GRAM, a novel domain in glucosyltransferases, myotubularins and other putative membrane-associated proteins

UGT51/52 glucosyltransferases are essential for the biosynthesis of sterol glucosides, which are membrane-bound lipids present in many eukaryotes[1]. The catalytic domain of yeast and *Dictyostelium discoideum* enzymes is preceded by an N-terminal extension that is not found in plant homologues[1].

PSI-BLAST (Ref. 2) searches against NRDB with a conserved region (see Fig. 1) of the N-terminal extension (residues 207–274) from *Dictyostelium discoideum* reveal significant similarity ($E = 10^{-4}$) to other yeast glucosyltransferases. A second iteration retrieves a region in TBC-domain-containing proteins ($E = 2 \times 10^{-8}$), which are predicted to activate Rab-like GTPases[3], and has additional matches in hypothetical proteins ($E = 3 \times 10^{-9}$) (see Fig. 2). In further iterative searches we found a second copy of the new domain in some putative Rab-like GTPase activators ($E = 2 \times 10^{-7}$) and a truncated duplication in yeast glucosyltransferases ($E = 7 \times 10^{-4}$). This truncated domain is directly followed by a pleckstrin homology (PH) domain (see Fig. 2). On the fifth iteration of searching, the domain is also retrieved in C2-domain-containing proteins ($E = 2 \times 10^{-5}$) and in some small formin-binding and abscisic acid (ABA)-responsive-element-binding proteins ($E = 1 \times 10^{-4}$). These proteins play a role in environmental stress responses of higher plants[4,5]. The same results were found with complementary hidden Markov model searches[6].

Further PSI-BLAST searches indicate similarity just above the default threshold to MTM1/MTMR1 myotubularins[7,8] ($E = 0.064$), a family of dual-specificity phosphatases and to Sbf [for SET (suvar3–9, enhancer-of-zeste, trithorax)-domain-binding factor] proteins ($E = 1.4$). Sbf-family members are myotubularin-like proteins that lack phosphatase activity[9]. However, mutations in the newly detected domain (and other sequence regions) of

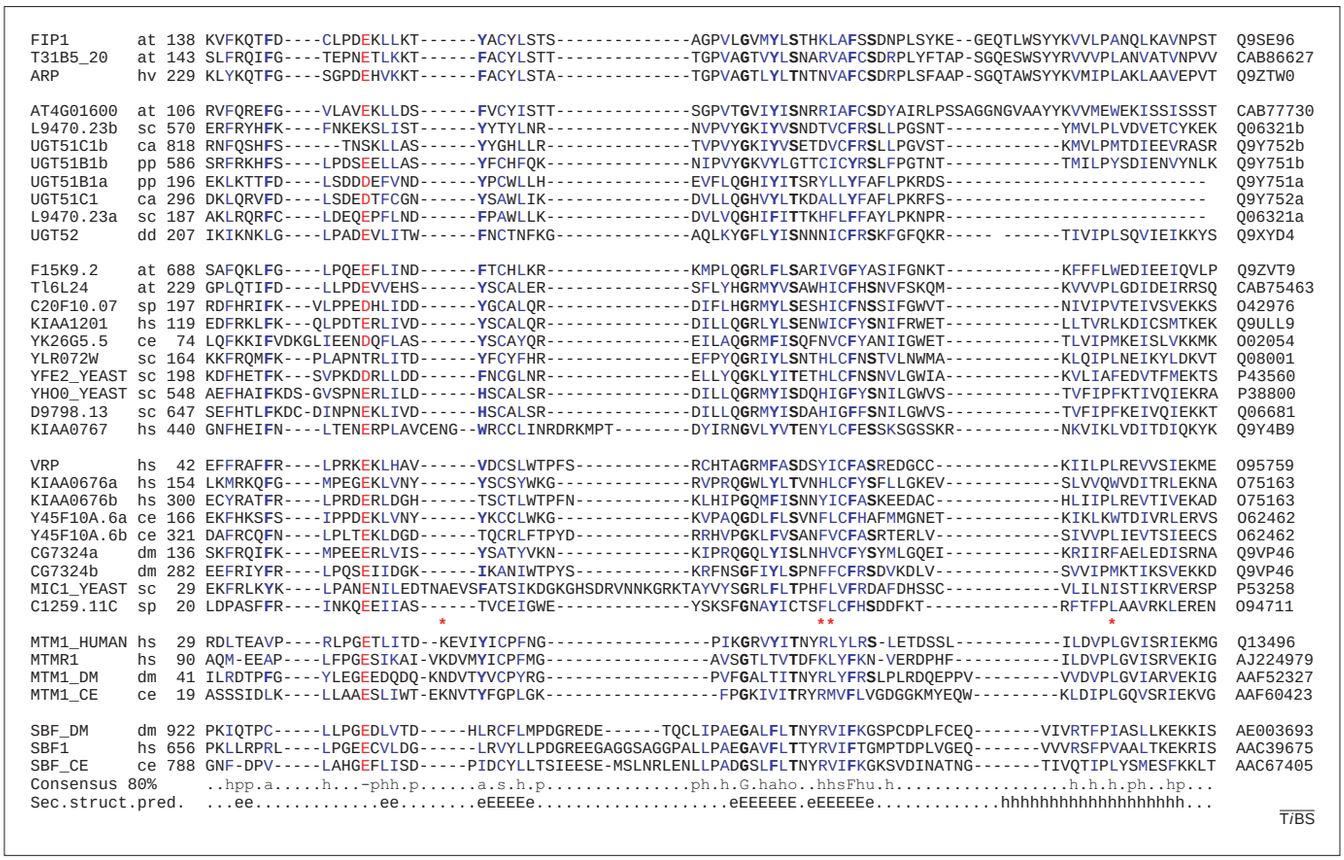

Figure 1

Multiple sequence alignment of GRAM domains of abscisic acid (ABA)-responsive-element-binding proteins (FIP1, T31B5_20, ARP, AT4G01600), PH-domain-containing glucosyltransferases (L9470.23, UGT51B1, UGT51C1), FYVE-domain-containing glucosyltransferase (UGT52), hypothetical proteins (F15K9.2, T16L24, C20F10.07, KIAA1201, YK26G5.5, YLR072W, YFE2_YEAST, YH00_YEAST, D9798.13, KIAA0767), TBC-domain-containing Rab-like GTPase activators (VRP, KIAA0676, Y45F10A.6, CG7324, MIC1_YEAST, C1259.11C), myotubularin dual-specificity phosphatases (MTM1_HUMAN, MTMR1, MTM_DM, MTM_CE) and Sbf1-like dual-specificity phosphatases (SBF_DM, SBF1, SBF_CE). First column: protein names (repeated domains in the same protein are labeled by a or b); second column: species names (at: *Arabidopsis thaliana*; ca: *Candida albicans*; ce: *Caenorhabditis elegans*; dd: *Dictyostelium discoideum*; dm: *Drosophila melanogaster*; hs: *Homo sapiens*; hv: *Hordeum vulgare*; pp: *Pichia pastoris*; sc: *Saccharomyces cerevisiae*; sp: *Schizosaccharomyces pombe*); third column: start of the domain in the respective sequences; rightmost column: database accession numbers. Conserved charged residues are shown in red; conserved hydrophobic residues are shown in blue; other conserved residues are shown in bold. Mutations in the GRAM domain of gene MTM1 are labeled with red asterisks above the sequence MTM1_HUMAN. The consensus sequence (conserved in 80% of the sequences) shown below; h, p, a, u, s, o and '-' indicate hydrophobic, polar, aromatic, tiny, small, alcoholic and negatively charged residues, respectively. The predicted secondary structure taken from the consensus of the alignment. H, helix predicted with expected average accuracy >82%; h, helix predicted with expected average accuracy <82%; E, β sheet predicted with expected average accuracy >82%; e, β sheet predicted with expected average accuracy <82%[20].

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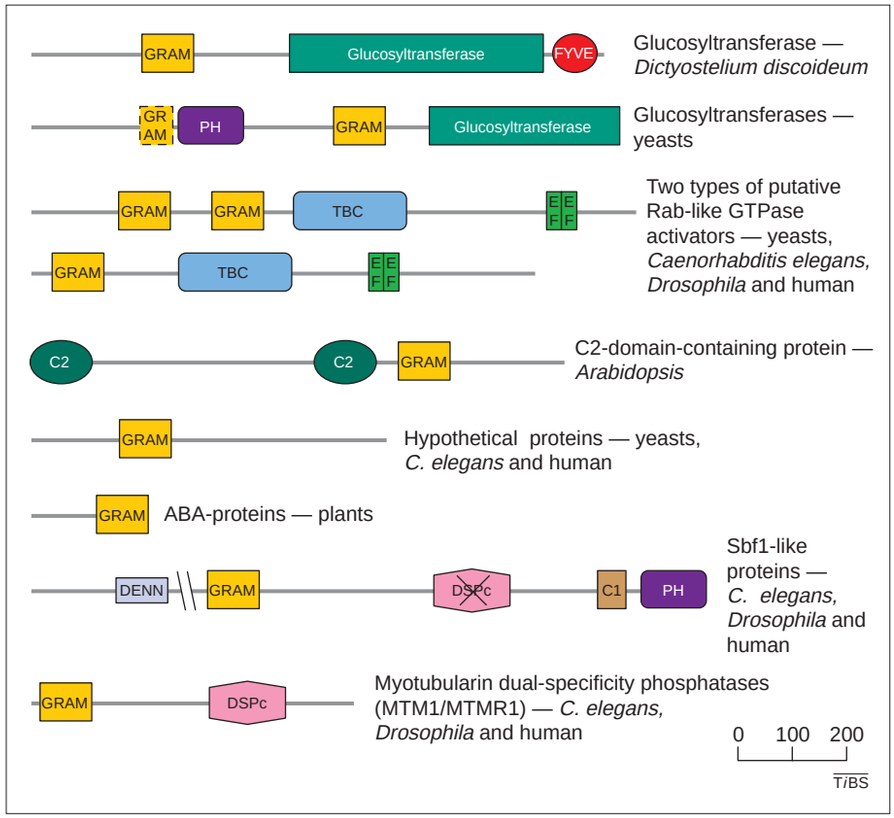

Figure 2

Domain architecture of proteins containing the GRAM domain. Only proteins with distinct modular organizations are shown. The domain names are according to the Simple Modular Architecture Research Tool[23] (http://smart.embl-heidelberg.de). Abbreviations and explanations: C1, protein kinase C conserved region 1; C2, protein kinase C conserved region 2 (CalB); EF, EF-hand, calcium-binding motif; FYVE, domain present in Fab1, YOTB, Vac1 and EEA1; PH, pleckstrin-homology domain; DSPc, catalytic domain of dual-specificity phosphatases; TBC, domain in Tre-2, BUB2p and Cdc16p. The DSPc domain in Sbf1-proteins lacks the catalytic function, marked by an X. The C1 domain is only present in Sbf1-like protein of *Drosophila melanogaster*. The DENN domain is defined by Pfam[24], and the glucosyltransferase domain borders have been published[1].

the human *MTM1* gene (see Fig. 1) are responsible for an X-linked congenital myopathy characterized by hypotonia and respiratory insufficiency[10,11]. The significance of these latter homologies is confirmed by MACAW alignment analysis (*P* values between 10^{-11} and 10^{-50})[12].

We named the newly discovered region the GRAM domain (after the better-characterized glucosyltransferases, Rab-like GTPase activators and myotubularins). It should be noted that the GRAM domain is present in only one of six biochemically identified Rab-like GTPase activators[13], implying that the new domain is not essential for GTPase activation.

Interestingly, all noncatalytic domains (PH domain[14,15], TBC domain[3], C2 domain[16,17], FYVE domain[18], C1 domain[19] and others) that co-occur with GRAM in the same proteins are predominantly associated with membrane-coupled processes.

The GRAM domain is generally ~70 amino acids (50 in the truncated version) in length. Secondary-structure prediction with PHD (Ref. 20) shows four β strands, which suggests that the core of the domain is a β sheet. Each predicted strand contains a conserved aromatic position. Other features include conserved charged residues, but only one glycine is invariable in the sequences identified so far (Fig. 1). The C-terminal α helix appears to be absent in the PH-domain-associated, truncated GRAM domain. Such a truncation is not unusual; it occurs, for example, in members of the ubiquitin-conjugating enzyme family[21,22].

In summary, we predict that the GRAM domain is likely to be an intracellular protein-binding or lipid-binding signalling domain, which has an important function in membrane-associated processes. In myotubularins, mutations in GRAM cause a muscle disease, thus suggesting that this domain is essential for the full function of the enzyme.

Although it is present in a variety of species, it only appears to be ubiquitious in putative Rab-like GTPase activators, myotubularins (MTM1/MTMR1), Sbf1 proteins and some hypothetical proteins. However, it seems to be used also in numerous taxon-specific proteins and pathways. The delineation of the GRAM domain and its borders allows the testing of these hypotheses experimentally.

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Condensing the RNA world

The track record of X-rays in revealing life's inner secrets is the envy of other photons. The most profound mysteries that have been investigated with them have been revealed first to the bravest and the most persistent people on earth. Few dared to purify a 1 500 000 Da complex containing 30 or more polypeptides and 990 000 Da of RNA. Fewer succeeded in crystallizing the large ribosomal subunit, and many turned to more sensible projects when they obtained poor crystals. By the time Ban *et al.*[1] obtained improved crystals of the large subunit from *Haloarcula marismortui* and found 132 heavy-atom positions for an osmium pentamine derivative and 84 heavy-atom positions for an iridium hexamine derivative, there was no turning back. The absence of three of the 31 polypeptides from the SwissProt database no longer elicited fear.

In the 20 years since Ada Yonath and co-workers reported the first crystals of the large subunit, new scientific and engineering disciplines, such as whole-genome sequencing, phylogenetic RNA alignment and secondary-structure prediction, and synchrotron X-ray crystallography came into practice; without these, a model of the large subunit could not have been built. Dominating the assembly is the 23S RNA macromolecule, whose six subdomains assemble into a large single domain. Of the 27 protein structures solved in the assembly, 21 were solved *de novo*; 13 have a nonglobular domain or are entirely extended in their native conformation. The nonglobular protein structures make no sense apart from the large subunit assembly and, in fact, resisted crystallization on their own.

Although Francis Crick proposed as early as 1968 that 23S RNA might catalyse peptide-bond formation, a huge body of genetic and biochemical work has been devoted to testing this hypothesis. Indeed, the notion that RNA once 'did it all' depends more significantly on determining which enzymes in modern cells still 'do it' with RNA catalytic centers than on evolutionary speculation and reconstruction. Difference electron density maps of the large subunit bound to two substrate analogs now demonstrate that the N3 nitrogen of adenosine at position 2486 of 23S RNA is positioned to abstract a proton from the N terminus of the incoming aminoacyl tRNA (Ref. 2). In these X-ray structures, no amino acid side chain is within 15 Å of the condensation. The lack of direct involvement of protein in the reaction underscores the first impression of the large-subunit structure: that polypeptides are largely there to stabilize 23S RNA.

There is an amusing turnabout in the large-subunit story. Crystal structures of substrate analogs for peptide transfer are bound by RNA, proving that the ribosome is a ribozyme. The proposed mechanism (adenosine 2486 as a general base) is the reverse of the mechanism for hydrolysis of an acylenzyme by serine proteases (histidine as a general base). Can the history of protein enzyme evolution be distilled to a counter attack of ribosomal protein synthesis? Revolutionary ribosomal RNAs make proteins, which then deploy an enhanced set of side chains as RNA mimics and function to break the bonds condensed in the RNA world.

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Fringe gives a saccharine to Notch

Post-translational modifications like phosphorylation, acetylation and fatty acid acylation of proteins are well-known strategies that cells use to regulate the activity of enzymes and maintain the binding properties of ligands and receptors. By contrast, glycosylation, where carbohydrates are attached to the side chains of amino acids, is important in the synthesis of many secreted and cell-surface proteins.

Moloney *et al.*[1] and Brückner *et al.*[2] now demonstrate that elongation of *O*-linked fucose on glycosylated proteins can act as an as-yet-unknown post-translational regulatory mechanism for modulating receptor–ligand interactions in signal transduction. Both groups studied the influence of glycosylation on the activity of Notch-induced signalling, which is important for the formation of tissue boundaries during development. Notch receptors are transmembrane proteins with an extracellular domain of epidermal growth factor (EGF)-like repeats and are activated by two conserved families of ligand proteins, Jagged/Serrate and Delta.

Molony *et al.* show that Fringe, a modulator of Notch, possess a fucose-specific glycosyltransferase activity that catalyses the elongation of carbohydrates on the EGF repeats of the receptor protein. Using tritium-labelled saccharides they found that EGF–*O*-fucose is a highly specific target for Fringe. The elongation of the glycans on Notch led to an inhibitory effect on the activation of its ligand, Jagged1, as monitored by a luciferase-reporter assay. Brückner *et al.* demonstrate that Fringe displays its ability to modify Notch within the Golgi apparatus and that modulation of the receptor increases the binding activity to its ligand Delta. Both groups show that the Notch–ligand interaction is not affected when replacing the DxD motif (aa 236–238) in Fringe, required for the catalytic activity in many glycosyltransferases, by either NNN or DEE.