RepA-WH1 prionoid: Clues from bacteria on factors governing phase transitions in amyloidogenesis

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ABSTRACT. In bacterial plasmids, Rep proteins initiate DNA replication by undergoing a structural transformation coupled to dimer dissociation. Amyloidogenesis of the ‘winged-helix’ N-terminal domain of RepA (WH1) is triggered in vitro upon binding to plasmid-specific DNA sequences, and occurs at the bacterial nucleoid in vivo. Amyloid fibers are made of distorted RepA-WH1 monomers that assemble as single or double intertwined tubular protofilaments. RepA-WH1 causes in E. coli an amyloid proteinopathy, which is transmissible from mother to daughter cells, but not infectious, and enables conformational imprinting in vitro and in vivo; i.e. RepA-WH1 is a ‘prionoid’. Microfluidics allow the assessment of the intracellular dynamics of RepA-WH1: bacterial lineages maintain two types (strains-like) of RepA-WH1 amyloids, either multiple compact cytotoxic particles or a single aggregate with the appearance of a fluidized hydrogel that it is mildly detrimental to growth. The Hsp70 chaperone DnaK governs the phase transition between both types of RepA-WH1 aggregates in vivo, thus modulating the vertical propagation of the prionoid. Engineering chimeras between the...
Sup35p/\([PSI^+]\) prion and RepA-WH1 generates \([REP-PSI^+]\), a synthetic prion exhibiting strong and weak phenotypic variants in yeast. These recent findings on a synthetic, self-contained bacterial prionoid illuminate central issues of protein amyloidogenesis.

**KEYWORDS.** amyloid proteinopathy, amyloid polymorphism, bacterial prionoid, Hsp70 chaperone, phase transitions, RepA-WH1

**INTRODUCTION**

Along the last few years, the field of functional amyloids, i.e., amyloid assemblies with a physiological role and no neuropathic side effects, has attracted much attention.\(^1,2\) Bacterial functional amyloids are invariantly extracellular, acting as scaffolding devices in building biofilms, three-dimensional networks that serve to colonize solid surfaces and confer to bacteria persistence against chemotherapy with antibiotics.\(^3\) Amyloids also serve as inactive storage deposits of the antimicrobial microcins.\(^4\) In eukaryotic cells, functional amyloids are either epigenetic determinants of non-Mendelian transmissible characters, as in the case of yeast prions,\(^5\) or scaffolding modules, as for mammalian Pmel17 in melanocytes;\(^6\) or even regulators of long-term synaptic potentiation, as proteins of the CPEB family from mollusks to mice.\(^7\) Among functional amyloids, the ability of the *Escherichia coli* curli system (CsgABC/CsgDEFG) to export nearly any protein tagged to a CsgA-derived secretion signal has enabled the extracellular screening of the amyloidogenic potential of whole proteomes and also of anti-amyloidogenic compounds.\(^8,9\)

The lack of natural intracellular amyloid proteinopathies in bacteria and the fact that expressing any amyloidosis-related human protein in *E. coli* leads to the formation of inclusion bodies, as many other heterologous mammalian proteins do, initially provoked the dismissal of bacteria as model organisms for studies on protein amyloidoses. Furthermore, although inclusion bodies exhibit amyloid features, they do not hamper bacterial viability in a significant way.\(^10\) Interestingly, aggregation is a natural resource in some bacterial proteins in which irreversible structural changes are used to transit across distinct, mutually exclusive, functional states.\(^1\) This is the case of the replication protein RepA of the *Pseudomonas* plasmid pPS10,\(^11\) which undergoes a transition through three association states, each of them linked to a defined function: from stable soluble dimers (transcriptional repressors of *repA* gene expression), RepA dissociates into metastable monomers (acting as plasmid DNA replication initiators), which then aggregate as oligomers that inhibit new rounds of DNA replication (by holding together two plasmid molecules through their replication origins).\(^12-15\)

In all these cases, binding to distinct DNA sequences in the plasmid triggers allosteric conformational changes that affect the structure of the N-terminal dimerization winged-helix domain (WH1).\(^16\) While tracking the molecular basis for the functional aggregation of RepA, in 2007 we reported that its isolated WH1 domain, in its mutant variant A31V, was able to assemble into amyloid fibers, built on the core amyloidogenic stretch L\(_{26}^\text{VLCAVSL}_{34}\) and upon a conformational change promoted by transient WH1 binding to a short plasmid-specific dsDNA sequence.\(^17\) We proved that, similarly to its action enabling DNA replication initiation through RepA monomerization,\(^13,16\) dsDNA acted as an allosteric effector on RepA-WH1 amyloidogenesis by enhancing its assembly into amyloid fibers.\(^17\) Furthermore, we showed that a small organic molecule (S4-indigo) was able to inhibit such a process by competing with DNA for the binding to RepA-WH1 *in vitro*.\(^18\) Aiming to characterize RepA-WH1 amyloidogenesis *in vivo*, we discovered that expressing fusions of the bacterial protein to a fluorescent protein tracer (mCherry/mRFP), thus replacing the natural C-terminal WH2 domain in RepA,\(^12,13\) generated in *E. coli* a synthetic amyloid proteinopathy that severely reduced
bacterial proliferation and finally led to cell death. These *in vivo* generated aggregates templated the amyloid conformation on soluble RepA-WH1 molecules *in vitro*, as they also did in a mutant variant-specific way *in vivo*, a signature for prion and prion-like proteins. These findings were already discussed in another publication in this journal dated in 2011 and thus will not be treated extensively again here. Instead, we will now review novel results recently reported on the RepA-WH1 prionoid and provide additional insight on the topic.

**Structural Polymorphism in RepA-WH1: from Amyloid Nano-Springs and Tubes to Chimeric Yeast Prions**

Structural polymorphism, i.e. the alternative ways in which a particular macromolecule can arrange its tertiary and/or quaternary structures, is an intrinsic feature of amyloid assemblies. Polymorphism has been described from the subtleties of the packing of protein side chains and strands in cross-β assemblies to the pitch of twisted amyloid fibers. Moreover, the ways and strengths in which phenotypes associated to a particular amyloidogenic protein show up, either in disease or within a physiological function, bear an intimate relation with polymorphism.

Recent electron microscopy (EM) and atomic force microscopy (AFM) studies on the amyloid fibers, assembled *in vitro* by templating on soluble RepA-WH1 molecules the amyloid conformation present in the RepA-WH1-mCherry aggregates purified from bacteria, revealed that the characteristic ≈25 nm-wide amyloid fibers are actually composed of several coiled filaments, each of them with ≈4 nm width (Fig. 1A). In turn, these filaments consist either in a single or a double thread of RepA-WH1 molecules that, with the limitations imposed by the low resolution of the EM reconstruction, are built by distorted monomers. This was inferred from the loose fit of a model based on the crystal structure of a replication-competent monomeric WH1 domain into the EM volume, as well as from circular dichroism (CD) spectra showing an increase in β-sheet structure upon RepA-WH1 assembly, as expected in any amyloidogenesis. Polymorphism is thus
manifested at three levels of increasing complexity: the number of threads that constitute the amyloid filaments (either one or two); the number of filaments per fiber bundle (mode value, 6); and variations in the pitch (on average, 64 nm) of the superhelix that results from twisting several filaments into the mature fibers. Disregarding if the assembly of the fibers was triggered by dsDNA or by templating with purified RepA-WH1-mCherry seeds, and by either leaving the samples to stand in the fridge or agitating them at higher temperatures, which respectively retards or accelerates amyloidogenesis, the fibers generated exhibit...
the aforementioned polymorphism.27 Interestingly, the overall architecture of the twisted RepA-WH1 amyloid filaments can be described as hollow springs (single) or tubules (double filaments) with an axial cavity with a diameter around 2.5 nm. The latter is close to the average dimension of a similar cavity in the short tubular proteotoxic oligomers assembled by α-synuclein.28 If this is a mere coincidence or if it points to a common mechanism of cytotoxicity for both proteins (e.g., the assembly of pores at membranes) remains to be determined.

Regarding the lateral association of RepA-WH1 filaments, it is known for other amyloid fibers that divalent anions, in particular SO₄²⁻, play a role in bridging them together, side-by-side.28-30 In the crystal structure of a RepA-WH1 dimer (PDB entry 1HKQ),13 two symmetry-related molecules were in contact through a PO₄³⁻ ion, which established a triple salt bridge between Lys85 in one molecule and Lys62 and His63 in the other. During the crystallization attempts, it became evident that incubation with monovalent anions (such as Cl⁻ or acetate) did not result in crystal growth, whereas SO₄²⁻ rendered poorer diffracting crystals, although this anion yielded the best fibers. The dependence on monovalent anions for RepA-WH1 crystallization matches their requirement in the lateral association of amyloid filaments to build the mature fibers. This could be interpreted as resulting from the overall electro-positive charge of the RepA-WH1 molecule,31 because lowering the ionic strength enhances the dissociation of the fiber ends to the point of releasing their constituent filaments.27

What we have learnt on the structure of RepA-WH1 fibers opens an intriguing possibility: that the kind of assembly that monomers of this protein build upon amyloidogenesis could bear some relation to the regulatory complexes of the full-length RepA protein that inhibit new rounds of amplification in recently replicated plasmids.15 This hypothesis, that needs experimental support, would imply that such regulatory complexes would be a new case of functional amyloid and so the cytotoxic RepA-WH1 prionoid would be the outcome of decoupling amyloidogenesis from its natural linkage to WH2 and plasmid replication,12,13 thus unveiling the proteotoxic potential typical of amyloids.

Aiming to test in vivo the amyloidogenic potential of the L₂⁶VLCASVL₁₃₄ stretch in RepA, 1-5 repeats of this sequence were used to replace, following the design of a parallel superpleated β-structure,32 the oligopeptide Q/N-rich repeats at the N-terminal domain yeast protein Sup35p that, in its aggregated state, constitutes the [PSI⁺] prion.33 As previously proved for a number of other mutant and heterologous sequences,34-36 the resulting chimeric protein enables an epigenetically inheritable prion, named [REP-PSI⁺],33 capable of reading-through the stop codon UAG, the phenotype characteristic of [PSI⁺],5 provided that at least three repeats of the bacterial-related sequence were present.33 The simultaneous presence of hydrophobic (RepA-WH1 derived) and polar (from the Sup35p scaffold) sequences in [REP-PSI⁺] results in a gradation of weak prion phenotypes/variants, as assessed by the pink color of colonies and the generation of very large aggregates, as evident in semi-denaturing detergent agarose gel electrophoresis (SDD-AGE).33 Interestingly, biophysical analyses of these functional prion variants revealed that the presence of the hydrophobic RepA-WH1 repeats within the Sup35p prion domain increased the β-sheet structure while resulting in the formation of oligomers, not fibers as for the wild-type prion. The generation of [REP-PSI⁺] variants, which are mitotically stable in terms of propagation, illustrates the feasibility of engineering phenotypic traits in prions by fully exploiting the structural polymorphism of amyloids, from oligomers to fibers, as a constructive resource.37

DNA and Hsp70-Modulated Phase Transitions in RepA-WH1 Amyloidogenesis

The effect of dsDNA as an allosteric effector of protein amyloidogenesis in vitro was a seminal discovery in RepA-WH1 research.17 However, the evidence for a similar process in vivo was indirect, i.e., a role of DNA in promoting RepA-WH1 amyloidogenesis in E. coli cells was inferred from an increase in the number and size of protein aggregates when the vector...
expressing the prionoid carried tandem repeats of the DNA sequence that efficiently promoted amyloidogenesis \textit{in vitro}.\textsuperscript{19} The development of a monoclonal antibody (termed B3h7), characterized \textit{in vitro} as specific for a pre-amyloid oligomeric conformation on-pathway to mature RepA-WH1 fibers (Fig. 1A),\textsuperscript{38} has recently made possible to unveil, through immuno-electron microscopy (iEM) (Fig. 1B), that in \textit{E. coli} cells expressing the prionoid the very first amyloid oligomeric precursors are found at the bacterial nucleoid,\textsuperscript{38} as expected from the known ability of DNA to promote RepA-WH1 amyloidogenesis \textit{in vitro}\textsuperscript{17,18} and possibly from the original function of the WH1 domain when, in the whole RepA, controls plasmid DNA replication.\textsuperscript{15} It was found, also by iEM, that amyloid particles exceeding \(\approx\)100 nm in diameter are placed out of the nucleoid territory,\textsuperscript{39} as expected if entropic exclusion by the nucleoid were playing a mayor role in the localization of the aggregates. These findings could have implications for the preferential or alternative tropism of amyloid aggregates toward the cytoplasm or the nucleus in some human neurodegenerative proteinopathies, or in the case of proteins such as FUS, whose ability to assemble functional ribonucleoparticles in either location becomes impaired in amyotrophic lateral sclerosis and frontotemporal dementia.\textsuperscript{40-42}

Concerning the intracellular inclusions formed by amyloidogenic proteins either in the cytoplasm or in the nucleus of eukaryotic cells, it is remarkable the recent description of the existence of phase transitions between soluble, hydrogel and fibrillar states, in some cases through intermediates with the appearance of liquid droplets.\textsuperscript{40-44} Such partitions imply changes in the association state beyond the quaternary structure, generating the so-called quinary structural level, having consequences for the functionality and bioavailability of the macromolecules involved. In many instances, these are proteins bearing domains with amino-acid compositions biased toward low complexities. In the case of Q/N/G-rich domains, phase transitions have been related with the package of mRNA in stress/P-granules and are relevant in amyloidogenesis.\textsuperscript{40-42} Interestingly, albeit RepA-WH1 lacks such simplified amino-acid composition and has a very stable fold in its native state, when assembled as cytoplasmic aggregates \textit{in vivo}, exhibits similar phase transitions, the first instance in which this has been observed in bacteria.\textsuperscript{39} In these experiments, a combination of fluorescence microscopy and microfluidics was used, because this enabled monitoring bacterial proliferation and the dynamics of RepA-WH1 aggregation at the level of multiple single cell lineages, for many generations (up to 200) and under controlled environmental conditions. For studies on amyloidosis, surveying the physiology of bacteria in microfluidic devices surpasses the approaches based on agarose pads in terms of the number of generations achievable before the cessation of growth, the feasibility to track lineages and the ease to inject into, or extract from, the cultures diverse molecules of interest. Two clearly distinct RepA-WH1 aggregates became evident at the microfluidic device (Fig. 1C): i) multiple globular, compact cytotoxic (G) particles that inhibit cell division; ii) a single, comet-shaped (C) aggregate that appears to be fluidized, i.e. elongates, contracts and undulates along the cell axis and readily splits between the daughter cells on cytokinesis.\textsuperscript{39} The outcome is that while bacteria expressing the G-type aggregates show a tendency toward filamentation and cells die after a few generations, those bearing the C-type aggregates keep dividing indefinitely at a reasonable rate. In an isogenic population expressing RepA-WH1, bacteria at distinct microfluidic channels propagate for generations one or the other type of aggregates, although interconversion (more commonly C to G) occurs at low rates.\textsuperscript{39} We also explored the effect of the Hsp70 chaperone DnaK on RepA-WH1 amyloidogenesis, because it is central in counteracting protein aggregation in bacteria and a role for DnaK in the activation of RepA-type proteins for plasmid replication was recognized for long.\textsuperscript{11} It is the activity of DnaK, i.e., its expression induced from a regulated promoter vs. its specific inhibition with myricetin, what determines the balance and switching (phase transition) between both kinds of aggregates: DnaK promotes the conversion of most cells to the elongated phenotype, whereas its inactivation results in the population dominated by the globular phenotype.\textsuperscript{39} iEM studies revealed that DnaK is preferentially located at the aggregate with an elongated and fluidized appearance, which
constitutes a territory with a clearly different density toward the electrons compared with that observed for the globular aggregates. Therefore this Hsp70 chaperone, most likely through its aggregate remodeling activity, is the factor determining quinary structural phase transitions for RepA-WH1 in the bacterial cytoplasm. On the contrary, the bacterial ClpB (Hsp100) chaperone has no apparent contribution to this effect. Coming to the point, the work carried out with our synthetic bacterial model prionoid suggests that the long sought cell factor(s) that would ameliorate amyloid cytotoxicity in human proteinopathies might be Hsp70 chaperones by promoting the assembly of pro-amyloidogenic proteins as non-fibrilar hydrogels.

**FURTHER PERSPECTIVES**

Ongoing research on the bacterial prionoid RepA-WH1 aims to trace a scheme of the molecular basis for amyloid cytotoxicity in such a minimal host as *Escherichia coli*, with the ambition of providing clues on the undeniably more complex mammalian amyloid proteinopathies. Such endeavor spans from a systems biology perspective, to highlight gene networks and metabolic pathways related to amyloid disease, to bottom-up approaches based on reconstructive, cytomimetic biochemistry performed with lipid vesicles.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

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**REFERENCES**

[1] Otzen D. Functional amyloid: Turning swords into plowshares. Prion 2010; 4:256-64; PMID:20935497; http://dx.doi.org/10.4161/pri.4.4.13676
[2] Romero D, Kolter R. Functional amyloids in bacteria. Int Microbiol 2014; 17:65-73; PMID:26418850
[3] DePas WH, Chapman MR. Microbial manipulation of the amyloid fold. Res Microbiol 2012; 163:592-606; PMID:23108148; http://dx.doi.org/10.1016/j.resmic.2012.10.009
[4] Bieler S, Estrada L, Lagos R, Baeza M, Castilla J, Soto C. Amyloid formation modulates the biological activity of a bacterial protein, J Biol Chem 2005; 280:26880-5; PMID:15917245; http://dx.doi.org/10.1074/jbc.M502031200
[5] Liebman SW, Chernoff YO. Prions in yeast. Genetics 2012; 191:1041-72; PMID:22879407; http://dx.doi.org/10.1534/genetics.111.137760
[6] Berson JF, Theos AC, Harper DC, Tenza D, Rapos G, Marks MS. Proprotein convertase cleavage liberates a fibrillogenic fragment of a resident glycoprotein to initiate melanosome biogenesis. J Cell Biol 2003; 161:521-33; PMID:12732614; http://dx.doi.org/10.1083/jcb.200302072
[7] Stephan JS, Fioriti L, Lamba N, Colnaghi L, Karl K, Derkatch IL, Kandel ER. The CPEB3 protein is a functional prion that interacts with the actin cytoskeleton. Cell Rep 2015; 11:1772-85; PMID:26074072; http://dx.doi.org/10.1016/j.celrep.2015.04.060
[8] Van Gerven N, Klein RD, Hulthgren SJ, Remaut H. Bacterial amyloid formation: Structural insights into curli biogenesis. Trends Microbiol 2015; 23:693-706; PMID:26439293; http://dx.doi.org/10.1016/j.tim.2015.07.010
[9] Sivanathan V, Hochschild A. Generating extracellular amyloid aggregates using E.coli cells. Genes Dev 2012; 26:2659-67; PMID:23166018; http://dx.doi.org/10.1101/gad.205310.112
[10] García-Fruitós E, Sabate R, de Groot NS, Villaverde A, Ventura S. Biological role of bacterial inclusion bodies: A model for amyloid aggregation. FEBS J 2011; 278:2419-27; http://dx.doi.org/10.1111/j.1742-4658.2011.08165.x
[11] Giraldo R, Fernández-Tresguerres ME. Twenty years of the pPS10 replicon: insights on the molecular mechanism for the activation of DNA replication in iteron-containing bacterial plasmids. Plasmid
Giraldo R, Andreu JM, Díaz-Orejas R. Protein domains and conformational changes in the activation of RepA, a DNA replication initiator. EMBO J 1998; 17:4511-26; PMID:9687517; http://dx.doi.org/10.1093/emboj/17.15.4511

Giraldo R, Fernández-Tornero C, Evans PR, Díaz-Orejas R, Romero A. A conformational switch between transcriptional repression and replication initiation in the RepA dimerization domain. Nat Struct Biol 2003; 10: 565-71; PMID:12766757; http://dx.doi.org/10.1038/nsb937

Díaz-López T, Dávila-Fajardo C, Blaessing F, Lillo MP, Giraldo R. Early events in the binding of the pPS10 replication protein RepA to single iteron and operator DNA sequences. J Mol Biol 2006; 364:909-29; PMID:16006506; http://dx.doi.org/10.1016/j.jmb.2006.09.013

Gasset-Rosa F, Díaz-López T, Lurz R, Prieto A, Fernández-Tresguerres ME, Giraldo R. Negative regulation of pPS10 plasmid replication: Origin pairing by zipping-up DNA-bound RepA monomers. Mol Microbiol 2008; 68:560-72; PMID:18284592; http://dx.doi.org/10.1111/j.1365-2958.2008.06166.x

Díaz-López T, Lages-Gonzalo M, Serrano-López A, Alfonso C, Rivas G, Giraldo R. Structural changes in RepA, a plasmid replication initiator, upon binding to origin DNA. J Biol Chem 2003; 278:18606-16; http://dx.doi.org/10.1074/jbc.M212024200

Giraldo R. Defined DNA sequences promote the assembly of a bacterial protein into distinct amyloid nanostructures. Proc Natl Acad Sci USA 2007; 104:17388-93; PMID:17959784; http://dx.doi.org/10.1073/pnas.0702006104

Gasset-Rosa F, Maté MJ, Dávila-Fajardo C, Bravo J, Giraldo R. Binding of sulphonated indigo derivatives to RepA-WH1 inhibits DNA-induced protein amyloidogenesis. Nucl Acids Res 2008; 36:2249-56; PMID:18285361; http://dx.doi.org/10.1093/nar/gkn067

Fernández-Tresguerres ME, Moreno-Díaz de la Espina S, Gasset-Rosa F, Giraldo R. A DNA-promoted amyloid proteinopathy in Escherichia coli. Mol Microbiol 2010; 77:1456-69; http://dx.doi.org/10.1111/j.1365-2958.2010.07299.x

Molina-García L, Giraldo R. Aggregation interplay between variants of the RepA-WHI prionoid in Escherichia coli. J Bacteriol 2014; 196:2536-42; http://dx.doi.org/10.1128/JB.01527-14

Giraldo R, Moreno-Díaz de la Espina S, Fernández-Tresguerres ME, Gasset-Rosa F. RepA-WHI prionoid: A synthetic amyloid proteinopathy in a minimalist host. Prion 2011; 5:60-4; PMID:21293179; http://dx.doi.org/10.4161/prion.5.2.14913

Tycko R. Physical and structural basis for polymorphism in amyloid fibrils. Prot Sci 2014; 23:1528-39; http://dx.doi.org/10.1002/pro.2544

Díaz-Avalos R, King CY, Wall J, Simon M, Caspar DLD. Strain-specific morphologies of yeast prion amyloid fibrils. Proc Natl acad Sci USA 2005; 102:10165-70; PMID:16006506; http://dx.doi.org/10.1073/pnas.0504599102

Wiltzius JJW, Landau M, Nelson R, Sawaya MR, Apostol MI, Goldschmidt L, Soriaga AB, Cascio D, Rajashankar K, Eisenberg D. Molecular mechanisms for protein-encoded inheritance. Nat Struct Mol Biol 2009; 16:973-8; PMID:19684598; http://dx.doi.org/10.1038/nsmb.1643

Lu JX, Qiang W, Yau WM, Schwieders CD, Meredith SC, Tycko R. Molecular structure of β-amyloid fibrils in Alzheimer disease brain tissue. Cell 2013; 154:1257-68; PMID:24034249; http://dx.doi.org/10.1016/j.cell.2013.08.035

Frederick KK, Debelouchina GT, Kayatekin C, Dorminy T, Jacavone AC, Griffin RG, Lindquist S. Distinct prion strains are defined by amyloid core structure and chaperone binding site dynamics. Chem Biol 2014; 21:295-305; PMID:24485763; http://dx.doi.org/10.1016/j.chemb.2013.12.013

Torreira E, Moreno-del Álamo M, Fuentes-Perez ME, Fernández C, Martín-Benito J, Moreno-Herrero F, Giraldo R, Llorca O. Amyloidogenesis of bacterial prionoid RepA-WHI recapitulates dimer to monomer transitions of RepA in DNA replication initiation. Structure 2015; 23:183-9; PMID:25543255; http://dx.doi.org/10.1016/j.str.2014.11.007

Chen SW, Drakulic S, Deas E, Oubearai M, Aprile FA, Arranz R, Ness S, Roodveldt C, Guiliains T, De-Genst EJ, et al. Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation. Proc Natl Acad Sci USA 2015; 112:E1994-2003; PMID:25855634; http://dx.doi.org/10.1073/pnas.1421204112

Campioni S, Mannini B, Lopez-Alonzo JP, Shalova IN, Penco A, Mulvihill E, Laurents DV, Relini A, Chiti F. Salt anions promote the conversion of HypF-N into amyloid-like oligomers and modulate the structure of the oligomers and the monomeric precursor state. J Mol Biol 2012; 424:132-49; PMID:23041425; http://dx.doi.org/10.1016/j.jmb.2012.09.023

Marek PJ, Patsalo V, Green DF, Raleigh DP. Ionic strength effects on amyloid formation by amylin are a complicated interplay among Debye screening, ion selectivity, and Hofmeister effects. Biochemistry 2012; 51:8478-90; PMID:23016872; http://dx.doi.org/10.1021/bi300574r
[31] Buell AK, Hung P, Salvatella X, Welland ME, Dobson CM, Knowles TP. Electrostatic effects in filamentous protein aggregation. Biophys J 2013; 104:1116-26; PMID:23473495

[32] Kajava AV, Baxa U, Steven AC. b arcades: recurring motifs in naturally occurring and disease-related amyloid fibrils. FASEB J 2010; 24:1311-9; PMID:20032312

[33] Gasset-Rosa F, Giraldo R. Engineered bacterial hydrophobic oligopeptide repeats in a synthetic yeast prion. [REP-PSI+]. Front Microbiol 2015; 6:311

[34] Crist CG, Kakayashiki T, Kurahashi H, Nakamura Y. [PHI+], a novel Sup35-prion variant propagated with non-Gln/Asn oligopeptide repeats in the absence of the chaperone protein Hsp104. Genes Cells 2003; 8:603-18; PMID:12839621

[35] Toombs J.A, Petri M, Paul KR, Kan GY, Ben-Hur A, Ross ED. De novo design of synthetic prion domains. Proc Natl Acad Sci USA 2012; 109:6519-24; PMID:22474356

[36] Bondarev SA, Shchepachev VV, Kajava A, Zhourvavleva GA. Effect of charged residues in the N-domain of Sup35 protein on prion [PSI+] stability and propagation. J Biol Chem 2013; 288:28503-13; PMID:23965990

[37] Giraldo R. Amyloid assemblies: Protein Legos at a crossroads in bottom-up synthetic biology. Chem Bio Chem 2010; 11:2247-357; PMID:20922739

[38] Moreno-del Álamo M, Moreno-Díaz de la Espina S, Fernández-Tresguerres ME, Giraldo R. Pre-amyloid oligomers of the proteotoxic RepA-WH1 prionoid assemble at the bacterial nucleoid. Sci Rep 2015; 5:14669; PMID:26423724

[39] Gasset-Rosa F, Coquel AS, Moreno-del Álamo M, Chen P, Song X, Serrano AM, Fernández-Tresguerres ME, Moreno-Díaz de la Espina S, Lindner AB, Giraldo R. Direct assessment in bacteria of prionoid propagation and phenotype selection by Hsp70 chaperone. Mol Microbiol 2014; 91:1070-87; PMID:24417419

[40] Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Heim MY, Stoynov S, Mahamid J, Saha S, Franzmann TM, et al. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. Cell 2015; 162:1066-77; PMID:26317470

[41] Hennig S, Kong G, Mannen T, Sadowska A, Kobelke S, Blythe A, Knott GJ, Iyer KS, Ho D, Newcombe EA, et al. Prion-like domains in RNA binding proteins are essential for building subnuclear paraspeckles. J Cell Biol 2015; 210:529-39; PMID:26283796

[42] Murakami T, Qamar S, Lin JQ, Schierle GS, Rees E, Miyashita A, Costa AR, Dodd RB, Chan FT, Michel CH, et al. ALS/FTD Mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. Neuron 2015; 88:678-90; PMID:26526393

[43] Escusa-Toret S, Vonk WI, Frydman J. Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. Nat Cell Biol 2013; 15:1231-43; PMID:24036477

[44] Wallace EW, Kear-Scott IL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, Katanski CD, Riback JA, Dion MF, Franks AM, et al. Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. Cell 2015; 162:1286-98; PMID:26359986