Correspondence

The fukutin protein family – predicted enzymes modifying cell-surface molecules

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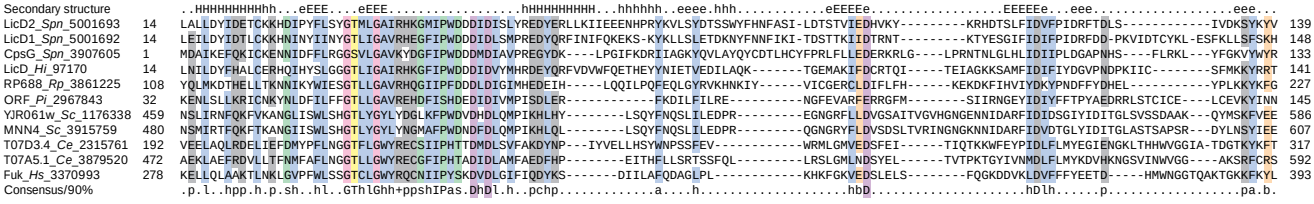
Fukuyama type congenital muscular dystrophy (FCMD) is an autosomal recessive disorder that is observed predominantly in Japanese populations [1]. Recently, the cause of this syndrome was discovered to be lesions in the gene encoding the protein fukutin; these lesions involve retroposon insertion and point mutations, which result in a truncated protein [2]. Consistent with the phenotypic patterns of the disease, the fukutin mRNA has been found in skeletal muscles, heart, brain and pancreas [2]. Fukutin contains a signal peptide and is localized to the Golgi and secretory granules [2]. Here, we report on a detailed computer analysis of the fukutin protein sequence, resulting in the prediction that it is an enzyme that modifies cell-surface glycoproteins or glycolipids.

A gapped BLASTP [3] search of the non-redundant (NR) database at the National Center for Biotechnology Information using the fukutin sequence as the query revealed significant hits (with e-values < 10^{-9}) not only to *Caenorhabditis elegans* proteins, such as T07D3.4 and T07A5.1, but also to the uncharacterized protein RP688 from the intracellular parasitic bacterium *Rickettsia prowazekii* (e < 10^{-4}). The RP688 sequence was used for further analysis of this protein family by iteratively searching the NR database using the PSI-BLAST program, which was run with the cut-off of e = 0.001 [3]. At convergence, not only fukutin and its *C. elegans* homologs, but also bacterial proteins involved in polysaccharide/phosphorylcholine modification and a yeast protein involved in mannosyl phosphorylation of oligosaccharides were retrieved from the database. Reverse searches with these sequences retrieved the original members of the fukutin family without any false positives. Fukutin, therefore, belongs to a family of proteins associated with the modification of the cell surface.

A multiple alignment of the fukutin protein family was constructed using Gibbs sampling, as implemented in PROBE [4], in conjunction with the –m4 option of PSI-BLAST (Figure 1). The alignment shows prominent conservation in an amino-terminal block, followed by a weakly conserved carboxy-terminal region. The most notable feature of the amino-terminal region is the presence of the strictly conserved signature G[TS]hhGhhx4hhxaxxDxD (in single-letter amino acid code and in which 'h' is a hydrophobic amino acid, 'a' is an aromatic amino acid and 'x' denotes any amino acid). A pattern search with this motif recovers from the NR database the fukutin family in its entirety without any false positives. The carboxy-terminal region contains a motif with a conserved aspartate residue flanked by hydrophobic residues (Figure 1). Secondary structure prediction using the PHD program [5] suggests a compact α/β fold for the fukutin domain (Figure 1).

LicD2 from *Streptococcus pneumoniae* is involved in the addition

Figure 1



Current Biology

A multiple alignment of fukutin family proteins. The proteins designations include: gene name_species abbreviation_GenBank identification number. The CpsG protein is encoded by the operon involved in capsular polysaccharide biosynthesis in *S. pneumoniae*. The species abbreviations are as follows: *Spn*, *S. pneumoniae*; *Rp*, *Rickettsia prowazekii*; *Pi*, *Prevotella intermedia*; *Hi*, *H. influenzae*; *Sc*, *Saccharomyces cerevisiae*; *Ce*, *C. elegans*; and *Hs*, *Homo sapiens*. The alignment is colored according to the 90% consensus with the following consensus terms: p (RKHEDNQST), polar; b (FLIMYWKREQ), big; s (STCVNDPGAS), small; h (LIVMFWYAC), hydrophobic; l (LVIMA), aliphatic; c (RKHED), charged; G,A or S, tiny; a (FYWH), aromatic; + (RKH), positive; S or T, alcohol. The coloring scheme used is: grey, charged; green, small; blue, hydrophobic/ aromatic; pink, tiny; yellow, alcohol; purple, polar; orange, big. Protein secondary structure was predicted using the PHD program [4], with the multiple alignment as the query, and is shown above the alignment (H indicates α helix and e indicates β strand).

of phosphorylcholine residues to lipoteichoic acid, an important component of the cell wall in Gram-positive bacteria [6]. It has been proposed that LicD2 is an enzyme that catalyzes the transfer of phosphorylcholine to its teichoic acid substrates from a CDP-choline donor [6]. Similarly, the *Haemophilus influenzae* homolog — LicD is required for the addition of phosphorylcholine to the Gram-negative bacterial lipopolysaccharide [7]. The yeast protein MNN4 participates in a similar reaction in which a mannosyl phosphate residue is added from a GDP–mannose donor to both N-linked and O-linked oligosaccharides on proteins ([8] and references therein). These studies, taken together with the sequence conservation pattern, suggest that the fukutin family proteins are phosphoryl-ligand transferases. The presence of the DxD motif and a distal aspartate residue that is conserved in most of the sequences suggests that these enzymes coordinate a divalent cation, which is similar to a number of nucleotidyltransferases (for example, see [9]).

Drawing from knowledge of the functions of these proteins and the subcellular localization of fukutin, we predict that this protein modifies cell-surface molecules, most probably through the attachment of phosphoryl-sugar moieties. The brains of individuals with FCMD show an abnormal pattern of gangliosides [10], which suggests that the transferase activity of fukutin could participate in one of the many steps of glycolipid modifications. Glycolipid modifications are an important factor in determining the adhesive properties of cells [11]. Thus, fukutin might regulate neural migration and muscle organization by affecting the biogenesis of crucial adhesion molecules. In this regard, it might be of interest that disruption of the basement membrane, linked to an M-laminin deficiency, has been reported in FCMD patients [12]. All the bacterial homologs of fukutin are from pathogenic bacteria and experimental evidence from *H. influenzae* [7] and *S. pneumoniae* [6] shows that the surface modifications catalyzed by these proteins (LicD and LicD2, respectively) are necessary for the adhesion of the bacteria to mammalian lung cells and pathogenesis. The sequence conservation described here, together with the apparent absence of fukutin family members in non-pathogenic bacteria, might suggest that the genes coding for these proteins have been horizontally transferred from eukaryotes to the pathogenic bacteria. A member of the fukutin family was detected in an expressed sequence tag from *Trypanosoma brucei* (AI077222), which suggests a possible role for this protein in the surface modifications of this eukaryotic pathogen (data not shown).

The proposed function and the phylogenetic distribution of the fukutin family members resemble those of the glycosyl transferases of the Fringe family that are involved in the modification of surface molecules of the Notch pathway in development [13]. Like the fukutin family proteins, Fringe homologs are seen in pathogenic but not in free-living bacteria. Thus horizontal gene transfer from eukaryotes might be a common theme in the origin of the enzymes involved in surface modification in bacterial pathogens, resulting in similarities in adhesion mechanisms between these bacteria and eukaryotic cells.

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