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Getting a handle on glycogen synthase – Its interaction with glycogenin

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Abstract

Glycogen is a polymer of glucose that serves as a major energy reserve in eukaryotes. It is synthesized through the cooperative action of glycogen synthase (GS), glycogenin (GN) and glycogen branching enzyme. GN initiates the first enzymatic step in the glycogen synthesis process by self glucosylation of a short 8–12 glucose residue primer. After interacting with GN, GS then extends this sugar primer to form glycogen particles of different sizes. We discuss recent developments in the structural biology characterization of GS and GN enzymes, which have contributed to a better understanding of how the two proteins interact and how they collaborate to synthesize glycogen particles.

1 Introduction

Glycogen is a branched polymer of glucose joined through an α1,4 glycosidic linkage with intersecting α1,6 linked glucose residues that serve as branch points. Glycogen is synthesized through cooperative actions of glycogen synthase (GS), glycogenin (GN) and glycogen branching enzyme (GBE) (Roach, 2002). GN sits at the core of the glycogen particle (Whelan, 1986) and initiates the process of particle formation by first transferring glucose residues onto itself, leading to an α-1,4 linked chain of 8-12 glucose units (Pitcher et al., 1987). The resulting oligosaccharide, still attached to GN, serves as the primer that is converted into a full-size glycogen particle through the combined action of GS and GBE (Roach, 2002).

Below we provide a brief review of the structural features of GS and GN and how these two enzymes interact to initiate glycogen biogenesis.

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2 Glycogenin is an autoglucosylating dimer

Humans contain two tissue specific GN isoforms. GN1 is expressed ubiquitously but found predominantly in muscle, whereas GN2 is expressed specifically in liver. GN contains a conserved Rossmann fold domain of approximately 250 amino acids at its N-terminus that is required for uridine diphosphate glucose (UDP-G) binding and catalysis. Additionally, GN also contains a highly conserved region of 30–35 residues at its C-terminus that is sufficient and required for binding to GS (Fig. 1A). The N-terminal Rossmann fold domain and the conserved C-terminal region of GN are connected by a linker region that is not conserved in either length or amino acid composition across various species and isoforms (Fig. 1A). This linker varies in length from 2–3 residues in *C. elegans* CeGN-b isoform (Uniprot ID H2KYQ5-2; generated through alternative splicing) to ~250 in *S. cerevisiae* ScGN1 (Uniprot ID P36143 (Fig. 1A)), and its precise function is unknown.

GN (EC 2.4.1.186) also has a glycosyl transferase A (GT-A) fold with glycosyltransferase (GT) activity. GN belongs to the GT-8 family of glycosyl transferases. A Mn^{2+} ion is required in the active site and plays the role of a Lewis acid catalyst that facilitates leaving group departure (Lairson et al., 2008).

The first structural analysis of rabbit muscle GN revealed a tight homo-dimer with approximately 1300 $Å^2$ of buried surface area (Fig. 2A) (Gibbons et al., 2002). Within the GN dimer, the autoglucosylated tyrosine residue is present in a flexible region and is within reach of the active site of both GN protomers (i.e. a glucose chain can be extended in an intersubunit and intrasubunit manner).

Glucose chains of various lengths and number of UDP and Mg^{2+} ions have been solved in GN crystal structures. The structural snapshots of GN catalytic cycles have shown that the first four glucose residues can be added and accommodated by the same catalytic GN protomer in an intrasubunit reaction, but catalysis of longer glucose chains requires the catalytic action of the opposing GN protomer in an intersubunit reaction (Chaikuad et al., 2011).

Interestingly, even in crystals of full length GN, no electron density has been observed for the variable linker region or the conserved C-terminal region that mediates interaction with GS, suggesting that both are disordered in the absence of interacting partners.

3 GS forms a tetramer regulated by G6P binding

GS contains two tandem Rossmann fold domains flanked by Nand C-terminal regulatory regions that contain phosphorylation sites (Fig. 1B). GS has a GT-B fold and belongs to the GT-3 family of glycosyl transferases. Like all eukaryotic GT-3 enzymes, GS is inhibited by protein phosphorylation and activated by binding to glucose 6-phosphate (G6P). Like GN, GS also uses UDP-G as the nucleotidesugar donor and is a retaining enzyme that catalyzes extension of α1,4-linked oligosaccharides. However, unlike GN, GS does not require the presence of divalent metal ions for catalysis.

Despite the importance and intense interest surrounding GS, its structure was not solved until 2010 when Baskaran et al. reported structures of yeast GS in the presence and absence of the allosteric activator G6P (Baskaran et al., 2010). The GT-B fold is conserved between eukaryotic, archaeal and bacterial GS enzymes. However only eukaryotic GS is allosterically regulated by phosphorylation and G6P binding.

Interestingly, GS forms a higher order structure consisting of four protomers. The tetrameric GS structure is responsive to G6P levels, as evidenced by the structural changes seen with respect to the bound vs. non-bound G6P states (Fig. 3). When GS is bound to G6P, the tetramer exists in its relaxed form (R-state; Fig. 3, left panels), resulting in the active site of each GS protomer being more open and possibly poised to better accommodate a substrate or a growing glucose chain. In contrast, the tetramer in the absence of G6P is in the tense state (T-state; Fig. 3, right panels) and this imparts each GS protomer with a less accessible active site in comparison to the G6P-bound R-state (Baskaran et al., 2010; Roach et al., 2012).

4 GS–GN interaction

Since GN provides the initiator glucose chain that GS then extends by addition of other α1,4-linked glucose residues, the question arises how GS and GN come in close proximity for such a reaction to take place. The affinity between GS and GN was previously presumed to be high [with a dissociation constant (K_d) in the nanomolar range] since the two proteins elute as a complex from a size-exclusion chromatography column, regardless of if they were purified from tissues (Pitcher et al., 1987) or through recombinant expression systems (Hunter et al., 2015; Khanna et al., 2013).

A potential explanation for the high affinity complex was that the glucose chain, which was covalently attached to GN, was promoting a tight GS–GN interaction. However, this possibility was ruled out, since a GN Tyr to Phe mutant lacking the glucose acceptor site interacted with comparable affinity with GS (Skurat et al., 2006; Zeqiraj et al., 2014).

The region at the extreme C-terminus of GN was identified as necessary and sufficient for binding to GS. In addition, the sequence within this region is highly conserved across metazoan species (Skurat et al., 2006). The structure of *C. elegans* GS (CeGS) complexed to a C-terminal peptide comprising the last 34 residues of GN (GN^{34}) was recently solved (Zeqiraj et al., 2014). CeGS forms a similar tetrameric higher order structure displayed by yeast GS, and the GN C-terminal peptide binds to the first of the two Rossmann fold domains of each GS protomer (Fig. 4A). The co-crystal structure revealed that the C-terminal GN^{34} motif, while unstructured in the absence of GS, forms a helix-turn-helix motif when bound to GS (Fig. 4). The interaction is mainly hydrophobic in nature with the extreme C-terminal helix (binding helix 2) making the most significant interactions with GN (Zeqiraj et al., 2014). While the entire length of GN^{34} is well conserved across metazoan species, only few of the residues when mutated to alanine caused a severe loss of binding between CeGS and *C. elegans* (CeGN) *in vitro* (Fig. 4B). More importantly, mutation of residues in binding helix 2 of mouse GN also reduced GS–GN interaction and glycogen content in cells (Zeqiraj et al., 2014), indicating that the binding mode is generally

conserved across species. CeGN^{34} interacts with CeGS with a K_d of 2 μM while full length CeGN interacts with a K_d of 0.2 μM. The enhanced binding affinity of the full length protein is attributable in part to an avidity effect arising from the multimeric nature of both GS (tetramer) and GN (dimer). In addition, an interaction between the N-terminal globular domain of GN with GS cannot be excluded as contributing to the binding interaction. However, structural and mutagenesis data have revealed that the ability of GS to initiate normal glycogen particles is dependent on GN^{34}, which acts as a docking motif for the recruitment of GS to the GN initiating particle (Zeqiraj et al., 2014).

The GN dimer can provide two GN^{34} docking motifs and it is possible that in its tetrameric form, GS is capable of extending glucose chains from two GN dimers (equivalent to four GN protomers) simultaneously (Fig. 5).

5 Normal versus aberrant forms of glycogenin

Recently, a pathogenic GN1 mutation was identified in humans with a newly described muscle glycogen storage disease that leads to skeletal muscle myopathy (Malfatti et al., 2014). A nonsense mutation leading to a premature stop codon resulted in the deletion of the majority of the GN^{34} docking motif (Fig. 4B). Interestingly, this particular patient (as well as other patients with GN mutations that resulted in almost complete deficiency of GN1) displayed polyglucosan bodies, which were positive for staining with a periodic acid-Schiff reagent (Malfatti et al., 2014). These polyglucosan bodies appeared to be aberrant forms of glycogen that did not fulfill all normal glycogen functions. These GN mutations suggest that some polyglucosan bodies resembling glycogen can be made in the absence of glycogenin. However, it appears that GN is required for the formation of a normal glycogen particle with the right 3-dimensional (3D) structure that is capable of fulfilling all its functions. It is possible that GN provides a role beyond the priming function of supplying the initiating glucose polymer to GS by providing necessary constrains or “instructions” to GS and GBE to synthesize a normal glycogen particle.

6 Role of GN variable length linker

Achieving normal forms of glycogen with the correct 3D structure is important as evidenced by the examples of patients with muscle myopathies where polyglucosan bodies are formed instead of normal glycogen. In addition, poorly branched, hyper-phosphorylated and mis-folded glycogen can form insoluble particles that accumulate in neurons and eventually lead to neuronal cell death causing Lafora disease (Monaghan and Delanty, 2010). Interestingly, glycogen particles can range in size from 10 to 290 nm, and this variation is most pronounced between muscle and liver tissues. The distribution of larger glycogen particles is greatly diminished when glycogen deposits have been depleted. One cannot help but wonder if GN, the key enzyme in glycogen particle initiation and shape determination, may also be required to regulate glycogen particle size. Indeed, *in vitro* studies of glycogen particles produced from different forms of CeGN consisting of variable lengths of the linker region between the N-terminal globular domain and the C-terminal GN^{34} docking motif showed that GN may be capable of regulating glycogen particle size and distribution (Zeqiraj et al., 2014). The length of the GN “variable length linker” directly correlated with the maximum

size of the glycogen particle synthesized by GS. Longer “variable length linkers” resulted in larger glycogen particles as well as particles with wider size distributions, whereas shorter linkers resulted in smaller glycogen particles and tighter particle size distributions (Zeqiraj et al., 2014). While more work is required to relate this *in vitro* observation to a biologically relevant function, it is tantalizing to speculate that the ability to differentially regulate glycogen particle size may be beneficial for more efficient packing. Bigger particles, which contain more non-reducing glucose residues on the surface and ready for release, may also be more beneficial in cases where glucose is needed rapidly. It is worth noting that GN products with variable length linkers are generated through differential splicing during gene expression.

7 Concluding remarks

Glycogen is widely seen as one of the most important molecules in glucose metabolism, and despite its detailed study there is much more to be learned about glycogen synthesis, its precise spatio-temporal role, and its additional functions as an organelle rather than a glucose depot. Since GN is not the rate-limiting enzyme in the glycogen synthesis reaction, its role has been overlooked in the past decade. It will be interesting to study the quality of the glycogen being made through the actions of GN, GS and GBE and not just the quantity. The fact that different forms of glycogen can be produced and that GN is required in making normal and functional glycogen, it is clear that GN is a fascinating enzyme with additional functions beyond providing the initiating glucose polymer. How the different structures of normal and aberrant forms of glycogen are synthesized is perhaps one of the most interesting questions for the future.

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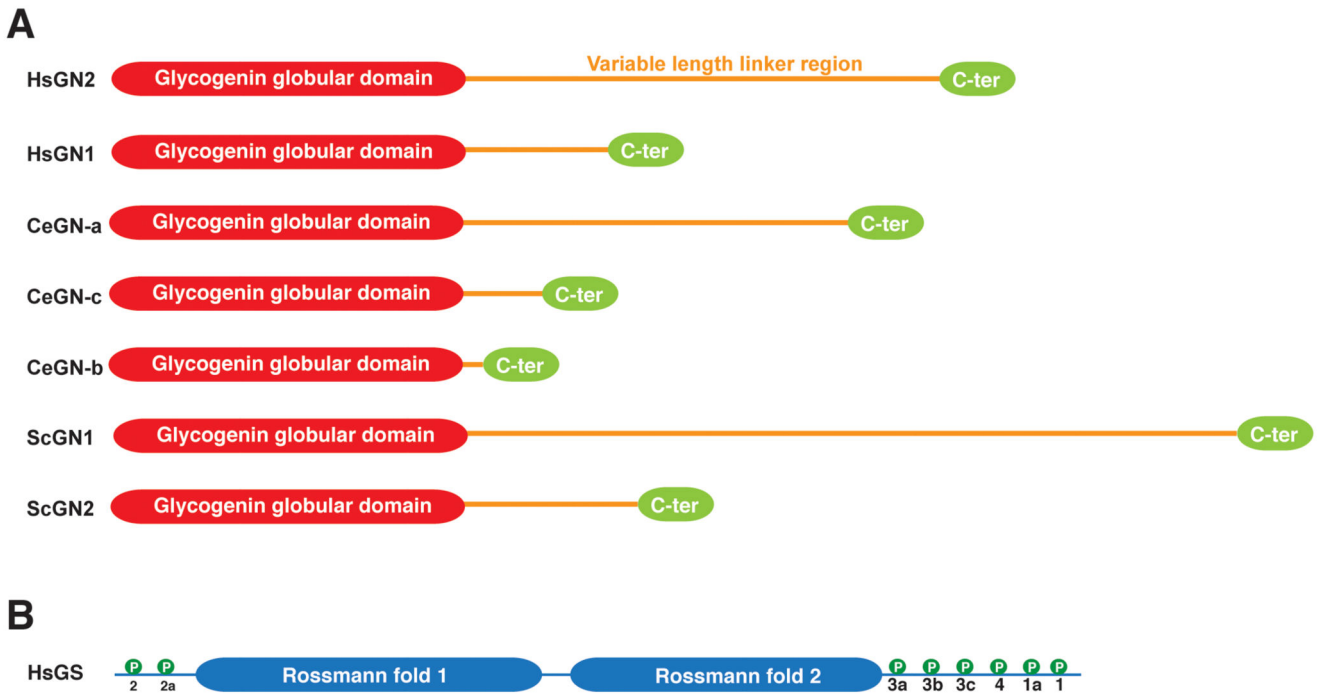

Figure 1. Domain architecture of glycogenin (GN) and glycogen synthase (GS)

A) Domain architecture of GN from different species. In the case of CeGN three different splice variants are shown. Hs = *H. Sapiens*, Ce = *C. elegans*, Sc = *S. cerevisiae*.

B) Domain architecture of HsGS. Sites of regulatory phosphorylation sites (P) are shown in green.

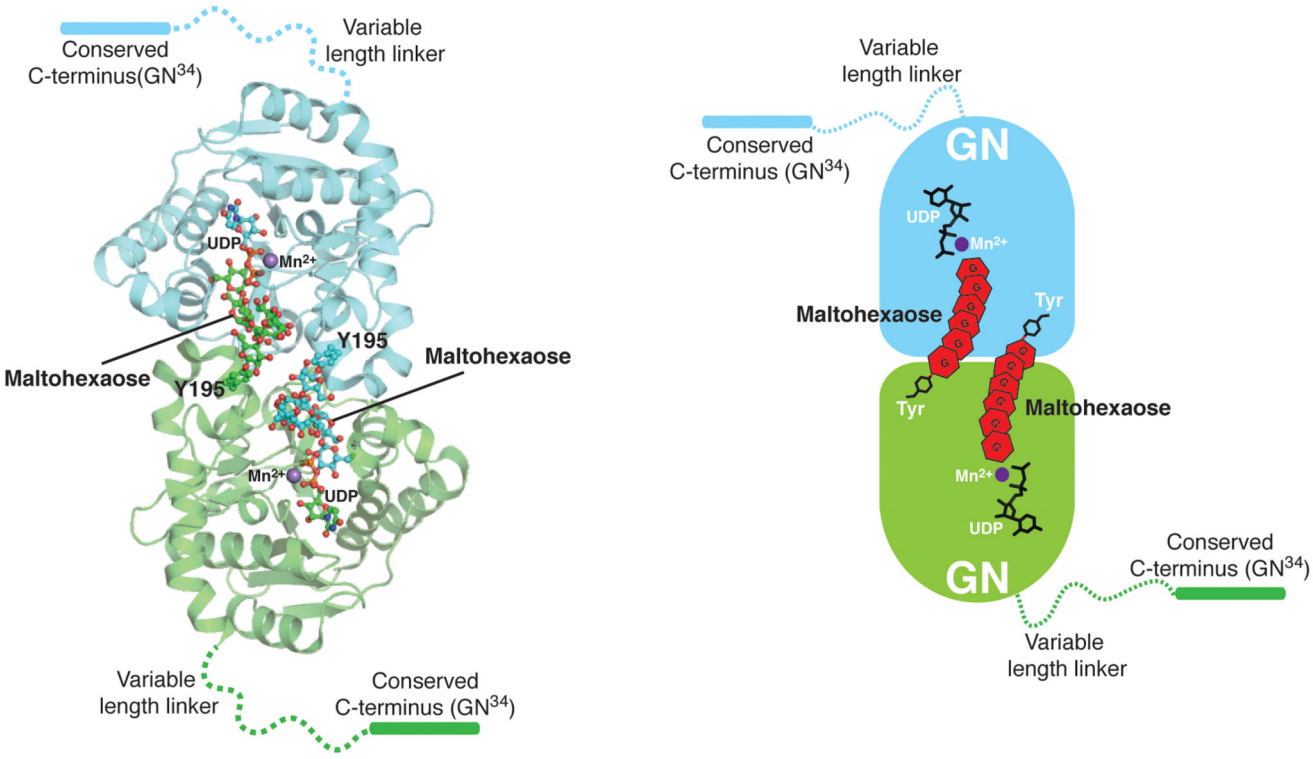

Figure 2. Structure of glycogenin

Left) Ribbons representation of a GN dimer (PDB ID 3U2V). Disordered C-terminal regions not present in the structure are shown as dashed and filled lines. UDP-G, Mn^{2+} and covalently attached sugars are shown as ball and stick models.

Right) Cartoon representation of the left figure (not to scale).

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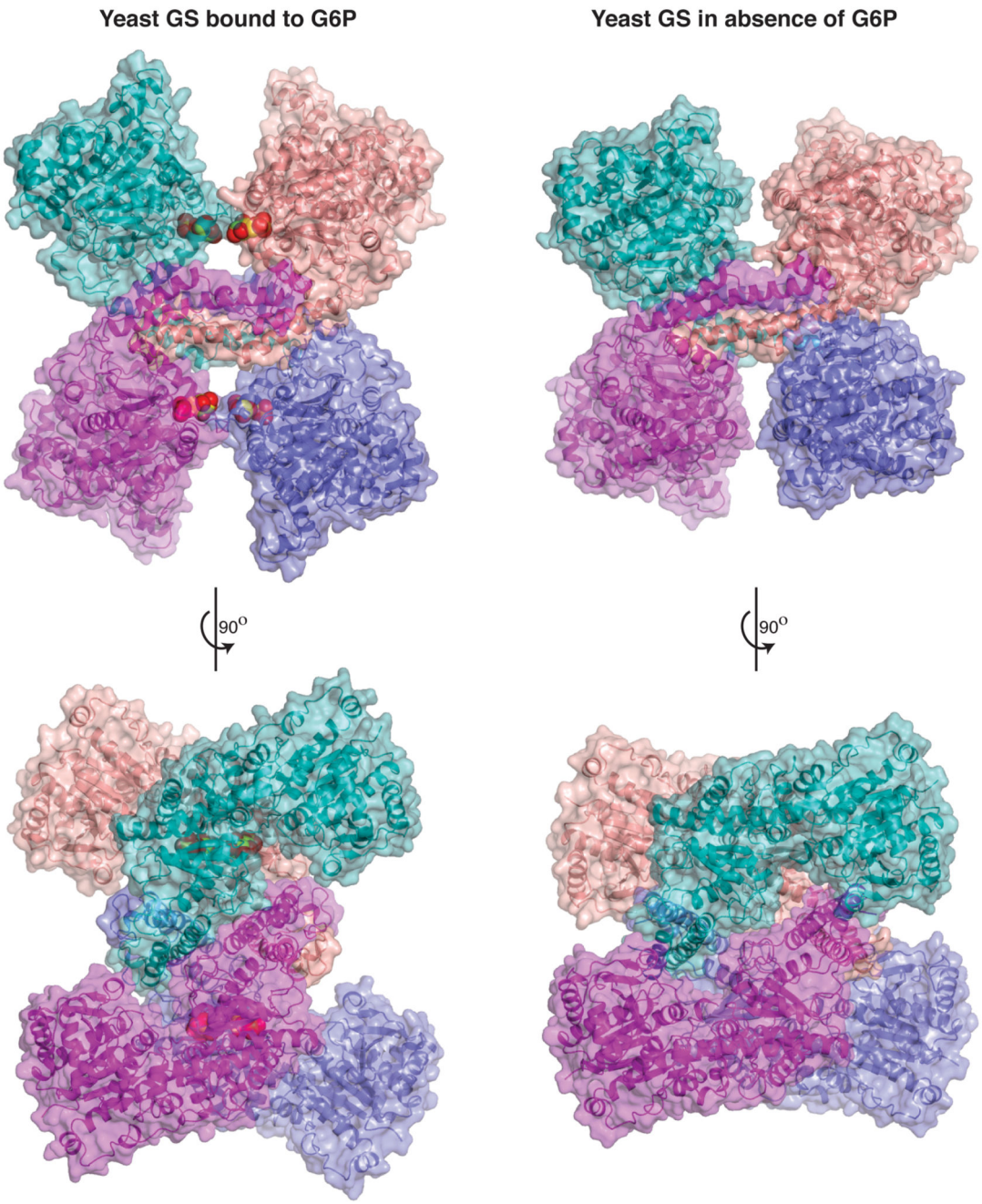

Figure 3. G6P induces a conformational change in the GS tetramer

Left) Ribbons and transparent surface representation of the yeast GS structure bound to the allosteric activator G6P (PDB ID 3NBO). G6P is shown with yellow and red colored atoms. **Right)** Ribbons and transparent surface representation of the yeast GS structure in the absence of the allosteric activator G6P (PDB ID 3NAZ).

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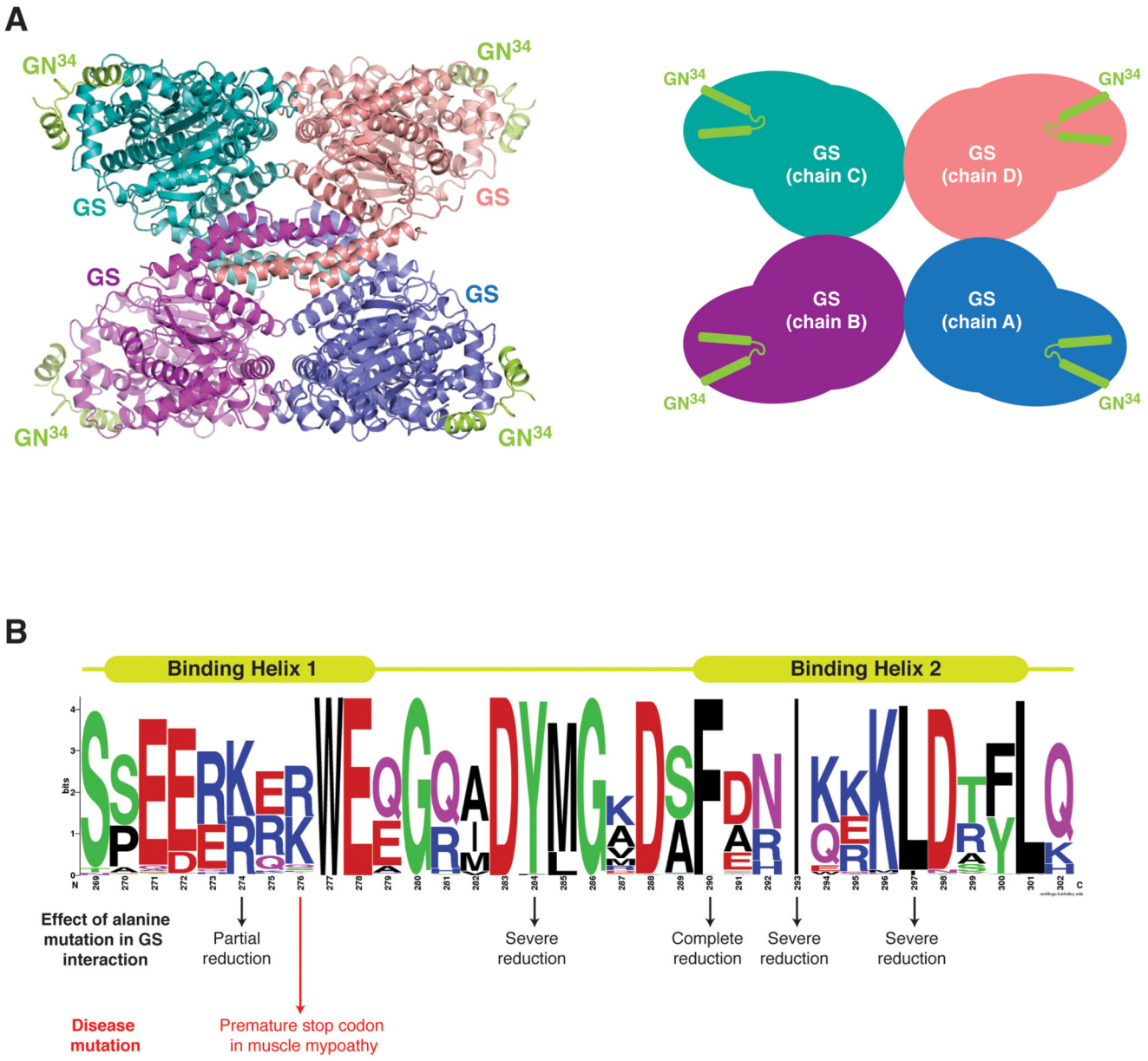

Figure 4. Structure of GS complexed to the GN^{34} docking motif

A) Left - Ribbons representation of *C. elegans* GS (CeGS) complexed to the GN C-terminal peptide GN^{34} (PSB ID = 4QLB). Right - Cartoon representation of the left figure (not to scale).

B) Sequence logo of GN^{34} from 648 GN sequences from metazoan species. Sequences were downloaded from the NCBI Blast database (blast.ncbi.nlm.nih.gov) and aligned using MUSCLE (www.ebi.ac.uk/Tools/msa/muscle/). The sequence logo was generated using the server available at: weblogo.berkeley.edu

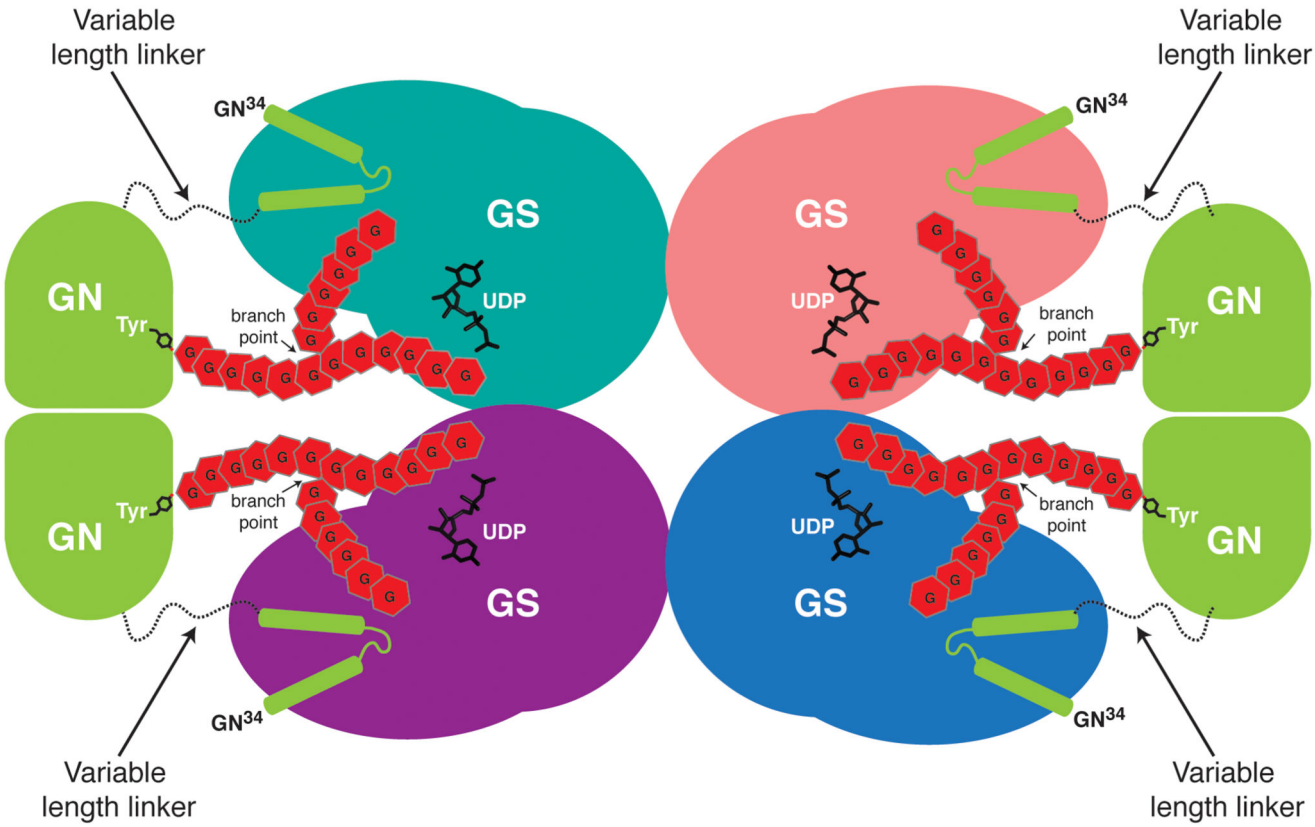

Figure 5. Possible model of how the GS–GN complex initiates glycogen synthesis

Dimeric GN can bind two GS protomers each of which is capable to extend an a1,4-linked glucose chain. Branch-points of α1,6-linked glucose are generated by the glycogen branching enzyme (GBE). Glucose residues are depicted as red hexagons labelled with "G". Not to scale.

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