Inventory of Supplemental Material

Figure S1. Expands data shown in Figure 1 to include analyses of other mouse and yeast genetic models and includes in vitro experiments with recombinant yeast and human glycogen synthases.

Figure S2. Shows separation of phospho-oligosaccharides by anion exchange chromatography and analyses of other fractions than those shown in Figure 2.

Figure S3. Provides the complete set of NMR data that led to the asignments of phosphoesters at C2 and C3.

Table S1. Defines the acquisition parameters for the various NMR experiments leading the assignments of Table 1.

Supplemental Information
Cell Metabolism, *Volume XX*

Phosphate Incorporation during Glycogen Synthesis and Lafora Disease

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Figure S1, related to Figure 1. Incorporation of phosphate into glycogen by cell extracts and purified glycogen synthases.

(A) The incorporation of ^{14}C or ^{32}P, as indicated, into exogenous glycogen (6.67 mg/mL) by cell extracts in the presence of UDP-[U-^{14}C]glucose or [β-^{32}P]UDP-glucose. Reaction products were separated by SDS-PAGE and radioactivity detected by phosphorimaging. The arrow indicates the bottom of the well where glycogen accumulates and the arrowhead the top of the stacking gel where smaller polysaccharides migrate. The upper panel shows analyses of extracts from muscle of wild type (WT) or MGSKO mice; the middle panel shows analyses of extracts from wild type yeast (WT) or yeast lacking glycogen synthase (*gsy1 gsy2*); the lower panel shows extracts from muscle of wild type (WT) or *Epm2a-/-* mice, which lack laforin. B, blank reaction without extract; 0, reactions boiled immediately after addition of UDP-glucose; 30, reactions terminated after 30 min; +, treatment of synthesized polysaccharide with glucosidases. (B) Time dependent incorporation of ^{14}C or ^{32}P, as indicated, into exogenous glycogen (6.67 mg/mL) by purified glycogen synthase. Reaction products were separated by SDS-PAGE and radioactivity detected by phosphorimaging. Upper panel, rabbit muscle glycogen synthase; middle panel, *S. cerevisiae* Gsy2p; lower panel, human glycogen synthase, muscle isoform.

Supplemental Figure 1

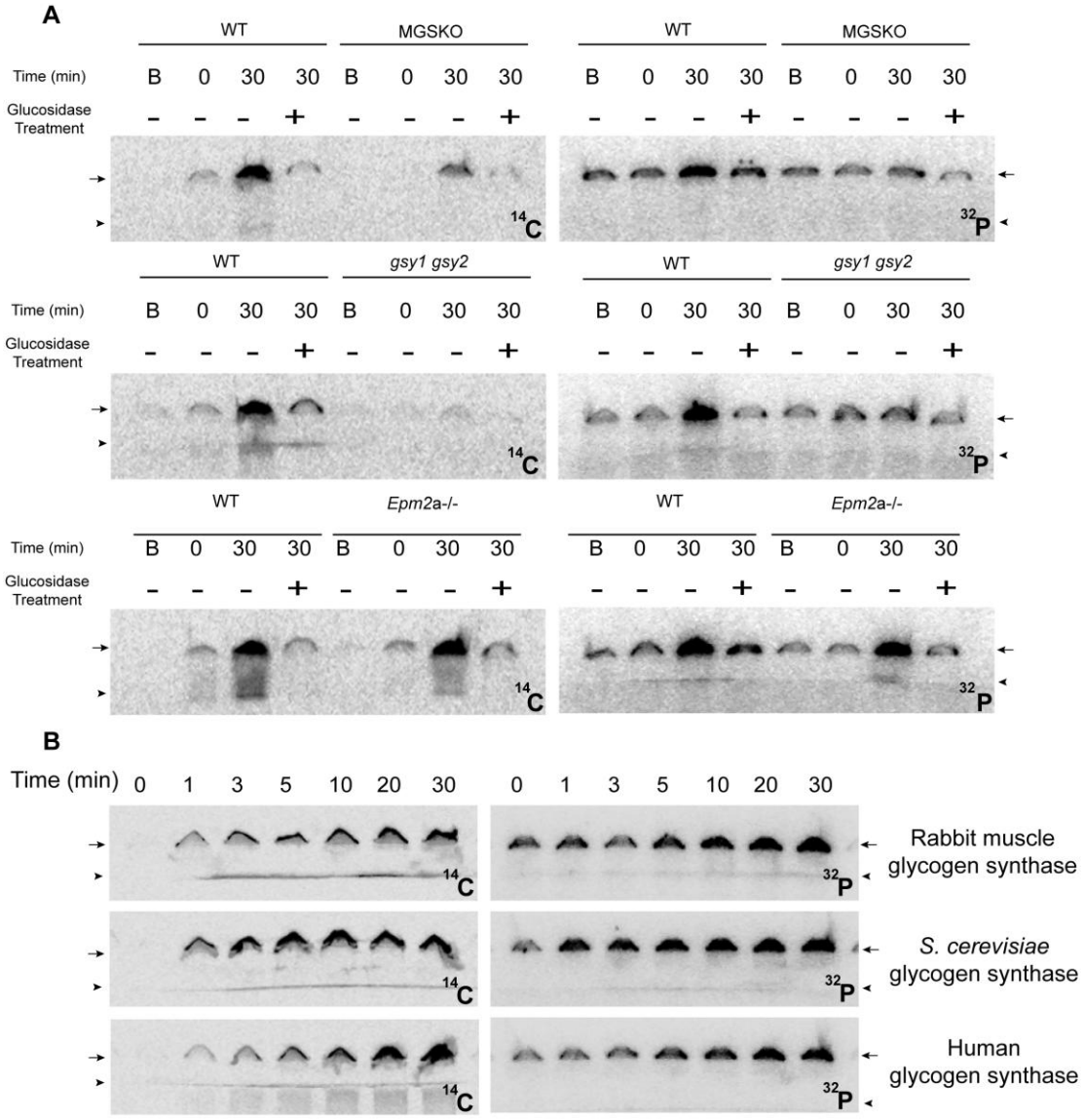


Figure S2, related to Figure 2. Purification and analysis of phosphorylated oligosaccharides from rabbit muscle glycogen.

(A) Rabbit muscle glycogen was digested with α-amylase and amyloglucosidase. The anionic species were bound to DEAE sepharose, eluted with ammonium bicarbonate and the associated phosphate measured after acid hydrolysis as described in Experimental Procedures. Of the twenty fractions collected, seven contained significant amounts of covalent phosphate. (B) HPAEC analysis of Fraction I. (C) HPAEC analysis of Fraction II (D) HPAEC analysis of Fraction III (E) HPAEC analysis of Fraction IV (F) HPAEC analysis of Fraction V (G) HPAEC analysis of Fraction VI (H) HPAEC analysis of Fraction VII. (I) MALDI-TOF-MS analysis of Fractions II + III + IV. (J) MALDI-TOF-MS analysis of fraction VI.

Supplemental Figure 2

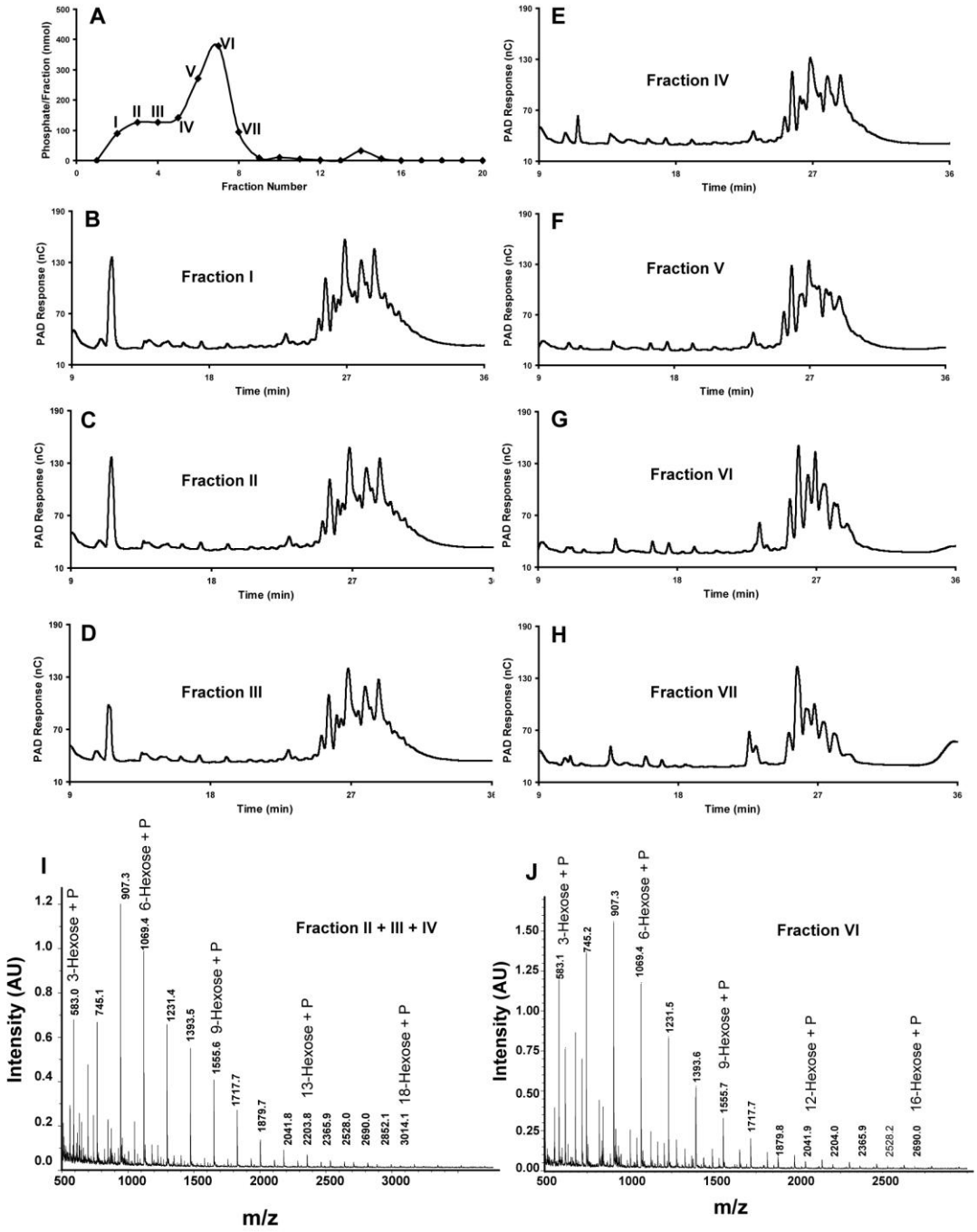
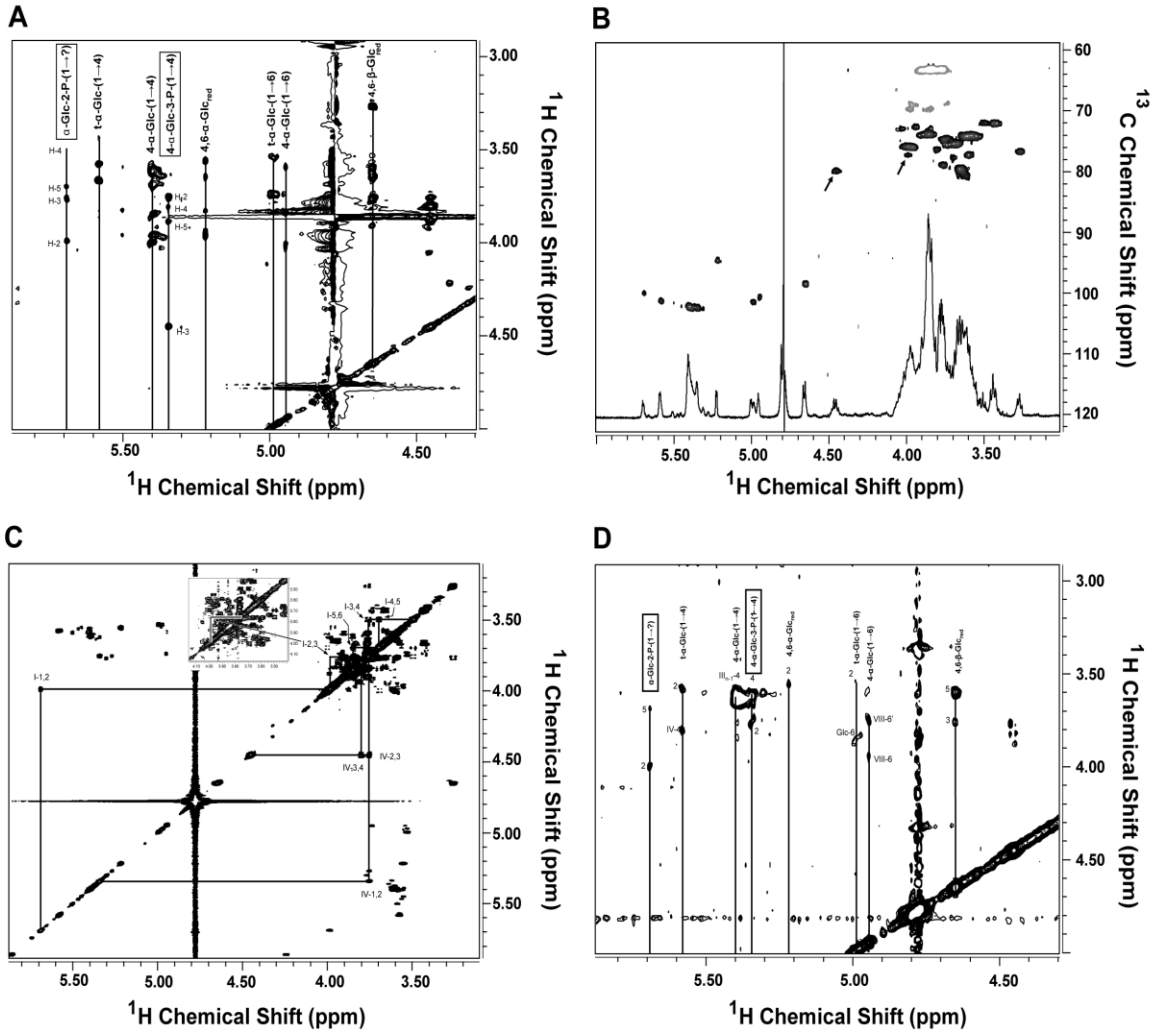


Figure S3, related to Figure 3. NMR spectroscopy of phosphorylated oligosaccharides from glycogen.

(A) Anomeric region of the ^{1}H-^{1}H TOCSY NMR spectrum of phosphorylated oligosaccharides. The spectrum was acquired at 25 °C and 18.8 T with a 1-s relaxation delay and 3.7 kHz spectral width, 100 ms spinlock time, 0.138 s acquisition time, 128 increments, and 32 transients per increment. Spin systems are indicated by vertical lines. The labels of the phosphorylated species are boxed and their cross peaks assigned based on COSY and HSQC experiments. (B) One-dimensional ^{1}H-NMR spectrum and 2-dimensional ^{1}H-^{13}C gradient-enhanced, multiplicity-edited HSQC (gHSQC) spectrum of phosphorylated oligosaccharides. In the gHSQC experiment, methyl and methine groups give rise to positive peaks (black contours), and methylene groups give rise to negative peaks (grey contours). The two phosphorylated positions are indicated by arrows. The spectra were acquired at 25 °C and 14.1 T. The 1-D spectrum was acquired with a 1-s relaxation delay and 5.0 kHz spectral width, 2 s acquisition time and 32 transients. The 2-D gHSQC spectrum was acquired with 5.0 kHz and 10.6 kHz spectral width in the proton and carbon dimensions, respectively, 0.15 s acquisition time, 100 increments, and 256 transients per increment. (C) Gradient enhanced ^{1}H-^{1}H COSY NMR spectrum of phosphorylated oligosaccharides. The spectrum was acquired at 25 °C and 18.8 T with a 1-s relaxation delay and 3.7 kHz spectral width, 0.138 s acquisition time, 1000 increments, and 16 transients per increment. The spin systems of phosphorylated residues I and IV are connected by lines. The inset displays a portion of the spectrum at lower contour level to show the cross peak between H-2 and H-3 of Residue I. (D) Anomeric region of the ^{1}H-^{1}H ROESY NMR spectrum of phosphorylated oligosaccharides. The spectrum was acquired at 25 °C and 18.8 T with a 1-s relaxation delay and 3.7 kHz spectral width, 200 ms mixing time, 0.138 s acquisition time, 128 increments, and 32 transients per increment. Spin systems are indicated by vertical lines. Intra-residue NOEs are labeled by arabic numerals only, inter-residue NOEs are labedled with roman numerals designating the residue (see Table S1) and an arabic numeral designating the position of the proton giving the NOE. The label "III$_{n-1}$-4" refers to the next residue of the same type in the direction of the reducing end. The label "Glc-6" refers to H-6 of an unidentified glucose residue.

Supplemental Figure 3



Supplemental Table 1

Table S1, related to Table 1.

experiment	sfrq	dfrq	sw	sw1	nt	ni	at	mix	np
proton	600		5040		32		1998		10070
^{1}H-^{1}H gCOSY	600		5040	5040	8	512	203		1024
^{1}H-^{1}H TOCSY	800		3721	3721	32	128	138	100	512
^{1}H-^{1}H ROESY	800		3721	3721	32	128	138	300	512
^{1}H-^{13}C gHSQC	600	150	5040	10555	256	100	150		756
^{1}H-^{31}P gHMQC	500	200	3470	8000	360	32	400		1388
^{1}H-^{31}P HMQC-TOCSY	500	200	3470	3000	400	24	300	40	1041

Abbreviations used:
sfrq: spectrometer frequency for proton nucleus in Hz
dfrq: decoupler frequency for heteronucleus (^{13}C or ^{31}P) in Hz
sw: spectral width in the directly detected dimension (^{1}H) in Hz
sw1: spectral width in the indirectly detected dimension (^{13}C or ^{31}P) in Hz
nt: number of transients
ni: number of increments
at: acquisition time in ms
mix: Mixing (spinlock) time in ms
np: number of points in the directly detected dimension