of Jesaitis and coworkers (5), indicated that G proteins and fMet-Leu-Phe–receptor complexes became physically and functionally dissociated from each other due to a lateral segregation of these proteins in the plasma membrane. At time of publication, the results from these two studies appeared contradictory. However, these data can now be put into a scenario that satisfies the model of G protein organization discussed above. In nonstimulated neutrophils, $G_{n\alpha}$ is either closely associated with the cytoskeleton and/or with $G_{\beta\gamma}$ in the plasma membrane. Binding of the chemotactic factor fMet-Leu-Phe to its receptor and the subsequent transfer of G_α to its active GTP-bound form, will trigger the dissociation of G_α from $G_{\beta\gamma}$ as well as the release of the G_α from the cytoskeleton. Each free G_α can then interact with and activate its target enzyme. The release of G_α from the cytoskeleton would allow ligand–receptor complexes to associate with the cytoskeleton and account for the segregation of G proteins from the fMet-Leu-Phe–receptor complexes.

In conclusion, the present results suggest a role of the cytoskeleton in modulating the transducing properties of G proteins in cellular signaling. The observation that $G_{n\alpha}$ is specifically released from the cytoskeleton upon activation with a chemtotactic factor may account for previous results regarding the termination of second-messenger generation derived from the chemotactic factor–receptor complex. The functional importance of the finding that $G_{n\alpha}$ is released from the cytoskeleton upon activation remains to be further elucidated.

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