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The small G-protein $ARF1_{GDP}$ binds to the $G_t\beta\gamma$ subunit of transducin, but not to $G_t\alpha_{GDP}$-$G_t\beta\gamma$

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Abstract AlF_4^- **activates heterotrimeric G-proteins Gα subunits but not small GDP/GTP-binding proteins like ARF1. On retinal membranes containing holotransducin ($G_t\alpha_{GDP}$-$G_t\beta\gamma$) and incubated with ARF_{GDP}, AlF_4^- induced $G_t\alpha_{GDP\text{-}AlF4}$ release and ARF_{GDP} binding, probably to the remaining membrane-attached $G_t\beta\gamma$. On phospholipid vesicles reconstituted with $G_t\beta\gamma$, ARF_{GDP} bound in proportion to $G_t\beta\gamma$, and was released upon subsequent $G_t\alpha_{GDP}$ addition. Thus ARF_{GDP} competes with $G_t\alpha_{GDP}$ for binding to $G_t\beta\gamma$, probably through a conserved motif in the 'α2 helix' of $G_t\alpha$ and ARF. This motif is found in the C-terminal helix of PH domains that bind to G$\beta\gamma$.**

Key words: ARF; G-protein; G$\beta\gamma$; Aluminofluoride; PH domain

1. Introduction

ADP-ribosylation factors, or ARFs are a family of small (~20 kDa) monomeric guanine-nucleotide binding proteins, originally discovered as cofactors for cholera toxin-catalysed ADP-ribosylation of heterotrimeric G-protein Gα subunits [1]. But ARFs are localized at high concentration in the Golgi complex, and their main physiological function might be in vesicular transport [2]. ARF polypeptides are comparable in length and are weakly homologous to small G-proteins of the ras superfamily, but they display closer homologies with the guanine nucleotide binding domain of Gα subunits, taking into account that these Gα subunits have an additional α-helical domain distinct from the nucleotide binding domain [3,4]. Furthermore ARFs are acylated at the N-terminal, like most Gα subunits, rather than isoprenylated at the C-terminal like ras. However, like ras, ARFs are not activated by aluminium fluoride [5], which activates the $G\alpha_{GDP}$ subunit of heterotrimeric G-proteins by binding next to the β phosphate of GDP, and simulating the γ phosphate of GTP [6,7]. The observation that AlF_4^- could perturb the process of vesicle formation in the Golgi [8–11], thus led to speculations that an heterotrimeric G-protein might also be involved in the vesicular transport process in the Golgi.

We have previously analysed the interaction of ARF with phospholipid membranes [12,13], using recombinant ARF produced in *E. coli* and purified either in the unacylated form (rARF) or in the myristoylated form (myrARF). $myrARF_{GDP}$ partially binds to phospholipid membranes, but $rARF_{GDP}$ remains fully in solution. Upon exchanging GDP for GTPγS, both $rARF_{GTP\gamma S}$ and $myrARF_{GTP\gamma S}$ bind permanently to phospholipid membranes. As expected, AlF_4^- does not modify the binding of $rARF_{GDP}$ or myr ARF_{GDP}.

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We have also analysed the interaction of heterotrimeric G-proteins with phospholipid membranes, taking retinal transducin as model [14]. In the inactive GDP-bound state, transducin subunits are associated in $G_t\alpha_{GDP}$-$G_t\beta\gamma$ heterotrimer and are membrane-bound; upon exchanging GDP for GTPγS, the $G_t\alpha$ subunit dissociates from $G_t\beta\gamma$ and is released from the membrane as soluble $G_t\alpha$GTPγS; AlF_4^- induces also the dissociation of holotransducin and the release of activated $G_t\alpha_{GDP\text{-}AlF4}$ leaving $G_t\beta\gamma$ alone on the membrane.

Here we investigated the interaction of ARF1 with transducin on unilluminated retinal rod membranes, which contain very little endogenous ARF and a large amount of transducin in the associated form $G_t\alpha_{GDP}$-$G_t\beta\gamma$. The membranes were incubated with exogenous $rARF_{GDP}$. In the absence of GTP, this added $rARF_{GDP}$ bound only very weakly to the membrane. Treating these preparations with AlF_4^- had the expected effect of releasing soluble $G_t\alpha_{GDP\text{-}AlF4}$ from the membrane, but also the surprising effect of increasing the membrane binding of rARF. As this binding could not result from a direct action of AlF_4^- on $rARF_{GDP}$, we surmised that it revealed a specific interaction of $rARF_{GDP}$ with the $G_t\beta\gamma$ subunits that remained alone on the membrane. We inferred that in native membranes the $rARF_{GDP}$-$G_t\beta\gamma$ interaction was hindered by the binding of $G_t\alpha$GDP to $G_t\beta\gamma$. This hypothesis was tested by studying the binding of ARF to phospholipid vesicles reconstituted with various combinations of purified $G_t\alpha$ and $G_t\beta\gamma$ subunits.

2. Material and methods

2.1. Buffers

Isotonic buffer: 20 mM Tris-HCl, pH 7.5; 100 mM NaCl; 1.5 mM $MgCl_2$; 0.1 mM PMSF; 5 mM β-mercapto-ethanol. For the experiments with Retinal Membranes, this buffer was complemented with 15 μM GDP. Low ionic strength buffer: 5 mM Tris-HCl, pH 7.5; 0.1 mM $MgCl_2$; 0.1 mM PMSF; 5 mM β-mercapto-ethanol.

2.2. Phospholipid vesicles

Large unilamellar vesicles were prepared as described by Szoka [15]. 20 mg of azolectin (soybean lipids, Sigma) were dissolved in 6 ml of diethyl ether, 1 ml of aqueous buffer (50 mM HEPES, pH 7.5) was added, the mixture was sonicated 2 min at 0°C. The solvent was removed under reduced pressure, the aqueous suspension of vesicles was filtered through 0.8 μm filter and stored at 4°C under argon.

2.3. Bovine retinal rod outer segment membranes

Bovine retinal rod outer segment membranes (ROS) were prepared under dim red light as described by Kühn [16] and stored at −80°C.

2.4. Proteins

Non-myristoylated recombinant ARF1 (rARF) was expressed in *E. coli* and purified near homogeneity by a single anion exchange chromatography QAE Sepharose column (Pharmacia) as previously described [12].

Myristoylated recombinant ARF1 (myrARF) was obtained as

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described elsewhere [13]. Briefly, bacteria were cotransformed with the pET11d/gly2ARF1 and pBB131/yeast *N*-myristoyl transferase plasmids. After expression, cells were harvested and lysed. Myristoylated ARF was precipitated at 35% saturation of ammonium sulfate whereas most bacterial proteins and the non-myristoylated form of ARF remained in the soluble fraction. Then sequential chromatography on DEAE Sepharose and MonoS columns removed the remaining contaminants and allowed to obtain myrARF near homogeneity.

Transducin subunits $G_t\alpha_{GDP}$, $G_t\alpha_{GTP\gamma S}$ and $G_t\beta\gamma$ were extracted from ROS membranes and purified as previously described [14].

Quantitation of proteins binding to membranes or phospholipid vesicles was made by densitometry of Coomassie blue stained SDS PAGE of aliquot pellet and supernatant fractions of the sedimented membranes.

3. Results

Recombinant unmyristoylated $rARF_{GDP}$ was added to native, non illuminated retinal membranes, in which rhodopsin is inactive and transducin is in the basal $G_t\alpha_{GDP}$-$G_t\beta\gamma$ state. The membranes were incubated in isotonic buffer (which retains transducin on the membrane), with 25 μM of rARF, a concentration in excess to that of transducin (10 μM) in the membrane suspension. After sedimentation, the proteins were extracted from the membrane pellet by low ionic strength washing, and were analysed by SDS PAGE. As observed previously with phospholipid vesicles [12], $rARF_{GDP}$ did not bind significantly to these retinal membranes: only a small proportion (~3%) of the total added rARF was retained in the membrane pellet. An aliquot of the membrane suspension supplemented with rARF was treated with AlF_4^-, in the dark, to activate transducin without photoexciting rhodopsin. This AlF_4^- treatment induced the release in the supernatant of a large fraction of $G_t\alpha$, in the $G_t\alpha_{GDP\text{-}AlF4}$ form, and also the release of a small proportion of $G_t\beta\gamma$ which is less tightly attached to membrane phospholipids when isolated than when associated with $G_t\alpha_{GDP}$ [14]. Thus, as expected, the pellet of the AlF_4^- treated sample retained much less $G_t\alpha$ and a little less $G_t\beta\gamma$ than the untreated control pellet. But, unexpectedly, this membrane pellet retained also much more rARF than the control pellet, sedimented in the absence of AlF_4^- (Fig. 1). We have shown previously [12] that rARF binds to phospholipid membranes upon its activation by GTP or GTPγS, but it is well established [5], and we have also extensively checked (data not shown) that AlF_4^- does not activate $rARF_{GDP}$, and does not increase its binding to phospholipid vesicles. Thus the increased binding of $rARF_{GDP}$ to the retinal membranes upon AlF_4^- treatment could not be due to an activation of $rARF_{GDP}$ by AlF_4^-. We were compelled to assume an indirect effect of AlF_4^- through its action on another protein present on the retinal membrane, which would result in uncovering a site of interaction for ARF_{GDP}. The most likely candidate was the $G_t\alpha_{GDP}$ subunit of transducin, which is associated to $G_t\beta\gamma$ in the native membrane. Upon the activation by AlF_4^-, $G_t\alpha_{GDP\text{-}AlF4}$ dissociates from $G_t\beta\gamma$ which remains membrane-bound and could provide a site of interaction for ARF_{GDP}.

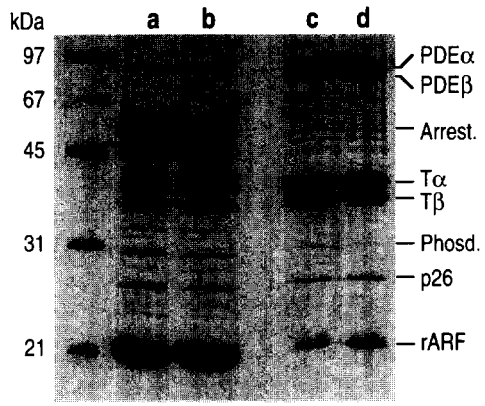

Fig. 1. Increased binding of $rARF_{GDP}$ to retinal membranes upon the activation of endogenous transducin by AlF_4. Retinal membranes (100 μM rhodopsin, ~ 10 μM transducin) suspended in isotonic saline buffer were incubated with 25 μM rARF; one aliquot was further incubated with AlF_4^- (50 μM $AlCl_3$, 5 mM NaF) and both samples were sedimented; a and b: SDS PAGE of soluble proteins in the supernatants of the untreated and AlF_4^- treated samples; c and d: proteins extracted by low ionic strength buffer from the corresponding membrane pellets. In (a) one recognises soluble retinal proteins (arrestin, phosducin, p26), small fractions of transducin $G_t\alpha$ and $G_t\beta\gamma$, and most of the added rARF that remained in solution (more than 90% of total, compare with c); the pellet (c) has retained most of the cGMP-phosphodiesterase (PDE) and of transducin, and a very small proportion of the added rARF. In (b), AlF_4^- has significantly increased the solubility of $G_t\alpha$ (and a little that of $G_t\beta\gamma$); the major soluble pool of rARF does not seem significantly decreased, but in (d), correlated with the loss of $G_t\alpha$ to the solution, one sees a 3–4-fold increase of the minor pool of rARF retained with the pellet. These data were reproduced over 5 independent experiments.

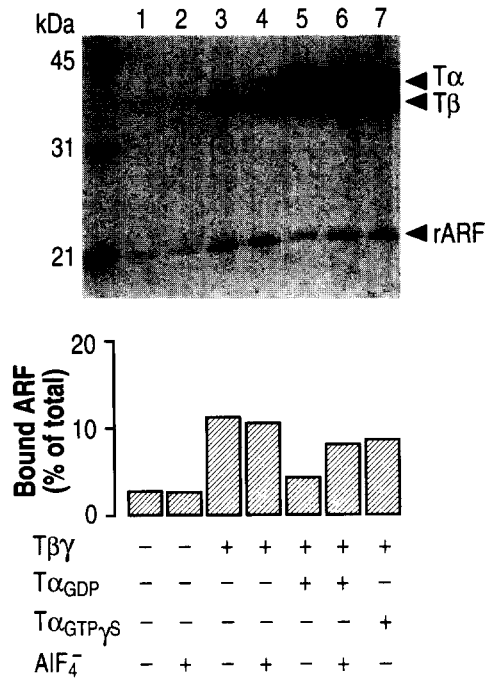

Fig. 2. Binding of $rARF_{GDP}$ to phospholipid vesicles reconstituted with $G_t\beta\gamma$ and $G_t\alpha$ subunits. Suspensions of large unilamellar phospholipid vesicles (2 mg azolectin/ml) in saline buffer were supplemented with $G_t\beta\gamma$ (6 μM), $G_t\alpha_{GDP}$ (6 μM), $G_t\alpha_{GTP\gamma S}$ (5 μM) and AlF_4 (50 μM $AlCl_3$, 5 mM NaF) as indicated; $rARF_{GDP}$ was added to 5 μM final concentration. The vesicles were sedimented, and the protein content of supernatant (not shown) and pellet were analysed by SDS PAGE and densitometry of the Coomassie blue stained gels. In the absence of any transducin subunit (lanes 1 and 2), the vesicle-bound rARF amounts to only 3% of the total added. The addition of $G_t\beta\gamma$ induced a marked increase in rARF binding (lane 3), which was unsensitive to AlF_4^- (lane 4). Further addition of $G_t\alpha_{GDP}$ induced a decrease of rARF binding (lane 5), which was now sensitive to AlF_4^- (lane 6). Addition of $G_t\alpha_{GTP\gamma S}$ did not induce a decrease of the binding induced by $G_t\beta\gamma$ (lane 7). These data were reproduced over 3 independent experiments.

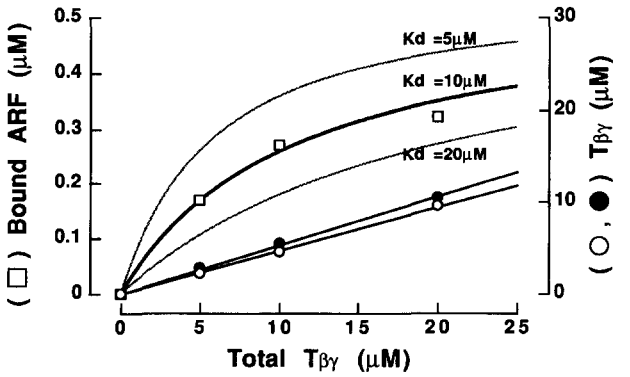

Fig. 3. Concentration dependence of $G_t\beta\gamma$ and $rARF_{GDP}$ binding to phospholipid vesicles. Suspensions of phospholipid vesicles (5 mg azolectin/ml) in saline buffer were supplemented with 1 μM $rARF_{GDP}$ and increasing concentrations of $G_t\beta\gamma$. After sedimentation, $T\beta\gamma$ content of pellet (●) and supernatant (○), and ARF content of pellet (□) were analyzed by SDS-PAGE and quantified by densitometry of Coomassie blue stained gels. $G_t\beta\gamma$-vesicle binding is strictly linear with ($G_t\beta\gamma$) in this concentration range. ARF binding was modeled with a bimolecular interaction scheme assuming the same affinity for $G_t\beta\gamma$ in solution and vesicle-bound $G_t\beta\gamma$. The best fit (thick line) was obtained for k_d = 10 μM.

To investigate this hypothesis we studied the binding of rARF to model phospholipid vesicles supplemented with purified $G_t\beta\gamma$ and/or $G_t\alpha$ subunits (Fig. 2). We used the same sedimentation assay as with retinal membranes. We had previously documented the binding of transducin subunits added at micromolar concentrations to these phospholipid vesicles [14]: under our standard incubation and sedimentation conditions, native, isolated $G_t\alpha_{GDP}$ subunits, as well as $G_t\alpha_{GTP\gamma S}$ and $G_t\alpha_{GDP\text{-}AlF4}$, bound only in a small proportion (~15%); native isolated $G_t\beta\gamma$ subunits bound more (~60% in the vesicle pellet); when both $G_t\alpha_{GDP}$ and $G_t\beta\gamma$ were added together, they bound cooperatively as $G_t\alpha_{GDP}$-$G_t\beta\gamma$ heterotrimers, 80% of both subunits sedimenting in the vesicle pellet. In contrast $G_t\alpha_{GTP\gamma S}$ or $G_t\alpha_{GDP\text{-}AlF4}$, when added together with $G_t\beta\gamma$, remained mostly in solution and did not increase the binding of $G_t\beta\gamma$.

As observed previously [12], a very small proportion (~3%) of the rARF that was incubated with the phospholipid vesicle suspension sedimented with the vesicle pellet. An addition of purified $G_t\alpha_{GDP}$ to the suspension did not induce any supplementary binding of $rARF_{GDP}$ to the vesicle pellet (not shown). By contrast, the addition to the suspension of purified $G_t\beta\gamma$ increased the binding of $rARF_{GDP}$ to the pelleted vesicles by a factor of 3 to 4 (Fig. 2). As expected, this $G_t\beta\gamma$-dependent binding of $rARF_{GDP}$ to the phospholipid vesicles was unsensitive to AlF_4^-.

The further addition of $G_t\alpha_{GDP}$, in stoichiometric amount to $G_t\beta\gamma$, to the phospholipid vesicle suspension incubated with $rARF_{GDP}$, induced the expected increase of $G_t\beta\gamma$ binding to the vesicles, concurrent with a stoichiometric binding of $G_t\alpha$, and induced also a very marked decrease of $rARF_{GDP}$ binding, down to the low level observed initially on the phospholipid vesicles before the addition of any of the transducin subunits. But now, an AlF_4^- treatment on these phosholipid vesicles supplemented with $rARF_{GDP}$ and both $G_t\alpha_{GDP}$ and $G_t\beta\gamma$, had effects on $G_t\alpha$ and rARF binding: the pool of bound $G_t\alpha$ was substantially decreased, as expected from the solubilisation of $G_t\alpha_{GDP\text{-}AlF4}$, and the binding of $rARF_{GDP}$ was increased up to the level that had been observed on vesicles supplemented with $G_t\beta\gamma$ alone: this duplicated exactly the observation made initially on retinal membranes. As a control, an aliquot of the vesicle suspension incubated with $rARF_{GDP}$ and $G_t\beta\gamma$ was supplemented with a stoichiometric amount of $G_t\alpha_{GTP\gamma S}$. A fraction of this added $G_t\alpha_{GTP\gamma S}$ bound to the vesicles, but this did not increase the amount of bound $G_t\beta\gamma$, nor decreased the binding of ARF_{GDP}. Thus like $G_t\alpha_{GDP\text{-}AlF4}$, $G_t\alpha_{GTP\gamma S}$ did not interact with $G_t\beta\gamma$ on the membrane, and did not interfere with the binding of ARF_{GDP} to the membrane. All these observations concurred to suggest that $rARF_{GDP}$ binds specifically to $G_t\beta\gamma$ on phospholipid vesicles, and that $G_t\alpha_{GDP}$ inhibits this binding, probably by competing for a common site on $G_t\beta\gamma$.

Under all above conditions, that is with total concentrations of $G_t\beta\gamma$ and of $rARF_{GDP}$ in the micromolar range, this $G_t\beta\gamma$ dependent membrane binding of $rARF_{GDP}$ remained limited (of the order of 10% of the added $rARF_{GDP}$), but it was proportional to the amount of vesicle-bound $G_t\beta\gamma$ and to the concentration of $rARF_{GDP}$ in the suspension. This gave an upper limit of 30–50 μM for the affinity of $rARF_{GDP}$ for $G_t\beta\gamma$, under the simplistic assumption that $G_t\beta\gamma$ would be totally membrane-bound, or that $rARF_{GDP}$ would bind exclusively to the membrane-bound $G_t\beta\gamma$. But neither assumption is correct: a constant fraction (45%) of $T\beta\gamma$ remained in solution, when $T\beta\gamma$ was added up to 20 μM in a 5 mg/ml phospholipid vesicle suspension (Fig. 3) and the fraction of vesicle-bound $T\beta\gamma$ was

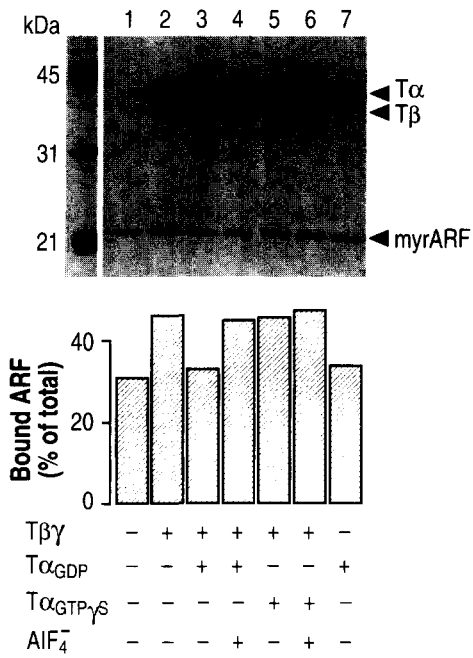

Fig. 4. Binding of $myrARF_{GDP}$ to phospholipid vesicles reconstituted with $G_t\alpha$ and $G_t\beta\gamma$ subunits. Same conditions as in Fig. 2 except for the lower concentration (1 μM) of myrARF added here to the vesicle suspension. In the absence of any transducin subunit ~30% of the total added myrARF was retained in the vesicle pellet (lane 1). The addition of $G_t\beta\gamma$ induced a 10% increase in myrARF binding (lane 2).This is comparable to the increase observed for rARF binding in Fig. 2, but is harder to see on the gel as the relative effect is much smaller. Further additions of $G_t\alpha$ and AlF_4^- have also effects comparable to that seen on Fig. 2 with rARF. Addition of $G_t\alpha_{GDP}$ alone (lane 7) had no effect on $myrARF_{GDP}$ binding. These data were reproduced over 3 independent experiments.

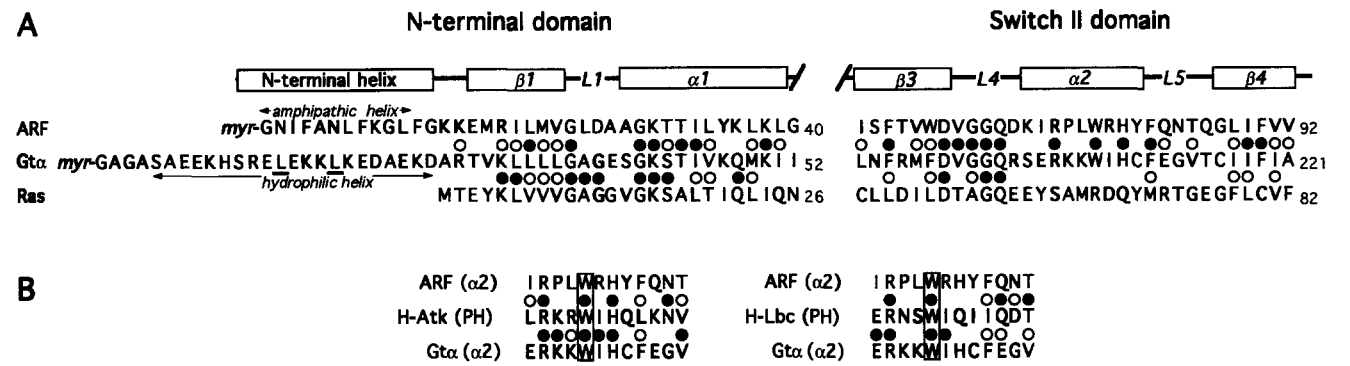

Fig. 5. (A) Sequence homologies between ARF, $G_t\alpha$ and ras in the N-terminal and 'Switch II' domains. (● = identity, ○ = analogy, secondary structure elements indexed as in [23]). The main site of interaction of $G_t\alpha_{GDP}$ with $G_t\beta\gamma$ is in the N-terminal hydrophilic helix of $G_t\alpha$, which has no homology with the N-terminal amphipathic helix of ARF. The conserved leucins of the coiled-coil segment in $G_t\alpha$ [21] are underlined. Another GDP-dependent site of interaction of $G_t\alpha$ with $G_t\beta\gamma$ is the 'α2' helix of $G_t\alpha$ in the 'switch II' domain. This domain is better conserved in ARF, which also binds $G_t\beta\gamma$, than in ras which does not bind $G_t\beta\gamma$. Thus the 'α2' helix might be the site of interaction of ARF_{GDP} with $G_t\beta\gamma$. (B) Comparisons of motifs around the conserved tryptophan in the 'α2' helix of ARF and of $G_t\alpha$, and in the PH domain C-terminal helix of two PH domain proteins, quoted as such in [25,26].

not modified in the presence of ARF. This strongly suggests that ARF binds with the same affinity to soluble and to membrane-bound $T\beta\gamma$ fractions. This is confirmed by the saturation of the concentration-dependent binding curve shown in Fig. 3, which was best fitted with the value of 10 μM for the affinity of $rARF_{GDP}$ for $T\beta\gamma$.

The binding experiments on phospholipid vesicles were repeated with recombinant myristoylated ARF ($myrARF_{GDP}$) in the incubation medium, instead of unacylated $rARF_{GDP}$ (Fig. 4). But $myrARF_{GDP}$ binds already significantly to phospholipid vesicles [13]: under our standard conditions, in the absence of any transducin subunit, 30% of the added $myrARF_{GDP}$ sedimented with the phospholipid vesicle pellet. The addition of $G_t\beta\gamma$ induced an increment of binding of $myrARF_{GDP}$ to the pellet, that seemed smaller in relative term but was comparable in absolute term to that observed under the same conditions with $rARF_{GDP}$. This indicated that myristoylated ARF_{GDP} has an affinity for $G_t\beta\gamma$ that is comparable to that of $rARF_{GDP}$, but this affinity cannot be determined accurately by our membrane-binding assay, due to the large 'background' $G_t\beta\gamma$-independent binding. The specificity of this $myrARF_{GDP}$-$G_t\beta\gamma$ binding was demonstrated by the full reversion of the $G_t\beta\gamma$-dependent increment of myrARF binding to the vesicles upon the stoichiometric addition of $G_t\alpha_{GDP}$ (Fig. 4), and its regeneration upon the further addition of AlF_4^-, as in the case of rARF.

Control experiments were also performed with ras. We used recombinant ras produced in *E. coli* [17], which is not farnesylated and very soluble. Ras_{GDP} did not bind to phospholipid vesicles, and additions of $G_t\beta\gamma$, or of $G_t\alpha_{GDP}$ or of both subunits did not induce any detectable binding (data not shown).

4. Discussion

Our reconstitution experiments demonstrate that recombinant unmyristoylated rARF1 as well as myristoylated ARF1, in the inactive GDP-bound form, bind to $G_t\beta\gamma$ subunits of transducin on phospholipid vesicles, but not to the $G_t\alpha_{GDP}$-$G_t\beta\gamma$ heterotrimer. Indeed the addition of $G_t\alpha_{GDP}$ inhibits ARF_{GDP} binding to $G_t\beta\gamma$, probably by competing for the same site on $G_t\beta\gamma$. By contrast $G_t\alpha_{GTP\gamma S}$ which does not bind to $G_t\beta\gamma$, does not inhibit the binding of ARF_{GDP} to $G_t\beta\gamma$. This accounts for the indirect action of AlF_4^- on ARF binding, that is observed only in the presence of both transducin subunits: AlF_4^- increases ARF_{GDP} binding to $G_t\beta\gamma$ by converting $G_t\alpha_{GDP}$ to $G_t\alpha_{GDP\text{-}AlF4}$ which loses its inhibitory effect on ARF_{GDP} binding to $G_t\beta\gamma$. The apparent binding affinity of $rARF_{GDP}$ for $G_t\beta\gamma$ is of 10 μM and is not dependent on the membrane binding of $G_t\beta\gamma$. $myrARF_{GDP}$ binds more to the phospholipid membrane, but its affinity for $G_t\beta\gamma$ is comparable to that of $rARF_{GDP}$. Therefore, neither the myristyl of ARF, nor the farnesyl of $G_t\beta\gamma$ seem to contribute to the protein-protein interaction between ARF and $G_t\beta\gamma$.

A major site of interaction of the Gα subunit of an heterotrimeric G-protein with $G\beta\gamma$ seems to be in the N-terminal helix of Gα [18–21]. The myristyl at the N-terminal end of the helix contributes to the $G\alpha_{GDP}$-$G\beta\gamma$ association [14] and it has been suggested on the basis of modeling [21], that a motif that includes two highly conserved Leucin residues near the other end of this helix, is involved in a coiled-coil association with the $G\beta\gamma$ subunit (see Fig. 5). The whole helix, which is very hydrophilic, is not visible in the cristallographic structure, which suggests that it is disordered and points away from the globular nucleotide-binding domain [22,23]. This makes it unlikely that the conformation of this N-terminal segment would be sensitive to GDP/GTP exchange in the nucleotide site.

The N-terminal helix of ARF1 has no sequence homology, and most probably no structural analogies with that of $G_t\alpha$. In the recent crystallographic structure of ARF_{GDP} [24], this amphipathic N-terminal helix is seen to fold back in the globular structure of the GDP binding domain, and to make tight hydrophobic contacts with other structural elements. There is therefore no indication that the N-terminal helix of ARF_{GDP} would play the same role as the N-terminal helix of $G_t\alpha$ and contribute to ARF_{GDP} binding to $G_t\beta\gamma$. Furthermore, this binding was observed as well in the absence of the myristyl, with rARF, as in its presence, with myrARF.

Conklin and Bourne [4] have suggested that another α-helical segment on the effector binding surface of Gα, may provide a GDP-dependent binding site for $G\beta\gamma$. This 'α2' helix is com-

prised between two flexible loops in the so called 'switch II' domain and it undergoes a dramatic conformational change between the GDP- and GTP-bound states: in the inactive GDP-bound state, the side chains of R204, W207 and F211 are exposed to the solvent (and may contact other proteins) whereas in the active GTPγS conformation they make tight contacts with another helical segment 'α3' of the same protein domain [23].

We noticed that the sequence of ARF1 'switch II' domain is highly homologous to that of $G_t\alpha$, particularly in the 'α2' helix, which has conserved the critical RxxWxxxF sequence (Fig. 5). By contrast this sequence is not conserved in Ras whose switch II domain has no significant homology with $G_t\alpha$. This might be correlated to our observation that ARF_{GDP} and $G_t\alpha_{GDP}$, but not ras_{GDP}, bind to $G_t\beta\gamma$. We therefore suggest that ARF_{GDP} interacts with $G_t\beta\gamma$, through its 'α2' helix, as does $G_t\alpha_{GDP}$. This would account for the inhibitory competition of $G_t\alpha_{GDP}$ against ARF_{GDP} binding, the higher affinity of $G_t\alpha_{GDP}$ for $G_t\beta\gamma$ being due to the existence on the N-terminal helix of $G_t\alpha$ of the coiled-coil interaction site with $G_t\beta\gamma$ [21].

We further suggest that the 'α2' helix of $G_t\alpha$, and the homologous one in ARF, might be conformational analogs of the C terminal helix of PH (Plekstrin Homology) domains [25,26], which include the critical sequence RxxWxxxI. PH domains have been identified in many signalling proteins, some of which bind $G\beta\gamma$ [27], possibly through their C-terminal section [28]. As shown on Fig. 5, homologies and analogies between the C terminal helix of some bona fide PH domains and the α2 helix of ARF, or of $G_t\alpha$, extend much beyond three conserved residues and are indeed stronger than homologies between two PH domains C-terminal helix or between two G-proteins 'α2' helix. We thus suggest more generally that the GDP-dependent $G\beta\gamma$-binding motif of the 'α2' helix of G proteins α subunit is conserved in the C-terminal helix of some 'PH' domains, and could be responsible for the binding of these PH domains to $G\beta\gamma$.

The physiological significance of the interaction of ARF with $G\beta\gamma$ remains uncertain as the affinity is low, but this interaction might be involved in the recently reported effect of aluminium-fluoride on ARF1-dependent (and probably $G\beta\gamma$-dependent) processes in Golgi membranes [29].

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References

[1] Kahn, R.A. and Gilman, A.G. (1984) J. Biol. Chem. 259, 6228–6234.
[2] Donaldson, J.G. and Klausner, R.D. (1994) Curr. Opin. Cell Biol. 6, 527–532.
[3] Kahn R.A. (1990) in: G-Proteins (Birnbaumer L. and Iyengar R. Eds.) pp. 201–214, Academic Press, New York.
[4] Conklin, B.R. and Bourne, H.R. (1993) Cell 73, 631–641.
[5] Kahn, R.A. (1991) J. Biol. Chem. 266, 15595–15597.
[6] Bigay, J., Deterre, P., Pfister, C. and Chabre, M. (1985) FEBS Lett. 191, 181–185.
[7] Sondek, J., Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Nature 372, 276–279.
[8] Donaldson, J.G., Kahn, R.A., Lippincott-Schwartz, J. and Klausner, R.D. (1991) Science 254, 1197–1199.
[9] Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R. and Rothman, J.E., (1991) Cell, 67, 239–253
[10] Donaldson, J.G., Lippincott-Schwartz, J. and Klausner, R.D. (1991) J. Cell. Biol. 112, 579–588.
[11] Robinson, M.S. and Kreis, T.E. (1992) Cell 69, 129–138.
[12] Franco, M., Chardin, P., Chabre, M. and Paris, S. (1993) J. Biol. Chem. 268, 24531–24534.
[13] Franco, M., Chardin, P., Chabre, M. and Paris, S. (1995) J. Biol. Chem. 270, 1337–1341.
[14] Bigay, J., Faurobert, E., Franco, M. and Chabre, M. (1994) Biochemistry 33, 14081–14090.
[15] Szoka, F. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198.
[16] Kühn, H. (1980) Nature 283, 587–589.
[17] Tucker, J., Sczakiel, G., Feuerstein, J., John, J., Goody, R.S. and Wittinghofer, A. (1986) EMBO J. 5, 1351–1358.
[18] Navon, S.E. and Fung, B.K.K. (1987) J. Biol. Chem. 262, 15746–15751.
[19] Neer, E.J., Pulsifer, L. and Wolf, L.G. (1988) J. Biol. Chem. 263, 8996–9000.
[20] Journot, L., Pantaloni, C., Poul, M.A., Mazarguil, H., Bockaert, J. and Audigier, Y. (1991) Proc. Natl. Acad. Sci. USA 88, 10054–10058.
[21] Lupas, A.N., Lupas, J.M. and Stock, J.B. (1992) FEBS Lett. 314, 105–108.
[22] Coleman, D.E., Berghuis, A.M., Lee, E., Linder, M.E., Gilman, A.G. and Sprang, S.R. (1994) Science 265, 1405–1412.
[23] Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Nature 369, 621–628.
[24] Amor, J.C., Harrison, D.H., Kahn, R.A. and Ringe, D. (1994) Nature 372, 704–708.
[25] Musacchio, A., Gibson, T., Rice, P., Thompson, J. and Saraste, M. (1993) Trends Biochem. Sci. 18, 343–348.
[26] Gibson, T.B., Hivönen, M., Musacchio, A., Saraste, M. and Birney, E. (1994) Trends Biochem. Sci. 19, 349–353
[27] Touhara, K., Inglese, J., Pitcher, J.A., Shaw, G. and Lefkowitz, R.J. (1994) J. Biol. Chem. 269, 10217–10220.
[28] Cohen, G.B., Ruibao, R. and D. Baltimore (1995) Cell 80, 237–248
[29] Finazzi, D., Cassel, D., Donaldson, J.G. and Klausner, R.D. (1994) J. Biol. Chem. 269, 13325–13330.