CellPress

Remodeling Amyloid Fibers: Baker's Yeast Shows Us the Way

Eva Asp,[1] Ming Proschitsky,[1] and Rajaraman Krishnan[1,*]
[1]Neurophage Pharmaceuticals, 222 Third Street, Suite 3120, Cambridge, MA 02142, USA
*Correspondence: rkrishnan@neurophage.com
http://dx.doi.org/10.1016/j.chembiol.2015.08.001

Proteopathies are a large and diverse group of human diseases that are caused by protein misfolding. Well-known examples of proteopathies are Alzheimer's and Parkinson's disease, which are both linked to amyloid fibril formation. In this issue of *Chemistry & Biology*, Castellano et al. (2015) describe the way to harness the power of a protein from baker's yeast, Hsp104, to disaggregate the fibrils.

Amyloid fibrils are highly ordered β sheet-rich protein aggregates associated with a large number of neurodegenerative diseases but also with normal biological functions such as storage of peptide hormones in secretory granules (Greenwald and Riek, 2010). The identification of amyloid-forming endogenous peptides in semen and their implications in HIV infectivity further demonstrate the diverse functions of amyloid fibrils (Münch et al., 2007). Despite an increased understanding of amyloid assembly both in vitro and in vivo, it has proven difficult to find effective therapeutics for diseases caused by amyloid proteins. In most neurodegenerative diseases, the progression and severity of the disease depends on the accumulation of several types of misfolded species (oligomers, protofibrils and fibrils) that form during the assembly of amyloids. Because these species have variable conformations and structural details that are not well understood, it has been challenging to develop therapeutics targeting all the conformers simultaneously. Hence, there has been a great effort to generate therapeutics targeting the common structural features in these misfolded aggregates. One approach has been to engineer conformational antibodies that recognize oligomers, protofibrils, and mature fibrils to block toxicity and fibril assembly (Haupt and Fändrich, 2014; Kayed et al., 2003).

Protein misfolding and aggregation is a common feature of cell physiology. To prevent the damaging effects of protein misfolding, cells have evolved various endogenous mechanisms such as chaperone pathways to ensure proper and rapid folding of nascent polypeptide chains and degradation of improperly folded proteins (Brandvold and Morimoto, 2015). The paper by Castellano and coworkers presents a novel approach in the use of a modified, yeast-specific chaperone Hsp104 as a possible therapeutic to remodel amyloids in semen and reduce HIV infectivity and transmission (Castellano et al., 2015). Hsp104 is a disaggregase involved in the recovery after stress-induced protein aggregation in *S. cerevisiae* and the only cellular component known to remodel amyloid fibrils (Glover and Lindquist, 1998). Hsp104 has been shown to have diverse substrate specificity and can disaggregate toxic oligomers, a pre-fibrillar state capable of self-templating, as well as amyloid fibrils formed from a large number of proteins (Shorter and Lindquist, 2004). The fact that its specificity toward different amyloids can be modulated by minor changes in the amino acid sequence makes it a very promising candidate for designing amyloid therapeutics (Jackrel and Shorter, 2015).

Castellano et al. describe in vitro Hsp104 remodeling and aggregate clustering of amyloid fibrils from proteolytic fragments of prostatic acid phosphatase (PAP) and semenogelin 1 (SEM1). PAP248-286 (SEVI) fibrils incubated with Hsp104 were remodeled into non-amyloid aggregates with reduced binding to the amyloid binding dye Thioflavin-T (ThT). The transformation of cross β sheet-rich structures to amorphous aggregates was also visualized by transmission electron microscopy (TEM). The remodeling does not generate increased levels of monomers and the resulting non-amyloid aggregated material had lost its seeding capacity. Next, the authors explore the remodeling activity of an enhanced variant of the disaggregase Hsp104^{A503V} that has previously been shown to remodel TDP-43 and α-synuclein protein aggregates implicated in frontotemporal lobar dementia (FTLD), amyotrophic lateral

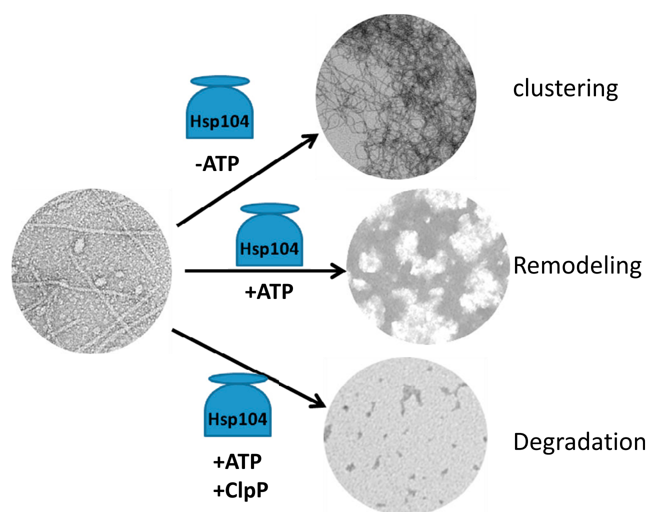

Figure 1. Hsp104 Mediated Clustering and Remodeling of Seminal Amyloids
Hsp104 mediated remodeled aggregates can be degraded efficiently in the presence of cellular proteases.

CrossMark

sclerosis (ALS), and Parkinson's disease, respectively. Hsp104[A503V] reduced ThT binding to SEVI fibrils by 50% after 2 hr incubation with an EC_{50} of 36 nM, a 20-fold decrease compared to wild-type Hsp104. Similar results were observed with an additional proteolytic fragment from the same protein, PAP85-120. The fibril remodeling activity of Hsp104 does not appear to be conserved between different amyloid forming peptides in semen because the semenogelin (SEM145-107) fragment was not remodeled by either Hsp104 or the enhanced variant (Figure 1).

In addition, Hsp104 was found to promote the formation of larger aggregates of seminal amyloids in an ATP-independent manner. The dual activity of Hsp104 to either remodel fibrils into large amorphous aggregates with less cross β sheet-rich structure or cluster fibrils into larger aggregates appeared to be fibril specific. Various shorter versions of the protein analyzed for remodel and clustering activity showed that amyloid remodeling requires Hsp104 to be in the active hexameric form as well as ATP hydrolysis whereas the backbone of Hsp104 is sufficient for the clustering of amyloid into larger aggregates. Further, the remodeled amorphous material was readily degraded by the bacterial peptidase, ClpP associated with HAP, a modified Hsp104 variant. A cell-based assay was used to evaluate the effect of various Hsp104 variants on seminal fibril remodeling, clustering, degradation and HIV infectivity. As expected, the remodeled material with less β sheet-rich protein aggregates had reduced HIV infectivity and treatment with the enhanced Hsp104 yielded the most significant reduction. Interestingly, fibrils that are not remodeled by Hsp104 but clustered together forming larger aggregates also show reduced HIV infectivity positively correlated with aggregation size.

How do we apply this information to design therapeutic molecules, specifically to treat amyloid diseases? The large size of the disaggregase and lack of prior knowledge about manufacturing and administering large protein complexes makes it difficult to consider Hsp104 as a therapeutic candidate against amyloids. Further, ensuring proper assembly of the active chaperone complex in the complex milieu of aggregating proteins and providing a constant supply of nucleotides would be challenging.

Alternatively, we suggest two more feasible strategies to design and develop therapeutic molecules against amyloids: grafting the substrate binding domain to therapeutic scaffolds and engineering amyloid-specific nanomachine disaggregators.

Hsp104 is a suitable candidate for grafting the substrate binding domain to therapeutic scaffolds because these studies show that Hsp104 is capable of binding a wide range of substrates in a sequence-independent or conformation-dependent manner. A large fraction of the potentiated variants that bind unnatural substrates only show alterations in their ATPase activity and altered interdomain communication. Therefore, if the precise substrate-binding domain is identified, transferring this to an antibody or smaller more stable structure like the nanobody or single chain antibody (Perchiacca et al., 2012) could allow generation of new class of potent amyloid-specific conformational binders.

Hsp104 is also a suitable candidate for engineering amyloid-specific nanomachine disaggregators because it can both bind to a diverse group of misfolded structures and remodel them. To do this, Hsp104 uses the peristaltic mechanism of substrate threading facilitated by a sequential hydrolysis of ATP molecules and an associated re-arrangement of the different domains in the active hexamer. If this movement is replicated in a monomeric protein with 2 domains, one for binding substrates transiently and the other to induce conformational change in the substrate-bound complex, then it could be engineered to bind amyloid substrates and induce its remodeling. Hsp104 variants that fail to multimerize but retain substrate binding and ATPase activity (partial or complete) could be good scaffolds to begin with. Other molecules like the prolyl isomerases SlyD and FkpA or the TolA binding phage protein g3p could also function as good scaffolds (Schmidpeter and Schmid, 2015). All these molecules trigger conformational changes in their domains by a *cis-trans* proline isomerization. This would also eliminate the need for ATP to fuel the conformational switch. Interestingly, the phage protein g3p binds a large number of amyloid proteins in a conformation-dependent manner with very high affinity (Krishnan et al., 2014) (Figure 2).

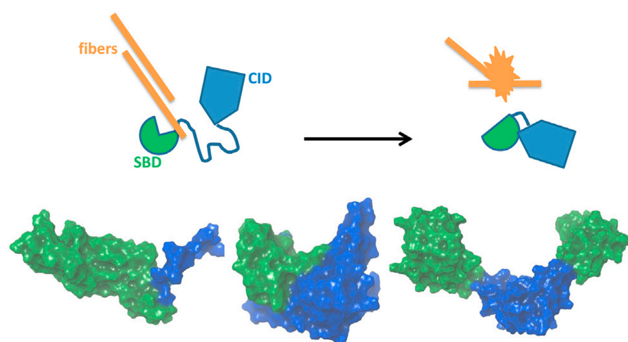

Figure 2. Schematic Representation of Amyloid Remodeling Nanomachines
The substrate binding domain (SBD) and conformational change inducing domain (CID) work in tandem to bind and remodel fibers. SlyD, g3p, and FkpA are alternate scaffolds that can be engineered to bind and remodel aggregates.

S. cerevisiae harbors a large number of self-replicating protein aggregates. These aggregates act as heritable epigenetic elements that allow the fungi to survive in diverse and often harsh environmental conditions. The chaperone networks in these organisms have been evolved to maintain protein aggregates without causing any detrimental effects to the cells. With most traditional approaches to make therapeutic molecules against amyloids failing over the last two decades, we should probably realign our approach to select therapeutic molecules based on conformational binders like the metazoan Hsp104 that remodel amyloids without any toxic side effects.

REFERENCES

Brandvold, K.R., and Morimoto, R.I. (2015). J. Mol. Biol. Published online May 21, 2015. http://dx.doi.org/10.1016/j.jmb.2015.05.010.

Castellano, L.M., Bart, S.M., Holmes, V.M., Weissman, D., and Shorter, J. (2015). Chem. Biol. *22*, this issue, 1074–1086.

 ©2015 Elsevier Ltd All rights reserved

CellPress

Glover, J.R., and Lindquist, S. (1998). Cell *94*, 73–82.

Greenwald, J., and Riek, R. (2010). Structure *18*, 1244–1260.

Haupt, C., and Fändrich, M. (2014). Trends Biotechnol. *32*, 513–520.

Jackrel, M.E., and Shorter, J. (2015). Prion *9*, 90–109.

Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W., and Glabe, C.G. (2003). Science *300*, 486–489.

Krishnan, R., Tsubery, H., Proschitsky, M.Y., Asp, E., Lulu, M., Gilead, S., Gartner, M., Waltho, J.P., Davis, P.J., Hounslow, A.M., et al. (2014). J. Mol. Biol. *426*, 2500–2519.

Münch, J., Rücker, E., Ständker, L., Adermann, K., Goffinet, C., Schindler, M., Wildum, S., Chinnadurai, R., Rajan, D., Specht, A., et al. (2007). Cell *131*, 1059–1071.

Perchiacca, J.M., Ladiwala, A.R., Bhattacharya, M., and Tessier, P.M. (2012). Proc. Natl. Acad. Sci. USA *109*, 84–89.

Schmidpeter, P.A., and Schmid, F.X. (2015). J. Mol. Biol. *427*, 1609–1631.

Shorter, J., and Lindquist, S. (2004). Science *304*, 1793–1797.

New Griselimycins for Treatment of Tuberculosis

Ulrike Holzgrabe[1,*]
[1]Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, 97074 Wuerzburg, Germany
*Correspondence: ulrike.holzgrabe@pharmazie.uni-wuerzburg.de
http://dx.doi.org/10.1016/j.chembiol.2015.08.002

Griselimycin (GM), a natural product isolated a half century ago, is having a bit of a renaissance. After being known for more than 50 years, it is now being pursued as a treatment for tuberculosis. With the new mechanism of action, excellent in vitro and in vivo activity against sensitive and drug-resistant *Mycobacterium tuberculosis*, and the improved pharmacokinetic properties, the cyclohexyl derivative of GM demonstrates a high translational potential.

Tuberculosis (Tb), caused by *Mycobacterium tuberculosis* (Mtb), is one of the major global infectious diseases. Even though 9 million cases were reported in 2013, with 1.5 million deaths per year (World Health Organization, 2014), the treatment of the infection is managed by old drugs discovered in the 1950s and 1960s. The regimen starts with a four drug combination consisting of isoniazid, rifampicin, ethambutol, and pyrazinamide for 2 months, followed by the combination of isoniazid and rifampicin for another 4 months. In 2012, after some 40 years of almost no progress in anti-Tb drug discovery, two new drugs were approved: bedaquiline, which targets the ATP synthase, and delamanid, a nitroimidazole that releases toxic nitric oxide when metabolized. Both bedaquiline and delamanid are active against multidrug-resistant (MDR) tuberculosis and thus are able to tackle the huge resistance problem. Some additional compounds are in the pipeline in different stages of clinical trials.

Along with the urgent need to solve the problems with MDR and extremely drug-resistant (XDR) strains, there is also a real necessity to find new antibiotics, which will allow a far shorter treatment with a lower number of drugs. Moreover, the new anti-infectives must work well with other drugs because Tb is often associated with HIV, especially in sub-Saharan Africa (where there were approximately 350,000 death in 2013), and diabetes. Finally, because Tb occurs mostly in low-income countries, the new drugs should be cheap. The bottom line is that new, effective, safe, and cheap anti-Tb drugs with different mechanism of action must be developed to address the significant challenge that Tb poses for public health.

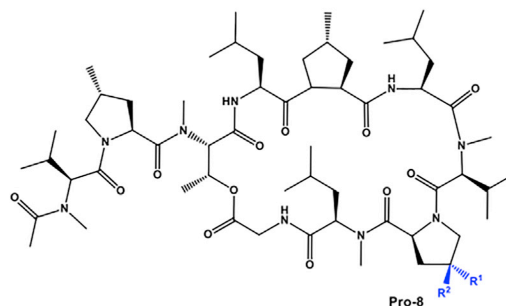

Figure 1. Structural Formula of Griselimycin
Alkylation of the proline ring in position 8 reveals an improvement of the pharmacokinetics.

Most molecules that are currently being explored as anti-Tb treatments options fall into the small molecule category, although there is an increasing interest in exploring natural products in this context. Cyclic peptide griselimycin (GM, Figure 1) was isolated in the 1960s from *Streptomyces* and its antibacterial and antimycobacterial activity was evaluated in the early 1970s (Terlain and Thomas, 1971a, 1971b). The early work established that GM has unfavorable pharmacokinetic properties, and, given that rifampicin was approved at that time, the development of GM as an anti-Tb drug was terminated. Recently, Rolf Müller and colleagues decided to revisit GM, given its high activity (Kling et al., 2015). First, they increased the metabolic stability of GM by alkylation of the proline residue in position 8. A cyclohexyl GM (CGM) derivative, obtained via a newly developed total synthesis, was metabolically stable, and the increased lipophilicity enhanced the penetration of the thick

 ©2015 Elsevier Ltd All rights reserved