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Oligomer formation by Na^+–Cl^--coupled neurotransmitter transporters

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Abstract

Na^+–Cl^--dependent neurotransmitter transporters (or neurotransmitter:Na^+ symporters, NSS) share many structural and functional features, e.g. a conserved topology of 12 transmembrane spanning α-helices, the capacity to operate in two directions and in an electrogenic manner. Biochemical and biophysical experiments indicate that these transporters interact in oligomeric quaternary structures. Fluorescence resonance energy transfer (FRET) microscopy has provided evidence for a constitutive physical interaction of NSS at the cell surface and throughout the biosynthetic pathway. Two interfaces for protein–protein interaction have been shown to be important in NSS; these comprise a glycophorin-like motif and a leucine heptad repeat. Upon mutational modification of the latter, surface targeting is considerably impaired without concomitant loss in uptake activity. This supports a role of oligomer formation in the passage of the quality control mechanisms of the endoplasmic reticulum and/or Golgi. In contrast, oligomerisation is dispensable for substrate binding and translocation.

Keywords: Na^+–Cl^--coupled neurotransmitter transporter; Neurotransmitter: Na^+ symporter; FRET (fluorescence resonance energy transfer); Microscopy; Oligomer; Dimer

1. Introduction

Neurotransmitters are released from the presynaptic specialisation to carry information to postsynaptic neurotransmitter receptors. Neurotransmitter transporters terminate the action of neurotransmitters by virtue of coupling their retrieval most economically to the steep sodium gradient over the plasma membrane (Rudnick and Clark, 1993; Chen et al., 2003). These membrane proteins are organised in distinct subfamilies with known substrate specificity (Amara and Kuhar, 1993); the members of the Na^+–Cl^--coupled neurotransmitter transporters or neurotransmitter:Na^+ symporter subfamily (NSS; according to the recently published transporter classification: Busch and Saier, 2002) comprise transporter proteins for monoamines (i.e. dopamine, norepinephrine and serotonin), amino acids (i.e. γ-amino butyric acid or GABA, glycine, proline and taurine) as well as osmolytes (i.e. betaine and creatine); in addition, there are the ‘‘orphan’’ transporters with to-date unknown substrate specificity (Masson et al., 1999). These proteins all share a common structural motif of 12 transmembrane helices. NSS proteins are of obvious clinical relevance: antidepressants, for instance, block the reuptake of noradrenaline and serotonin at their respective transporters. This was originally demonstrated some 40 years ago for the action of uptake inhibitors on noradrenaline reuptake (Axelrod et al., 1961). In the meantime, it has been amply documented that the norepinephrine and/or of the serotonin transporter are the site of action of several structurally diverse classes of inhibitors (Kent, 2000; Iversen, 2000). Drugs of abuse such as amphetamines and congeners like methylenedioxi-methamphetamine (better known as ‘ecstasy’) and metamphetamine induce reverse transport by the serotonin (Scholze et al., 2000) and dopamine transporter (Scholze et al., 2002a) and this is believed to underlie their psychotropic and addictive properties (Schloss and Williams, 1998). Cocaine is a powerful psychomotor stimulant and has rewarding/reinforcing properties, because it blocks the reuptake of dopamine in the *nucleus accumbens* (Carroll et al., 1999). The antiepileptic drug tiagabine blocks reuptake of GABA, the principal inhibitory neurotransmitter in the brain (Iversen, 2000).

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2. Neurotransmitter transporters are organised in oligomeric complexes

Many different membrane proteins are organised in complex quaternary structures (Klingenberg, 1981; Arkin, 2002). Increasing evidence suggests that this is also true for transporters (Veenhoff et al., 2002). Earlier approaches relied on rather harsh treatments to estimate the size of NSS and hence distinguish between a monomeric form and an oligomeric assembly. The methods were per se disruptive, because they relied e.g. on ionising radiation or on solubilisation by detergents. Radiation inactivation studies are based on the ability of high-energy electrons to irreversibly inactivate the target that is in this case to abolish transport activity or inhibitor binding. Target size analysis thus estimates the minimum size of the protein that is still functionally active. Upon exposure to X-rays, the human serotonin transporter (SERT; Plenge et al., 1990) displays a range of target sizes that is consistent with the monomer. In contrast, the size that was extracted from radiation inactivation data was indicative of a di- or tetrameric complex of the rat dopamine transporter (DAT; Berger et al., 1994; Milner et al., 1994). The estimated molecular mass based on the deduced amino acid sequence of the cloned DAT-cDNA is approximately 70,000 Da. Berger et al. (1994) reported values of 94,000 and 143,000 Da, whereas the experiments conducted by Milner et al. (1994) yielded values of 278,000 Da. Moreover, radiation inactivation studies yielded a variety of different target sizes of the sodium glucose transporter 1 (SGLT-1; Lin et al., 1984; Beliveau et al., 1990; Stevens et al., 1990; Veyhl et al., 1993). These estimates were in contrast to the conclusions reached using freeze-fracture electron microscopy; this approach visualized an asymmetrically shaped monomer of SGLT-1 (Eskandari et al., 1998). Clearly, these discrepancies highlight the limitations inherent in radiation inactivation. In fact, it is difficult to measure with high precision the range that is most relevant for the determination of the target size; that is the range of energy, where the bulk of the protein is being inactivated and hence their little signal to measure over the blank. Typically, it is therefore necessary to rely on extrapolation to extract an estimate of the target size. Given these limitations, it is not surprising that there are conflicting data.

If a hydrophobic membrane protein is solubilised by a detergent, the portions of the proteins that are not miscible with water are covered by the detergent micelle. Detergent micelles may thus invade the hydrophobic core of the protein and inactivate it. This phenomenon is commonly observed. Hence the choice of the correct detergent is in most cases done by trial and error. It is also obvious that, by this mechanism, detergents may dissociate oligomeric complexes. Alternatively, when used at amounts that do not suffice to cover the hydrophobic areas adequately, detergents may actually cause membrane proteins to aggregate. Thus, while in theory it is straightforward to determine the molecular mass of a protein by gel permeation chromatography, a brief practice with membrane proteins rapidly reveals its limitations and the imponderabilities arising from the properties of detergent micelles. Similarly, coimmunoprecipitation of membrane proteins is prone to analogous artefacts. Nevertheless, meaningful data have been obtained by gel filtration and these proposed an oligomeric state of the rat and human SERT some 10 to 20 years ago (Cesura et al., 1983; Habert et al., 1986; Ramamoorthy et al., 1993). These findings were supported by studies employing cross-linking of the rat SERT (Jess et al., 1996). Several recent studies showed the existence of oligomeric complexes in DAT and SERT by use of differently epitope-tagged constructs: coimmunoprecipitation experiments suggest the existence of functionally interacting di-, tetra- and higher order oligomers of the SERT (Kilic and Rudnick, 2000), DAT (Sorkina et al., 2003) and the norepinephrine transporter (NET; Kocabas et al., 2003).

If an inactivating mutation has a dominant negative effect, this can—by definition—only arise if the protein forms a complex. Dominant negative effects are exerted by naturally truncated forms (Kitayama et al., 1999; Hahn et al., 2003) and by engineered mutations (Ramsey and DeFelice, 2002; Torres et al., 2003) of NET and DAT oligomers. Finally, it has been shown that specific cross-linking of a cysteine^{306} on top of the sixth transmembrane domain of the human DAT increased its apparent molecular mass about twofold in non-reducing sodium dodecyl sulphate/polyacrylamide gel electrophoresis; this higher molecular weight complex is most plausibly explained as a symmetrical homo-dimeric complex of the human DAT (Hastrup et al., 2001). This conjecture has been supported by a zinc-site engineered on top of transmembrane domain 6 that mediated a potent inhibition of dopamine uptake upon high-affinity binding of Zn^{2+} (Norgaard-Nielsen et al., 2002). The enumeration listed up to here tacitly implies that all NSS-family members are oligomers. Therefore, it is worth noting that the glycine transporter (GLYT) has repeatedly been shown to be a monomer (Lopez-Corcuera et al., 1993; Haugeto et al., 1996; Horiuchi et al., 2001).

Oligomeric assembly is not confined to members of the NSS-family; it has also been described for members of other neurotransmitter transporter families. Oligomerisation is the rule, if the other pharmacologically relevant neurotransmitter transporter family is considered, i.e. the excitatory amino acid transporter or glutamate transporter family (Seal and Amara, 1999). However, the number of subunits per oligomer differs, if individual members are investigated: a mixed dimeric/trimeric form has been described for rat excitatory amino acid transporter 1/glutamate transporter (rEAAT1/GLAST; Haugeto et al., 1996), a trimeric form for rEAAT2/glutamate transporter (rEAAT2/GLT-1Haugeto et al., 1996), a pentameric assembly for hEAAT3 (Eskandari et al., 2000) and a dimeric form in hEAAT4 (Dehnes et al., 1998).

2.1. Constitutive oligomer formation of NSS in living cells

Thus, the data summarized above indicate that the disruptive biochemical and biophysical approaches yield divergent results. As will be argued below, some of the discrepancies are only spurious and they can be reconciled if the data are reinterpreted with hindsight. This is particularly true for target size analysis. In addition, conclusive evidence remained scant for oligomer formation in situ. A method has recently been developed that allows to study the dynamic association of proteins in living cells (Pollok and Heim, 1999). This approach relies on fluorescence resonance energy transfer (FRET) which was first described by Förster (1948). FRET is a quantum physical phenomenon which occurs only if two fluorophores are in sufficiently close proximity (< 100 Å) and in an appropriate relative orientation allowing an excited fluorophore (donor) to transfer its energy to a second, longer-wavelength fluorophore (acceptor) in a non-radiative manner (Pollok and Heim, 1999; Schmid and Sitte, 2003). Upon excitation of the donor at its specific wavelength, distinct light emission from the acceptor ensues at a substantial loss of emission from the donor. Suitable FRET pairs comprise the cyan and yellow fluorescent proteins (Wouters et al., 2001; Schmid and Sitte, 2003). FRET-based approaches have been successfully applied to monitor the physical association of membrane proteins like G-protein coupled receptors (Milligan and White, 2001) or ion channels (Riven et al., 2003) in the membrane of living cells, and this constitutes the major advantage of the method. It has been introduced into the field of NSS to visualise the oligomeric state of several members of the NSS subfamily in living cells: the human SERT and the rat GAT-1 (Schmid et al., 2001), and, most recently, the human DAT (Sorkina et al., 2003). These studies unequivocally prove that SERT, GAT-1 and DAT form constitutive oligomers in the membranes of living cells and throughout the biosynthetic and recycling pathway. However, the potential of FRET-based techniques has not yet been exhausted. It is to be expected that additional donor–acceptor pairs will be developed. It is also likely that additional information will be extracted by determining the extent of fluorescence anisotropy (Riven et al., 2003).

2.2. Structural basis/motifs of NSS oligomerisation and stoichiometry of the complex

Glycophorin A and phospholamban are among the best characterised α-helical membrane proteins that form oligomeric complexes. Glycophorin A (Dieckmann and DeGrado, 1997) resides as a dimer in the erythrocyte membrane. Phospholamban is a myocardial protein that regulates the efficiency of the Ca^{2+}-pumping ATPase in the sarcoplasmic reticulum and that can exist in a pentameric form (Arkin et al., 1997). The elucidation of the structural motifs that underlie the oligomerisation of these two prototypic membrane proteins have been comprehensively reviewed elsewhere (White and Wimley, 1999; Popot and Engelman, 2000; Arkin, 2002; DeGrado et al., 2003). The minimal structural motif found in Glycophorin A that drives dimerisation is "GxxxG" (MacKenzie et al., 1997; Gurezka et al., 1999) in a manner described metaphorically as knob-in-the-hole or groove-to-ridge interaction (White and Wimley, 1999). A leucine heptad repeat, i.e. four leucine side chains spaced by six intervening amino acids, is the structural motif that drives oligomerisation in phospholamban. This leucine heptad repeat supports the formation of a 'leucine zipper' which was originally described for transcription factors (Heldin, 1995). The potential importance of these two motifs is underscored by the fact that they both occur in the amino acid sequence of NSS-family members; the degree of conservation is obviously a matter of debate (see Fig. 1A). The GxxxG-motif has been described in the sixth transmembrane domain of the DAT (Hastrup et al., 2001), however, it is also present in other NSS family members (Fig. 1B). A perfect leucine heptad repeat is found in transmembrane domain 2 of

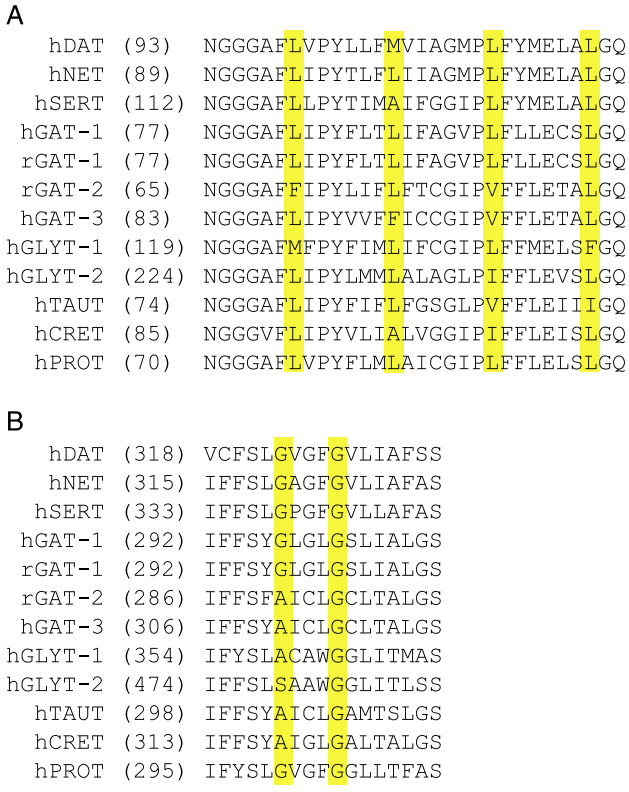

Fig. 1. Sequence alignments of transmembrane domains 2 and 6. The sequences of NSS-family members are shown for transmembrane domain 2 (Panel A) and transmembrane domain 6 (Panel B) to indicate the relative conservation of the leucine heptad repeat-motif (LxxxxxxL, Panel A) and the glycophorin motif (GxxxG, Panel B): Note that the first glycine of the GxxxG-motif may be substituted by an alanine (Lemmon et al., 1994). The number of the first residue of each sequence shown is given in parentheses. DAT: human dopamine transporter, NET: norepinephrine transporter, SERT: serotonin transporter, GAT: GABA transporter, GLYT: glycine transporter, TAUT: taurine transporter, CRET: creatine transporter, PROT: proline transporter; 'h' and 'r' denote the species: human and rat, respectively.

the rat GAT-1 (leucines in positions 83, 90, 97 and 104; (Scholze et al., 2002b). Other family members like the DAT, NET or SERT contain only leucine heptad repeats where one leucine has been replaced by other amino acid side chains (third repeat, in position 103 of the DAT = methionine; second repeat, in position 125 of the SERT = alanine; see Fig. 1A; Amara and Kuhar, 1993). It is interesting to note that an incomplete leucine heptad repeat has also been described in a bacterial homologue of the NSS (Androutsellis-Theotokis et al., 2003). This supports the notion that the LxxxxxxL-motif represents a highly conserved motif throughout the evolution of the transporter proteins. However, it is obvious that the interaction motifs important for oligomerisation of NSS may not be limited to these two: other motifs (Liu et al., 2002) have to be tested for their usefulness in the search for the complete structural basis of oligomer formation.

It has been convincingly shown that the Glycophorin A-like motif GxxxG supports formation of a symmetrical dimer in the DAT (Hastrup et al., 2001). This is bona fide evidence for a homophilic interaction in the DAT oligomer. We reported that the leucine heptad repeat supported oligomer formation in a NSS family member, namely the rat GAT-1. The evidence was provided by progressively exchanging the leucines with alanine residues and by documenting the oligomeric state of the resulting mutants by FRET microscopy (Scholze et al., 2002b). Our conclusion has been confirmed for the hDAT (Torres et al., 2003). Recently, we recognised a third, discontinuous interaction domain in the human SERT that is located in transmembrane domain 11/12 (Just et al., 2003; and submitted). The structural motif that supports the formation of this third contact site is still enigmatic. However, the fact that a minimum of three interaction sites have been recognised (transmembrane domains 2 and 6, which is homophilic, and transmembrane domains 11 and 12) now casts strong doubts on the existence of a self-sustained oligomeric complex in which all interaction sites engage in homophilic contacts. Several studies support a significant contribution of the leucine heptad repeat in oligomer formation of transmembrane domain proteins (Arkin et al., 1994; Cornea et al., 2000; Gurezka et al., 1999; Choma et al., 2000; Zhou et al., 2000, 2001; Scholze et al., 2002b). Thus, by analogy with soluble proteins, a leucine zipper-like structure may exist that forms the interaction domain in transmembrane domain proteins. Nevertheless, this leucine zipper-like basis for an oligomeric interaction in NSS must be refuted in a definitive manner if one considers that oligomers have to be arranged in self-limiting structures. This has been suggested on the basis of a logical argument already more than 20 years ago (Klingenberg, 1981). Furthermore, several additional objections argue against a leucine-zipper in transmembrane domain proteins: (i) polyleucine segments show per se limited propensity to associate into complexes within the lipid bilayer (Zhou et al., 2000); (ii) leucine heptad repeats have been examined in ion channels, such as the *shaker* K^+-channel or L-type calcium channels (McCormack et al., 1991; Garcia et al., 1997, respectively). In these instances, the leucine heptad repeats were found to support gating rather than oligomer formation. Thus, we conclude that a classical leucine zipper is unlikely to represent the structural basis for oligomer formation in NSS. Furthermore, because of the existence of three contact sites, we deduce that heterophilic interaction domains stabilize the first dimer (presumably via interaction of transmembrane domain 2 with transmembrane domains 11/12). Analogous heterophilic interaction domains explain the contact points of the bacterial EmrE-oligomer (Soskine et al., 2002). Furthermore, in this model, the homophilic interaction between two transmembrane domains 6 will then drive the formation of a "dimer of dimers" (Fig. 2; Just et al., 2003; and submitted). This arrangement has the added attractiveness that is does not violate fundamental topological rules (Klingenberg, 1981).

Obviously, one is tempted to generalise this model. This temptation is further augmented by the fact that the vast majority of helical membrane proteins, for which a high-resolution structure is available, are organised in oligomers (Arkin, 2002); see also http://blanco.biomol.uci.edu. However, the available high-resolution data also underscore the danger inherent in generalisation: The multidrug resistance transporter AcrB is a trimer (Murakami et al., 2002); in

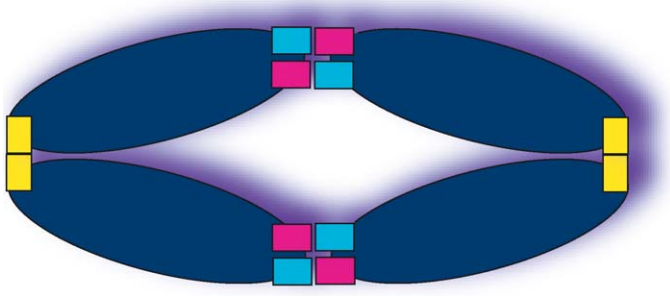

Fig. 2. A putative model for a tetrameric form of NSS. To illustrate the findings about interaction domains so far, we construct a putative tetrameric model of a given NSS. The heterophilic interaction domains transmembrane domain 2 and transmembrane domain 12 are coloured in blue and red, the homophilic interaction domain in transmembrane domain 6 is marked in yellow.

contrast, the Na^+/H^+-antiporter NahA exists in dimeric form (Williams, 2000). Finally, as mentioned earlier, there is little evidence to suggest that the glycine transporter GlyT1 is anything but a monomer.

3. The biological significance of oligomer formation

3.1. Impact on activity

It has been hypothesised more than two decades ago that most membrane proteins form oligomeric complexes at the cell surface (Klingenberg, 1981). Although this notion has been corroborated for most but not all NSS, the role of NSS oligomerisation still remains enigmatic. Moreover, it has not been firmly established thus far whether transport activity relies on the organisation of the transporter in a quaternary structure. The evidence obtained with bacterial transporters suggests that the quaternary structure only plays a modest role in controlling the transport cycle (Veenhoff et al., 2001) and that the monomer suffices for transport.

However, differently epitope-tagged hSERT with distinct sensitivity to uptake inhibition induced by methanethiosulfonate compounds have been used to infer a functional interaction between the subunits of hSERT dimers (Kilic and Rudnick, 2000). Furthermore, evidence for the functional oligomerisation of the human NET and DAT has been described using dominant-negative mutants (Kitayama et al., 1999; Torres et al., 2003).

Oligomerisation is frequently encountered in ion channels (Rosenmund et al., 1998; Jentsch, 2002); channel-like properties have been attributed to a subset of the members of the NSS-family (Sonders and Amara, 1996). Thus, it has been speculated that pore-structures are formed by several monomeric entities in a higher order SERT oligomer; in this complex, the pore-lining residues are thought to be contributed from several transmembrane domain 1 segments (Adams and DeFelice, 2002). However, such a structural arrangement is not likely to be supported by the experimental evidence gathered so far (see above and Fig. 2).

Both, evidence for and against the multimeric protein representing the functional unit of the transport complex has been reported for other transport proteins. Band 3/AE1, the erythrocyte anion exchanger 1, is a dimeric protein at the cell surface (Jennings and Smith, 1992; Cuppoletti et al., 1985). However, inhibition of one subunit in the dimer does not affect the transport properties of the other subunit; thus, the monomer is the functional unit within the dimeric quaternary organisation (Macara and Cantley, 1981). A dominant-negative approach revealed no significant interactions of wild type glucose transporter 3 (GLUT3) coexpressed in *Xenopus laevis* oocytes with an up to threefold greater amount of a functionally inactive GLUT3-mutant (Burant and Bell, 1992). Furthermore, biochemical studies revealed that the members of the Na^+/H^+-exchanger (NHE) family are organised as oligomers, but the minimal functional unit is the monomer (Fafournoux et al., 1994; Gerchman et al., 2001). This has been confirmed by two-dimensional crystallography (Williams et al., 1999). For the Na^+/P_i-cotransporter, a similar conclusion was reached in an eletrophysiological approach by coexpression of the wild type and a functionally distinguishable mutant thereof (Köhler et al., 2000).

3.2. Impact on sorting/targeting

As mentioned above, mutations in the leucine heptad repeat of GAT-1 abolished oligomer formation. These mutants therefore provided the opportunity to address the question, if this transporter functions as a monomer or as an oligomer. The observations were clearcut; mutational exchange of the leucines to alanines abolished FRET and led to intracellular retention of the mutant transporters (Scholze et al., 2002b). Nevertheless, in vesicles prepared from human embryonic kidney cells expressing the mutant transporters, the leucine to alanine mutations did neither affect the affinity for the substrate GABA nor the turnover number of uptake (Scholze et al., 2002b). Thus, we conclude that oligomer formation is not needed to confer uptake activity by GAT-1. Rather, oligomerisation is important to pass the quality control of the endoplasmic reticulum and/or the Golgi to be targeted to the cell surface. This notion has been introduced as a general concept for membrane proteins (Ellgaard and Helenius, 2003). Most recently, a similar conclusion was reached for the human DAT (Torres et al., 2003).

4. The benefit associated with oligomerisation—a speculative explanation

It is at present unclear in which compartment the transporters are being retained if they accumulate as monomers. Proofreading is thought to occur primarily in the endoplasmic reticulum. Misfolded membrane proteins are thought to be retained (Ellgaard and Helenius, 2003), because they do not adopt a stable minimum energy conformation; the latter is a prerequisite for function (Pankevych et al., 2003). Eventually, the misfolded membrane protein may be cleared from the endoplasmic reticulum by retrograde transport through the translocation pore and by degradation in the proteasome (Petäjä-Repo et al., 2001). This degradation obviously requires ubiquitination. There is an additional proofreading step that occurs at the level of the Golgi stacks. In fact, the Golgi contains a specialized ubiquitin ligase (Tul1 = transmembrane ubiquitin ligase 1), which specifically recognises misfolded membrane proteins or membrane proteins with short transmembrane segments that contain exposed hydrophilic residues. Ubiquitination by this enzyme targets the proteins to the lysosomal compartment (Reggiori and Pelham, 2002). Alternatively, membrane proteins that escape from the endoplasmic reticulum may

be recognized by Golgi proof-reading and retrieved by retrograde transport to the endoplasmic reticulum. These quality control systems appear to operate in a stringent mode, because collectively they mediate the retention of membrane proteins that can otherwise function normally, if they reach the plasma membrane. The most conspicuous example is the ΔF508 mutation in the cystic fibrosis transmembrane regulator (CFTR; Pasyk and Foskett, 1995; Egan et al., 2002). The fact that the quality control system is set to be overprotective can be understood based on the following consideration: misfolded membrane proteins are particularly detrimental; because of their large hydrophobic segments, they are prone to aggregation. At the level of the plasma membrane, this may interfere with the function of adjacent "normal" membrane proteins. We propose that oligomerisation has evolved as an additional level of quality control; a given membrane protein must not only stabilize its individual folding domains to adopt a stable tertiary structure; the final conformation must also support the assembly of the oligomer. This hypothetical model is also supported by the following observation: mutations can be introduced into the carboxy terminus of DAT and these cause intracellular retention of DAT (Lee et al., 1996; Bjerggaard et al., 2002). The same is true for GAT-1 (Scholze et al., 2002b; Farhan et al., in preparation). However, in GAT-1 the truncation of the carboxy terminus does not eliminate FRET (Scholze et al., 2002b). Thus, oligomerisation is necessary but not sufficient for export from the endoplasmic reticulum and/or Golgi. There is an additional signal that is provided for by discontinuous segments of the transporter and that may possibly allow for the binding of proteins of the quality control machinery. Because of the topology of the transporters, these must be intracellular proteins; in other words they must be distinct from the well known endoplasmic reticulum chaperones such as calnexin.

5. Conclusion/outlook

Oligomerisation of transmembrane domain proteins has been documented for a plethora of membrane proteins. Its importance has just begun to be appreciated. Thus far, several examples exist that either suggest a role for oligomer formation within the tightly controlled cellular homeostasis (e.g. endoplasmic reticulum-quality control) or a deleterious effect: homo-oligomer formation of a mutant receptor tyrosine-kinase encoded by the *neu*-oncogene on the basis of a single point mutation (valine to glutamate) leads to constitutive activation (Weiner et al., 1989). In cystic fibrosis, mutations within the transmembrane domain segments of the cystic fibrosis transmembrane conductance regulator enhance intracellularly retained misfolded protein (Wigley et al., 2002). Hence, at the current early stage it is easy to define the future direction of research in this field: if the mechanisms underlying oligomer formation in membrane proteins are understood in greater detail, our expanded knowledge will allow us to analyse the role that protein–protein interactions play in disease. There are many diseases that arise from defective oligomerisation of membrane proteins. Thus, we may ultimately hope to efficiently counteract detrimental processes and restore normal function to these membrane proteins (Golemis et al., 2002).

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