Conformational disease

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A large and diverse number of diseases are now recognized as 'conformational diseases', caused by adoption of non-native protein conformations that lead to aggregation. The recent conference, 'Alpha$_1$-antitrypsin deficiency and other conformational diseases', held in Airlie, Virginia, USA (27–30 June, 2000) focused on some of the common pathways by which cells protect themselves from toxicity associated with protein misfolding and aggregation.

Many diseases are caused by inherited or acquired modifications in protein structure. Recessively inherited genetic diseases like cystic fibrosis and familial hypercholesterolaemia, for example, are caused by mutations that cause a loss-of-function of the mutant gene product by interfering with its synthesis, transport, stability or enzymatic activity. In contrast, conformational diseases (Carrell, R. W. & Lomas, D. A. *Lancet* **350,** 134–138; 1997), which include such diverse disorders as Alzheimer's and Parkinson's diseases, amyloidoses, α_1-antitrypsin deficiency and the prion encephalopathies, arise when a specific protein undergoes a conformational rearrangement that endows it with a tendency to aggregate and become deposited within tissues or cellular compartments. Conformational diseases can be inherited, usually as dominant traits, or can be induced, as in the case of prions. The vast majority of conformational diseases are, however, of unknown etiology. How cells respond to the production of these abnormal protein conformers and how these misfolded proteins cause cytotoxicity are central unanswered questions that were the subject of the recent conference, which was organized by Richard N. Sifers and David Lomas and funded by the NIH, the Alpha One Foundation and the Fundacion Leopoldo Fernandez Pujals. This meeting broke new ground in bringing together researchers focusing on common questions relating to this extremely diverse group of disorders.

α_1-antitrypsin (α_1-AT) deficiency serves as an excellent model for conformational disease because it is one of the few members of this class for which detailed structural data are available on both the wild-type and mutant proteins. α_1-AT is an abundant serum glycoprotein, secreted by the liver, the normal function of which is to bind to and inactivate the neutrophil protease, elastin. α_1-AT-deficiency mutations interfere with the folding of α_1-AT, preventing its secretion from the hepatocyte endoplasmic reticulum (ER). The resulting loss-of-function phenotype is lung damage/emphysema as a result of destruction of the lung parenchyma from uncontrolled elastase activity. α_1-AT deficiency is also the leading genetic cause of liver disease in children, because of the hepatotoxic effect of abnormally folded α_1-AT molecules that accumulate in the ER lumen. α_1-AT deficiency is therefore unusual in that it is both a classical recessive disorder (lung disease) and a gain-of-function conformational disease.

Robin Carrell and David Lomas (Univ. Cambridge, UK) described work on the structural rearrangements that take place when α_1-AT meets and inactivates its target, the serine proteases. The ability to undergo this marked conformational changes comes at price — in their active, inhibitory state, serine-protease inhibitors (serpins) such as α_1-AT are in a non-compact metastable configuration. In the case of α_1-AT, this inherent instability allows the proteins to undergo loop-sheet polymerization, creating an abnormal structure in which the loop from the active site of one α_1-AT molecule inserts itself as another β-strand into a pre-existing β-sheet of an adjacent molecule (Fig. 1). This intrinsic propensity of wild-type α_1-AT to undergo this structural transformation is markedly enhanced in mutant forms. Mutant α_1-AT is better able to accommodate the extraneous strand from an adjacent molecule because the mutation destabilizes the sheet, allowing increased mobility of its constituent strands. This loop–sheet insertion is but one example of pathological conversion of a loop to a β-strand through interactions with a pre-existing β-sheet. The propensity to undergo loop–sheet polymerization is not restricted to α_1-AT, as other serpins can undergo the same transformation. David Lomas provided a dramatic example of this. The rare form of familial encephalopathy with neuronal inclusion bodies (FENIB) is caused by a mutant neuroserpin that undergoes loop-sheet polymerization. Structural modelling of the neuroserpin mutation indicates that it too may lead to instability of the β-sheet structure, increasing its propensity to accept an extraneous strand. Robin Carrell noted that such β-promiscuity may account not only for the pathogenic properties of the serpins, but may also help to explain the 'infectious' property of β-sheets in prion disorders that also seem to be caused by an induced transition from α-helix to β-strand.

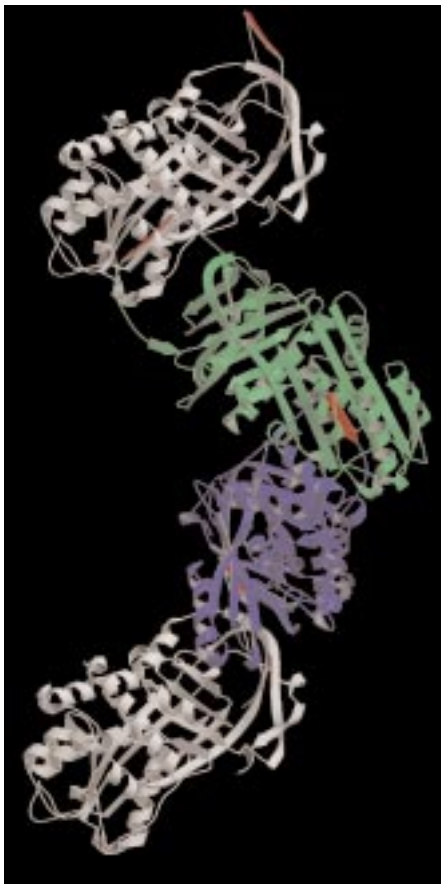

Figure 1 Loop–sheet polymers of α-1-antitrypsin. The severe Z-deficiency variant perturbs the structure of α-1-antitrypsin, allowing the reactive centre loop of one molecule to insert into β-sheet A of a second. This dimer then extends to form chains of polymers, which are retained within the endoplasmic reticulum of hepatocytes to form inclusions. Each antitrypsin molecule is illustrated in a different colour with the linking reactive centre loop shown in red. (Courtesy of T. Dafforn, Dept of Haematology, Univ. Cambridge, UK).

β-strand promiscuity may also be involved in the etiology of another class of conformational diseases, the polyglutamine expansion disorders such as Huntington's disease and spinocerebellar ataxia. These dominantly inherited diseases are caused by the presence of long (>35) tracts of polyglutamine in huntingtin and ataxin-3, respectively, which have been proposed to function as polar 'zippers' that entrap important regulatory factors by stabilizing β-strands into sheets composed of heterogeneous proteins (Perutz, M. F. *et al. Proc. Natl Acad. Sci. USA* **91,** 5355–5358; 1994). In support of this hypothesis, Henry Paulson (Univ. Iowa) reported that green fluorescent protein fused to Gln_{19}, which is soluble when expressed in cells alone, is recruited to inclusion bodies when co-expressed with proteins containing pathogenic length polyglutamine expansions. Paulson also reported that the transcription factor CREB-binding protein (CBP), which contains an endogenous Q_{19} tract, was also trapped in inclusion bodies when co-expressed with full-length ataxin-3 containing expanded $polyQ_{84}$.

How cells deal with the abnormal protein structures associated with conformational disease was a major focus of the meeting. Interaction of nascent secretory proteins in the ER with molecular chaperones, such as BiP or the lectin chaperones calnexin and calreticulin, is likely to play a central role in the decision between secretion, retention and degradation. Maurizio Molinari (ETH, Zürich) reviewed the features of the calnexin/calreticulin–glucosyl transferase cycle. In the ER, calnexin and calreticulin bind to monoglucosylated glycoproteins. Proteins are then deglucosylated and leave the ER if correctly folded. In contrast, misfolded proteins are recognized and reglucosylated by the UDP–glucose glycoprotein glycosyl transferase (UGT), which allows them to enter another cycle of calnexin/calreticulin binding and folding. Molinari described the crucial feature of the newly-synthesized polypeptide that determines whether it will be engaged by the chaperone BiP or by the calnexin/calreticulin lectin system, which seems to be the length of the nascent chain at which the first asparagine-linked glycosylation event occurs. BiP binding seems to be directly proportional and calnexin/calreticulin binding inversely proportional to this parameter. John Bergeron (McGill, Montreal) described studies showing that exposed hydrophobic residues situated carboxy-terminal to the glucosylation site seem to be part of the recognition code used by the glucosyl transferase to identify misfolded proteins and to tag them for re-engagement by the calnexin/calreticulin system.

Prolonged chaperone association may be sufficient to prevent secretion of proteins that have not yet acquired native tertiary and quartenary structures, but most misfolded proteins are eventually degraded. Degradation of integral membrane proteins and secretory proteins that are unable to fold in the ER, a process known as ER-associated degradation (ERAD; Werner, E. D. *et al. Proc. Natl Acad. Sci. USA* **93,** 13797–13801; 1996) is mediated by cytoplasmic proteasomes, and requires retrotranslocation or 'dislocation' of the polypeptide chains across the Sec61 translocon in the ER membrane. Karin Römisch (Univ. Cambridge, UK) reported the characterization of *sec61* mutants that differentially affect protein import into the ER and protein and glycopeptide export from the ER, indicating that retrotranslocation does not occur by a simple reversal of the translocation pathway. Römisch also presented evidence for a role for the peptide-binding activity of the lumenal chaperone protein disulfide isomerase (PDI) in retrotranslocation of a cysteine-free substrate.

How do chaperones discriminate between proteins that fold slowly and those that are misfolded? Richard Sifers (Baylor, Houston, Texas) presented a model in which ER mannosidase I mediates trimming of mannose residues from proteins engaged by the calnexin/calreticulin-glucosyl transferase cycle, which serves to tag misfolded protein for degradation by a proteasome-mediated pathway. A similar dependence on mannosidase I activity was reported for the degradation of folding-defective thyroglobulin molecules (Peter Arvan, Albert Einstein Medical School, New York). At this point it is not known whether glycan trimming by ER mannosidase I is a stochastic process that is influenced simply by the duration for which a protein is engaged in the calnexin/calreticulin-glucosyl transferase cycle, or whether the system is also sensitive to threshold effects and has deterministic features that discriminate between slow folding and misfolding. Sifers also described studies using selective inhibitors of ER mannosidase I and II, the results of which indicate that mannosidase II-mediated glycoprotein trimming may tag specific substrates (such as α1-AT polymers) for degradation by a non-proteasomal pathway. Ingrid Haas (BZH, Heidelberg) presented data implicating mannose trimming in the proteasome-mediated degradation of an immunoglobulin κ-light-chain engineered to contain one or two glycosylation sites, even when engagement of the substrate with calnexin/calreticulin is blocked by deoxynojerimycin or castanospermine.

The ER mannosidase I inhibitor kifunensine blocked degradation of both the membrane and the secreted forms of immunoglobulin μ-chain (Roberto Sitia, DiBiT, Milan), which supports the idea that ER mannosidase activity may serve as a timer for committing proteins to dislocation/degradation pathways. Interestingly, the requirement for mannose trimming could be bypassed by attaching soluble μ-chains to the transmembrane and cytosolic domains of the T-cell receptor α-chain, indicating that more than one pathway can lead to dislocation and proteasomal degradation of ER-synthesized proteins, and that these pathways may have a measure of substrate specificity.

Evidence for diversity in pathways leading to ERAD was also noted in the function of components of the heat-shock protein 70 (Hsp70) class of chaperones. Jeff Brodsky (Univ. Pittsburgh, Pennsylvania) used genetic analysis in yeast to contrast the requirements for degradation of soluble lumenal proteins (mutant α1-AT or pro-α mating factor), with an integral membrane protein (the cystic-fibrosis transmembrane conductor, CFTR). Activity of the lumenal Hsp70-like chaperone, BiP, and its lumenal DNA-J-like co-chaperones, Jem1p and Scj1p, were required for proteasome-mediated degradation of the lumenal proteins, but not for the degradation of integral membrane CFTR. On the other hand, BiP activity was dispensable for proteasome-mediated degradation of CFTR, whereas the cytosolic Hsp70-like chaperone Ssa1p was required. These observations may reflect differences in the mechanism of dislocation of soluble and integral membrane proteins to cytoplasmic proteasomes, or may simply be indicative of differences in the topological accessibility of these different proteins to chaperones in different cellular compartments.

Cells have several responses to the accumulation of misfolded proteins in the ER. The 'ER-overload response' induces the nuclear transcription factor NF-κB, a central mediator of the human immune response. Heike Pahl (Univ. Hospital, Freiburg, Germany) reported that expression of mutant CFTR induces NF-κB expression and activates transcription of the κB gene. Levels of the inflammatory cytokine IL8 are increased in lungs from patients of cystic fibrosis, and NF-kB was found to be constitutively active in mice in which the wild-type CFTR gene had been replaced with the ΔF508 mutant, supporting the hypothesis that ER overload contributes to the chronic inflammation that often contributes to the high morbidity in cystic fibrosis.

The 'unfolded-protein response' triggered by the presence of misfolded protein in the ER activates the kinase IRE1, leading to the transcription of genes encoding chaperones and many components of the secretory pathway, and through a parallel process suppresses protein synthesis by modulating the phosphorylation state of eukaryotic initiation factor-2α (eIF2α). Randy Kaufman (Univ. Michigan) described the phenotype of a mouse 'knock-in' of a mutant allele of

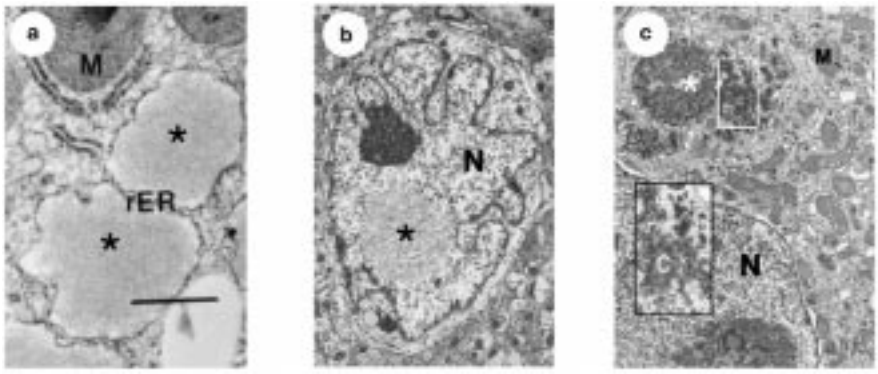

Figure 2 Inclusion bodies in different cellular compartments. Inclusion bodies, consisting of highly aggregated, often ubiquitinated protein, are characteristic of conformational diseases. a, Electron micrograph of a liver cell from an α1-antitrypsin-deficient patient, showing a prominent inclusion body composed of polymerized α1-AT (asterisk) within a distended ER lumen. b, Intranuclear-inclusion body (asterisk) from the brain of a transgenic mouse expressing human huntingtin. c, Cytoplasmic aggresome (asterisk) from a cell expressing mutant CFTR. Inset, higher magnification of the boxed region, showing the proximity of aggregates to a centriole. M, mitochondrion; N, nucleus; C, centriole; rER, rough endoplasmic reticulum. Image in a courtesy of J. Teckman (Washington Univ., St Louis, Missouri); image in b courtesy of S. Davies, Univ. College London, UK); image in c provided by R. Kopito.

eIF2α that cannot be phosphorylated by the stress-induced kinase PERK. Embryonic development of homozygous eIF2α-mutant mice is apparently normal, but homozygous eIF2α-mutant cells are unable to activate transcription of the stress-activated transcription factor CHOP. David Ron (Skirball Inst., New York) described the phenotype of a mouse mutation that inactivates PERK. In addition to complete loss of translational inhibition by ER stress, *Perk*$^{-/-}$ cells are also unable to activate CHOP expression. Thus, both studies support the role of eIF2α phosphorylation in activated gene expression by ER stress, and provide the first evidence for the existence of a signalling pathway that operates in parallel with the well-characterized IRE1 pathway to regulate gene expression in the unfolded-protein response. The downstream mediators of this new pathway remain to be discovered.

One feature that is common to all conformational diseases is the tendency of mutant protein to aggregate. Where and how these aggregates are deposited seems to be highly specific and tightly linked to pathogenesis. Nuclear-inclusion bodies are characteristic of the CAG expansion diseases such as spinocerebellar ataxia type 3 (H. Paulson; Fig 2a). Mutant serpins accumulate in the dilated ER lumen of hepatocytes and neurons from individuals affected with α1-AT deficiency and FENIB, respectively (D. Lomas and H. Carrell; Fig 2b). Cytoplasmic-inclusion bodies are prominent in several conformational diseases including Parkinson's, Alzheimer's and amyotrophic lateral sclerosis. Ron Kopito (Stanford Univ., California) discussed the biogenesis of cytoplasmic-inclusion bodies, emphasizing the distinction between aggregation (assembly of proteins into non-native oligomers) and formation (sequestration of aggregates at a specific cellular location). Aggregated forms of mutant protein form high-molecular-mass, SDS-insoluble complexes that are delivered, by retrograde transport on microtubules, to a pericentriolar region called the 'aggresome', indicating a role for the microtubule cytoskeleton in the elimination of misfolded protein (Fig. 2c). Kopito proposed that sequestration of aggregated proteins at a single locus may facilitate their capture and destruction by the autophagocytic pathway.

David Perlmutter and Jeffrey Teckman (Washington Univ., St. Louis, Missouri) reported that mutant α1-AT accumulates within an expanded and dilated ER lumen and that this accumulation is accompanied by the appearance of multiple autophagosomes that surround the dilated ER in several different cell-culture systems, in the livers of PiZ (a mutant allele of α1-AT) transgenic mice, and in liver biopsies of α1-AT-deficient patients. Similarly, Roberto Sitia (DiBiT, Milan) reported that mutant or unassembled immunoglobulin M μ-heavy chains accumulate within ER-derived vesicles, called Russell bodies, that are ultrastructurally related to autophagosomes. Therefore, one of the more important themes to emerge from this conference is that autophagy may represent a hitherto underappreciated mechanism by which cells eliminate potentially toxic protein aggregates.

At present, there are no mechanism-based therapies available for any conformational disease, even though the prevalence of these diseases increases as human life expectancy rises. Current research into therapeutic strategies are focused on efforts to prevent or reverse the conformational changes that lead to the formation of pathogenic proteins. Stephen Bottomley (Monash Univ., Australia) reported that chemical chaperones like trimethylamine-*N*-oxide and citrate markedly retard the rate of α1-AT polymerization *in vitro* by specifically inhibiting the formation of intermediates that lead to polymerization, and have no effect on the ability of the serpin to undergo the structural rearrangements required for inhibition of serine proteases. At present, these modifiers only work *in vitro* and at very high concentration, but these studies indicate the possibility of one day modifying the process of polymerization with drugs *in vivo*. Another promising approach is the use of small, reactive loop peptides, which insert into β-sheet A of α1-AT thereby blocking polymerization. Identifying the crystal structures of serpin–peptide complexes should facilitate progress in the design of antipolymerization peptidomimetics (D. Lomas). A further encouraging strategy is the use of 4-phenylbutyrate (4-PBA), which seems to increase the cell-surface expression of CFTR(ΔF508), the most common cystic-fibrosis-causing allele (P. Zeitlin, Johns Hopkins Univ., Baltimore, Maryland). Similarly, treatment of alveolar macrophages expressing the α1-AT PiZ allele with 4-PBA was reported to increase the secretion of the mutant protein (M. Brantly, Univ. Florida, Gainesville), but the mechanism of 4-PBA action remains unknown. Although these approaches to suppress the pathogenic conformational changes are promising, it was clear from this meeting that understanding the cell-biological mechanisms by which abnormal protein structures lead to conformational diseases will be essential for the development of effective therapies.

The quest for the cure of a human disease nucleated a multidisciplinary meeting that integrated recent advances in structural biology, biophysics and cell biology. It was a successful demonstration of the synergism that can be realized by bringing together basic and clinical scientists, patients, pharmaceutical representatives and NIH officials working in different areas but sharing a common goal. □

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