BCHS 6229
Protein Structure and Function

Lecture 12 (Nov 17, 2011)

Special topics II
(project papers # 2, 4, 6, & 9)

Paper # 2

Aggregation of γ-crystallins associated with human cataracts via domain swapping at the C-terminal β-strands

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Edited by B. J. Berne, Columbia University, New York, NY, and approved May 12, 2011 (received for review December 20, 2010)

The prevalent eye disease age-onset cataract is associated with aggregation of human γD-crystallins, one of the longest-lived proteins. Identification of the γ-crystallin precursors to aggregates is crucial for developing strategies to prevent and reverse cataract. Our microseconds of atomistic molecular dynamics simulations uncover the molecular structure of the experimentally detected aggregation-prone folding intermediate species of monomeric native γD-crystallin with a largely folded C-terminal domain and a mostly unfolded N-terminal domain. About 30 residues including a, b, and c strands from the Greek Key motif 4 of the C-terminal domain experience strong solvent exposure of hydrophobic residues as well as partial unstructuring upon N-terminal domain unfolding. Those strands comprise the domain–domain interface crucial for unusually high stability of γD-crystallin. We further simulate the intermolecular linkage of these monomeric aggregation precursors, which reveals domain-swapped dimeric structures. In the simulated dimeric structures, the N-terminal domain of one monomer is frequently found in contact with residues 135–164 encompassing the a, b, and c strands of the Greek Key motif 4 of the second molecule. The present results suggest that γD-crystallin may polymerize through successive domain swapping of those three C-terminal β-strands leading to age-onset cataract, as an evolutionary cost of its very high stability. Alanine substitutions of the hydrophobic residues in those aggregation-prone β-strands, such as L145 and M147, hinder domain swapping as a pathway toward dimerization. These findings thus provide critical molecular insights onto the initial stages of age-onset cataract, which is important for understanding protein aggregation diseases.

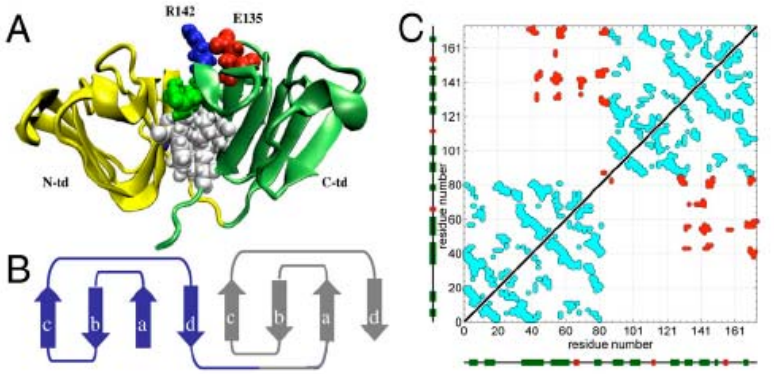

Fig. 1. (*A*) A cartoon representation of human γD-crystallin. The N-terminal domain (N-td) and the C-terminal domain (C-td) are shown in yellow and green, respectively. The heavy side chain of the residues at the interdomain surface is shown in ball-stick representation. The E135–R142 residue pair is also shown that forms a stabilizing salt-bridge interaction, as predicted in earlier simulations. White color is used for nonpolar residues, while polar residues are shown in green. Acidic residues are colored in red and basic residues are colored in blue. (*B*) The complex topology of a crystallin domain consisted of two intercalated antiparallel β-sheet Greek Key motifs. Each motif is colored differently and the naming of the strands is illustrated. (*C*) The residue–residue contact map of the crystal structure of human γD-crystallin. A contact between residue *i* and *j* has been considered if any heavy atom of residue *i* is within 6.5 A of residue *j* in the crystal structure. The intradomain contacts are colored in cyan, whereas the interdomain contacts are colored in red. The secondary elements of the protein are also shown along the axes, with β-strands in green and helices in red.

homologous domains (4). Each domain is composed of interca-

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Progress in Biophysics & Molecular Biology 86 (2004) 407–485

Progress in Biophysics & Molecular Biology

www.elsevier.com/locate/pbiomolbio

Review

Ageing and vision: structure, stability and function of lens crystallins

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Cataract: the most common cause of blindness - of enormous medical (and economical) relevance worldwide.

Many causes:

- mutation in one of the lenticular proteins (usually already present at birth);
- one of the symptoms of systemic disease, for example diabetes is a risk factor for cataract;
- the result of mere ageing.

Lenticular proteins, such as the abundant water-soluble proteins, the crystallins, cannot be replaced and thus have to last the lifetime of the organism.

Crystallins in mammals:

αA, and αB;
βB1, βB2, βB3, βA3/A1, βA2, and βA4;
γA, γB, γC, γD, γE, γF, and γS),

Table 1
Some crystallin parameters

Crystallin	Residues	Size (kDa)	pI	SwissProt	PDB	MIM
Human lens crystallins						
αA	173	19 909	5.6	P02489	—	123 580
αB	175	20 159	6.8	P02511	—	123 590
βB1	251	27 892	8.6	P53674	10ki	600 929
βB2	204	23 249	6.5	P43320	—	123 620
βB3	211	24 230	5.9	P26998	—	123 630
βA1	198	23 191	6.4	P05813	—	123 610
βA2	196	21 964	5.9	P53672	—	600 836
βA3	215	25 150	5.7	P05813	—	123 610
βA4	195	22 243	5.8	P53673	—	123 631
γS	177	20 875	6.4	P22914	1ha4[a]	123 730
γA	173	20 761	7.8	P11844	—	123 660
γB	174	20 776	7.0	P07316	—	123 670
γC	173	20 747	7.0	P07315	—	123 680
γD	173	20 607	7.2	P07320	1hk0	123 690

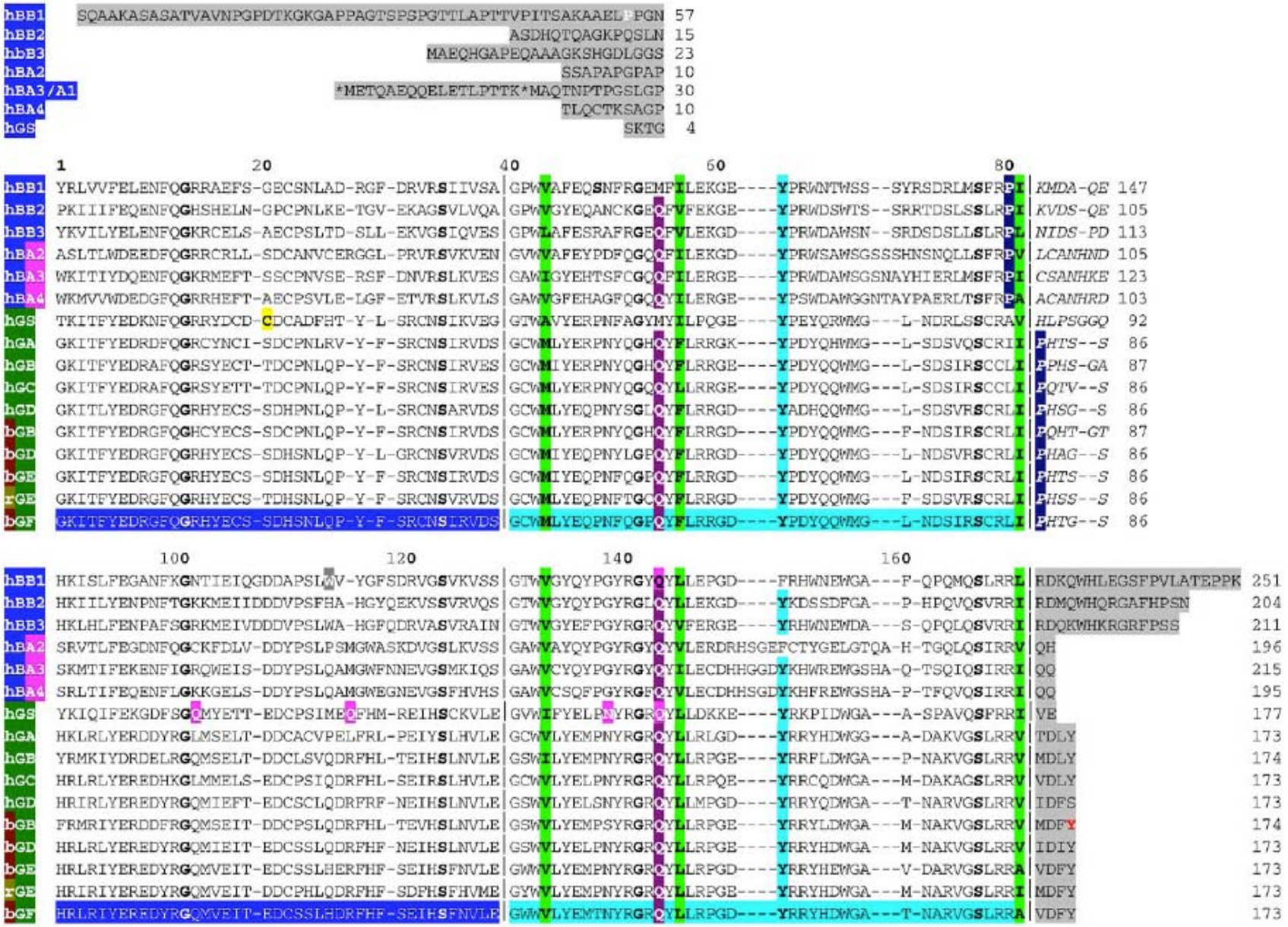
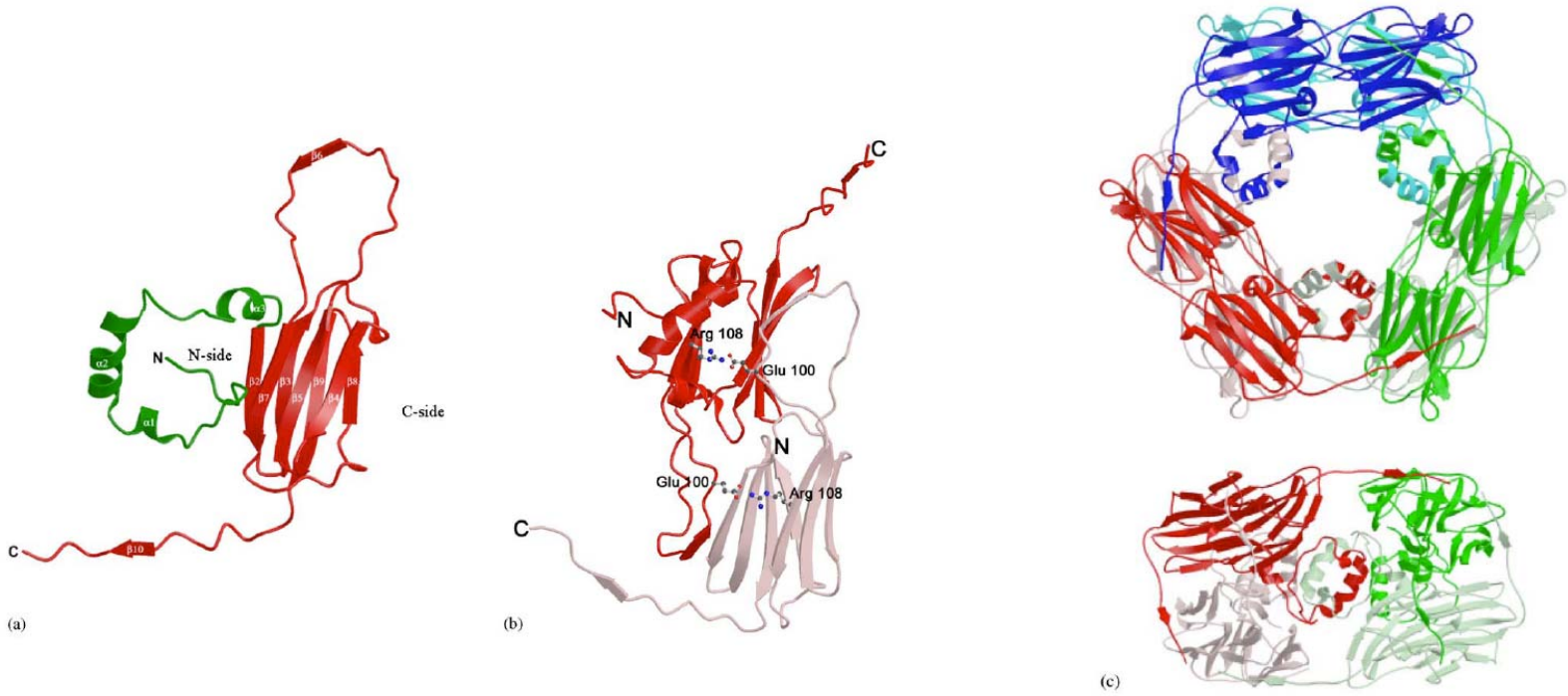


Fig. Sequence alignment of the βγ-crystallin family.

Structure of crystallins and their relatives: two families

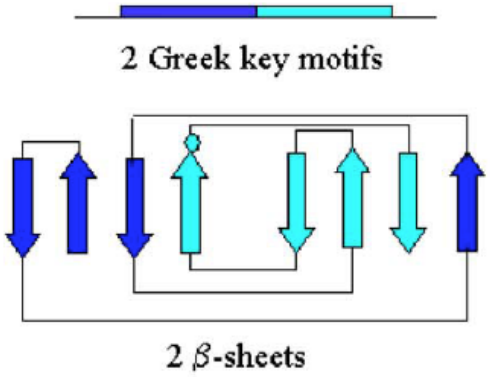
1.The α-crystallins

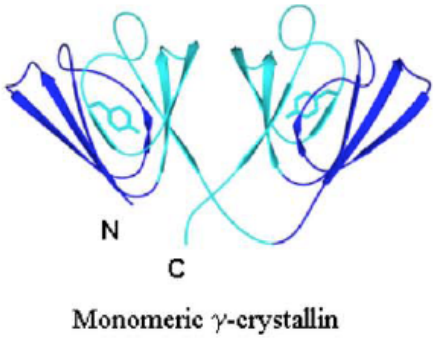


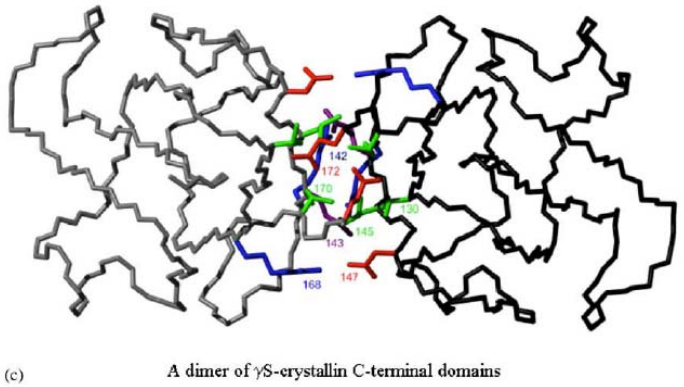
Structure of a plant small heat shock protein assembly

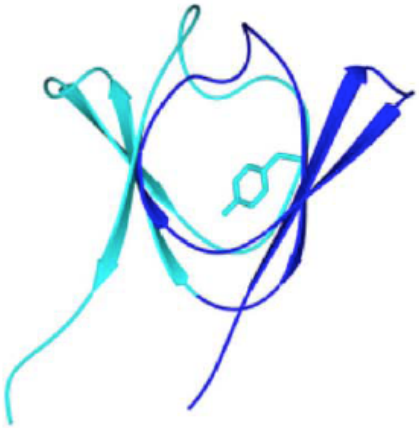
2. Structure of the two-domain β- and γ-crystallins

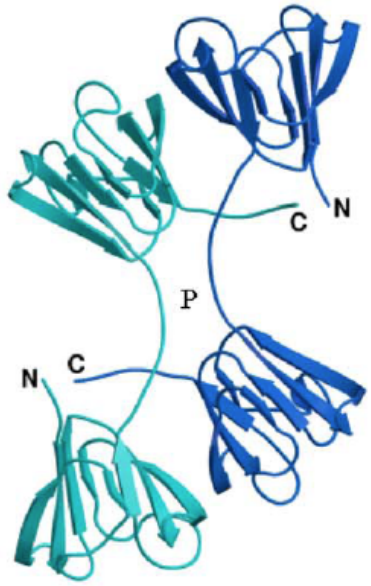
The βγ domain is an independent fold



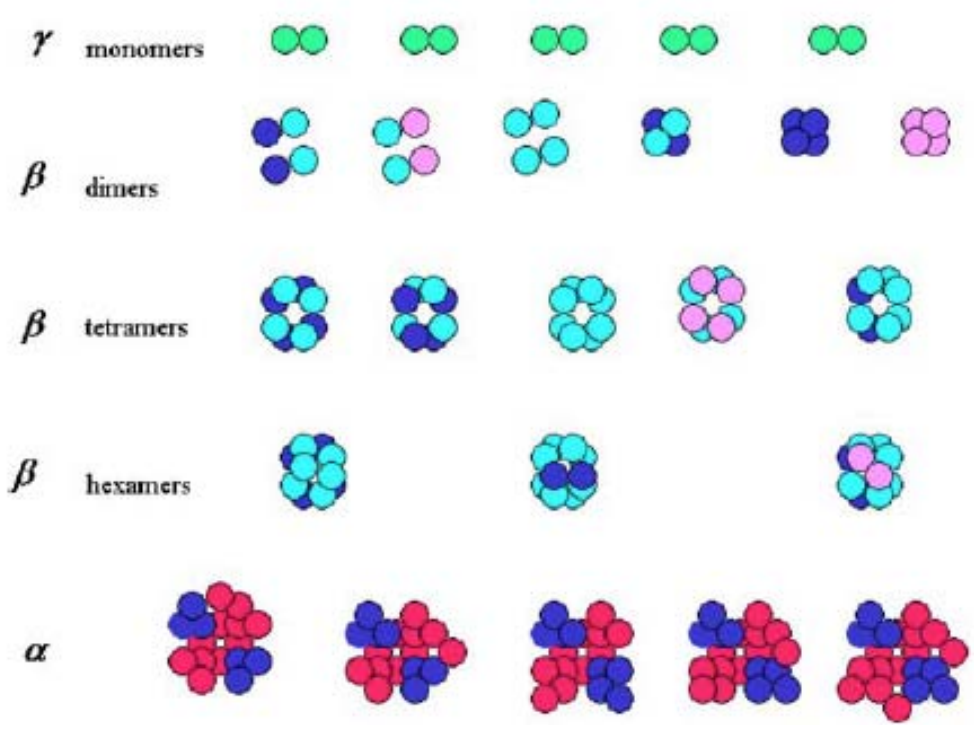








The modular structure of the βγ crystallins. (a) Each βγ-crystallin domain is made from two linear sequence related Greek key motifs that intercalate on folding to form two β-sheets.



Structural polydispersity in the lens crystallins. The lens αβγ-crystallins constitute an array of differently sized proteins. Although the γ-crystallin family members are individually monodisperse, the oligomeric crystallins have the potential of greater polydispersity.

Paper # 4

doi:10.1038/nature09638

Structure of human *O*-GlcNAc transferase and its complex with a peptide substrate

Michael B. Lazarus[1,4]*, Yunsun Nam[2,3]*, Jiaoyang Jiang[4], Piotr Sliz[2,3] & Suzanne Walker[4]

The essential mammalian enzyme O-linked β-*N*-acetylglucosamine transferase (*O*-GlcNAc transferase, here OGT) couples metabolic status to the regulation of a wide variety of cellular signalling pathways by acting as a nutrient sensor[1]. OGT catalyses the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine (UDP-GlcNAc) to serines and threonines of cytoplasmic, nuclear and mitochondrial proteins[2,3], including numerous transcription factors[4], tumour suppressors, kinases[5], phosphatases[1] and histone-modifying proteins[6]. Aberrant glycosylation by OGT has been linked to insulin resistance[7], diabetic complications[8], cancer[9] and neurodegenerative diseases including Alzheimer's[10]. Despite the importance of OGT, the details of how it recognizes and glycosylates its protein substrates are largely unknown. We report here two crystal structures of human OGT, as a binary complex with UDP (2.8 Å resolution) and as a ternary complex with UDP and a peptide substrate (1.95 Å). The structures provide clues to the enzyme mechanism, show how OGT recognizes target peptide sequences, and reveal the fold of the unique domain between the two halves of the catalytic region. This information will accelerate the rational design of biological experiments to investigate OGT's functions; it will also help the design of inhibitors for use as cellular probes and help to assess its potential as a therapeutic target.

Paper # 3

CAZy ~ Carbohydrate-Active enZymes
(http://www.cazy.org).

Glycosyltransferases:Structures, Functions, and Mechanisms

Lairson et al., Annu. Rev. Biochem. 2008. 77:521–55

Sugar nucleotide-dependent GT

GT-A fold: glycosyltransferase protein topology consisting of two closely abutting β/α/β Rossmann domains

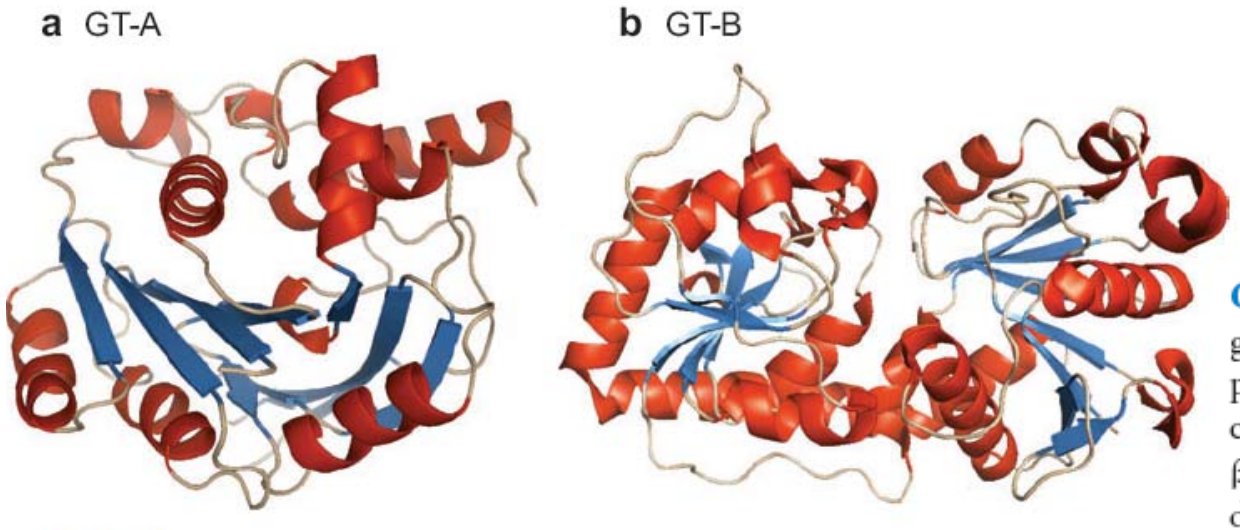
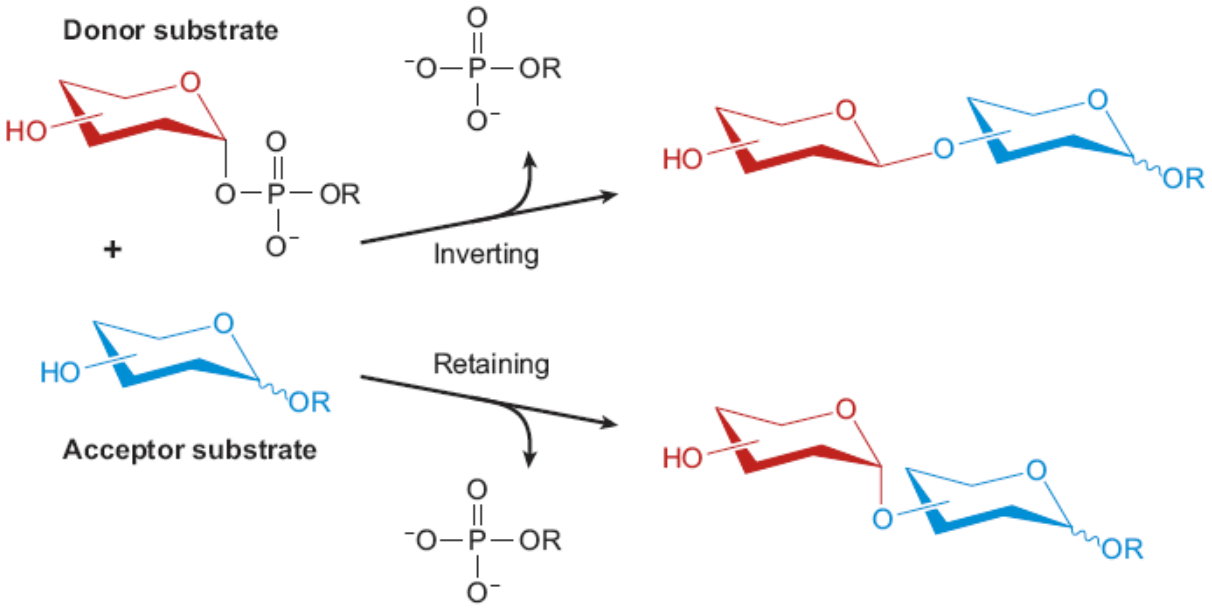


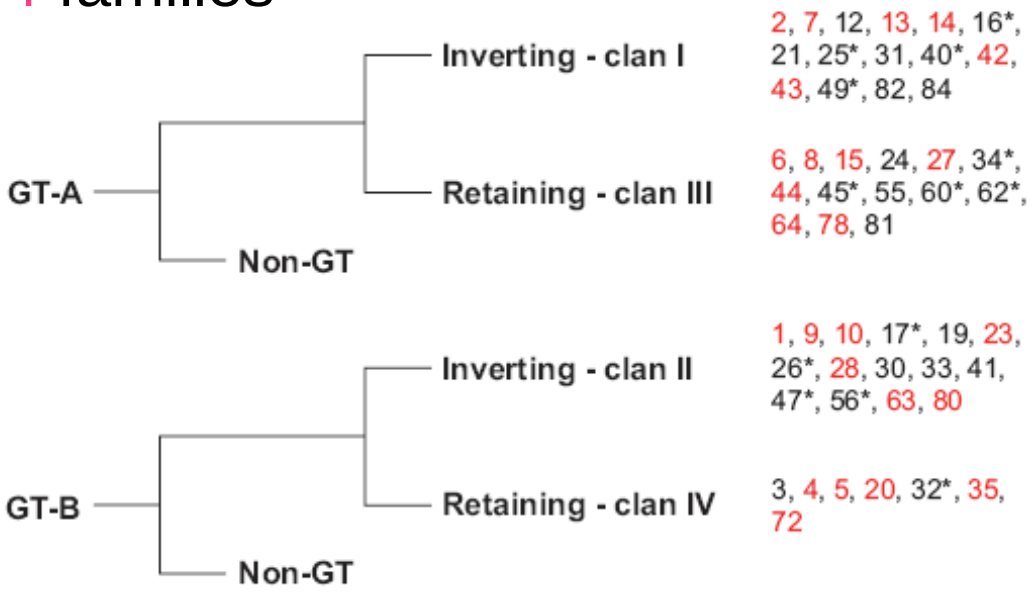
Figure 1

Overall folds observed for glycosyltransferase enzymes. (*a*) The GT-A fold is represented by the inverting enzyme SpsA from *Bacillus subtilus*, Protein Data Bank (pdb) 1qgq, and (*b*) the GT-B fold, by bacteriophage T4 β-glucosyltransferase, pdb 1jg7.

GT-B fold: glycosyltransferase protein topology consisting of two β/α/β Rossmann domains that face each other and are linked flexibly



Currently 94 families



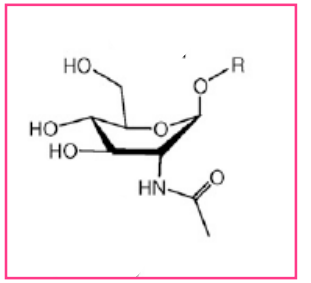
Trends in Biochemical Sciences 35 (2010) 547–555

Review

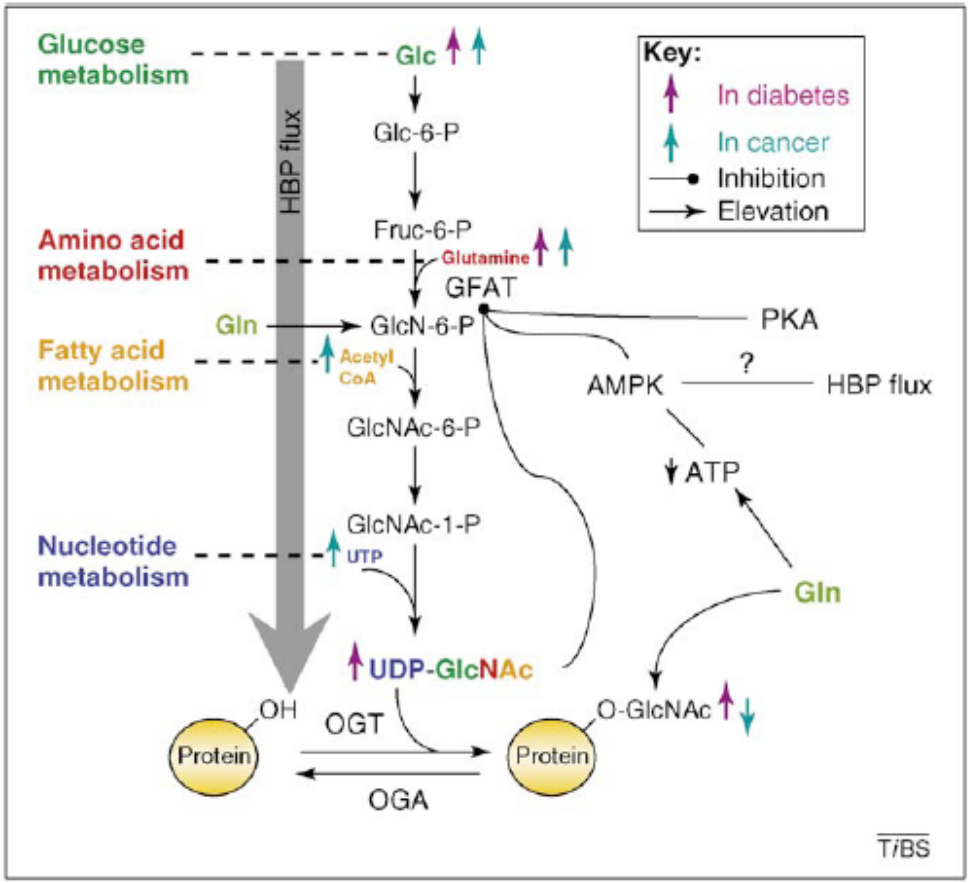
O-GlcNAc signaling: a metabolic link between diabetes and cancer?

C. Slawson, R.J. Copeland and G.W. Hart

The Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 N. Wolfe St. Baltimore, MD 21205, USA



O-linked β-N-acetylglucosamine (O-GlcNAc) is a sugar attachment to serine or threonine hydroxyl moieties on nuclear and cytoplasmic proteins. In many ways, O-GlcNAcylation is similar to phosphorylation because both post-translational modifications cycle rapidly in response to internal or environmental cues. O-GlcNAcylated proteins are involved in transcription, translation, cytoskeletal assembly, signal transduction, and many other cellular functions. O-GlcNAc signaling is intertwined with cellular metabolism; indeed, the donor sugar for O-GlcNAcylation (UDP-GlcNAc) is synthesized from glucose, glutamine, and UTP via the hexosamine biosynthetic pathway. Emerging research indicates that O-GlcNAc signaling and its crosstalk with phosphorylation are altered in metabolic diseases, such as diabetes and cancer.



Paper # 6

Structural basis of photosensitivity in a bacterial light-oxygen-voltage/helix-turn-helix (LOV-HTH) DNA-binding protein

Abigail I. Nash[a,1], Reginald McNulty[b,1], Mary Elizabeth Shillito[b], Trevor E. Swartz[c,2], Roberto A. Bogomolni[c], Hartmut Luecke[b,3], and Kevin H. Gardner[a,3]

[a]Departments of Biochemistry and Pharmacology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390–8816; [b]Center for Biomembrane Systems, Department of Molecular Biology and Biochemistry, Department of Physiology and Biophysics, Department of Information and Computer Science, University of California, Irvine, CA 92697–3900; and [c]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

Edited by J. Clark Lagarias, University of California, Davis, CA, and approved April 22, 2011 (received for review January 11, 2011)

Light-oxygen-voltage (LOV) domains are blue light-activated signaling modules integral to a wide range of photosensory proteins. Upon illumination, LOV domains form internal protein-flavin adducts that generate conformational changes which control effector function. Here we advance our understanding of LOV regulation with structural, biophysical, and biochemical studies of EL222, a light-regulated DNA-binding protein. The dark-state crystal structure reveals interactions between the EL222 LOV and helix-turn-helix domains that we show inhibit DNA binding. Solution biophysical data indicate that illumination breaks these interactions, freeing the LOV and helix-turn-helix domains of each other. This conformational change has a key functional effect, allowing EL222 to bind DNA in a light-dependent manner. Our data reveal a conserved signaling mechanism among diverse LOV-containing proteins, where light-induced conformational changes trigger activation via a conserved interaction surface.

allosteric regulation | photosensing | PER-ARNT-SIM domain

α-helix, β-strand extension of its LOV domain upon illumination (18). In both cases, the external structures interact with the β-sheet surface of the LOV domain, suggesting a site for signal propagation common between them. The functional importance of regulated interactions at this site have been validated by the ability of point mutations on the β-sheet or interacting effector surfaces to decouple changes in effector activity from adduct formation (18, 19).

Among the known LOV-containing proteins are several transcription factors, such as the zinc-finger containing *N. crassa* white collar-1 (WC-1) (20) and the algal basic leucine zipper AUREOCHROMEs (21). Although light controls the binding of these proteins to DNA, the mechanism(s) of this regulation is not understood at a molecular level. Here we address this shortcoming by examining how a LOV domain directly regulates DNA binding, establishing the generality of LOV signaling. Our studies focus on EL222, a 222 amino acid protein isolated from the marine bacterium *Erythrobacter litoralis* HTCC2594. In addition to

Trends in Microbiology September 2011, Vol. 19, No. 9

Review

Cell PRESS

Light helps bacteria make important lifestyle decisions

Mark Gomelsky[1] and Wouter D. Hoff[2]

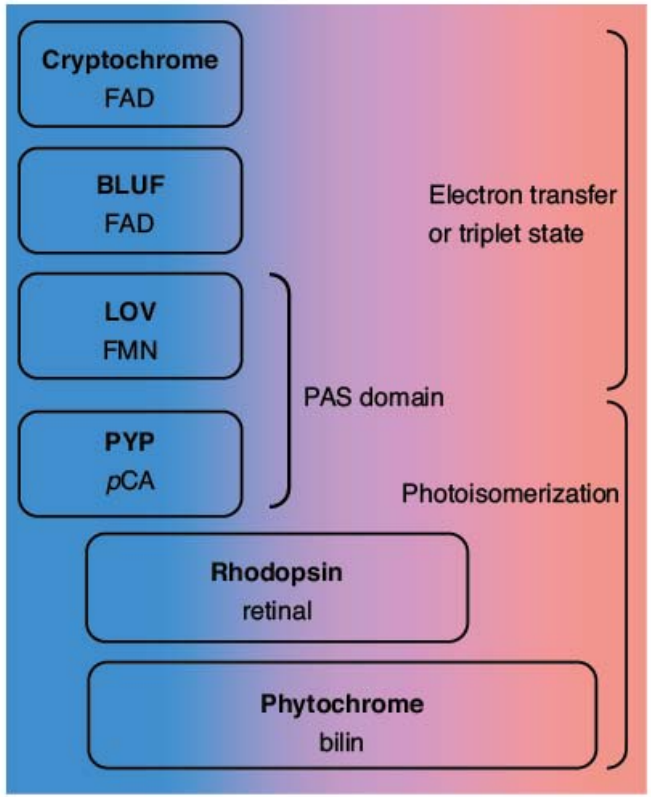


Figure I. The six types of photoreceptors.

Photoreceptor protein families.

Six distinct types of photoreceptors (chromophore). Photoactivation triggers a characteristic series of events unique to each of the six photosensory protein types, and can be based on initial photochemical events involving C=C double bond photoisomerization, electron transfer or the formation of a chemically reactive triplet state. Light induces conformational changes in the photoreceptor domain that are transduced to a downstream output domain, which can either be fused to the photoreceptor in the same protein, or be represented by a separate protein.

Paper # 9

Structural Basis of Cell Wall Cleavage by a Staphylococcal Autolysin

Sebastian Zoll[1], Bernhard Pätzold[1☯], Martin Schlag[2☯], Friedrich Götz[2], Hubert Kalbacher[1,3], Thilo Stehle[1,4*]

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Abstract

The major autolysins (Atl) of *Staphylococcus epidermidis* and *S. aureus* play an important role in cell separation, and their mutants are also attenuated in virulence. Therefore, autolysins represent a promising target for the development of new types of antibiotics. Here, we report the high-resolution structure of the catalytically active amidase domain AmiE (amidase *S. epidermidis*) from the major autolysin of *S. epidermidis*. This is the first protein structure with an amidase-like fold from a bacterium with a gram-positive cell wall architecture. AmiE adopts a globular fold, with several α-helices surrounding a central β-sheet. Sequence comparison reveals a cluster of conserved amino acids that define a putative binding site with a buried zinc ion. Mutations of key residues in the putative active site result in loss of activity, enabling us to propose a catalytic mechanism. We also identified and synthesized muramyltripeptide, the minimal peptidoglycan fragment that can be used as a substrate by the enzyme. Molecular docking and digestion assays with muramyltripeptide derivatives allow us to identify key determinants of ligand binding. This results in a plausible model of interaction of this ligand not only for AmiE, but also for other PGN-hydrolases that share the same fold. As AmiE active-site mutations also show a severe growth defect, our findings provide an excellent platform for the design of specific inhibitors that target staphylococcal cell separation and can thereby prevent growth of this pathogen.

peptidoglycan hydrolase AmiE