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AMPK β Subunit Targets Metabolic Stress Sensing to Glycogen

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Summary

AMP-activated protein kinase (AMPK) is a multisubstrate enzyme activated by increases in AMP during metabolic stress caused by exercise, hypoxia, lack of cell nutrients [1], as well as hormones, including adiponectin and leptin [2, 3]. Furthermore, metformin and rosiglitazone, frontline drugs used for the treatment of type II diabetes, activate AMPK [4]. Mammalian AMPK is an αβγ heterotrimer with multiple isoforms of each subunit comprising α1, α2, β1, β2, γ1, γ2, and γ3, which have varying tissue and subcellular expression [5, 6]. Mutations in the AMPK γ subunit cause glycogen storage disease in humans [7], but the molecular relationship between glycogen and the AMPK/Snf1p kinase subfamily has not been apparent. We show that the AMPK β subunit contains a functional glycogen binding domain (β-GBD) that is most closely related to isoamylase domains found in glycogen and starch branching enzymes. Mutation of key glycogen binding residues, predicted by molecular modeling, completely abolished β-GBD binding to glycogen. AMPK binds to glycogen but retains full activity. Overexpressed AMPK β1 localized to specific mammalian subcellular structures that corresponded with the expression pattern of glycogen phosphorylase. Glycogen binding provides an architectural link between AMPK and a major cellular energy store and juxtaposes AMPK to glycogen bound phosphatases.

Results and Discussion

A putative domain of the AMPK β subunit contained within residues 42–183 was identified by using sequence searches and secondary structure predictions (Figure 1A). Limited proteolysis of AMPK-β1(42–183) revealed a protease-resistant fragment corresponding to residues 68–163 (β-GBD). The β-GBD sequence (AMPK-β1(68–163)) is most closely related to isoamylase domains [8] found in glycoside hydrolase family 13, which includes glycogen and starch branching enzymes [9], and is more remotely related to glycogen and starch binding proteins found in the carbohydrate binding module-containing families 20 and 21 (http://afmb.cnrs-mrs.fr/CAZY/) (Figure 1B).

Bacterially expressed β-GBD bound glycogen in a saturating manner (Figure 2A) (at 75 μg/ml in the presence of 5 mg/ml glycogen). β-GBD binding to glycogen was inhibited by increasing concentrations of β-cyclodextrin (Figure 2B), and half maximal inhibition occurred at ~1.5 mM β-cyclodextrin, as is the case for starch binding proteins [10]. Isoamylase domains, although found in glycogen and starch branching enzymes, have not been shown formally to associate with either carbohydrate, but we demonstrate that β-GBD belongs to this family and show that it binds glycogen. Thus, β-GBD represents a new subfamily of glycogen binding modules.

The β-GBD sequence is highly conserved across the AMPK/SNF1 β subfamily (Figure 3A). We constructed a 3D homology model of β-GBD (Figure 3B) based on aligning residues 68–163 of the rat AMPK β1 subunit with the three known isoamylase domain structures [8, 11, 12]. Inspection of the model revealed two surface-exposed tryptophans and a lysine (W100, K126, and W133) that together formed a putative carbohydrate binding site (Figure 3C) that is strikingly similar to binding site 1 in the glucoamylase and cyclodextrin glycosyltransferase starch binding domains [13, 14]. When maltotriose was modeled into the glycogen binding site, the apolar faces of two sugars stacked against the aromatic rings of W100 and W133 (Figure 3C). The dual specificity phosphatase laforin (Figure 1B) contains a glycogen binding domain belonging to the more distally related CBM family 20 and shares the equivalent residues of W100, K126, and W133. Mutation of these residues also prevents laforin binding to glycogen [15].

We show that mutation of putative key binding residues of β-GBD, W100G and K126Q, completely abolished β-GBD binding to glycogen, whereas the W133L mutation only partially inhibited binding (Figure 3D). Mutation of the AMPK β autophosphorylation site S108 contained within the β-GBD [16] to glutamic acid partially inhibited glycogen binding (Figure 3D), whereas alanine had no effect. In yeast, the β homolog, Gal83p, contains a mutation (G235R) within its glycogen binding domain [17] that causes the Snf1p substrate Sip4p (a transcriptional activator) to become insensitive to glucose suppression [18]. We find that the equivalent G147R mutation in β-GBD partially inhibited glycogen binding (Figure 3D). Arg at position 147 would be expected to distort the glycogen binding site by electrostatic repulsion of Lys-126 in the β-GBD model structure (Figure 3C). Gal83p may therefore be important for recruiting Sip4p to glycogen to allow inactivation by glycogen bound phosphatases in the presence of glucose.

We used purified rat liver AMPK to confirm that the

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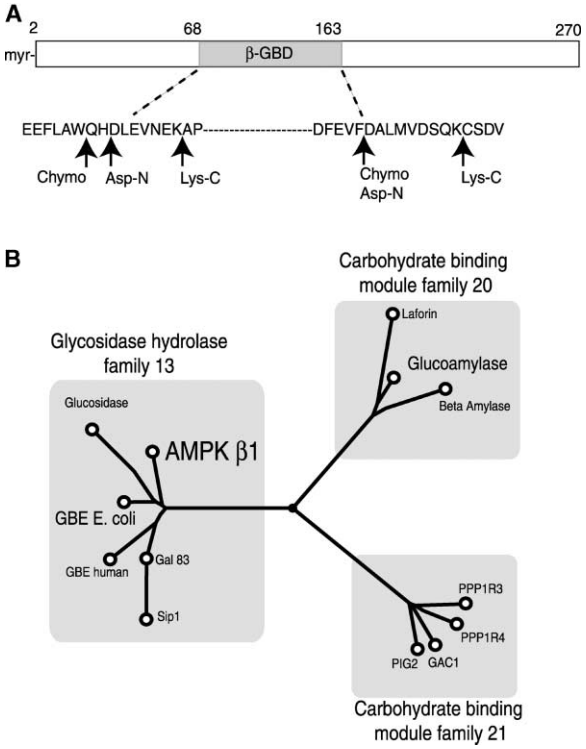

Figure 1. AMPK β1 Contains a Proteolysis-Resistant Domain that Is Related to the Starch and Glycogen Binding Domain Superfamily

(A) Bioinformatic analysis identified a potential midmolecule domain in the AMPK β subunit. Limited proteolysis analysis with the specific proteases chymotrypsin (Chymo), Asp-N, and Lys-C identified the domain between residues 68 and 163.

(B) The relationship between AMPK β1(68–163) and other known starch or glycogen binding domains was determined by unrooted phylogenetic tree analysis as determined by the AllAll server (http://cbrg.inf.ethz.ch/Server/AllAll.html).

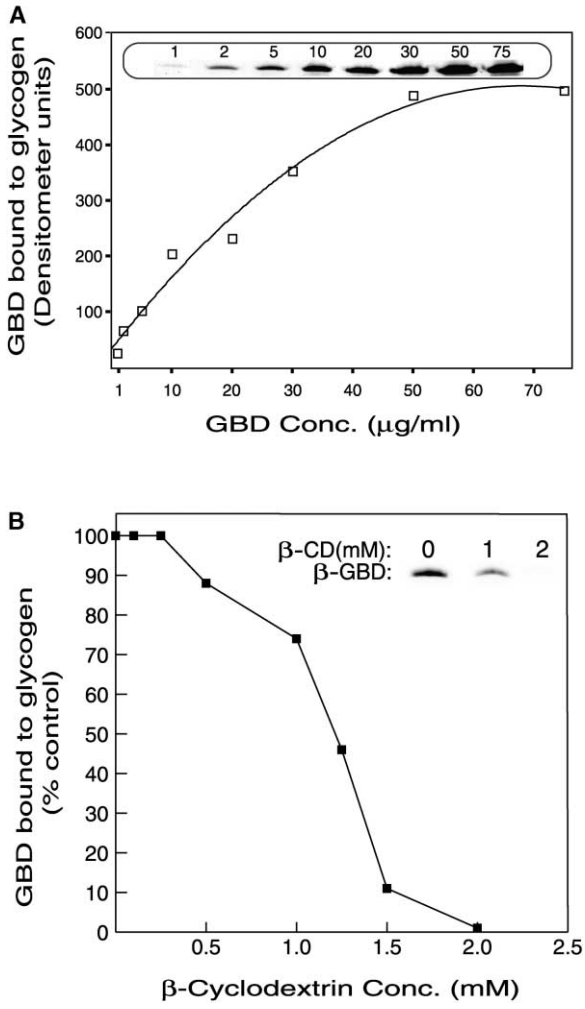

Figure 2. Interaction of β-GBD (AMPK β1(68–163)) with Glycogen

(A) β-GBD associates with glycogen. The inset shows the relative amount of β-GBD protein bound to glycogen for the corresponding amounts of added β-GBD (μg), as assessed by Coomassie staining of a Tris-tricine gel.

(B) β-cyclodextrin (β-CD) competes for binding of glycogen to β-GBD, and half maximal inhibition occurs with ~1.5 mM β-cyclodextrin. The insert shows Coomassie staining of a Tris-tricine gel from this experiment.

holoenzyme binds to glycogen (Figure 4). But, AMPK binding to glycogen required 10-fold higher concentrations of β-cyclodextrin to prevent binding (data not shown), indicating that its binding is stronger than β-GBD alone. This may be due to more favorable positioning of the β-GBD within the context of the holoenzyme and may reduce the AMPK dissociation rate from glycogen. Since several studies have reported that activation of AMPK is reduced in tissues containing high levels of glycogen [19, 20], we tested the effect of glycogen and found that it did not affect the activity of AMPK directly (Figure 4). To study the subcellular localization of the AMPK β1 subunit, we transiently expressed β1-GFP in COS-7 cells together with glycogen phosphorylase (GP) tagged to *Discosoma sp*. red fluorescent protein. AMPK β1-GFP colabeled glycogen bodies with GP, as shown in Figure 5, and thereby demonstrated that AMPK localizes to glycogen in vivo. The glycogen bodies were similar in size and shape to those reported when the dual-specificity phosphatase laforin was overexpressed [15] and to those reported in the companion paper [21], which reports that AMPK β1/β2 colocalized with glycogen synthase.

In summary, we show that the AMPK β subunit contains a glycogen binding domain that targets AMPK to glycogen (Figure 5). Several lines of evidence now link AMPK to glycogen metabolism. The yeast homolog Snf1p is important for maintaining glycogen levels [22]. Glycogen synthase, an AMPK substrate [19], and glycogen phosphorylase coimmunoprecipitate with AMPK [6]. Mutation in AMPK γ3 (R200Q) causes glycogen accumulation and reduced meat quality in Hampshire pigs [23]. By contrast, reduced muscle glycogen and improved meat yields are linked to the adjacent V199I γ3 variant [24]. In humans, six mutations in AMPK γ2 have been found in individuals exhibiting cardiac hypertrophy due to glycogen accumulation in cardiomyocytes and ventricular preexcitation typical of the Wolff-Parkinson-White (WPW) syndrome [7]. Thus, just as the AMPK

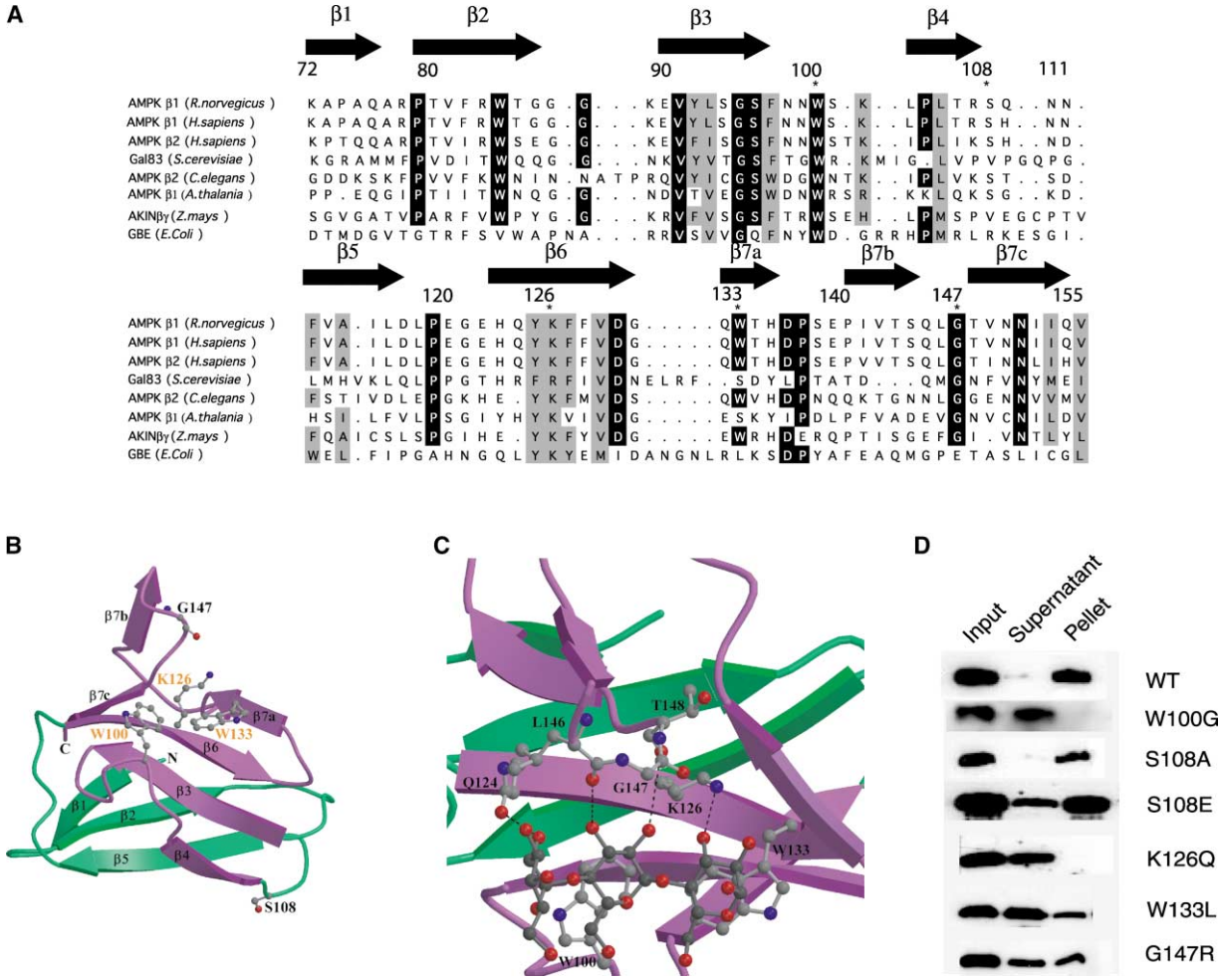

Figure 3. Molecular Basis for Glycogen Binding to β-GBD

(A) Structural alignment of the Snf1 family of β subunits based on the structure of the *E. coli* glycogen branching enzyme (GBE, accession number P07762) [11]. Rat AMPK β1 β-GBD (accession number P80386), human AMPK β1 (accession number Q9Y478), human AMPK β2 (accession number NP_005390), *S. cerevisiae* GAL83 (accession number Q04739), *C. elegans* AMPK β2 (accession number NP_499446), *A. thalania* AMPK β1 (accession number NP_197615), and *Z. mays* AKINbetagamma-1 (accession number AAG31751) are shown. Amino acid sequences were aligned with Clustalw and were formatted by an Excel macro as previously described [5]. Conserved amino acids are shaded black, and conserved substitutions are shaded gray. Residues highlighted by an asterix have been mutated (see [D]). The arrows above the sequences indicate predicted β strands 1–7c of the model.

(B) A ribbon representation of the GBD model shown as a β sandwich, with two antiparallel β sheets (colored green and purple), was based on the structural alignment of N-isoamylase domains (PDB entries 1M7X, 1EH9, and 1BF2). Residues conserved across the larger starch and glycogen binding domain family are labeled orange, while AMPK-specific residues, including an autophosphorylation site at Ser-108, are labeled black.

(C) Potential sugar binding mode for the GBD based on the crystal structure of cyclodextrin glycosyltransferase complexed with carbohydrate (PDB entry 2DIJ). Maltotriose, a small polysaccharide (shown with dark bonds), and relevant residues are shown in ball-and-stick representation. The dashed lines indicate possible hydrogen bonds.

(D) Identification of residues critical for binding of β-GBD to glycogen. Residues important for binding to glycogen were predicted by the structural alignment (shown in [A]) and the model (shown in [B]). Wild-type and mutant β-GBD were incubated with glycogen; Western blots of Tris-tricine gels of input, supernatant (glycogen unbound), and pellet (glycogen bound) β-GBD are shown. As predicted from the model shown in (B), essential residues include W100 and K126; W133 and G147 also contribute to glycogen binding.

γ subunit mutations can cause disorders of glycogen metabolism [7], mutations in the AMPK β subunit can be anticipated to interfere with glycogen metabolism.

Glycogen plays an important role in targeting many different proteins [25], including glycogenin, glycogen phosphorylase, glycogen synthase, phosphatases, and now AMPK, as depicted in Figure 6. Further studies will be required to understand the regulation and precise mechanism of how AMPK associates with glycogen. To begin to explore these questions, we have crystallized recombinant AMPK β1(68–163) with crystals diffracting to beyond 2.2 Å resolution.

Supplemental Data

Supplemental Data including the Experimental Procedures are available at http://images.cellpress.com/supmat/supmatin.htm.

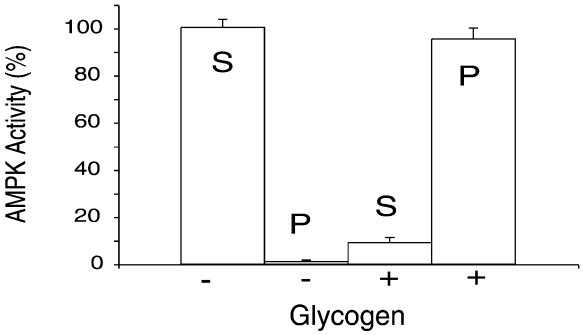

Figure 4. Effect of Glycogen on AMPK Activity

Purified rat liver AMPK ($\alpha\beta\gamma$) was incubated in the presence and absence of 30 mg/ml glycogen and was then centrifuged; AMPK activity was measured in the supernatant (S) and pellet (P). AMPK bound to glycogen, but AMPK activity was not inhibited by glycogen. Data are shown as mean ± SEM, n = 6.

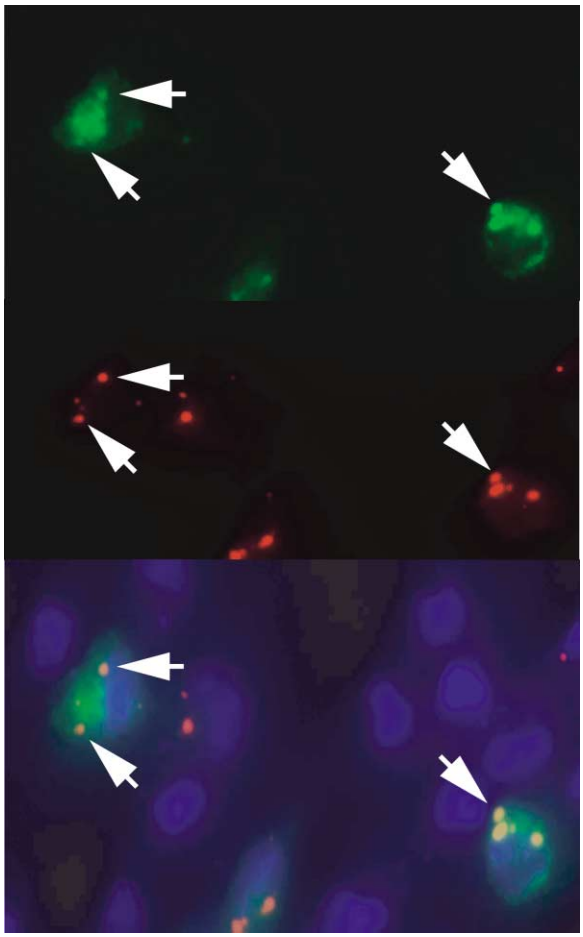

Figure 5. The AMPK β Subunit Colocalizes with Glycogen Phosphorylase

GP-RFP and AMPK β1-GFP were coexpressed in COS-7 cells. AMPK β1-GFP (green) is shown in the top panel, GP-RFP (red) is shown in the middle panel, and colocalized AMPK β1-GFP and GP-RFP (colored orange) are shown in the bottom panel. Nuclei (blue) were stained with Hoechst stain 33258. The arrows indicate the presence of glycogen bodies that both AMPK β1-GFP and GP-RFP colocalize to.

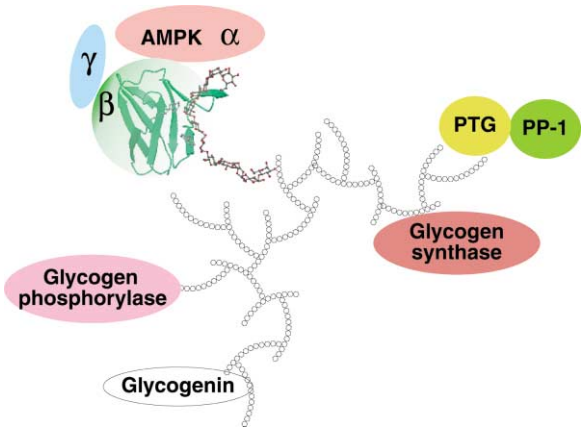

Figure 6. Schematic Representation of Glycogen Acting as a Scaffold that Links AMPK to a Major Cellular Energy Store as Well as Juxtaposes AMPK to Glycogen Bound Phosphatases, Glycogen Phosphorylase, and Glycogen Synthase

Glycogen is shown as a branched structure. PP1, protein phosphatase 1; PTG, protein targeting to glycogen [26].

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