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Review

Molecular insights into the WW domain of the Golabi-Ito-Hall syndrome protein PQBP1

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ABSTRACT

The WW domain-containing PQBP1 (polyglutamine tract-binding protein 1) protein regulates mRNA processing and gene transcription. Mutations in the PQBP1 gene were reported in several X chromosome-linked intellectual disability (XLID) disorders, including Golabi-Ito-Hall (GIH) syndrome. The missense mutation in the GIH syndrome maps within a functional region of the PQBP1 protein known as the WW domain. The causative mutation of PQBP1 replaces the conserved tyrosine (Y) at position 65 within the aromatic core of the WW domain to cysteine (C), which is a chemically significant change. In this short review, we analyze structural models of the Y65C mutated and wild type WW domains of PQBP1 in order to infer potential molecular mechanisms that render the mutated PQBP1 protein inactive in terms of ligand binding and its function as a regulator of mRNA splicing.

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1. Introduction

The WW domain-containing PQBP1 (polyglutamine tract-binding protein 1) gene encodes a protein that regulates mRNA processing and RNA polymerase II-driven transcription [1–5]. The PQBP1 gene is highly conserved in evolution and its orthologs in plants, worms and mammals share a high degree of sequence similarity. In mammals, the PQBP1 gene is widely expressed, but its transcript is enriched in the brain [6]. Originally, the PQBP1 protein was identified as a protein partner of poly-glutamine-(poly-Q)-containing proteins, including huntingtin, ataxin-1 and neuronal transcription factor Brn-2. Mutations in the PQBP1 gene were reported in several X chromosome-linked intellectual disability (XLID) disorders, such as Renpenning, Sutherland-Haan, Hamel, Porteous, and Golabi-Ito-Hall (GIH) syndromes [7,8]. Interestingly, although caused by different mutations that affect the coding sequence of the PQBP1 gene – frame shifts that result in truncated protein products or a missense point mutation – these syndromes share similar clinical features [7]. In addition to severe intellectual disability, the patients also have a lean body, short stature, small head, and are frequently diagnosed with cardiac abnormalities [9,10]. The missense mutation in the GIH syndrome maps within a functional region of the PQBP1 protein known as the WW domain (Fig. 1) [8,11–13]. In contrast to other mutations reported for PQBP1, the genetic lesion of GIH syndrome does not affect the size of the mutated protein, which normally migrates on SDS–PAGE as a singlet of 38 kDa [14].

The WW domain is one of the smallest protein modules mediating specific protein–protein interactions with short proline-rich or proline-containing motifs [11–13]. The WW domain mutation implicated in the GIH syndrome replaces the conserved tyrosine (Y) at position 65 within the aromatic core of the domain to cysteine (C), which is a chemically significant change to the protein that we would like to discuss here in detail [14].

Recently, we have documented that the binding of the Y65C mutant of PQBP1 protein to its cognate ligand, WBP11 (also known as SIPP1), which regulates mRNA processing, was abrogated compared to that of the control wild-type protein [14]. More importantly, we have verified that in lymphoblasts derived from a GIH patient, the complex between the endogenous PQBP1 and endogenous WBP11 proteins was compromised compared with the intact complex between these two proteins found in lymphoblasts derived from a normal individual who was matched for race and

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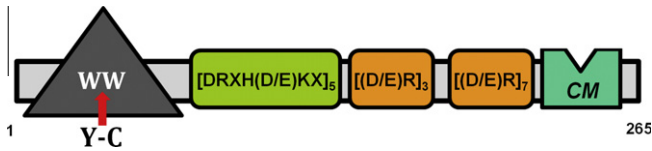E-mail address: msudol1@geisinger.edu (M. Sudol).

Fig. 1. Schematic of the organization of the PQBP1 protein. PQBP1 contains only one discernable modular domain, the WW domain located at the N-(amino)-terminus. The causative Y-C PQBP1 mutation of the GIH syndrome is located within the middle of the WW domain sequence and it is indicated with a red arrow. There are multiple copies of three distinct amino acid repeats found in PQBP1: five consecutive copies of DRXH(D/E)KX and ten copies of (D/E)R, which occur consecutively in repeats of three and seven separated by a small stretch of intervening amino acids. At the C-(carboxy)-terminal region there is a stretch of highly conserved amino acids – GPLFQQRPYPSPG –, which are identical among PQBP1 proteins of man (*Homo sapiens*), a sweet water polyp (*Hydra magnipapilata*) and a small flowering plant (*Arabidopsis thaliana*). We indicate the relative location of this sequence as a green box with *CM*, after "conserved motif". An indentation on the box is to suggest a possibility that the *CM* motif interacts with the WW domain, shown as a gray triangle.

sex with the GIH patient [14]. Using a cell culture model and a reporter gene construct that monitors efficiency of mRNA splicing, we have shown that the missense mutation in the PQBP1 WW domain resulted in a substantial decrease in pre-mRNA splicing [14].

2. Y65 of the WW domain of PQBP1 is conserved among the family of WW domains

In an effort to understand the importance of tyrosine at position 65 (Y65) in the function of PQBP1, we performed amino acid sequence alignment of the entire human family of WW domains (Fig. 2). Our analysis reveals that Y65 is predominantly conserved across all WW domains and that it is frequently substituted by its chemical analogue phenylalanine, further implicating its central role in the function of WW domains. In addition to Y65, a number of other residues in the WW domain of PQBP1 are also predominantly conserved across the entire human family of WW domains.
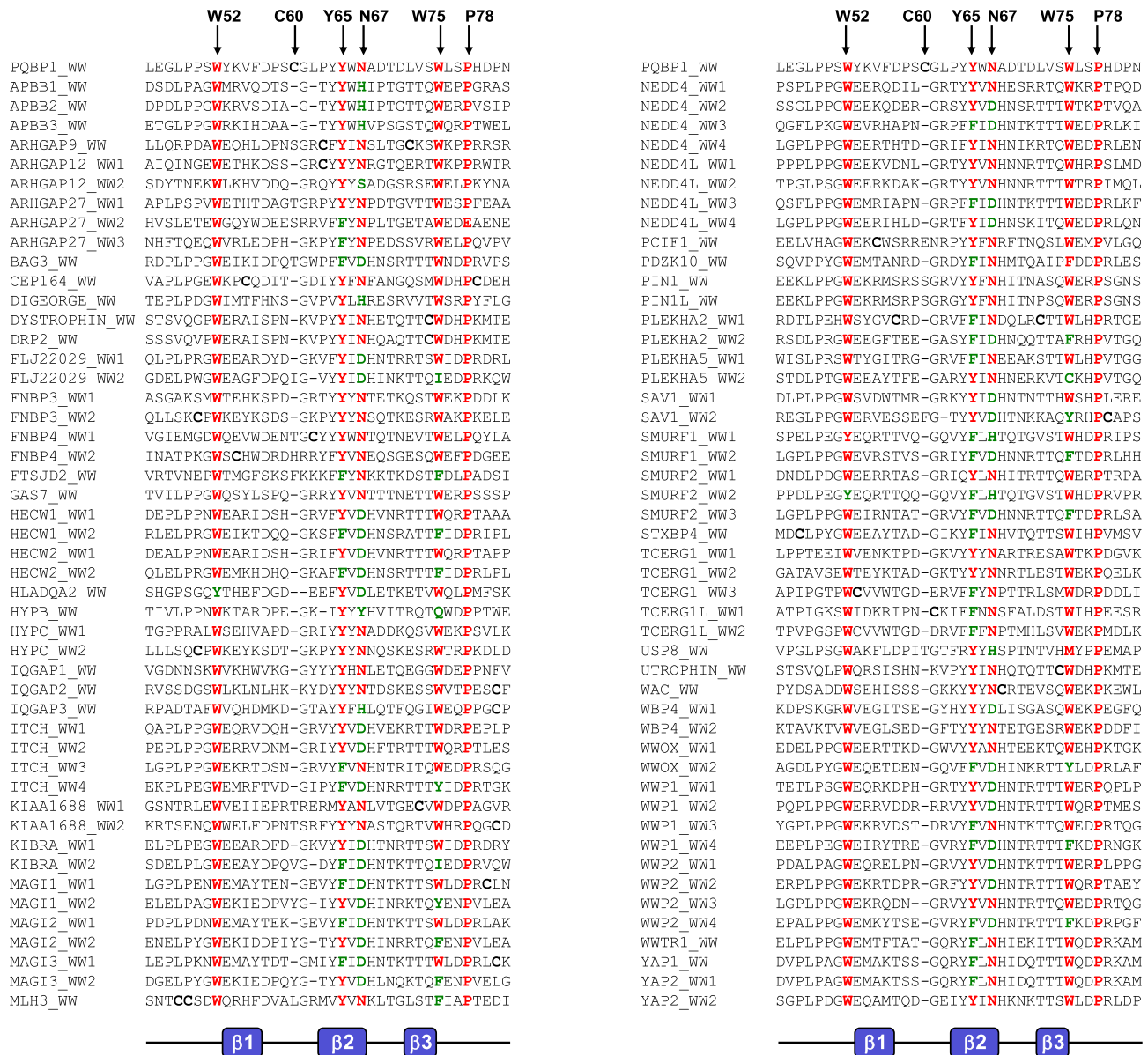


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                      W52      C60   Y65 N67  W75   P78
PQBP1_WW        LEGLPPSWYKVFDPSCGLPYYWNADTDLVSWLSPHDPN
APBB1_WW        DSDLPAGWMRVQDTS-G-TYYWHIPTGTTQWEPPGRAS
APBB2_WW        DPDLPPGWKRVSDIA-G-TYYWHIPTGTTQWERPVSIP
APBB3_WW        ETGLPPGWRKIHDAA-G-TYYWHVPSGSTQWQRPTWEL
ARHGAP9_WW      LLQRPDAWEQHLDPNSGRCFYINSLTGCKSWKPPRRSR
ARHGAP12_WW1    AIQINGEWETHKDSS-GRCYYNRGTQERTWKPPRWTR
ARHGAP12_WW2    SDYTNEKWLKHVDDQ-GRQYYSADGSRSEWELPKYNA
ARHGAP27_WW1    APLPSPVWETHTDAGTGRPYYYNPDTGVTTWESPFEAA
ARHGAP27_WW2    HVSLETEWGQYWDEESRRVFFYNPLTGETAWEDEAENE
ARHGAP27_WW3    NHFTQEQWVRLEDPH-GKPYFYNPEDSSVRWELPQVPV
BAG3_WW         RDPLPPGWEIKIDPQTGWPFFVDHNSRTTTWNDPRVPS
CEP164_WW       VAPLPGEWKPCQDIT-GDIYYFNFANGQSMWDHPCDEH
DIGEORGE_WW     TEPLPDGWIMTFHNS-GVPVYLHRESRVVTWSRPYFLG
DYSTROPHIN_WW   STSVQGPWERAISPN-KVPYYINHETQTTCWDHPKMTE
DRP2_WW         SSSVQVPWERAISPN-KVPYYINHQAQTTCWDHPKMTE
FLJ22029_WW1    QLPLPRGWEEARDYD-GKVFYIDHNTRRTSWIDPRDRL
FLJ22029_WW2    GDELPWGWEAGFDPQIG-VYYIDHINKTTQIEDPRKQW
FNBP3_WW1       ASGAKSMWTEHKSPD-GRTYYYNTETKQSTWEKPDDLK
FNBP3_WW2       QLLSKCPWKEYKSDS-GKPYYYNSQTKESRWAKPKELE
FNBP4_WW1       VGIEMGDWQEVWDENTGCYYYWNTQTNEVTWELPQYLA
FNBP4_WW2       INATPKGWSCHWDRDHRRYFVNEQSGESQWEFPDGEE
FTSJD2_WW       VRTVNEPWTMGFSKSFKKKFFYNKKTKDSTFDLPADSI
GAS7_WW         TVILPPGWQSYLSPQ-GRRYYVNTTTNETTWERPSSSP
HECW1_WW1       DEPLPPNWEARIDSH-GRVFYVDHVNRTTTWQRPTAAA
HECW1_WW2       RLELPRGWEIKTDQQ-GKSFFVDHNSRATTFIDPRIPL
HECW2_WW1       DEALPPNWEARIDSH-GRIFYVDHVNRTTTWQRPTAPP
HECW2_WW2       QLELPRGWEMKHDHQ-GKAFFVDHNSRTTTFIDPRLPL
HLADQA2_WW      SHGPSGQYTHEFDGD--EEFYVDLETKETVWQLPMFSK
HYPB_WW         TIVLPPNWKTARDPE-GK-IYYYHVITRQTQWDPPTWE
HYPC_WW1        TGPPRALWSEHVAPD-GRIYYYNADDKQSVWEKPSVLK
HYPC_WW2        LLLSQCPWKEYKSDT-GKPYYYNNQSKESRWRTRPKDLD
IQGAP1_WW       VGDNNSKWVKHWVKG-GYYYYHNLETQEGGWDEPPNFV
IQGAP2_WW       RVSSDGSWLKLNLHK-KYDYYYNTDSKESSWVTPESCF
IQGAP3_WW       RPADTAFWVQHDMKD-GTAYYFHLQTFQGIWEQPPGCP
ITCH_WW1        QAPLPPGWEQRVDQH-GRVYYVDHVEKRTTWDRPEPLP
ITCH_WW2        PEPLPPGWERRVDNM-GRIYYVDHFTRTTTWQRPTLES
ITCH_WW3        LGPLPPGWEKRTDSN-GRVYFVNHNTRITQWEDPRSQG
ITCH_WW4        EKPLPEGWEMRFTVD-GIPYFVDHNRRTTTYIDPRTGK
KIAA1688_WW1    GSNTRLEWVEIIEPRTRERMYANLVTGECVWDPPAGVR
KIAA1688_WW2    KRTSENQWWELFDPNTSRFYYYNASTQRTVWHRPQGCD
KIBRA_WW1       ELPLPEGWEEARDFD-GKVYYIDHNTRTTSWIDPRDRY
KIBRA_WW2       SDELPLGWEEAYDPQVG-DYFIDHNTKTTQIEDPRVQW
MAGI1_WW1       LGPLPENWEMAYTEN-GEVYFIDHNTKTTSWLDPRCLN
MAGI1_WW2       ELELPAGWEKIEDPVYG-IYYVDHINRKTQYENPVLEA
MAGI2_WW1       PDPLPDNWEMAYTEK-GEVYFIDHNTKTTSWLDPRLAK
MAGI2_WW2       ENELPYGWEKIDDPIYG-TYYVDHINRRTQFENPVLEA
MAGI3_WW1       LEPLPKNWEMAYTDT-GMIYFIDHNTKTTTWLDPRLCK
MAGI3_WW2       DGELPYGWEKIEDPQYG-TYYVDHLNQKTQFENPVELG
MLH3_WW         SNTCCSDWQRHFDVALGRMVYVNKLTGLSTFIAPTEDI
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                      W52      C60   Y65 N67  W75   P78
PQBP1_WW        LEGLPPSWYKVFDPSCGLPYYWNADTDLVSWLSPHDPN
NEDD4_WW1       PSPLPPGWEERQDIL-GRTYYVNHESRRTQWKRPTPQD
NEDD4_WW2       SSGLPPGWEEKQDER-GRSYYVDHNSRTTTWTKPTVQA
NEDD4_WW3       QGFLPKGWEVRHAPN-GRPFFIDHNTKTTTWEDPRLKI
NEDD4_WW4       LGPLPPGWEERTHTD-GRIFYINHNIKRTQWEDPRLEN
NEDD4L_WW1      PPPLPPGWEEKVDNL-GRTYYVNHNNRTTQWHRPSLMD
NEDD4L_WW2      TPGLPSGWEERKDAK-GRTYYVNHNNRTTTWTRPIMQL
NEDD4L_WW3      QSFLPPGWEMRIAPN-GRPFFIDHNTKTTTWEDPRLKF
NEDD4L_WW4      LGPLPPGWEERIHLD-GRTFYIDHNSKITQWEDPRLQN
PCIF1_WW        EELVHAGWEKCWSRRENRPYYFNRFTNQSLWEMPVLGQ
PDZK10_WW       SQVPPYGWEMTANRD-GRDYFINHMTQAIPFDDPRLES
PIN1_WW         EEKLPPGWEKRMSRSSGRVYYFNHITNASQWERPSGNS
PIN1L_WW        EEKLPSGWEKRMSRPSGRGYYFNHITNPSQWERPSGNS
PLEKHA2_WW1     RDTLPEHWSYGVCRD-GRVFFINDQLRCTTWLHPRTGE
PLEKHA2_WW2     RSDLPRGWEEGFTEE-GASYFIDHNQQTTAFRHPVTGQ
PLEKHA5_WW1     WISLPRSWTYGITRG-GRVFFINEEAKSTTWLHPVTGG
PLEKHA5_WW2     STDLPTGWEEAYTFE-GARYYINHNERKVTCKHPVTGQ
SAV1_WW1        DLPLPPGWSVDWTMR-GRKYYIDHNTNTTHWSHPLERE
SAV1_WW2        REGLPPGWERVESSEFG-TYYVDHTNKKAQYRHPCAPS
SMURF1_WW1      SPELPEGYEQRTTVQ-GQVYFLHTQTGVSTWHDPRIPS
SMURF1_WW2      LGPLPPGWEVRSTVS-GRIYFVDHNNRTTQFTDPRLHH
SMURF2_WW1      DNDLPDGWEERRTAS-GRIQYLNHITRTTQWERPTRPA
SMURF2_WW2      PPDLPEGYEQRTTQQ-GQVYFLHTQTGVSTWHDPRVPR
SMURF2_WW3      LGPLPPGWEIRNTAT-GRVYFVDHNNRTTQFTDPRLSA
STXBP4_WW       MDCLPYGWEEAYTAD-GIKYFINHVTQTTSWIHPVMSV
TCERG1_WW1      LPPTEEIWVENKTPD-GKVYYYNARTRESAWTKPDAPP
TCERG1_WW2      GATAVSEWTEYKTAD-GKTYYYNNRTLESTWEKPQELK
TCERG1_WW3      APIPGTPWCVVWTGD-ERVFFYNPTTRLSMWDRPDDLI
TCERG1L_WW1     ATPIGKSWIDKRIPN-CKIFFNNSFALDSTWIHPEESR
TCERG1L_WW2     TPVPGSPWCVVWTGD-DRVFFFNPTMHLSVWEKPMDLK
USP8_WW         VPGLPSGWAKFLDPITGTFRYYHSPTNTVHMYPPEMAP
UTROPHIN_WW     STSVQLPWQRSISHN-KVPYYINHQTQTTCWDHPKMTE
WAC_WW          PYDSADDWSEHISSS-GKKYYYNCRTEVSQWEKPKEWL
WBP4_WW1        KDPSKGRWVEGITSE-GYHYYYDLISGASQWEKPEGFQ
WBP4_WW2        KTAVKTVWVEGLSED-GFTYYYNTETGESRWEKPDDFI
WWOX_WW1        EDELPPGWEERTTKD-GWVYYANHTEEKTQWEHPKTGK
WWOX_WW2        AGDLPYGWEQETDEN-GQVFFVDHINKRTTYLDPRLAF
WWP1_WW1        TETLPSGWEQRKDPH-GRTYYVDHNTRTTTWERPQPLP
WWP1_WW2        PQPLPPGWERRVDDR-RRVYYVDHNTRTTTWQRPTMES
WWP1_WW3        YGPLPPGWEKRVDST-DRVYFVNHNTKTTQWEDPRTQG
WWP1_WW4        EEPLPEGWEIRYTRE-GVRYFVDHNTRTTTFKDPRNGK
WWP2_WW1        PDALPAGWEQRELPN-GRVYYVDHNTKTTTWERPLPPG
WWP2_WW2        ERPLPPGWEKRTDPR-GRFYYVDHNTRTTTWQRPTAEY
WWP2_WW3        LGPLPPGWEKRQDN--GRVYYVNHNNRTTQWEDPRTQG
WWP2_WW4        EPALPPGWEMKYTSE-GVRYFVDHNTTTTFKDPRPGF
WWTR1_WW        ELPLPPGWEMTFFAT-GQRYFLNHIEKITTWQDPRKAM
YAP1_WW         DVPLPAGWEMAKTSS-GQRYFLNHIDQTTTWQDPRKAM
YAP2_WW1        DVPLPAGWEMAKTSS-GQRYFLNHIDQTTTWQDPRKAM
YAP2_WW2        SGPLPDGWEQAMTQD-GEIYYINHKNKTTSWLDPRLDP
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Fig. 2. Amino acid sequence alignment of the human family of WW domains. Alignment is divided into two columns for clarity and each column is headed by the WW domain of PQBP1. Highly conserved amino acid residues across the entire family of WW domains are colored red and green, and the equivalent residues in the WW domain of PQBP1 are indicated by vertical arrows. Cysteines are in bold font.

These include the signature tryptophan residues at positions 52 and 75 (W52 and W75), an asparagine at position 67 (N67) and a proline at position 78 (P78). It is notable that while W52 is substituted by tyrosine in only a few WW domains, W75 displays more versatility and is replaced by amino acids as diverse as tyrosine, phenylalanine, isoleucine, glutamine and also cysteine. In a manner akin to the structural versatility of W75, the N67 residue can also be replaced by amino acids as diverse as aspartate, serine, tyrosine and histidine. In contrast, WW domains appear to have an obligate requirement for P78 as it is only absent from the WW2 domain of ARHGAP27 GTPase. The highly conserved nature of W52, Y65, N67, W75 and P78 residues in PQBP1 across the entire family of WW domains and their key role in the structural and functional integrity of WW domains is further corroborated by site-directed mutagenesis studies on the WW domain of Pin1 [15]. The mutagenesis of the WW domain of Pin1 revealed that W52, N67, Y65 and P78 form a hydrophobic core of the domain, which may be important in stabilizing the structure [15].

3. Y65C PQBP1 mutant and intramolecular disulfide bonds

In order to gain better insight about the effect of Y65C mutation on the WW domain fold, we generated 3D atomic models of wt and Y65C-mutant WW domains of PQBP1 (Fig. 3). As discussed in our recent study, the hydrophobic core that is located beneath the ligand-binding pocket of the wt WW domain is composed of a highly conserved quartet of hydrophobic residues W52, Y65, N67 and P78 (and perhaps to a lesser extent by W75). While Y65 constitutes a key component of the hydrophobic core within the wild type domain, the placement of cysteine at this position within the Y65C-mutant WW domain may not only result in the collapse of the triple-stranded β-sheet "roof" supporting the ligand-binding "cradle" [16,17], but under oxidative conditions, the formation of an intramolecular disulfide bond between C60 and C65 residues within the Y65C-mutant WW domain may also be favored (Fig. 4). This scenario is conceivable due to the inherent flexibility of the β1-β2 loop allowing it to curve away from the ligand-binding "cradle" toward the hydrophobic core of the WW domain, so as to

bring the C60 and C65 residues in close proximity and thereby drive their covalent linkage via a disulfide bond. As we have shown recently in an in vitro assay, the binding of Y65C-mutant WW domain to its cognate peptide ligands is indeed compromised, but perhaps not as dramatically as would be expected from the replacement of a conserved tyrosine with a cysteine within its hydrophobic core. This salient observation argues in favor of an alternative mechanism by which the Y65C mutation could lead to loss-of-function in PQBP1. Given that the in vitro binding studies, which we recently reported, were conducted under reducing conditions in the presence of a reducing agent, 5 mM β-mercaptoethanol [14], it is thus conceivable that the Y65C-mutant WW domain may behave differently toward its cognate ligands within the milieu of the cell, than in an in vitro binding assay. Thus, under non-reducing conditions in response to cellular oxidative stress, for instance, it is conceivable that the Y65C-mutant WW domain may undergo intramolecular disulfide bond formation as proposed above. Accordingly, such intramolecular covalent modification would destroy the "cradle" optimized for ligand binding and the resulting Y65C-mutant domain would not be expected to bind to its cognate ligands in a physiologically-relevant manner, leading to loss-of-function in PQBP1, as we observed in co-immunoprecipitation assays performed on full length proteins expressed in cultured cells. It is also important to note that C60 occupies a unique position in the WW domain of PQBP1 and is largely absent from other WW domains. Moreover, it is intriguing that the position of C60 in the WW domain of PQBP1 is either absent in other WW domains or substituted for by other amino acids, with the exception of cysteine (Fig. 2). Of particular interest is the observation that the occurrence of more than one cysteine in the WW domains is a rare event. Of ∼100 human WW domains, only four contain more than one cysteine. These include the WW domains of ARHGAP9, CEP164, MLH3 and PLEKHA2 (Fig. 2). Notably, these cysteine residues are located in the following areas: within the β2 and β3 strands (ARHGAP9), the β1 strand and the C-terminus following the β3 strand (CEP164), within the N-terminus preceding the β1 strand (MLH3), and within the β1-β2 loop and β3 strand (PLEKHA2). The markedly differential location of cysteine residues
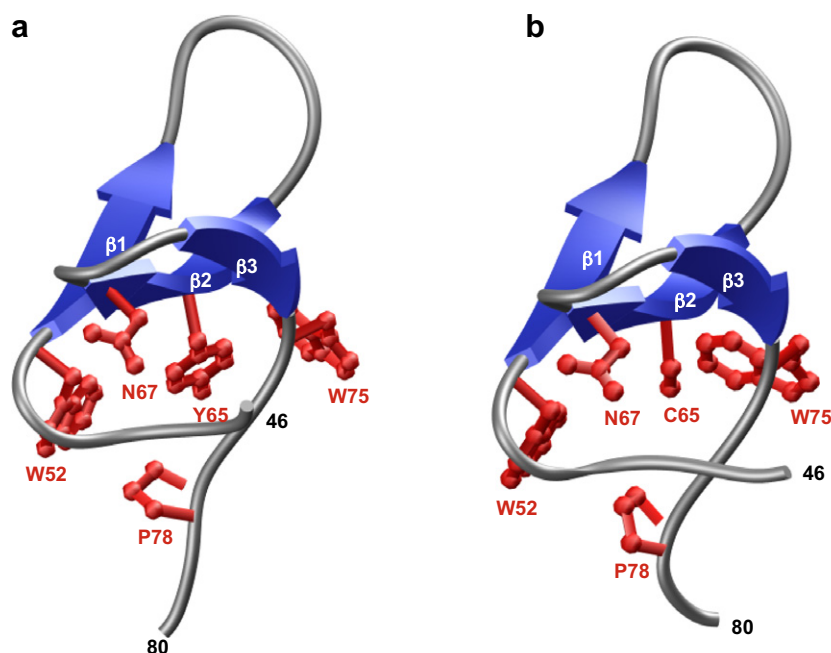

Fig. 3. 3D atomic models of wild type (A) and Y65C-mutant (B) WW domains of human PQBP1. The triple-stranded β-sheet of the WW domains is shown in blue and the intervening loops in gray. The sidechains of residues W52, Y65/C65, N67, W75 and P78 that constitute the hydrophobic core of the domains are colored red.

with the WW domain family suggests that they contribute to WW domain structure in distinct manners and may modulate their function via distinct mechanisms. Although the presence of more than one cysteine within a protein opens up the possibility of intra-molecular disulfide bond formation, it should be noted that this remains an unlikely scenario in the case of WW domains of ARHGAP9, CEP164 and PLEKHA2 proteins on structural grounds. Thus, unlike the Y65C-mutant WW domain of PQBP1, the cysteine residues are either located within one of the three rigid β-strands or away from the flexible β1-β2 loop in the WW domains of ARH-GAP9, CEP164 and PLEKHA2. Taken together, it seems that the Y65C mutation may predispose the WW domain of PQBP1 to loss-of-function via the formation of an intramolecular disulfide bond. However, it is important to mention that in some cases intra-molecular disulfide bond formation could lead to a gain of protein function, as exemplified by the bridge between cysteines 377 and 330 in Cdc25C phosphatase, which results in enhanced binding to 14-3-3 protein and the cytoplasmic sequestration of the protein [18]. Fittingly, in our in vitro binding studies, we did observe a small number of peptide ligands that bound more tightly to the Y65C PQBP1 WW domain compared to the wild type control. Therefore, it is possible, that in the network or systems view, the Y65C mutations may result in both loss and gain of function changes that in concert deregulate mRNA processing.

4. Y65C PQBP1 regulation via oxidation

Although in general, proteins that signal in the cytoplasm are embedded in a reducing environment, the reversible oxidation of cysteines of cytoplasmic proteins is a well-established regulatory mechanism for various signaling proteins, including protein–tyrosine phosphatases [19]. The general cellular redox state and the extracellular-ligand stimulated production of reactive oxygen or reactive nitrogen species (ROS or RNS) affects oxidation of cysteines in proteins, in a reversible manner. Reaction of H_2O_2 oxidizes cysteine thiols at first to sulfenic acid (SOH), and at higher concentrations of the oxidant, to sulfinic (SO_2H) or sulfonic acid (SO_3H) derivatives [19]. The sulfenic acid (SOH) derivative of C65 of PQBP1 could form sulfenylamides by reaction with other amino acids of the PQBP1 that are located in the proximity of the WW domain, in addition to forming an intra-molecular disulfide bridge with C60. Interestingly, the wt PQBP1 protein does not contain any other cysteine residues except for the WW domain-located C60.

We cannot exclude other possibilities by which oxidation of C65 could regulate the function of PQBP1 protein. The sulfinic (SO_2H) or sulfonic acid (SO_3H) derivatives of C65 in PQBP1 may lead to conformational changes that also render the domain inactive in terms of ligand binding. Moreover, the cysteine thiols in a local environment with pK_a below 6 could form a reactive thiolate anion that is permissive for posttranslational modifications such as phosphorylation, glutathionylation or even adduct formation with other endogenous electrophilic molecules [19]. Such modifications could also render the mutated WW domain inactive in terms of ligand binding and signaling of the PQBP1 complex in mRNA processing.

5. Concluding remarks

The atomic details of the Y65C lesion of the PQBP1 protein will hopefully come soon from crystal structures of the wild type and mutant proteins. Apart from the intrinsic changes that the mutation causes to the WW domain itself, it will be very important to see the relative location of the carboxy-terminal tail of the protein with its well-conserved motif (*CM*) (Fig. 1). Since most of the PQBP1 mutations that result in XLID syndromes have the carboxy-terminal region of the protein missing, including the very *CM* sequence, we presume that somehow the WW domain and the *CM* region collaborate together to regulate mRNA splicing.

Apart from X-ray crystallography, two other approaches represent attractive ways to study the effect of the Y65C mutation on the molecular architecture of the PQBP1 protein. These are protein folding assays in the periplasm of bacteria [20] and atomic force microscopy [21]. Both of these approaches are able to interrogate the PQBP1–Y65C mutant and wild type proteins under reducing and non-reducing conditions. These two approaches could quickly validate several regulatory scenarios proposed here.

In sum, our recent studies on PQBP1 and various plausible mechanisms discussed here for the inactivation of PQBP1 provide the framework for conducting future studies to unlock the molecular basis of XILD disorders at atomic level with important consequences on the rational design of therapeutic approaches.
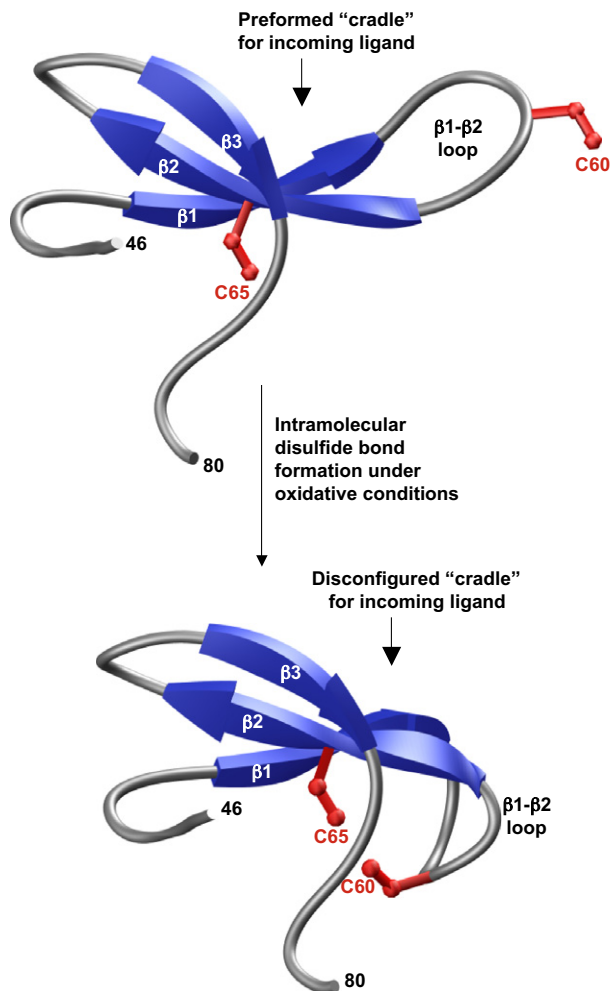

Fig. 4. A hypothetical model for the loss-of-function in the Y65C mutant form of human PQBP1 in Golabi-Ito-Hall syndrome. Under oxidative conditions, the formation of an intra-molecular disulfide bond between C60 and C65 residues within the Y65C-mutant WW domain may be favoured. This scenario is conceivable due to the inherent flexibility of the β1-β2 loop, allowing it to curve away from the ligand-binding "cradle" toward the hydrophobic core of the WW domain, so as to bring the C60 and C65 residues in close proximity and thereby drive their covalent linkage via an S–S bond. Note that the S–S bond between the sidechain SG atoms of C60 and C65 residues is omitted for clarity.

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