High-resolution structure of a BRICHOS domain and its implications for anti-amyloid chaperone activity on lung surfactant protein C

Hanna Willander^{a,1}, Glareh Askarieh^{b,c,1}, Michael Landreh^{d,1}, Per Westermark^e, Kerstin Nordling^{a,f}, Henrik Keränen^g, Erik Hermansson^{a,f}, Aaron Hamvas^h, Lawrence M. Nogeeⁱ, Tomas Bergman^d, Alejandra Saenz^j, Cristina Casals^j, Johan Åqvist^g, Hans Jörnvall^d, Helena Berglund^k, Jenny Presto^{a,f}, Stefan D. Knight^{a,b,2}, and Jan Johansson^{a,f,2}

^aDepartment of Anatomy, Physiology, and Biochemistry, and ^bDepartment of Molecular Biology, Swedish University of Agricultural Sciences, S-751 24 Uppsala, Sweden; ^cDepartment of Chemistry, Oslo University, 1033 Blindern, 0315 Oslo, Norway; ^dDepartment of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden; ^eDepartment of Immunology, Genetics and Pathology, Uppsala University, 751 85 Uppsala, Sweden; ^fKI-Alzheimer Disease Research Center, NVS (Neurobiology, Care Sciences, and Society) Department, Karolinska Institutet, S-141 86 Stockholm, Sweden; ^gDepartment of Cell and Molecular Biology, Uppsala University, S-751 24 Uppsala, Sweden; ^hDepartment of Pediatrics, Washington University School of Medicine and St. Louis Children's Hospital, St. Louis, MO 63110; ⁱDepartment of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21287-2533; ^jDepartment of Biochemistry and Molecular Biology I and CIBER (Centro de Investigación Biomédica en Red) Enfermedades Respiratorias, Complutense University of Madrid, 28040-Madrid, Spain; and ^kStructural Genomics Consortium, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden

Edited by* David Eisenberg, University of California, Los Angeles, CA, and approved December 22, 2011 (received for review September 7, 2011)

BRICHOS domains are encoded in >30 human genes, which are associated with cancer, neurodegeneration, and interstitial lung disease (ILD). The BRICHOS domain from lung surfactant protein C proprotein (proSP-C) is required for membrane insertion of SP-C and has anti-amyloid activity in vitro. Here, we report the 2.1 Å crystal structure of the human proSP-C BRICHOS domain, which, together with molecular dynamics simulations and hydrogen-deuterium exchange mass spectrometry, reveals how BRICHOS domains may mediate chaperone activity. Observation of amyloid deposits composed of mature SP-C in lung tissue samples from ILD patients with mutations in the BRICHOS domain or in its peptide-binding linker region supports the in vivo relevance of the proposed mechanism. The results indicate that ILD mutations interfering with proSP-C BRICHOS activity cause amyloid disease secondary to intramolecular chaperone malfunction.

interstitial lung disease | SFTPC mutations | β -sheet aggregates | transmembrane segment | discordant helix

Surfactant protein C (SP-C) is produced in the alveolar type II cell from an endoplasmic reticulum (ER) integral membrane protein precursor. Surfactant protein C proprotein (proSP-C) contains four regions; a short N-terminal segment (residues 1–23) facing the cytosol and important for intracellular trafficking, a transmembrane (TM) region constituting the main part of mature SP-C (residues 24–58) eventually secreted with phospholipids into the alveoli, a linker region (residues 59–89), and a BRICHOS domain (residues 90–197), defined from the structure presented here and localized to the ER lumen (1) (Fig. 1A). We refer to the linker region plus the BRICHOS domain as the C-terminal part of proSP-C (CTC). Maturation of proSP-C involves proteolytic processing in several steps at different intracellular locations, following insertion of the SP-C part as a TM helix (2). Mature SP-C (PDB ID 1spf) (3) adopts a TM helical conformation, but its valine-rich sequence (Fig. 1A) is far from optimal for TM helix formation. In model TM segments, poly-Leu variants insert into the ER membrane in a helical conformation, while poly-Val variants get trapped in an extended conformation, suggesting that helix propensities influence the ability to insert into the ER membrane (4). Consistent with this hypothesis, engineered SP-C with a poly-Leu repeat rather than the native poly-Val variant yields a stable TM helix, whereas wild-type (WT) SP-C is metastable and forms β -sheet aggregates and amyloid-like fibrils in vitro (5). The reason for using such a discordant sequence for a segment destined to end up as a TM helix is not known, but this sequence is highly conserved throughout proSP-Cs (6).

Since the first identification of an interstitial lung disease (ILD)-associated mutation (7) in the proSP-C gene (*SFTPC*), several additional mutations have been described. More than 50 *SFTPC* mutations, about half of which are previously not described, are summarized in *SI Appendix, Table S1*. The vast majority of these mutations are located in the linker and BRICHOS domains, with the linker mutation I73T being the most prevalent (8). The BRICHOS domain consists of approximately 100 amino acids and was initially identified from sequence alignments of *Bri* related to familial British and Danish dementia, *chondromodulin* associated with chondrosarcoma, and proSP-C associated with ILD or respiratory distress syndrome (9). The BRICHOS domain has thus far been found as a constituent of 12 otherwise different protein families associated with degenerative and proliferative disease. Only two Cys residues and one Asp residue are strictly conserved in all BRICHOS domains (6).

The proSP-C BRICHOS domain has been suggested to act as a chaperone that targets the SP-C region of proSP-C and prevents its aggregation while assisting its safe membrane insertion as a TM helix (10). Transgenic expression of proSP-C with two different BRICHOS mutations linked to ILD in a mammalian cell line generates Congo red positive inclusions and abundant aggregates of proSP-C, while expression of the I73T mutation only gave rise to low amounts of aggregated proSP-C (11). In vitro data thus support the notion that the ILD-associated mutations could give rise to SP-C amyloid formation, but there are no earlier reports of amyloid found in ILD. In this study, we determined the crystal structure of the BRICHOS domain of human proSP-C, analyzed BRICHOS-peptide interactions using hydrogen deuterium exchange mass spectrometry (HDX-MS), mapped ILD related mutations in *SFTPC* to the 3D structure, and performed molecular dynamics (MD) simulations of WT and mutant BRICHOS. We

Author contributions: S.D.K. and J.J. designed research; H.W., G.A., M.L., P.W., K.N., H.K., E.H., A.H., L.M.N., A.S., C.C., J.Ä., H.B., and J.P. performed research; A.H. contributed new reagents/analytic tools; H.W., G.A., M.L., P.W., T.B., C.C., J.Ä., H.J., H.B., J.P., S.D.K., and J.J. analyzed data; and H.W., G.A., M.L., S.D.K., and J.J. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 2YAD).

¹H.W., G.A., and M.L. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: janne.johansson@ki.se or stefan.knight@molbio.slu.se.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1114740109/-DCSupplemental.

Fig. 1. ProSP-C sequence and 3D structure of its BRICHOS domain. (A) Sequence of full-length human proSP-C. The N-terminal, situated in the cytosol, is presented in yellow. The TM and mature SP-C parts are in green. The C-terminal part of proSP-C (CTC) is shown in gray for the linker and blue for the BRICHOS domain. HDX rate constants in CTC are shown as colored lines above the sequence where red is fast, yellow is intermediate and blue is slow exchange. Secondary structure elements are shown as rectangles (helices) and arrows (β -strands). Starting position of the BRICHOS domain is labeled. Green dots, below the sequence, represent strictly conserved residues. Asterisks mark ILD mutations; the black are point mutations, the highlighted red correspond to the $\Delta 91-93$ deletion, the highlighted yellow are frameshift mutations, the two red asterisks correspond to start and end points of the Δ exon 4 deletion, and the unfilled asterisk corresponds to an 18 base pair insertion. Residues in the trimer interface are labeled with black triangles. Open and filled circles identify residues on face A and B of the β -sheet, respectively. (B) Ribbon diagram representation of one subunit, with secondary structure elements $\beta 1$ - $\beta 2$ - $\beta 3$ - $\beta 4$ - $\alpha 1$ - $\alpha 2$ - $\beta 5$ labeled. A dashed line indicates the missing region between helices $\alpha 1$ and $\alpha 2$.

further found the presence of amyloid composed of mature SP-C in lung tissues from ILD patients with *SFTPC* BRICHOS and linker mutations.

Results

Structure of the BRICHOS Domain. Crystals suitable for structure determination were obtained from recombinant CTC subjected to proteolysis with trypsin. The size of the crystallized protein has an average mass of 11,540 Da determined by MS, compatible with a product covering L82-K160 and D168-Y197 (Fig. 1A). The trypsin treatment of CTC has not significantly altered its critical structured part or its interaction with short substrate peptides, based on circular dichroism spectra and VVV tripeptide binding analyses (*SI Appendix, Fig. S1*).

There are two trimers (*SI Appendix, Fig. S2*) in the asymmetric unit of the crystals (pdbID 2yad). Size-exclusion chromatography and analytical ultracentrifugation show that recombinant CTC mainly forms trimers (12). However, chemical cross linking of proSP-C expressed in transfected A549 cells suggests that it does

not oligomerize (13) and peptide binding experiments show that peptide substrates bind to monomeric BRICHOS domains (14). Hence, the active form of BRICHOS appears to be the monomer. The fold of the proSP-C BRICHOS domain has not been previously seen and no structural homologs are present in the structure database. The domain encompasses residues 90–197 of proSP-C and has an overall architecture where two α -helices enclose a central five-stranded β -sheet (Fig. 1B). In the N-terminal half of the domain, four consecutive strands form an anti-parallel β -sheet. A fifth, C-terminal strand is parallel to β_4 , and the two helices following β_1 – β_4 stretch diagonally across each side of the β -sheet. We use “face A” to denote the face of the β -sheet that packs against helix 1, and “face B” for the face packing against helix 2 (Fig. 1B). The two helices are amphiphilic, with the hydrophobic side packing against the β -sheet to contribute to the hydrophobic core, and the polar side solvent accessible (α_1) or buried in the interface between subunits (α_2) (*SI Appendix, Fig. S2*). Residues 149–180 and 82–88, corresponding to the disordered regions defined by HDX-MS of intact CTC (Fig. 1A) and encompassing the proteolyzed 161–167 segment, have little visible electron density in our maps and were not modeled (Fig. 1B).

BRICHOS β -Sheet Face A Is a Likely Peptide Binding Surface with Accessibility Regulated by Strictly Conserved Asp105. Conserved residues in the BRICHOS domain of proSP-C were mapped on the crystal structure to identify structurally important positions and potential peptide binding surfaces (Figs. 1A, 2A). The strictly conserved disulfide bridge between C121 and C189 that links $\beta 4$ and $\alpha 2$ might be important for stability, and conserved Gly and Pro residues located in loop regions may be important for the fold and for dynamical properties of the domain. The remaining conserved residues in proSP-C BRICHOS are located primarily on face A and B of the β -sheet. Many of the CTC point mutations identified in patients with ILD (*SI Appendix, Table S1*) coincide with strictly conserved amino acid positions (Figs. 1A and 2, *SI Appendix, Fig. S3*).

Many of the hydrophobic core residues in the β -sheet (in particular on face A) are strictly conserved in proSP-C (Figs. 1A and 2A, *SI Appendix, Fig. S3*), while corresponding helix residues show a wider distribution of hydrophobic side chains, as expected for core residues. This pattern suggests that the β -sheet side chains are conserved not because they are strictly required for formation of the hydrophobic core, but because they are involved in some other function, such as peptide binding. Peptide binding would, however, require substantial reorganization of the structure to expose one or both of the β -sheet faces and allow binding.

The aspartic acid residue at position 105 of proSP-C is the only strictly conserved nondisulfide residue in all known BRICHOS sequences, and two mutations of D105 are known to associate with ILD (*SI Appendix, Table S1*). D105 is the first of four conserved residues at the end of strand $\beta 2$ and beginning of strand $\beta 3$. The side chain is located in a partially hydrophobic surrounding in contact with the N-terminal end of $\alpha 2$. We investigated the possibility of a structural role for Asp105 by carrying out MD simulations on the WT and the D105N substituted monomer from the crystal structure, in both cases at successively higher temperatures to monitor structural stability. Monomeric WT and D105N behave very differently in the simulations. Whereas there are only minor conformational changes in the mutant, several large-scale changes occur in WT at moderately elevated temperatures. The N-terminal part of $\alpha 2$ unwinds and this region communicates via the β -sheet and two disulfide bridges with $\alpha 1$ and the connecting loop from strand $\beta 4$, which undergo a conformational change that moves helix 1 out from face A by 5–7 Å (Fig. 3, *Movie S1*). This repositioning is accompanied by many of the hydrophobic core residues on face A becoming solvent accessible (Fig. 3; *SI Appendix, Table S2 and Fig. S3B*). More than 500 Å² hydrophobic surface area on face A is exposed when $\alpha 1$ moves away from the

Fig. 2. Structural conservation and location of ILD-associated mutations in the BRICHOS domain of human proSP-C. (A) Stereo view showing the BRICHOS domain as a cartoon with conserved residues as sticks. (B) As (A) but showing the targets for ILD-associated mutations. Point mutations are shown as sticks labeled with the proSP-C residue number and residue type, the Δ exon4 and Δ 91-93 deletion mutations are shown in red, frame shift mutations are colored yellow and identified by residue number.

sheet. Hence, the strictly conserved Asp side chain appears to tune the stability of the structure, thereby providing a mechanism for exposing the central β -sheet, and in particular the highly conserved face A, which would make it accessible for binding to peptide substrates as predicted above.

Both the BRICHOS Domain and Linker Region Bind to Substrate Pep-

tides. The BRICHOS mutation $\Delta 91-93$ and the linker mutation I73T give rise to ILD and amyloid deposits with similar immunoreactivity (*SI Appendix, Table S3* and see further below). This observation indicates that both these mutations result in improper chaperoning of the proSP-C TM segment, but in vitro experi-

Fig. 3. Conformational changes after MD simulations. The two structures after MD simulations are superimposed on the starting X-ray structure (green). The D105N mutant in blue remains unchanged compared to the distorted WT monomer in magenta.

ments suggest that BRICHOS and linker mutations can result in different extents of proSP-C aggregation (11). The first half of the linker region is highly conserved through evolution, but is flexible and lacks ordered secondary structure (Fig. 1A). In HDX experiments, addition of the substrate peptides (10, 15) KKVVVVVVVKK (V_7) or KKVVVVVK (V_5) to CTC had no measurable effect on the deuteration pattern of the BRICHOS domain. However, a part of the linker region (residues 68–71) shows a significant decrease in deuteration in the presence of V_5 or V_7 , while no such effect is observed in a control experiment with the nonsubstrate peptide KKAATAAAAKK (A_7) (Fig. 4). Correspondingly, V_7 and V_5 , but not A_7 , become protected against HDX in the presence of CTC, but not in the presence of proSP-C BRICHOS alone, which lacks the N-terminal half of the linker region (Fig. 4). Coincubation of a free peptide corresponding to the linker region and V_7 did not result in any effect on deuteration of any of the peptides. These data indicate that the linker region interacts with peptides bound to proSP-C BRICHOS.

Lung Tissue from ILD Patients with SFTPC Mutations Contain Amyloid

of Mature WT SP-C. Lung tissue obtained at lung transplantation ($n = 6$) or autopsy ($n = 1$) of children with end-stage ILD due to a mutation in *SFTPC* was analyzed histologically for the presence of amyloid, defined by the presence of deposits that stain with Congo red and show green birefringence under polarized light (16). In order to avoid Congo red staining of non-amyloid, particular care was taken (17). In all but one ILD case, amyloid deposits with typical amyloid staining properties were identified. The amyloid appeared as small extracellular, irregular deposits mostly interstitially but sometimes in alveolar lumina

Fig. 4. The linker region stabilizes substrate peptides bound to BRICHOS. HDX-MS spectra show that the presence of V₇ induces significant protection from deuterium labeling in the VLEM fragment from the N-terminal linker region (top left). Similarly, a subpopulation of the V₇ peptide is significantly protected from exchange when CTC is present (bottom right). The schematic model (bottom left) shows how bound target peptides can interact with the linker to form a β -hairpin, see text for details.

(Fig. 5, *SI Appendix, Table S3*), the latter then often roundish. Such deposits are not found in healthy tissue. As expected, the amyloid deposits were labeled with antibodies to serum amyloid P component (SAP) (Fig. 5).

Immunolabeling experiments were performed on three materials with amyloid associated with the linker mutation I73T or the BRICHOS mutation Δ 91–93. Antibodies against mature SP-C labeled alveolar epithelium. In addition there was a diffuse and uneven background staining. Double staining with Congo red was necessary to identify the small amyloid deposits, which for all three cases showed a clear-cut but somewhat uneven immunolabeling (Fig. 5, *SI Appendix, Table S3*). Control experiments support the presence of mature SP-C in the deposits; (i) preabsorption with peptide corresponding to proSP-C residues 24–41 abolished all immunoreactivity, (ii) antibodies against the N-terminal segment of proSP-C, or against CTC, labeled alveolar epithelium strongly in some areas but the amyloid deposits were completely nonreactive, and (iii) incubation with antibodies against the acute phase serum protein AA, which forms amyloid secondary to chronic inflammatory states, showed no immunoreactivity in any case.

Further support for the notion that WT SP-C can form amyloid comes from *in vitro* studies showing that incubation of a synthetic peptide corresponding to the first 21 residues of mature SP-C (i.e., proSP-C residues 24–44), results in formation of amyloid-like fibrils, as judged by light microscopy after staining with Congo red (*SI Appendix, Fig. S4*). These results show that ILD due to mutations in CTC can be associated with formation of amyloid, and that the region that forms amyloid deposits is derived from the mature SP-C region, localized outside CTC. We suggest that in ILD due to mutations in CTC, proSP-C fibrils are formed in-

Fig. 5. Amyloid in lung tissue. (A) The amyloid was strongly stained with Congo red and showed a bright green birefringence in polarized light (arrows), diagnostic of amyloid. (B) An amyloid deposit, labeled with an antibody against mature SP-C, visualized with 2,2'-diamino benzidine (brown) and then stained with Congo red and examined in polarized light. Staining with Congo red is evident in the periphery of the deposit (arrow). (C) Small amyloid deposits close to a vessel immunolabeled for SAP and in addition stained with Congo red for visualization of amyloid. Congo red staining and SAP labeling colocalize (black arrows) but SAP is also present in elastic structures (green arrow). (D) Same material as in (C), but visualized between crossed polars. [Scale bars (A, C, and D), 50 μ m and (B) 20 μ m.]

tracellularly, likely in the loosely packed ER membranes, and then processed out of the cell.

Discussion

Available data suggest that CTC acts as a chaperone for the extremely hydrophobic and β -structure-prone TM proSP-C segment (18). Based on the present results a model for the function of CTC during proSP-C biosynthesis can be suggested (*SI Appendix, Fig. S5*). The BRICHOS domain with the help of the linker region specifically captures peptides representative of the poly-Val TM part of proSP-C, explaining how mutations in the linker region or the BRICHOS domain can be associated with ILD and amyloid formation. The linker region may serve as a substitute β -strand that docks to the BRICHOS-bound proSP-C TM region, forming a β -hairpin structure (Fig. 4). Such a function of the linker would explain both why proSP-C is the only BRICHOS-containing protein with a highly conserved linker region and the only one with a target region composed of a single β -strand. For the other BRICHOS proteins, the putative target regions are β -hairpins located C-terminally to the domain (6, 18). β -Hairpin structures have been implicated in the formation of cytotoxic oligomers and fibrils from the amyloid β -peptide (A β) associated with Alzheimer's disease (19, 20). It has been reported that recombinant BRICHOS domains from proSP-C and Bri2 prevent fibril formation of A β *in vitro* (21, 22), suggesting that BRICHOS may bind β -hairpin intermediates that occur in amyloid formation. The conserved hydrophobic surfaces of the BRICHOS central β -sheet appear well suited for such a function and would parallel the steric chaperones of the chaperone/usher pathway where a hydrophobic platform is used to capture unfolded structures (23). Chaperones more or less invariably utilize a "capping" mechanism to shield their hydrophobic binding surfaces from solution in the absence of substrate, often by forming homocomplexes that bury these surfaces (24, 25). MD simulations using both the crystallographic WT and D105N mutant trimer model as starting structures show that none of the movements that occur in the WT monomeric structure can occur in

the trimer. The trimer thus stabilizes the subunit in a conformation that blocks the putative binding site, consistent with its role as a chaperone capping mechanism.

This study indicates that mutations in a domain with chaperone function, rather than in the amyloid material itself, can cause amyloid formation of its substrate, and disease in humans. Molecular chaperones have been implicated as potent antagonists of protein misfolding diseases, including amyloidoses (26, 27), but improper chaperone function directly resulting in amyloid disease have not been described. Our findings are potentially important as they suggest that many proteins and peptides so far only known to form fibrils *in vitro* may do so *in vivo*, and that more diseases than previously suspected may be related to amyloid formation. The deposits now found in ILD tissue were small and scarce, explaining why they have previously escaped detection.

Experimental Procedures

Protein Production and Structure Analysis. For WT CTC, a region from nucleotide 175 (His59) to nucleotide 591 (Ile197) of the proSP-C cDNA sequence was amplified from human lung cDNA. ProSP-C BRICHOS (residues 86–197) was created from CTC and expression and purification were performed essentially as described (15, 28).

The electron density maps resulting from MAD (multiple anomalous dispersion) phasing based on 18 selenium sites allowed us to model all residues in the proSP-C BRICHOS domain except residues 152–179, located between $\alpha 1$ and $\alpha 2$ and encompassing the peptide 161–167 removed by trypsin cleavage during crystallization. The final model consist of 470 amino acid residues (residues 89–149 and 180–197 in chain A, 82–149 and 181–197 in chain B, 88–151 and 180–197 in chain C, 89–148 and 180–197 in chain D, 89–125, 132–149 and 181–197 in chain E, 88–148, and 181–197 in chain F), and 137 water molecules. We modeled 19

protein residues with alternative conformations. All of the modeled chains can be pairwise superimposed with rmsd of 0.6 ± 0.1 Å for 76 superimposed C α atoms. All residues are within the allowed regions of the Ramachandran plot.

See *SI Appendix* for details about protein production, crystal structure determination, MD and HDX-MS.

Histological Examination of Lung Tissue and Fibrils. Lung tissue sections of 10 μ m thick were deparaffinized, stained with Congo red, and examined for amyloid in a polarization microscope. Sections from all the materials containing amyloid deposits were immunolabelled with rabbit antiserum against mature SP-C, N-terminal propeptide segment, CTC, or human SAP as described (29). The very pronounced chronic inflammation may raise the question whether observed amyloid deposits could be of AA origin and therefore other sections were immunolabelled with antibodies against protein AA. After development with 2,2'-diaminobenzidine tetrahydrochloride, the immunolabelled sections were stained with Congo red (30) for the simultaneous detection of amyloid and immunoreactivity.

A synthetic peptide, residues 24–44 of human proSP-C, was incubated for 7 d at 200 μ M in 10% formic acid at 37°C with shaking. Droplets (0.8 microliter) were applied to microscopical slides, air dried, and stained with Congo red B (30). After mounting under cover slips, the materials were examined in a polarization microscope for Congoophilia and green birefringence.

ACKNOWLEDGMENTS. We thank Eva Davey for help with immunohistochemistry and Drs. M. Siponen and S. Moche for collecting the first Se-Met dataset. We thank the ESRF (European Synchrotron Radiation Facility), and Diamond beam line staffs for help during data collection. This work was supported by the Swedish Research Council and the Spanish Ministry for Research and Innovation and NIH grants HL-082747 and HL-65174. The Structural Genomics Consortium is a registered charity (number 1097737).

- Mulugeta S, Beers MF (2006) Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung. *Microbes Infect* 8:2317–2323.
- Beers MF, Kim CY, Dodia C, Fisher AB (1994) Localization, synthesis, and processing of surfactant protein SP-C in rat lung analyzed by epitope-specific antipeptide antibodies. *J Biol Chem* 269:20318–20328.
- Johansson J, Szyperki T, Curstedt T, Wüthrich K (1994) The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α -helix. *Biochemistry* 33:6015–6023.
- Mingarro I, Nilsson I, Whitley P, von Heijne G (2000) Different conformations of nascent polypeptides during translocation across the ER membrane. *BMC Cell Biol* 1, Available at <http://www.biomedcentral.com/1471-2121/1/3>.
- Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J (2001) Prediction of amyloid fibril-forming proteins. *J Biol Chem* 276:12945–12950.
- Hedlund J, Johansson J, Persson B (2009) BRICHOS—a superfamily of multidomain proteins with diverse functions. *BMC Res Notes* 11, Available at <http://www.biomedcentral.com/1756-0500/2/180>.
- Nogee LM, et al. (2001) A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 344:573–579.
- Wert SE, Whitsett JA, Nogee LM (2009) Genetic disorders of surfactant dysfunction. *Pediatr Dev Pathol* 12:253–274.
- Sanchez-Pulido L, Devos D, Valencia A (2002) BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer. *Trends Biochem Sci* 27:329–332.
- Johansson H, Nerelius C, Nordling K, Johansson J (2009) Preventing amyloid formation by catching unfolded transmembrane segments. *J Mol Biol* 389:227–229.
- Nerelius C, et al. (2008) Mutations linked to interstitial lung disease can abrogate anti-amyloid function of prosurfactant protein C. *Biochem J* 41:201–209.
- Casals C, et al. (2008) C-terminal, endoplasmic reticulum-lumenal domain of prosurfactant protein C—structural features and membrane interactions. *FEBS J* 275:536–547.
- Wang WJ, Russo SJ, Mulugeta S, Beers MF (2002) Biosynthesis of surfactant protein C (SP-C). Sorting of SP-C proprotein involves homomeric association via a signal anchor domain. *J Biol Chem* 277:19929–19937.
- Fitzen M, et al. (2009) Peptide-binding specificity of the prosurfactant protein C Brichos domain analyzed by electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom* 184:3591–3598.
- Johansson H, Nordling K, Weaver TE, Johansson J (2006) The Brichos domain-containing C-terminal part of pro-surfactant protein C binds to an unfolded poly-val transmembrane segment. *J Biol Chem* 281:21032–21039.
- Sipe JD, et al. (2010) Amyloid fibril protein nomenclature: 2010 recommendations from the nomenclature committee of the International Society of Amyloidosis. *Amyloid* 17:101–104.
- Westermarck P (2011) Subcutaneous adipose tissue biopsy for amyloid protein studies. *Amyloid Proteins: Methods and Protocols*, eds EM Sigurdsson, M Calero, and M Gasset (Springer Science and Business Media, NY), in press, 2nd Ed.
- Willander H, Hermansson E, Johansson J, Presto J (2011) BRICHOS domain associated with lung fibrosis, dementia and cancer—a chaperone that prevents amyloid fibril formation? *FEBS J* 278:3893–3904.
- Sandberg A, et al. (2010) Stabilization of neurotoxic Alzheimer amyloid- β oligomers by protein engineering. *Proc Natl Acad Sci USA* 107:15595–15600.
- Yu L, et al. (2009) Structural characterization of a soluble amyloid β -peptide oligomer. *Biochemistry* 48:1870–1877.
- Peng S, Fitzen M, Jörnvall H, Johansson J (2010) The extracellular domain of Bri2 (ITM2B) binds the ABri peptide (1–23) and amyloid β -peptide (A β 1–40). Implications for Bri2 effects on processing of amyloid precursor protein and A β aggregation. *Biochem Biophys Res Commun* 393:356–361.
- Nerelius C, Gustafsson M, Nordling K, Larsson A, Johansson J (2009) Anti-amyloid activity of the C-terminal domain of proSP-C against amyloid β -peptide and medin. *Biochemistry* 48:778–786.
- Zavialov AV, et al. (2003) Structure and biogenesis of the capsular F1 antigen from *Yersinia pestis*: preserved folding energy drives fiber formation. *Cell* 113:587–596.
- Haslbeck M, Franzmann T, Weinfurter D, Buchner J (2005) Some like it hot: the structure and function of small heat-shock proteins. *Nat Struct Mol Biol* 12:842–846.
- Zavialov AV, Knight SD (2007) A novel self-capping mechanism controls aggregation of periplasmic chaperone Caf1M. *Mol Microbiol* 64:153–164.
- Barral JM, Broadley SA, Schaffar G, Hartl FU (2004) Roles of molecular chaperones in protein misfolding diseases. *Semin Cell Dev Biol* 15:17–29.
- Bonini NM (2002) Chaperoning brain degeneration. *Proc Natl Acad Sci USA* 99:16407–16411.
- Johansson H, Eriksson M, Nordling K, Presto J, Johansson J (2009) The Brichos domain of prosurfactant protein C can hold and fold a transmembrane segment. *Protein Sci* 18:1175–1182.
- Persson A, et al. (2006) Lactadherin binds to elastin—a starting point for medin amyloid formation? *Amyloid* 13:78–85.
- Westermarck GT, Johnson KH, Westermarck P (1999) Staining methods for identification of amyloid in tissue. *Meth Enzymol* 309:3–25.