MYRbase: analysis of genome-wide glycine myristoylation enlarges the functional spectrum of eukaryotic myristoylated proteins

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Abstract

We evaluated the evolutionary conservation of glycine myristoylation within eukaryotic sequences. Our large-scale cross-genome analyses, available as MYRbase, show that the functional spectrum of myristoylated proteins is currently largely underestimated. We give experimental evidence for in vitro myristoylation of selected predictions. Furthermore, we classify five membrane-attachment factors that occur most frequently in combination with, or even replacing, myristoyl anchors, as some protein family examples show.

Background

Myristoylation is an important lipid modification of a variety of eukaryotic and viral, and also a few bacterial, proteins [1-4] that can direct proteins to membranes. There, it can influence their submembrane localization (for example, in lipid raft or raft-like compartments [5]) as a function of additional factors (for example, subsequent palmitoylation or the possession of a polybasic region) [6,7]. Also, myristoyl anchors have been found to be involved in specific protein-protein interactions [8,9]. Complex conformational switches can trigger changes in accessibility of the lipid moiety [10-13]. The enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT) [14] recognizes a motif [15] at the amino terminus of substrate proteins (which can also become amino-terminal after proteolytic cleavage) and attaches myristic acid, a saturated 14-carbon fatty acid, to the amino-terminal glycine. As bacteria and viruses, with the exception of two predicted NMTs in entomopoxviruses [15], lack the myristoylating enzyme, their proteins are modified by eukaryotic host NMT.

A sensitive prediction method using position-specific, redundancy-corrected profiles of known substrates in combination with physicochemical constraints on enzyme-substrate interactions has been developed [16] and is available from the web [17]. The sensitivity (cross-validation performance over the learning set of known substrates) is above 95% and the rate of false-positive prediction is estimated as <0.5% for the general eukaryotic, and <0.3% for the fungal, parameter set for proteins starting with glycine. The authors acknowledge that taxon-dependent enzyme-substrate specificities might influence prediction performances to a larger extent than included in the current implementation of the prediction algorithm. Large-scale application of this NMT predictor over GenBank (from the National Center for Biotechnology Information...
To make these data more accessible and interpretable in terms of biological significance, we evaluated the evolutionary conservation of the predicted myristoylation motif among groups of homologous proteins. This approach ranks predicted myristoylated proteins according to the number of homologous sequences with a conserved motif for this common lipid modification. Although not an absolute requirement, the evolutionary conservation of a motif in large protein families can be used to postulate its functional importance. These results are accessible through MYRbase [18], an easily navigable, searchable, web-based collection of tables containing the multiply linked and annotated results of our large-scale predictions, ordered by their number of occurrences in clusters of closely related proteins.

A more detailed description of MYRbase is given in the next section and on the accompanying website [18]. The main part of this manuscript is dedicated to a comprehensive description of the results, which also include the in vitro experimental verification of predicted myristoylation for amino-terminal peptides derived from human homologs of several non-obvious substrates (for example, 47 kDa GTPase IIGP, ubiquitin hydrolase Ubq-M, lung cancer candidate FUS1, potassium channel Kirz.1 and potassium channel interacting protein KChIP1). From these results we suggest extending the previously known functional spectrum of myristoylated proteins, representing a first step towards a characterization of the entire set of myristoylated proteins.

### MYRbase

We applied the NMT predictor for glycine myristoylation to taxonomic subsets of publicly available databases (SWISS-PROT, GenBank). The NMT prediction methodology is described in detail elsewhere [16]. Then, we first removed redundancy within our predictions using the program med-hit [19,20] with a 40% amino-acid identity threshold. This procedure already results in a dramatic reduction of the prediction lists to a much smaller set of representative sequences (for example, from approximately 4,400 sequences to approximately 2,000 for the eukaryotic subset). So as not to lose the information in the removed sequences, they were assigned to groups according to their clustering by med-hit. The conservative threshold of 40% amino-acid identity allows the interpretation that sequences within the clusters are homologous to their representative sequence but also results in the appearance of more remotely related sequences in separate clusters. The size of these clusters was used to rank the representative sequences in the tables in the database and is linked to view the full set of sub-tables listing all cluster members.

We have also estimated the taxonomic distribution of sequences within a cluster, as occurrence in a larger set of organisms might reflect the functional need for conservation of the motif during the aeons of evolution. Special emphasis is given to the known experimental status of myristoylation to distinguish new predictions. Therefore, we indicate the annotation of a cluster member for myristoylation in the SWISS-PROT [21] database below the cluster size and add further details to the sequence description. Besides this short description of the protein, the predictions are linked to their corresponding GenBank entries, an RPS-Blast with CD-search [22] to view the domain architecture and PSI-Blast [23] to collect the set of homologous sequences.

Then, the position in the sequence of the glycine that is predicted to be myristoylated (position 2 often implies prior cleavage of a leading methionine) and the full-length motif follow. We highlight cysteines that could be subject to palmitylation in yellow and positive charges by a blue background. Both factors can not only influence membrane affinity but also induce changes in subcompartment-specific localizations [5]. Finally, the score assigned by the NMT predictor (yielding details of the single score components on mouse-over), the estimated probability of false-positive prediction and a simple prediction attribute are listed.

Entries in MYRbase can be accessed by simple browsing or entering the corresponding MYRbase identifier on the index page (MYRbase identifiers correspond to GenInfo numbers of entries from the latest database built). Alternatively, we recommended using our MYRbase-specific BLAST-engine (also
Results and discussion
Conservation of glycine myristoylation in the evolution of eukaryotes

When analyzing single eukaryotic genomes of human, mouse, fly, worm, yeast and plant, we found a conserved relative occurrence (around 0.5-0.8% of all proteins in the genome) of predicted myristoylated proteins, with an elevated percentage for Arabidopsis [16] that is also supported by combined theoretical/experimental data for this organism [24]. In our genome-spanning analysis, we find that the most prominent and taxonomically widespread groups of myristoylated proteins comprise GTP-binding proteins (several alpha subunits of G proteins and ADP ribosylation factors), serine/threonine kinases, calcium-binding EF-hand proteins with diverse functions (for example, recoverin, calcineurin B, guanylate cyclase activators) and tyrosine kinases (Table 2 for complete listing).

It is interesting that for proteins with conserved myristoylation there are a few families with a large number of members, and a large number of families with only a few members. An interpretation of this situation, which is reminiscent of a power-law distribution (see Figure 1 and legend), in evolutionary terms might be that the few larger families are the oldest cases of eukaryotic protein glycine myristoylation. The many smaller families, on the other hand, appear to be examples of functional specialization, as they are often confined to taxonomic subgroups. Most of the larger families with a conserved motif include proteins with experimentally verified myristoylation (Table 2). Hence, future research will focus on the role of the predicted lipid anchors for the specialized smaller groups.

The amino-terminal myristoylation motif is an exchangeable module in evolution

We do not only observe myristoylation of proteins specific to taxonomic subgroups but also examples of myristoylation of proteins from some species that have multiple orthologs in other species that lack the motif for this lipid modification. In several of these cases, the myristoyl anchor has been substituted by other factors that can facilitate membrane association, such as other lipid anchors (palmitoyl, farnesyl, geranylgeranyl), transmembrane regions or specific protein-lipid or protein-membrane protein interaction domains. Such substitutions can have occurred not only in orthologs and paralogs but even in isoforms of the same protein.

For example, several Gα subunits have either a myristoyl-, both myristoyl- and palmitoyl-, or only palmitoyl-anchors [25]. While the Arabidopsis Rab5 ortholog Ara7 is geranylgeranylated on carboxy-terminal cysteines just like Rab5 in other species, its closely related paralog Ara6 lacks the carboxy-terminal cysteines but has an experimentally verified amino-terminal myristoylation motif instead [26]. Furthermore, it has been shown that an artificially introduced transmembrane helix can substitute for acyl-mediated membrane targeting of eNOS [27]. While most phospholipase C-gamma’s are expected to be targeted to membranes by amino-terminal pleckstrin homology domains (PH, which bind phospholipids), a phospholipase C in Trypanosoma cruzi utilizes a myristoyl-anchor instead [28]. Indeed, myristoylation was also able to substitute for PH domain-mediated membrane targeting of the scaffolding protein Gab1 [29].

Diverse shuffling, similar to that of globular domains, of several membrane-attachment factors (MAFs) within homologous proteins apparently occurred during evolution (by substitution, addition or deletion). It should be noted, however, that most of these changes also imply drift in targeting specificity or strength, and that the contributions and importance of single MAFs for membrane attachment might differ.

The notion of evolutionary modularity should not be misunderstood as a common ancestry for all sequence segments comprising myristoylation motifs. On the contrary, sequence-based comparisons appear to favor a model of multiple independent evolution (for example, for the emergence of those single motifs; data not shown). The modularity we describe here is that targeting specificity is achieved by combining different modules for membrane attachment in arrangements that are not fixed but can change dynamically during evolution. The evolutionary steps required for these changes from one targeting motif to the other are most likely to involve intermediates that contain several of the interchangeable factors.

Myristoyl anchors mediate targeting of proteins to many locations inside and outside of cells

Such evolution has apparently led to lipid-anchor-mediated targeting to a wide range of subcellular localizations. The role of glycine myristoylation is not limited to the classical attachment of proteins to the plasma membrane. There, several myristoylated proteins are found to target cholesterol and sphingolipid-enriched detergent-resistant compartments, designated as lipid rafts [30]. In addition, the myristoyl anchor is involved in targeting proteins to membranes throughout the cell, ranging from endoplasmic reticulum (ER), Golgi and mitochondrial membranes to the nuclear envelope and even to extracellular localization (see Table 2). Extracellular localization is the result of an alternative myristoylation- and palmitoylation-dependent export mechanism identified in Leishmania and possibly conserved to higher eukaryotes [31].

Specificity and strength of targeting can be achieved by optimizing lipid interactions by the type and number of lipid anchors (for example, acyl (myristoyl, palmitoyl) [6], prenyl (farnesyl, geranylgeranyl) [32,33] and GPI [34,35]), possibly...
Table 2

Experimentally verified myristoylated proteins and their subcellular localizations

| Experimentally verified protein type(s) | Total number in MYRbase | Subcellular localization | References |
|----------------------------------------|--------------------------|--------------------------|------------|
| GTP-binding proteins                   |                          |                          |            |
| G-protein alpha subunits (Gαі, Gαз, Gαо, Gαt) | 201                      | Type-dependent (receptor-specificity); for example, cytosol/plasma membrane (lipid rafts) | [25,95]    |
| ADP ribosylation factors (ARF) and ARF-like (ARL) | 79                       | Type-dependent; cytosol, intracellular membranes, cytoskeleton, nucleus/nucleolus (for example, ARL4, ARL5) | [109,110]  |
| Rab5-related (Ar6)                     | 8                        | Cytosol, endosomes, ER, plasma membrane | [26]       |
| S/T-kinases                            |                          |                          |            |
| Ca\(^{2+}\)/calmodulin-dependent kinases |                          |                          |            |
| Ca\(^{2+}\)/calmodulin-dependent kinases | 82                       | Type-dependent; for example, cytosol, plasma membrane, ER membrane | [111]      |
| Fen, Pto and related S/T-kinases       | 60                       | Cytosol, plasma membrane (~) | [112]      |
| cAMP-dependent kinases (PKA catalytic subunit) | 51                      | (Nucleus)/cytosol/plasma membrane over anchoring proteins | [113,114]  |
| cGMP-dependent kinase II               | 13                       | (Cytosol)/plasma membrane | [115]      |
| Ca\(^{2+}\)-binding EF-hand proteins   |                          |                          |            |
| Recoverin, neuronal calcium sensor, visinin, frequin, neurocalcin, hippocalcin, | 66                       | Type-dependent, triggered by Ca\(^{2+}\)/myristoyl switch; for example, cytosol, plasma membrane, Golgi, neurofilament-rich structures | [78,116]  |
| Calcinurin B phosphatase subunit, p22  | 43                       | Cytosol, plasma membrane (phosphatidyserine-rich regions), microtubule cytoskeleton (p22) | [117,118]  |
| Guanylate cyclase activators           | 21                       | Cytosol/rod outer segment membranes of photoreceptor cells | [119]      |
| DNA-dependent kinase interacting protein KIP = calcium and integrin binding protein CIB = calmyrin | 11                       | Cytosol, plasma membrane, ER, nuclear envelope, nucleoplasm, cytoskeleton | [120,121]  |
| S/T protein phosphatase + EF hand (PPEF) | 12                       | Axons, dendrites, ciliar membranes | [132]      |
| Hydrophilic acylated surface protein B (HASPB) | 10                      | Extracellular (!) face of the plasma membrane | [31]       |
| Erythrocyte membrane protein band 4.2 (Pallidin) | 10                      | Plasma membrane, intracellular vesicles | [133]      |
aided by additional features (such as clusters of positive charges [36]), but also by specific protein-lipid (for example, PIP$_2$ [37]) and protein-protein (for example, PDZ domain [38]) interactions.

**Classification of five membrane-attachment factors frequently co-occurring with myristoyl anchors (coMAFs)**

As several factors in addition to myristoylation contribute to subcellular localization and membrane attachment, we have analyzed their distribution within our sets of known and predicted myristoylated proteins. We distinguish five major classes that are defined in Table 3 and depicted in Figure 2.

From our analysis, subsequent cysteine palmitoylation (class I) and clusters of positive charges (class II) seem to occur in comparable amounts in both known and predicted subsets, while there is a clear tendency for the remaining classes (class III-V) to be more common among the new predictions. The prediction of the myristoylation of eukaryotic transmembrane proteins (class IV), in particular, will have to be verified in the future, although it seems generally reasonable, as viral examples exist [39,40] and the very similar acyl modification with palmitoyl anchors is commonly found in combination with transmembrane regions [41].

We also expect that final targeting specificity will be determined by not one but a combination of several MAFs, and the subcellular localization will remain difficult to predict, especially in regard to the involvement of protein-protein interactions (class V). Reversibility of membrane attachment can be

| Experimentally verified myristoylated proteins and their subcellular localizations |
|----------------------------------------------------------------------------------|
| Vps15 (phosphatidylinositol 3-kinase-associated p150)                              | 10 | Cytosol, intracellular membranes (late Golgi) [134] |
| Neuronal axonal membrane protein NAP-22 (brain acid soluble protein 1 BASP1; CAP23) | 9  | Golgi, plasma membrane (lipid rafts) [97] |
| SSeCKS (A-kinase anchor protein 12)                                               | 9  | Cytosol, cytoskeleton, plasma membrane [135] |
| Sip2, beta subunits of S-AMP-activated protein kinase                               | 9  | Cytosol, plasma membrane [136] |
| T-lymphoma invasion and metastasis inducing protein 1 (TIAM1 protein)             | 9  | Cytosol, plasma membrane [137] |
| PSD-Zip70, FEZI                                                                   | 9  | Neuronal membranes (postsynaptic density, dendritic rafts), plasma membrane (lipid rafts) [138] |
| S/T protein phosphatase Z                                                          | 8  | Nucleus [139] |
| Annexin XIII                                                                    | 6  | Cytosol, Golgi, plasma membrane (apical, lipid rafts) [100] |
| Ezrin-binding partner PACE-1                                                     | 6  | Cytosol, Golgi, lamellipodia [140] |
| BH3 interacting domain death agonist (BID)                                       | 5  | Cytosol, mitochondrial membrane [45] |
| Calpastatin (testis-specific isoform tCAST)                                      | 5  | Cytosol, intracellular membranes [141] |
| A-kinase anchor protein 200                                                        | 5  | Cytosol, plasma membrane [142] |
| Golgi-associated PR-1 protein                                                     | 5  | Golgi, plasma membrane (lipid rafts/caveolae) [143] |
| Vps20 (vacuolar protein sorting)                                                  | 4  | Cytosol, endosomal membranes [144] |
| Adenylate kinase I                                                               | 4  | Cytosol, plasma membrane [145] |
| A-kinase anchor protein 7 (18)                                                     | 3  | Cytosol, plasma membrane [114] |
| 2’,5’-oligoadenylate synthetase splice form 2                                      | 3  | Cytosol, rough ER, ribosomes, nucleus [57] |
| NADH ubiquinone dehydrogenase B18                                                 | 3  | Mitochondrial membrane [146] |
| Hisactophilin 1 and 2 (Histidine-rich actin binding protein)                     | 2  | Cytosol, plasma membrane, nucleus [147] |
| Phosphoinositide-specific phospholipase C in T. cruzi                            | 2  | Cytosol, plasma membrane [28] |
| Actin-myosin network maintenance protein Nullo                                    | 2  | Plasma membrane (metaphase furrows during mitosis, cellularization front) [148] |
| t-Gelsolin (fragment after caspase cleavage)                                      | 2  | Not determined for myristoylated form, cytoskeleton(?) [124] |
| Igloo (growth associated protein)                                                  | 1  | Cytosol, neuronal membranes [149] |
| Flagellar creatine kinase (ATP:guanido phosphotransferase)                       | 1  | Sperm tail membranes [150] |

* Not yet in MYRbase; cleavage motif is not automatically detected but is predicted when submitted as fragment to the predictor.
regulated by deamidation, disruption of the cluster of positive charges through introduction of negative charges (phosphorylation, deamidation of asparagine to aspartate), a pH-dependent switch when there are histidines within the positive charge clusters, or conformational changes due to proteolytic cleavage, binding of small molecules or altered protein-protein interactions.

**The functional spectrum of myristoylated proteins is underestimated**

From Table 2 we can see that the functional spectrum of experimentally verified myristoylated proteins is much more diverse than their well-known involvement in signaling. However, complete analysis of eukaryotic sequences in GenBank suggests that, after subtraction of known examples and their homologs, a significant number of proteins remain whose function has not been implicated with myristoylation so far (Table 1). To investigate the spectrum of these predictions with functions ‘new’ to myristoylated proteins, we have systematically analyzed the domain composition of a non-redundant (90% identity) subset of the respective proteins (HMMer [42] against PFAM [43]; E-value 0.01) and summarize the results in Table 4 (ranked by the number of occurrences of domains and corrected for repeated occurrence of the same domain in one entry). As a rule, the proteins associated with domain hits in Table 4 do not belong to a unique protein family but to several families that differ in their overall domain architecture. Consequently, we also analyzed the set of new predictions in respect of clearly defined protein families, and summarize them in Table 5. This list only includes families with at least 10 different proteins that, furthermore, begin with a methionine in their sequence (for exclusion of possible non-amino-terminal fragments). Not surprisingly, as myristoylation of remotely related family members has already been established, we find additional examples of kinases, phosphatases and phosphodiesterases. Several ‘new’ predictions will be discussed in the following sections. If not listed in Tables 5 or 6, a MYRBase identifier (MI) that equals the GenInfo number of the predicted entry in the analyzed database will be given to access the corresponding entries in MYRBase.

**In vitro verification of selected predictions**

We also picked a few interesting predictions from MYRbase and investigated the capacity of the corresponding amino-terminal peptides (Table 6 and Additional data file 1) to be myristoylated. Our in vitro experiments with synthetic peptides and bacterially expressed NMT (see Materials and methods) confirm the computational results for specific immunoglobulin µ-heavy and λ-light chains, 47 kDa GTPase IIGP, ubiquitin hydrolase Ubq-M, lung cancer candidate FUS1, potassium channel Kir2.1 and potassium channel interacting protein KChIP1. Hence, we show that our predictor recognizes the capability of substrates to productively interact with NMT. However, it is clear that in vitro myristoylation of synthetic peptides only gives limited information about the situation of the full-length protein in vivo. Some positively predicted substrates might be myristoylatable in vitro, but whether they come into contact with NMT in vivo depends on the cellular context and needs to be proven or at least shown to be plausible.

**Immunoglobulins and possible non-amino-terminal fragments in MYRbase**

For example, MYRbase also includes a series of specific immunoglobulin chains. By our in vitro myristoylation experiments, we show for a µ-heavy and a λ-light chain (Table 6, and Additional data file 1) that peptides derived from the amino termini of the corresponding database entries are in principal agreement with the physicochemical requirements for productive substrate-NMT interaction as modeled by our predictor.

It is unclear whether the predicted entries only represent non-amino-terminal fragments in the database. Differing sequence constructs for that region could be attributable to V(D)J recombination [44]. However, we could not find convincing expressed sequence tag (EST) evidence for variants with a starting methionine just before the predicted glycines. Proteolytic cleavage is an alternative possibility for glycines to become amino-terminal (for example, BID [45] and several viral proteins). Immunoglobulin chains are normally targeted to the ER via signal peptides and the existence of NMT activity in the ER has been proposed [2]. None of the predicted cleavage sites [46] of immunoglobulin precursors after the removal of the signal peptide appeared to result in an amino-terminal glycine. However, a different subcellular targeting mechanism, as well as alternative cleavage sites [47]...
and cleavage by other proteases, cannot be excluded. Myristoylation at the predicted sites may occur \textit{in vivo} only if the sequences, starting with glycine as they appear in the database, can interact with NMT within the cell.

Early work exists describing \textit{in vivo} incorporation of radiola- beled myristate into the same specific immunoglobulin chains as those predicted [48]. In this light, the possible alternative of myristoylation of an internal lysine (a theoretical suggestion of Pillai and Baltimore [48]) has to be addressed experimentally. Reports of internal lysine myristoylation are rare in the literature [49,50]. These potentially myristoylated immunoglobulin chains should have a yet-unknown cellular function (possibly, in a cell-type and cell-state-dependent manner) [48,51] in which the lipid anchor influences subcellular targeting.

**Myristoylation and the innate immune response**

Plant-specific disease-resistance proteins, with the nucleo- tide binding motif NB-ARC and leucine-rich repeat domains (for protein-protein interactions), are prominent in Table 5. While this manuscript was in preparation, octapeptides derived from the amino terminus of respective proteins were shown to be myristoylatable by plant NMT [24]. Myristoylation of those proteins that are involved in the innate immune response [52] fits well into the scheme that they act through interaction with avirulence proteins that are injected into the plant host cell through the type III secretion system of the parasitic bacteria [53]. The lipid anchor may facilitate co-localization since a series of type-III secreted bacterial proteins are also myristoylated by host plant NMT [4].

In the immune response of mammals to intracellular pathogens, expression of families of 65 kDa and 47 kDa GTPases is induced by interferon-gamma [54]. In this work, we show \textit{in vitro} myristoylation (Table 6 and Additional data file 1) of the amino terminus of a human homolog of 47 kDa GTPase IIGP [55]. This protein seems to be the only family member, besides very close homologs, that has a myristoyl anchor. Lipid modifications are quite common within the large superfamily of GTP-binding proteins, for example, Ras and Rho-related as well as Rho and Rab small GTPases are most often either farnesylated or geranylgeranylated [32]. Also, members of the 65 kDa family of the interferon-inducible GTPases [54] share the CaaX box motif that most likely directs their geranylgeranylation. In the case of the 47 kDa GTPase IIGP, a myristoyl-anchor could be involved in localizing the protein to membranes of the ER and Golgi [55,56]. In the context of myristoylation and mammalian innate immune responses, it is noteworthy that interferon-inducible, double-stranded (ds) RNA-activated 2’-5’-oligoadenylate synthetase isoform 2 is also known to be myristoylated [57].

**Myristoylation and ubiquitination**

The amino terminus of the human ubiquitin-specific protease Ubp-M is myristoylated by NMT \textit{in vitro} (Table 6, and Additional data file 1). This enzyme is suspected to be involved in the deubiquitination of histone 2A, cell-cycle regulation and caspase-dependent apoptosis [58,59]. However, the observations were made with a protein amino-terminally tagged with green fluorescent protein (GFP), which questions the functional importance of a putative amino-terminal myristoyl anchor. Interestingly, only a GFP-tagged mutated inactive version was localized to the nucleus, while the GFP-tagged protein lacking inactivating mutations was found in the cytoplasm [58]. This indicates that several factors influence its localization, and it cannot be ruled out that a myristoylated amino terminus would result in a different localization pattern, possibly also targeting the ‘active’ wild-type protein to its actual place of action.

### Table 3

The five classes of co-occurring membrane attachment factors (coMAFs)

| coMAF class* | Palmitoylatable cysteine | Amino-terminal cluster of positive charges | PIP2-specific binding domain | Transmembrane segments | Other/not attributable (for example, protein-protein interactions) |
|--------------|-------------------------|------------------------------------------|----------------------------|------------------------|---------------------------------------------------------------|
| Experimentally verified homolog | 453 | 443/133/12 | 9 | 0 | 432 |
| Additional NEW prediction | 728 | 729/269/58 | 21 | 203 | 1,860 |

*Distribution of the five classes of coMAFs among the set of experimentally verified myristoylated proteins plus their homologs and the set of additional new predictions. Assignments are not necessarily unique, but can be combinations thereof. At least one palmitoylatable cysteine within the first five residues (starting with the myristoylated glycine). The content of positive charges (amino acids lysine (K), arginine (R) and histidine (H)) in the region of positions 6 to 35 is compared to the average composition in GenBank in the same region. The values correspond to hits occurring using threshold deviations of 1, 2 and 3σ from the GenBank average. Significant HMMER-hit (below E-value of 0.01) with phosphatidylinositol-bisphosphate (PIP2)-specific binding domains (PH, ENTH, FERM, PX or FYVE). A very conservative method was used to detect putative transmembrane segments. Besides requiring the attribute ‘trusted’ by the sensitive method DAS-TMfilter [151], we only list the hits that have more than three predicted TM regions and that are additionally filtered for their overall polarity measured by the content of positive charges. All other proteins that did not fulfill any of the above criteria.
In addition to Ubq-M, we predict myristoylation for another group of ubiquitin-specific proteases conserved in rat, fly, worm and plants (for example, MI 27683149). These proteins are substantially shorter, lack additional domains (such as an amino-terminal zinc finger in Ubq-M), and most probably belong to the ubiquitin carboxy-terminal hydrolase subfamily [60]. Furthermore, we predict that the Caeenorhabditis elegans cytokinesis defect protein 3 [61] has a myristoyl anchor followed by a calcium-binding EF-hand and an ubiquitin hydrolase homology domain (MI 16950531).

It might be interesting to check whether the predicted lipid modification for some of these ubiquitin-specific proteases could result in subcellular targeting similar to that of a 26S proteasome variant whose regulatory subunit 4 was predicted [16], and subsequently shown to be, myristoylated in vivo [62].

In this context, it is also interesting that we predict myristoylation for a group of proteins containing putative RING zinc finger domains (Tables 4 and 5) that are commonly found in ubiquitin ligases. Although RING finger domains do exist in some proteins known to be myristoylated (for example, rapsyn, see Table 2), most of the hits with this top-ranking domain in Table 4 seem to have ‘new’ functions in the context of myristoylated proteins. For example, a subgroup of the RING finger proteins are isoforms of the Notch pathway [63] protein neuralized [64], with a conserved myristoylation motif in human, mouse, fly and worm (for example, MI 15128197). Another example of a RING domain-containing protein with conserved myristoylation motif in human, mouse, fly and worm is mahogunin (MI 27229238), which is involved in spongiform neurodegeneration [65]. Also, the myristoylation motif of the RING-containing peripheral nerve injury gene nin283 (for example, MI 14150005) is conserved in human, mouse, fly and worm.

Furthermore, several F-box-containing proteins, parts of which could act as receptors for ubiquitination targets, are found within our set of proteins predicted to be myristoylated (for example, MI 6912466 and MI 7299840).

**Myristoylation of mitochondrial proteins**

As well as proteins involved in apoptosis or the mitochondrial respiratory chain that are known to be myristoylated (Table 2), we also predict amino-terminal myristoylation of a homolog of TOM40, a protein thought to be a central component of the mitochondrial import machinery [66], presumably acting as a pore-forming [67] sorting station [68]. Unexpectedly for a proposed all-beta integral membrane
Table 4

Domain composition of the set of new predictions

| Number of entries | ID       | Description                                                                 |
|-------------------|----------|-----------------------------------------------------------------------------|
| 57                | PF00097  | Zinc finger, C3HC4 type (RING finger)                                       |
| 43                | PF00069  | Protein kinase domain                                                       |
| 35                | PF00481  | Protein phosphatase 2C                                                      |
| 26                | PF00023  | Ankyrin repeat                                                               |
| 21                | PF00931  | NB-ARC domain                                                               |
| 20                | PF00646  | F-box domain                                                                |
| 18                | PF04782  | Protein of unknown function (DUF632)                                        |
| 18                | PF04783  | Protein of unknown function (DUF630)                                        |
| 16                | PF00036  | EF hand                                                                     |
| 14                | PF00135  | Carboxylesterase                                                            |
| 14                | PF00487  | Fatty acid desaturase                                                       |
| 13                | PF00651  | BTB/POZ domain                                                              |
| 13                | PF05049  | Interferon-inducible GTPase (IIGP)                                          |
| 12                | PF00233  | 3′-cyclic nucleotide phosphodiesterase                                     |
| 12                | PF00443  | Ubiquitin carboxy-terminal hydrolase                                        |
| 12                | PF00085  | Thioredoxin                                                                 |
| 11                | PF00001  | 7 transmembrane receptor (rhodopsin family)                                 |
| 11                | PF00047  | Immunoglobulin domain                                                       |
| 9                 | PF00622  | SPRY domain                                                                 |
| 9                 | PF00628  | PHD-finger                                                                  |
| 9                 | PF00096  | Zinc finger, C2H2 type                                                      |
| 8                 | PF01459  | Eukaryotic porin                                                            |
| 8                 | PF03011  | Plasmodium falciparum erythrocyte membrane protein (PFEMP)                  |
| 8                 | PF00400  | WD domain, G-beta repeat                                                    |
| 8                 | PF00595  | PDZ domain (Also known as DHR or GLGF)                                      |
| 8                 | PF00560  | Leucine rich repeat                                                         |
| 7                 | PF00520  | Ion transport protein                                                       |
| 7                 | PF01135  | Protein-L-isoadaspartate(D-aspartate) O-methyltransferase (PCMT)            |
| 7                 | PF00514  | Armadillo/beta-catenin-like repeat                                          |
| 7                 | PF00989  | PAS domain                                                                  |
| 7                 | PF00134  | Cyclin, amino-terminal domain                                                |
| 7                 | PF00169  | PH domain                                                                   |
| 6                 | PF00300  | Phosphoglycerate Phos Phosphoglycerate mutase family                        |
| 6                 | PF01145  | SPFH domain / Band 7 family                                                 |
| 6                 | PF01582  | TIR domain                                                                  |
| 6                 | PF02174  | PTB domain (IRS-1 type)                                                    |
| 6                 | PF00462  | Glutaredoxin                                                                |
| 6                 | PF03193  | Protein of unknown function, DUF258                                        |
| 6                 | PF00515  | TPR domain                                                                  |
| 6                 | PF00240  | Ubiquitin family                                                            |
| 6                 | PF01265  | Cytochrome c/c1 heme lyase                                                  |
| 6                 | PF00702  | haloacid dehalogenase-like hydrolase                                        |
| 6                 | PF04641  | Protein of unknown function, DUF602                                         |
| 6                 | PF0271   | Helicase conserved carboxy-terminal domain                                  |
| 5                 | PF00070  | Pyridine nucleotide-disulphide oxidoreductase                               |
| 5                 | PF01417  | ENTH domain                                                                 |
| 5                 | PF00270  | DEAD/DEAH box helicase                                                      |
| 5                 | PF02230  | Phospholipase/Carboxylesterase                                              |
protein of the outer mitochondrial membrane [69], sequences from human, mouse, rat, frog and fly reveal a conserved predicted myristoylation site at the amino terminus (Table 5).

Interestingly, the amino termini of cytochrome c-type heme lyase (CCHL) homologs in worm, fly, zebrafish, frog, mouse and human (for example, MI 7512486) also share a conserved predicted myristoylation motif. It should be noted that, in the human sequence, a proline at motif position 4 is rather unfavorable in terms of flexibility of the substrate to adopt the proper conformation in the NMT binding pocket. Fungal homologs also have amino-terminal glycines. Although possible myristoylation cannot be excluded, three large aromatic residues that are difficult to accommodate in the narrow NMT substrate-binding pocket follow these conserved glycines, which might also suggest a new, as yet unknown, role for the amino-terminal glycine conservation. CCHL catalyzes the conversion of apocytochrome c to holocytochrome c by attaching a heme group [70], which is an important step required for cytochrome c biogenesis and transport through the membranes [71]. Deletion of the mammalian gene encoding CCHL is associated with X-linked microphthalmia with linear skin defects syndrome [72].

**Myristoylation and potassium channels**

Myristoylation has been predicted for specific modulatory proteins interacting with voltage-gated potassium channels [77]. In this work, we demonstrate in vitro myristoylation of the human representative of the KChIP1 subgroup of Kv4 channel-interacting proteins (Table 6 and Additional data file 1). The myristoylation motif is conserved in mouse, rat and human and, in support, there exists homology to hippocalcin, frequenin and neuronal calcium sensor proteins [77], whose myristoylation has already been shown experimentally to be of functional importance [78]. However, amino-terminal truncation experiments with KChIP1 suggest that, in the investigated system, the modulatory activity is sufficiently represented by the more conserved central domain containing EF-hand Ca²⁺-binding motifs [79]. On the other hand, the role of a myristoyl lipid anchor for KChIP1 could also lie in subcellular targeting specificity. Voltage-gated potassium channel isoforms (for example, Kv2.1 and Kv1.5) can localize to distinct lipid-raft populations [80]. In the context of the similar targeting specificity of acyl chains [81], it is interesting that isoforms KChIP2 and KChIP3 that lack the predicted myristoylation motif are palmitoylated, and their lipid modification controls the plasma-membrane localization of the associated channels [82].

Furthermore, several mammalian and avian homologs of the strong inward rectifying potassium channel Kir2.1 are predicted to be myristoylated. The fact that the motif is evolutionarily retained over 11 organisms would rather support the functional importance of such conservation. The in vitro results show that the amino terminus of human Kir2.1 can productively interact with NMT (Table 6 and Additional data file 1). None of the other Kirs is predicted to be

Table 4 (Continued)

| Domain composition of the set of new predictions |
|------------------------------------------------|
| 5 | PF00891 O-methyltransferase |
| 5 | PF00046 Homeobox domain |
| 5 | PF05003 Protein of unknown function (DUF668) |
| 5 | PF00010 Helix-loop-helix DNA-binding domain |
| 5 | PF00170 bZIP transcription factor |
| 5 | PF00018 SH3 domain |
| 5 | PF00255 Glutathione peroxidase |
| 5 | PF00017 SH2 domain |
| 5 | PF00089 Trypsin |
| 5 | PF00153 Mitochondrial carrier protein |
myristoylated, and a lipid anchor unique to Kir2.1 could be responsible for differing subcellular and submembrane localizations of isoforms, similar to those observed among other potassium channels [80,83]. Consequently, it is not surprising that artificial introduction of a myristoylation motif into the isolated cytosolic carboxy terminus of another Kir (Kir3.1) is sufficient to co-localize the fragment with intact channels [84]. After synthesis in the ER, carboxy-terminal signals direct the export of Kir2.1 to the Golgi complex [85,86]. Stockklausner et al. [87] showed that a cluster of positive charges in the amino terminus that are conserved with Kir1.2/4.1 can be held responsible for post-Golgi trafficking of Kir2.1 to the plasma membrane. There, Kir2.1 is localized to lipid rafts [87]. It was hypothesized that interaction with phosphoinositides (PIP2) could be necessary for lipid-raft association but Stockklausner et al. [87] found that mutation of the corresponding residues had no effect on lipid-raft targeting. Other work suggests the involvement of PIP2 interaction in regulating gating processes [88].

The functional role of the predicted myristoyl anchor for these channel proteins with respect to submembrane localization (lipid rafts), conformational flexibility of the amino terminus or protein-protein interactions remains to be investigated.

**Myristoylation and targeting to lipid rafts**

Many pieces of evidence suggest that not only the many different membranes in the cell, but also the microcompartments therein, can be distinguished by their lipid and protein compositions [89,90]. Proteomic analyses of detergent-

### Table 5

**Examples of proteins predicted to be myristoylated**

| Protein(s)                                    | Total number in MYRbase | MI in MYRbase (number in cluster) | Taxonomic range |
|-----------------------------------------------|-------------------------|-----------------------------------|-----------------|
| ^Disease-resistance proteins                  | 32                      | 25300600 (27) NB-ARC+LRR, 25453543 (5) TIR+NB-ARC+LRR | Plants          |
| ^S/T protein phosphatase 2C gamma (1G)        | 30                      | 2130393 (6)(pc3), 26450759 (6), 4505999 (5)(1G), 25411959 (4), 22326510 (3), 6728987 (3), 6319415 (3)(pc2/3), 23172576 (3)Dm, 23615237 (2)Pf, 22326564 (2), 7488279 (2), 15239565 (1), 23483487 (1)(1Gp), 24417914 (1), 25341907 (1), 21626866 (1)(1Gdm), 6319601 (1)(pc4) | Mammals, insects, fungi, plants, apicomplexa |
| ^S/T-kinases (CrkI(cyclin-dependent kinase cdc2-related)-related) | 29                      | 22327464 (11), 27817936 (10), 14532736 (4), 25402555 (3), 20805217 (1) | Plants          |
| cGMP-specific 3',5'-cyclic phosphodiesterase 8A, 8B, 9A | 23                      | 6166014 (10) 9A, 27479159 (9) 8AB, 21626649 (2) Dm, 2706887 (2) | Mammals, insects |
| ^Thioredoxin                                  | 20                      | 15231958 (11), 1388078 (4), 28209505 (2), 28372832 (2), 28372834 (1) | Plants          |
| Putative RING zinc finger proteins (ubiquitin ligases?) | 17                      | 20279471 (10), 27500282 (4) Hs Mm, 7292914 (1) Dm, 3874246 (1) Ce, 23488532 (1) Pf | Plants, mammals, insects, apicomplexa |
| Interferon-inducible GTPase; 47 kDa GTPase IIIGP | 16                      | 25029534 (16) | Mammals          |
| Mitochondrial import receptor subunit TOM40   | 16                      | 12230369 (15), 6539563 (1) | Mammals, amphibians, insects, plants |
| ^Germ cell less GCL                           | 15                      | 21314704 (13), 7304006 (1), 6425507 (1) | Mammals, fish, insects, worms |
| Inward rectifier potassium channel Kir2.1     | 13                      | 26336911 (13) | Mammals          |
| Sphingolipid delta 4 desaturase DES1/DES2     | 12                      | 2771729 (12) | Mammals, insects, worms |
| ARF GTPase-activating protein (PH, ArfGAP, ankyrin domains) | 12                      | 22051029 (12) | Mammals          |
| Naked cuticle 1.2 homolog                     | 12                      | 22028145 (11), 27729789 (1) | Mammals          |
| Protein-L-isospartate carboxymethyltransferase | 12                      | 27882417 (10), 27713894 (1), 3133008 (1) | Mammals, amphibians, worms |
| Cyclin-box carrying protein I                  | 11                      | 1078903 (11) | Mammals, insects, worms |

Amino-terminal peptides of single representatives shown to be myristoylated in ^[24] and ^[152]. ^Amino-terminal glycine to alanine mutation prevents nuclear envelope localization [153].
resistant lipid-raft fractions from different cells [91-94] unveil partly overlapping but also cell-type specific populations of proteins. This includes several known myristoylated proteins that are involved in various signaling pathways (for example, Gα [95], Src-family tyrosine kinases [96], NAP-22 [97], endothelial nitric oxide synthase [98], rapsyn [99], annexin XIII [100], MARCKS [101], CAP23 [102] and Nef [103] or participate in viral budding (for example, HIV Gag [104]).

In addition, we predict myristoylation for two more proteins of unknown function that co-localized with detergent-resistant membrane fractions. Each appears in a separate proteomic analysis of lipid rafts in Jurkat T-cells [91,94]. The first (161 amino acids, MI 8923579) protein has a conserved myristoylation site plus potentially palmitoylated cysteines in human, rat and mouse homologs. Interestingly, the second protein (227 amino acid, MI 12834233), which has a predicted central coiled coil region, was not only detected in detergent-resistant fractions of T cells in a dynamic, receptor-activation-dependent manner [94], but also in a chaotrope-resistant fraction derived from nuclei of neuroblastoma N2a cells that contains clusters of proteins localizing to the outer nuclear membrane [105]. It will be interesting to establish possible dynamic trafficking between distinct subcellular compartments of this protein (with highly conserved orthologs in human, mouse and frog, and more diverged in zebrafish and fly).

Existing proteomic analyses focus on the most abundant protein representatives within membrane fractions and can also only identify a temporally limited subset of dynamically targeted proteins. Therefore, it would not be surprising to find several more myristoylated proteins to participate in highly specific signaling events by reversible trafficking to and from membrane microdomains.

Targeting experiments suggest differences in submembrane localization that depend on the anchor type. For example, membrane partition clustering of non-dimerizing green fluorescent protein variants fused to peptides containing double acylation (for example, myristoyl-palmitoyl) or prenylation (for example, farnesyl, geranylgeranyl) consensus signals was shown to differentially depend on lipid raft disruption by cholesterol depletion [81]. However, targeting experiments with a few artificial constructs varying only in lipid anchor type cannot take into account the complexity of the interplay between different MAFs that coexist in real proteins. Hence, it is difficult to generally attribute targeting specificity purely to occurrences of particular lipid anchors. Nevertheless, a myristoyl anchor followed by a palmitoyl anchor seems to be a strong indication for targeting to the plasma membrane and also eventually to lipid rafts.

Conclusions
We have evaluated the evolutionary conservation of predicted glycine myristoylation within sequences of the eukaryotic section of GenBank. We find that a small number of very large families of myristoylated proteins seems to be opposed to a large number of very small protein families (Figure 1). Our approach allows the summarizing of the currently available knowledge of experimentally verified (Table 2) as well as newly predicted myristoylated eukaryotic proteins (examples in Table 5, throughout the text and in MYRbase).

We estimate that a substantial fraction of new predictions is associated with proteins whose function has not been known in the context of myristoylation (Tables 1, 4, 5) so far. By demonstrating in vitro myristoylation of amino termini derived from several interesting functionally diverse proteins (Table 6 and Additional data file 1), we strengthen the prediction results for this lipid modification. Nevertheless, we also want to emphasize that the actual in vivo modification of respective proteins remains to be established.

The myristoylation motif typically acts together with several other MAFs but can also be fully replaced by them within...
protein families during evolution. The concerted effect of these MAFs defines subcellular localization and targeting specificity. We classify five coMAFs that most commonly co-occur with myristoyl lipid modifications (Table 3, Figure 2).

The results of this work are summarized and accessible through the web-based MYRbase [18] that aims to stimulate further experiments. A considerable amount of work will be necessary in the future to fully cover and understand the functional spectrum of the myristoylated proteins.

Materials and methods

In vitro myristoylation assay

The amino-terminal eight amino acids of selected predictions (Table 6) were synthesized with two minor modifications. To avoid racemization of the carboxy-terminal cysteine, one serine residue was added to the terminus of MI 4761381. Threonine and valine were added to the octapeptide of MI 5454156, as the dominance of positive charges was expected to result in difficulties in purification and handling. Peptides were purchased from Sigma Genosys (Japan). NMT was prepared as described previously [106] and the cDNA of yeast NMT was a gift from J. Gordon. Peptides were myristoylated in vitro essentially as described in [107] and analyzed by mass spectrometry. MALDI-TOF mass spectrometry was carried out on a Voyager DE Pro (PE Biosystems) and the matrix was 10 mg/ml alpha-cyano-4-hydroxycinnamic acid (Sigma) in 0.1% TFA-50% acetonitrile solution. The spectra were calibrated using calibration mixture 1 or 2 (PE Biosystems). The spectra are available in portable document format (PDF) from the MYRbase homepage [18]. As positive controls, peptides of proteins known to be myristoylated in vivo have been analyzed, as well as corresponding peptides without modification by NMT as negative controls. Differences between theoretical and experimental masses for peptides ending in serine are most likely due to carboxy-terminal sodium salt formation. MYRbase identifiers can be found in the upper left corner of each page.

Additional data files

The mass spectra of the investigated peptides are available (Additional data file 1).

Acknowledgements

The authors are grateful for support from Boehringer Ingelheim. This project was partly funded by the Fonds zur Förderung der wissenschaftlichen Forschung Österreichs (FWF grant P15037), by the Austrian National Bank (OeNB - Österreichische Nationalbank), by the Austrian Gen-AU bioinformatics integration network (BiN) sponsored by BM-BWK, by the bioinformatics contract study 2002-2004 for BM-WA, by the Asahi Glass Foundation, Japan (to N.H.), by grants-in-aid for Scientific Research on Priority Areas ‘Genome Information Science’ (14015231 and 15014230) (to N.H.) and Scientific Research (C) (to N.H.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a grant-in-aid for the Fujita Health University High-tech Research Center from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Gordon JI, Duronio RJ, Rudnick DA, Adams SP, Gokel GW: Protein N-myristoylation. J Biol Chem 1991, 266:8647-8650.
2. Bourin JA: Myristoylation. Cell Signal 1997, 9:15-35.
3. Farazi TA, Waksman G, Gordon JI: The biology and enzymology of protein n-myristoylation. J Biol Chem 2001, 276:39501-39504.
4. Nimchuk Z, Marois E, Kjemtrup S, Leister RT, Katagiri F, Dangl JL: Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from Pseudomonas syringae. Cell 2001, 101:353-363.
5. McCabe JB, Berthaume LG: N-terminal protein acylation confers localization to cholesterol, sphingolipid-enriched membranes but not to lipid rafts/caveolae. Mol Biol Cell 2001, 12:3601-3617.
6. Resh MD: Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1999, 1451:11-16.
7. McCabe JB, Berthaume LG: Functional roles for fatty acylated amino-terminal domains in subcellular localization. Mol Biol Cell 1999, 10:3771-3786.
8. Hayashi N, Masubara M, Jinbo Y, Titani K, Izumi Y, Matsushima N: Nef of HIV-1 interacts directly with calcium-bound calmodulin. Protein Sci 2000, 9:1905-1913.
9. Hayashi N, Izumi Y, Titani K, Matsushima N: The binding of myristoylated N-terminal nonapeptide from neuro-specific protein CAP-23/NAP-22 to calmodulin does not induce the globular structure observed for the calmodulin-nonmyristoylated peptide complex. Protein Sci 2000, 9:1905-1913.
10. Ames JB, Tanaka T, Stryer L, Ikura M: Portrait of a myristoyl switch protein. Curr Opin Struct Biol 1996, 6:432-438.
11. McLaughlin S, Aderem A: The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions. Trends Biochem Sci 1995, 20:272-276.
12. Zhou W, Resh MD: Differential membrane binding of the human immunodeficiency virus type 1 matrix protein. J Virol 1996, 70:8540-8548.
13. Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, Superti-Furga G: A myristoyl/phosphotyrosine switch regulates apoA-I. Cell 2003, 112:945-957.
14. Raju RV, Datla RS, Moyna TN, Kakkar R, Cartensen SA, Sharma RK: N-myristoyltransferase. Mol Cell Biochem 2000, 204:135-135.
15. Maurer-Stroh S, Eisenhaber B, Eisenhaber F: N-terminal N-myristoylation of proteins: refinement of the sequence motif and its taxon-specific differences. J Mol Biol 2002, 317:523-540.
16. Maurer-Stroh S, Eisenhaber B, Eisenhaber F: N-terminal N-myristoylation of proteins: prediction of substrate proteins from amino acid sequence. J Mol Biol 2002, 317:541-551.
17. NMT: MyristoylCoA:Protein N-Myristoyltransferase [http://mendel.imp.univie.ac.at/myristate]
18. MYRBase [http://mendel.imp.univie.ac.at/myristate/myrbase]
19. Li W, Jaroszewski L, Godzik A: Tolerating some redundancy significantly speeds up clustering of large protein databases. Bioinformatics 2002, 18:77-82.
20. Li W, Jaroszewski L, Godzik A: Clustering of highly homologous sequences to reduce the size of large protein databases. Bioinformatics 2001, 17:282-283.
21. Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O’Donovan C, Phan L, et al.: The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res 2003, 31:365-370.
22. Marchler-Bauer A, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, et al.: CDD: a curated Entrez database of conserved domain alignments. Nucleic Acids Res 2003, 31:383-387.
23. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25:3389-3402.
24. Boisson B, Giglione C, Meinell T: Unexpected protein families including cell defense components feature in the N-myristoylome of a higher eukaryote. J Biol Chem 2003, 278:43418-43429.
25. Chen CA, Manning DR: Regulation of G proteins by covalent modification. Oncogene 2001, 20:1643-1652.

26. Ueda T, Yamagata M, Uchiyama M, Nakano A, Araé, a plant-uniques novel type Rab GTPase, functions in the endocytic pathway of Arabidopsis thaliana. EMBO J 2001, 20:4730-4741.

27. Gonzalez E, Kou R, Lin AJ, Golan DE, Michel T: Subcellular targeting and agonist-induced site-specific phosphorylation of endothelial nitric-oxide synthase, J Biol Chem 2002, 277:39554-39560.

28. Furuya T, Kashuba C, Rocamora R, Moreno SN: A novel phosphatidylinositol-phospholipase C of Trypanosoma cruzi that is lipid modified and activated during trypomastigote to amastigote differentiation, J Biol Chem 2000, 275:6342-6348.

29. Maroun CR, Naujokas MA, Park M: Membrane targeting of Grb2-associated Binder-I (Gab) I scaffolding protein through Src myristoylation sequence substitutes for Gab1 pleckstrin homology domain and switches an epidermal growth factor receptor-dependent protein export in invasive morphogenic process. Mol Biol Cell 2003, 14:691-708.

30. Simons K, Toomre D: Lipid rafts and signal transduction, Nat Rev Mol Cell Biol 2000, 1:1-19.

31. Denny PW, Gokool S, Field MC, Smith DF: Acylation-dependent protein export in Leishmania. J Biol Chem 2000, 275:10177-1025.

32. Maurer-Stroh S, Washietl S, Eisenhaber F: Protein prenyltransferases. Genome Biol 2003, 4:212.

33. Chen CA, Manning DR, Mumenthaler C, Nishida E, Irie Y, Nakamura T: The on-off story of protein-conducting TOM channel in the outer membrane of Escherichia coli, J Biol Chem 2001, 276:26258-26266.

34. Hassainin A, Golub R, Lewis SM, Wu GE: Evolution of the recombinant signal sequences in the Ig heavy-chain variable region locus of mammals. Proc Natl Acad Sci USA 2000, 97:14151-14156.

35. Zha J, Weiler S, Oh KJ, Wei MC, Korsmeyer SJ: Posttranslational N-myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science 2000, 290:1761-1765.

36. Nielsen H, Brunak S, von Heijne G: Machine learning approaches for the prediction of signal peptides and other protein sorting signals, Protein Eng 1999, 12:3-9.

37. Ping J, Schildbach JF, Shaw SY, Quertermous T, Novotny J, Brucoleri R, Margolies MN: Effect of heavy chain signal peptide mutations and NH2-terminal chain length on binding of anti-digoxin antibodies, J Biol Chem 1993, 268:32000-32007.

38. Pillai S, Baltimore D: Myristoylation and the post-translational acquisition of hydrophobicity by the membrane immunoglobulin heavy-chain polypeptide in B lymphocytes. Proc Natl Acad Sci USA 1987, 84:7654-7658.

39. Stevenson FT, Forsman SL, Lockshin SM, Lovett DH: Myristylation of the tumor necrosis factor alpha precursor on specific lysine residues. J Exp Med 1992, 176:1053-1062.

40. Stevenson FT, Bursten SL, Fenton C, Locksley RM, Lovett DH: The 31-kDa precursor of interleukin 1 alpha is myristoylated on specific hydrophobic residues. J Biol Chem 1993, 268:7245-7249.

41. Meffre E, Casellas R, Nussenzweig MC: Antibody regulation of B cell development, Nat Immunol 2000, 1:379-385.

42. Dang JL, Jones JD: Plant pathogens and integrated defence responses to infection, Nature 2001, 411:826-833.

43. Costero A, Lindeberg M, Petnicki-Ocwieja T, Schneider DJ, Alfaro JR: Genomic mining type III secretion system effectors in Pseudomonas syringae yields new picks for all TTSS prospectors. Trends Microbiol 2002, 10:462-469.

44. Dendorf U, Guethlein L, Klamp T, Osbek K, Schaub A, Futterer A, Pfeffer K, Howard JC: Two families of GTPases dominate the complex cellular response to IFN-gamma. J Immunol 1998, 161:6715-6723.

45. Zerrahn J, Schalib VE, Brinkmann V, Guhlich U, Kaufmann SH: The IFN-gamma ROC GTPase-1 IIP1 is transiently expressed during listeriosis. J Immunol 2002, 168:3428-3436.

46. Uthaija R, Praefcke GJ, Howard JC, Herrmann C: IIP1, an interferon-gamma-inducible 47-kDa GTPase of the mouse, shows cooperative enzymatic activity and GTP-dependent multimerization. J Biol Chem 2003, 278:29336-29343.

47. Besse S, Dreuillet L, Marie I, Puvion-Dutilleul F, Hovanessian AG: Ultrastructural localization of interferon-ducible double-stranded RNA-activated enzymes in human cells. Exp Cell Res 1998, 239:379-392.

48. Cai SY, Babitz RW, Marchesi VT: A mutant deubiquitinating enzyme (Ubp-M) associates with mitotic chromosomes and blocks cell division. Proc Natl Acad Sci USA 1999, 96:2828-2833.

49. Minnaugh EG, Kayastha G, McGovern NB, Hwang SG, Marcu MG, Trepel J, Cai SY, Marchesi VT, Neckers L: Caspase-dependent deubiquitination of monoubiquitinated nucleosomal histone H2A induced by diverse apoptogenic stimuli. Cell Death Differ 2001, 8:1182-1196.

50. D'Andrea A, Pelman D: Deubiquitinating enzymes: a new class of biological regulators. Curr Rev Biochem Mol Biol 1998, 33:337-352.

51. Kaina B, Schnabl H, Schnabl N, Grum AA, Glotzer M: A ubiquitin C-terminal hydrolase is required to maintain osmotic balance and execute actin-dependent processes in the early C. elegans embryo. J Cell Sci 2002, 115:2293-2302.

52. Kunkele KP, Heins S, Dombowski M, Nargang FE, Benkert O, Haire D: Aral6, a plant-membrane associated PDZ domain protein, ANGELIN: A ubiquitin C-terminal hydrolase is required to maintain osmotic balance and execute actin-dependent processes in the early C. elegans embryo. J Cell Sci 2002, 115:2293-2302.

53. Pavlopoulos E, Pitsouli C, Klueg KM, Muskavitch MA, Moschonas NK, Pavlopoulos E, Pitsouli C, Klueg KM, Muskavitch MA, Moschonas NK: Lipid rafts and signal transduction. J Biol Chem 2003, 278:23800-23805.

54. Suzuki H, Okazawa Y, Komiya T, Saeki K, Mekada E, Kitada S, Ito A, Steiner H, Kispal G, Zollner A, Haid A, Neupert W, Lill R, Murrell JG: Regulation of G proteins by covalent modification. Proc Natl Acad Sci USA 1999, 96:1828-1833.

55. Suzuki H, Okazawa Y, Komiya T, Saeki K, Mekada E, Kitada S, Ito A, Steiner H, Kispal G, Zollner A, Haid A, Neupert W, Lill R, Murrell JG: Regulation of G proteins by covalent modification. J Biol Chem 1999, 274:37930-37936.

56. Doi D, Anzai T, Saito S, Matsumura M, Rosenbluh S, Murrell JG: Role of G proteins in receptormediated apical signaling. J Biol Chem 2001, 276:26711-26718.
Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. J Biol Chem 2002, 277:3399-3409.

93. Li N, Maki A, Richards DP, Naber C, Keller BO, Li L, Shaw AR: Mono- cyte lipid rafts contain proteins implicated in vesicular trafficking and phagosome formation. Proteomics 2003, 3:536-548.

94. Bini L, Pacini S, Liberatori S, Valenzini S, Pellegrini M, Raggiacchi R, Pal- lini V, Baldini CT: Extensive temporally regulated reorganization of the lipid raft proteome following T-cell antigen receptor triggering. Biochem J 2003, 369:301-309.

95. Moffett S, Brown DA, Linder ME: Lipid-dependent targeting of G proteins into rafts. J Biol Chem 2000, 275:2191-2198.

96. Keshavarzian M, Tolar P, Pletcher D, Rharouz L, Rivera J, Draber P: Structure-function analysis of Lyn kinase association with lipid rafts and initiation of early signaling events after Fcγ receptor I aggregation. Mol Cell Biol 2001, 21:8318-8328.

97. Terashita A, Funatsu N, Umeda M, Shimada Y, Ohno-Iwashita Y, Epplen JM, Mukawa S: Lipid binding activity of a neuron-specific protein NAP-22 studied in vivo and in vitro. J Neurosci Res 2002, 70:172-179.

98. Sowa G, Pypaert M, Sessa WC: Distinction between signaling mechanisms in lipid rafts vs. caveolae. Proc Natl Acad Sci USA 2001, 98:14072-14077.

99. Marchand S, Devillers-Thiery A, Pons S, Changeux JP, Cartaud J: Rapsyn escorts the nicotinic acetylcholine receptor along the exocytic pathway via association with lipid rafts. J Neurosci 2002, 22:8891-8900.

100. Lafont F, Lecat S, Verkade P, Simons K: Annexin VILib associates with lipid microdomains to function in apical delivery. J Cell Biol 1998, 142:1413-1427.

101. Arbozua A, Schmitz AA, Vergeres G: Cross-talk unfolded: MARCKS proteins. Biochem J 2002, 362:1-12.

102. Lafont F, Fukami K, Thelen M, Frey D, Caroni P: MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalem- mal rafts, and regulate cell cortex actin dynamics through a common mechanism. J Cell Biol 2000, 149:1453-1471.

103. Wang J,K, Yokokawa E, Verdin E, Trono D: The Nef protein of HIV-1 associates with rafts and primes T cells for activation. Proc Natl Acad Sci USA 2000, 97:394-399.

104. Lindwasser OW, Resh MD: Multimerization of human immunodeficiency virus type 1 Gag promotes its localization to barbed, raft-like membrane microdomains. J Virol 2001, 75:7913-7924.

105. Dreger M, Bengtsson L, Schoneberg T, Otto H, Hucho F: Nuclear envelope proteomics: novel integral membrane proteins of the inner nuclear membrane. Proc Natl Acad Sci USA 2001, 98:14072-14077.

106. Takasaki A, Hayashi N, Matsubara M, Yamauchi E, Taniguchi H: Identification of the calmodulin-binding domain of neuron-spe- cific protein kinase C substrate protein CAP-22/NAP-2. Direct involvement of protein myristoylation in calmodulin- binding and functional association with microsomal targeting protein (MTP) in yeast. J Biol Chem 1999, 274:11848-11853.

107. Gunaratne RS, Sajid M, Ling FT, Tripathi R, Pachebat JA, Holder AA: Characterization of N-myristoyltransferase from Plasmo- dium falciparum. Biochem J 2000, 348:459-463.

108. Guex N, Petisich MC: SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1998, 19:2174-2183.

109. Peperkok R, Horst-Wagenblatt A, Koning N, Giroud AM, Bossemeyer D, Kinzel V: Intracellular distribution of mammalian protein kinase A catalytic subunit altered by conserved Asn2 demethylation. J Cell Biol 2000, 148:715-726.

110. Fraser ID, Tavilion SJ, Lester LB, Langeberg LR, Westphal AM, Dean RA, Marrion NV, Scott JD: A novel lipid-anchored A-kinase anchoring protein facilitates CAM-responsive membrane interactions.
events. EMBO J 1998, 17:2261-2272.

115. Vaandrager AB, Smolenski A, Tilby BC, Houtsmuller AB, Ehler EM, Buz AG, Edikhoven M, Boomaars WE, Lohmann SM, de Jonge HR: Membrane targeting of C-17CD depends on a C-terminal protein kinase is required for cystic fibrosis transmembrane conductance regulator Cl-channel activation. Proc Natl Acad Sci USA 1998, 95:1466-1471.

116. Spilker S, Dresbach T, Brunewell KH: Reversible translocation and activity-dependent localization of the calcium-myristoyl switch protein VILIP-I to different membrane compartments in living hippocampal neurons. J Neurosci 2002, 22:7331-7339.

117. Pierino BA, Martin BA: Ca(2+)- and myristoylation-dependent association of calcineurin with phosphatidylserine. J Biochem (Tokyo) 2001, 129:835-841.

118. Timm S, Titus B, Berndt K, Barroso M: The EF-hand Ca(2+)-binding protein p22 associates with microtubules in an N-myristoylated substrate, SSeCKS. Biochemistry 2000, 41:13021-13028.

119. Hwang JY, Koch KW: Calcium- and myristoyl-dependent properties of guanylate cyclase-activating protein-1 and protein-2. Biochemistry 2002, 41:13021-13028.

120. Stabler SM, Ostrowski LL, Janicki SM, Monteiro MJ: A myristoylated case requiring myristic acid to preferentially interact with the Alzheimer’s disease presenilin 2 protein. J Biol Chem 1999, 145:1277-1292.

121. Naik UP, Naik MU: Association of CIB with GPIlb/IIla during outside-in signaling is required for platelet spreading on fibrinogen. J Biol Chem 2003, 180:1353-1362.

122. Resh MD: Myristylation and palmitoylation of Src family members: the fats of the matter. Cell 1994, 76:411-413.

123. Lang ML, Chen YW, Shen L, Gao H, Lang GA, Wade TK, Wade WF: Igα Fc receptor (FcαR2) cross-linking recruits tyrosine kinases, phosphotyrosine kinases and serine/threonine kinases to glyco lipid rafts. Biochem J 2002, 364:517-525.

124. Usutani T, Sakurai N, Nakano K, Ishisaka R: C-terminal 15 kDa fragment of cystoskeletal actin is posttranslationally N-myristoylated upon caspase-mediated cleavage and targeted to mitochondria. FEBS Lett 2003, 539:37-44.

125. Brooks P, Fuertes G, Murray RZ, Bose S, Knecht E, Rechsteiner MC, Hendil KB, Tanaka K, Dyson J, Rivett J: Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. EMBO J 2002, 21:9235-9243.

126. Liu J, Hughes TE, Sessa WC: The first 35 amino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: a green fluorescent protein study. J Cell Biol 1997, 137:1525-1535.

127. Michel T: Targeting and translocation of endothelial nitric oxide synthase. Braz J Med Biol Res 1999, 32:1361-1366.

128. Barr FA, Puppe M, Vanekerchovje H, Warren G: GRASP65, a protein involved in the stacking of Golgi cisternae. Cell 1997, 91:235-243.

129. Kuo A, Zhong C, Lane WS, Derynck R: Regulated membrane localization of the calcium-myristoyl switch protein VILIP-1 to different membrane compartments in living hippocampal neurons. J Neurosci 2002, 22:7331-7339.

130. Pierino BA, Martin BA: Ca(2+)- and myristoylation-dependent association of calcineurin with phosphatidylserine. J Biochem (Tokyo) 2001, 129:835-841.

131. Timm S, Titus B, Berndt K, Barroso M: The EF-hand Ca(2+)-binding protein p22 associates with microtubules in an N-myristoylated substrate, SSeCKS. Biochemistry 2000, 41:13021-13028.

132. Hwang JY, Koch KW: Calcium- and myristoyl-dependent properties of guanylate cyclase-activating protein-1 and protein-2. Biochemistry 2002, 41:13021-13028.

133. Naik UP, Naik MU: Association of CIB with GPIlb/IIla during outside-in signaling is required for platelet spreading on fibrinogen. J Biol Chem 2003, 180:1353-1362.

134. Resh MD: Myristylation and palmitoylation of Src family members: the fats of the matter. Cell 1994, 76:411-413.

135. Lang ML, Chen YW, Shen L, Gao H, Lang GA, Wade TK, Wade WF: Igα Fc receptor (FcαR2) cross-linking recruits tyrosine kinases, phosphotyrosine kinases and serine/threonine kinases to glycolipid rafts. Biochem J 2002, 364:517-525.

136. Liu J, Hughes TE, Sessa WC: The first 35 amino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: a green fluorescent protein study. J Cell Biol 1997, 137:1525-1535.

137. Michel T: Targeting and translocation of endothelial nitric oxide synthase. Braz J Med Biol Res 1999, 32:1361-1366.

138. Barr FA, Puppe M, Vanekerchovje H, Warren G: GRASP65, a protein involved in the stacking of Golgi cisternae. Cell 1997, 91:235-243.

139. Kuo A, Zhong C, Lane WS, Derynck R: Regulated membrane localization of the calcium-myristoyl switch protein VILIP-1 to different membrane compartments in living hippocampal neurons. J Neurosci 2002, 22:7331-7339.

140. Sullivan A, Uff CR, Isacke CM, Thorne RF: PACE-1, a novel protein that interacts with the C-terminal domain of ezrin. Exp Cell Res 2003, 284:224-238.

141. Li S, Goldberg A: A novel N-terminal domain directs membrane localization of mouse testis-specific calpastatin. Biol Reprod 2000, 63:1594-1600.

142. Rossi EA, Li Z, Feng H, Rubin CS: Characterization of the targeting, binding, and phosphorylation domains of an S rim kinase protein and a myristoylated alamine-rich C kinase substrate-like analog that are encoded by a single gene. J Biol Chem 1999, 274:27201-27210.

143. Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T, Emr SD: ESCRT-II: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev Cell 2002, 3:271-282.

144. Collavin L, Lazarevic D, Utrera R, Marzinotto S, Monte M, Schneider C: wt p53 dependent expression of a membrane-associated isofrom of adenylate kinase. Oncogene 1999, 18:5879-5888.

145. Walker JE, Ariizumi MJ, Depauw A, Fearney IH, Finel M, Miedd SM, Pilkingon SJ, Runswick MJ, Skehel JP: Sequences of 20 subunits of NADH-ubiquinone oxidoreductase from bovine heart mitochondria. Application of a novel strategy for sequencing proteins using the polymerase chain reaction. J Mol Biol 1992, 26:1051-1072.

146. Hanakam F, Allbrecht R, Eckersorn C, Matzner M, Gerisch G: Myristoylated and non-myristoylated forms of the pH sensor protein Lisactophillin II: intracellular shuttling to plasma membrane and nucleus monitored in real time by a fusion with green fluorescent protein. EMBO J 1998, 17:2693-2694.

147. Hunter C, Sugn P, Scheiter ED, Wieschaus E: Conserved domains of the Nullo protein required for cell-surface localization and formation of adherens junctions. Mol Biol Cell 2002, 13:46-157.

148. Neal VA, Young MIW: Igloo, a GAP-43-related gene expressed in the developing nervous system of Drosophila. Development 1994, 120:2235-2243.

149. Quest AF, Harvey DJ, McLinney RA: Myristoylated and nonmyristoylated pools of a sea urchin sperm flagellar creatine kinase exist side-by-side: myristoylation is necessary for efficient localization. Biochemistry 1997, 36:9937-9942.

150. Cserzo M, Eisenhaber F, Eisenhaber B, Simon I: On filtering false positive transmembrane protein predictions. Protein Eng 2002, 15:745-752.

151. Ashraf K, Farazi TA, Gordon JD: A role for Saccharomyces cerevisiae fatty acid synthesis 4 in regulating protein N-myristoylation during entry into stationary phase. J Biol Chem 1998, 273:25864-25874.

152. Robertson SE, Dockendorff TC, Leatherman JL, Faulkner DL, Jongens TA: germ cell-less is required only during the establishment of the germ cell lineage of Drosophila and has activities which are dependent and independent of its localization to the nuclear envelope. Dev Biol 1999, 215:288-297.