

Functional amyloids in bacteria

Diego Romero,^{1*§} Roberto Kolter^{2§}

¹Institute of Subtropical and Mediterranean Hortofruticultura “La Mayora”- CSIC, and Department of Microbiology, University of Malaga, Malaga, Spain.

²Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

Received 12 June 2014 · Accepted 28 June 2014

Summary. The term amyloidosis is used to refer to a family of pathologies altering the homeostasis of human organs. Despite having a name that alludes to starch content, the amyloid accumulations are made up of proteins that polymerize as long and rigid fibers. Amyloid proteins vary widely with respect to their amino acid sequences but they share similarities in their quaternary structure; the amyloid fibers are enriched in β -sheets arranged perpendicular to the axis of the fiber. This structural feature provides great robustness, remarkable stability, and insolubility. In addition, amyloid proteins specifically stain with certain dyes such as Congo red and thioflavin-T. The aggregation into amyloid fibers, however, it is not restricted to pathogenic processes, rather it seems to be widely distributed among proteins and polypeptides. Amyloid fibers are present in insects, fungi and bacteria, and they are important in maintaining the homeostasis of the organism. Such findings have motivated the use of the term “functional amyloid” to differentiate these amyloid proteins from their toxic siblings. This review focuses on systems that have evolved in bacteria that control the expression and assembly of amyloid proteins on cell surfaces, such that the robustness of amyloid proteins are used towards a beneficial end. [Int Microbiol 2014; 17(2):65-73]

Keywords: *Bacillus subtilis* · bacterial biofilms · extracellular matrix · TasA amyloid-like fibers

Amyloids in history

The term amyloidosis is used to refer to a family of pathologies altering the homeostasis of human organs. Written descriptions of what most likely were amyloidoses dates back to the late 17th century. An autopsy report describing a spleen full of white stones can be considered the first description of amyloidosis, which is now known as “sago spleen” to describe the

starch-like granules that grow in the organ [20]. The term amyloid, meaning resembling starch, was first used two centuries later. The German chemist Rudolph Virchow discovered that the *corporea amylacea* of the nervous system stained with Congo red in a similar way as did cellulose and starch [41]. There was a long-standing controversy on the chemical nature of such amyloid plaques with some maintaining that they were made of starch (thus amyloid) and others arguing that they were more akin to lard [20]. It was not until the 20th century that chemical analyses revealed that such accumulations consisted of protein. By then, however, the medical term had gained a stronghold and to date these proteins are referred to as amyloids, despite their having no starch content whatsoever. However, the early descriptions of amyloid proteins already revealed a peculiarity; under the electron microscope amyloids appeared as long and rigid fibers [20].

*Corresponding author: D. Romero
Departamento de Microbiología. Facultad de Ciencias
Universidad de Málaga-Instituto de Hortofruticultura
Subtropical y Mediterránea “La Mayora”(IHSM-UMA-CSIC)
Bulevar Louis Pasteur-Campus Universitario de Teatinos, s/n
29071 Málaga, Spain
Tel. +34-952134274
E-mail: diego_romero@uma.es

§ Both authors are equal contributors

Table 1. Amyloid proteins**1A.** Pathogenic amyloids

Name	Precursor	Disease	Ref.
AA	Serum amyloid	Systemic amyloidosis	[10]
A β	β -Protein (APP)	Alzheimer's disease	[10]
AIAPP	Islet amyloid polypeptide	(Type 2 diabetes and insuloma)	[10]
α -Synuclein	α -Synuclein	Parkinson's disease	[10]

1B. Functional amyloids

Species	Protein	Function	Ref.
Humans	Pmel17	Elimination of toxic intermediates during melanin synthesis	[19]
Fungi	Hydrophobins	Formation of fungal coat	[56]
<i>Escherichia coli</i> , <i>Salmonella enteritidis</i>	Curli	Interaction with host, biofilm formation	[9,15]
<i>Pseudomonas</i> sp.	FapC	Biofilm formation	[12]
<i>Streptomyces coelicolor</i>	Chaplins	Formation of aerial structures	[13]
<i>Bacillus subtilis</i>	TasA	Biofilm formation	[31]
<i>Klebsiella pneumoniae</i>	Microcin E492	Antimicrobial	[40]
<i>Staphylococcus aureus</i>	PSM	Biofilm formation	[37]
<i>Streptococcus mutans</i>	Adhesin P1	Biofilm formation	[29]
<i>Mycobacterium tuberculosis</i>	MTP	Host interaction	[1]
<i>Xanthomonas axonopodis</i>	Harpin	Virulence factor, multicellularity	[28,39]

The intense research on amyloid proteins has shown that even though they vary widely with respect to their amino acid sequences, they share similarities in their quaternary structure. Amyloid protein fibers are enriched in **β -sheets arranged perpendicular** to the axis of the fiber [50]. This structure provides great robustness, which is the defining feature of amyloid protein. Amyloids have remarkable stability, insolubility and specifically stain with certain dyes such as Congo red and thioflavin-T. The fact that amyloid fibers had always been associated with human pathologies led to the perception that the amyloid state was due to an erratic processing or misfolding of soluble and functional proteins [44]. However, the aggregation into amyloid fibers seems to be widely distributed among proteins and polypeptides, and in some cases, these amyloid fibers are important in maintaining the homeostasis of the organism [10,23]. Two outstanding examples of amyloids non-related to pathologies in humans are: The protein pMel17, which form amyloid fibers to eliminate intermediate aggregates that may be toxic to the organism, and proteins or peptides hormones of the

human endocrine system, which are efficiently stored in secretory granules in an amyloid-state, thus contributing to the normal physiology of cells [19,23]. Amyloid fibers are also present in insects, fungi and in bacteria, and they participate in protection, interaction with surfaces, and detoxification (Table 1). Prions, another group of proteins with propensity to fold into amyloid fibers but with the astonishing ability of self-propagating, are mostly known for their pathological implications, although exceptions to this rule are arising [27,49]. This happens to be with the translation regulation protein CPEB (cytoplasmic polyadenylation element binding protein) in the mollusk *Aplysia*. As other prions, this protein possesses the ability to acquire different functional conformations, and it appears that the prion dominant form contributes to stabilizing the long-term stimulated synapses in memory storage [42]. Another example of a non-pathological prion is the protein HET-s in the fungus *Podospora anserina* [26,55], which in the prion state seems to contribute to build a physical barrier that impede the transfer of deleterious elements between genetically in-

compatible strains [26]. All these findings have motivated the use of the term “functional amyloid” to differentiate these amyloid proteins from their toxic siblings [14]. In this review we focus on systems that have evolved in bacteria that control the expression and assembly of amyloid proteins on cell surfaces, such that the robustness of amyloid proteins are used towards a beneficial end.

Functional amyloids in bacteria

One of the first reports of functional amyloid proteins in bacteria was a study of the curli pili of an uropathogenic strain of *Escherichia coli*. These pili were initially described as fibronectin-binding organelles on the surface of cells [30]. Subsequently, Chapman and collaborators demonstrated that the curli fibers that emerged from the surfaces of *E. coli* cells had the same physical properties as the well-studied amyloid proteins responsible for human amyloidoses, e.g., staining with specific dyes (Fig.1) [9]. *Escherichia coli* uses curli amyloid-like fibers for a variety of physiological functions. Among these are interactions with host tissues, biofilm formation, and evasion of the immune system [4]. This first description of a bacterial functional amyloid opened the possibility that amy-

loid proteins could be present in other bacterial species. Indeed, Larsen and collaborators carried out an immunolabeling study with diverse bacterial species and suggested that amyloid proteins were present as constituents of bacterial biofilms in a variety of environmental samples [22]. However, there is still just a limited number of examples where the direct implication of amyloids in biofilm formation has been demonstrated: Tafi, the curli homolog in *Salmonella*, FapC in many *Pseudomonas* species, TasA in *Bacillus subtilis*, and the recently found phenol soluble modulins (PSMs) in *Staphylococcus aureus* and the adhesin protein P1 in *Streptococcus mutans* in dental plaque biofilms [12,15,29,31,37].

Amyloid proteins may be utilized to perform other physiological processes in bacteria. For example, chaplins of *Streptomyces coelicolor* not only serve to interact with surfaces, but they also facilitate the rising of the aerial hyphae [5,11]. In *Mycobacterium tuberculosis*, amyloid pili (MTP) are necessary to interact with the host during pathogenesis [1]. Another example of an amyloid having a role during pathogenesis involves the harpins, important virulence factors of *Xanthomonas axonopodis* and other plant pathogenic bacteria. The harpin Hpag of *Xanthomonas* has been demonstrated to form amyloid fibers in vitro, and to be related to the hypersensitive response (HR) caused in the host and most recently shown to

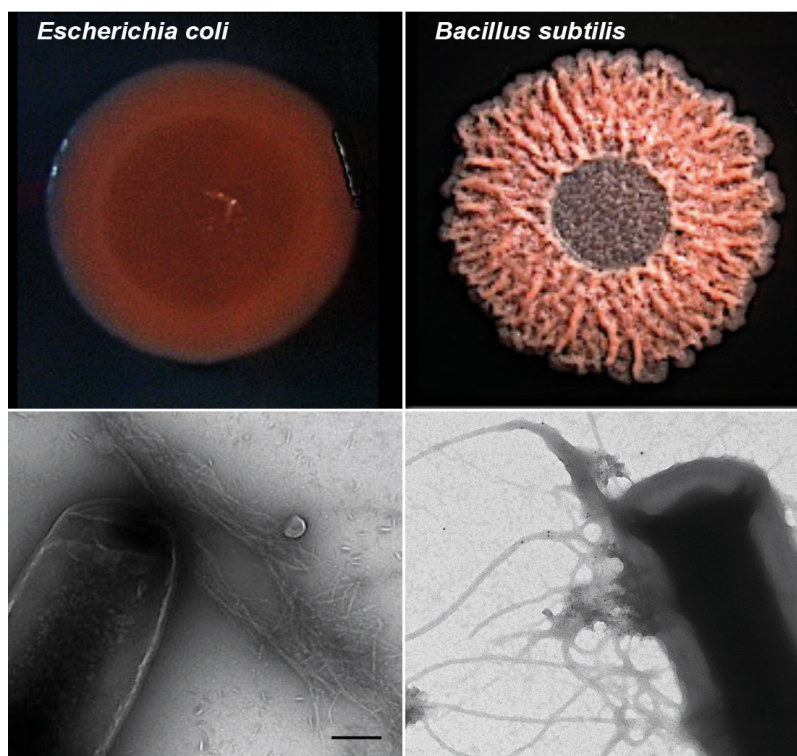


Fig. 1. Top row: *Escherichia coli* and *Bacillus subtilis* colonies stain with the amyloid specific dye Congo red. The pictures were taken after 72 h of growth in MSgg (minimal salts glycerol glutamate) agar (*B. subtilis*) or YESCA agar (*E. coli*) supplemented with Congo red. Bottom row: Transmission electron micrographs of curli amyloid fibers in *E. coli* (left) and TasA amyloid-like fibers in *B. subtilis* (right). Bars equal 200 nm.

participate in the multicellular behavior of this bacterial species [28,39]. In this case, it was demonstrated that tetrameric oligomers were the main protein species found in hypersensitive responses. These findings contribute to the debate on the toxicity of amyloids. Historically, fibers have been considered as the etiological agents of amyloidosis. However, intermediate aggregates have a high propensity to insert into biological membranes and induce structural destabilization [16,51]. Thus, it is now thought that the intermediate aggregates, rather than the fibers themselves, are responsible for disease development [51].

Other studies demonstrate that, besides their role in developmental programs or interaction with the host cells, amyloids can be exploited as a detoxifying system. An interesting example of how the toxicity of amyloids can be modulated in self-benefit is that of the toxin microcin E492 produced by *Klebsiella pneumoniae* [40]. This toxin is a small peptide that oligomerizes into the cytoplasmic membranes of Enterobacteriaceae, causing pores that result in cell death [21]. The toxicity of microcin E492 was observed to vary depending on the state of growth of the producing cells; it was higher during exponential growth and decreased progressively with the aging of the culture. Not that the reduction of toxicity was associated to the transition of the toxin from an oligomeric state to further polymerization into amyloid fibers. This observation led to the conclusion that the toxic oligomers of microcin E492 resemble the intermediate aggregates of the protein A β associated with Alzheimer's disease [2,24].

The inclusion bodies (IBs) that form during heterologous expression of proteins in bacteria are also an example of proteins with an amyloid conformation. The IBs have been historically considered the bottleneck that reduces the yield of overexpressed soluble and functional proteins. However, recent research demonstrates that peptides can be recovered from the IBs and still retain functionality. Besides the biological role of IBs as scavengers of putative toxic molecules for the bacterial cells, they offer an exciting new way for the efficient and controlled delivery of drugs in chemotherapy [52,53].

Amyloidogenesis as a sophisticated process of protein aggregation

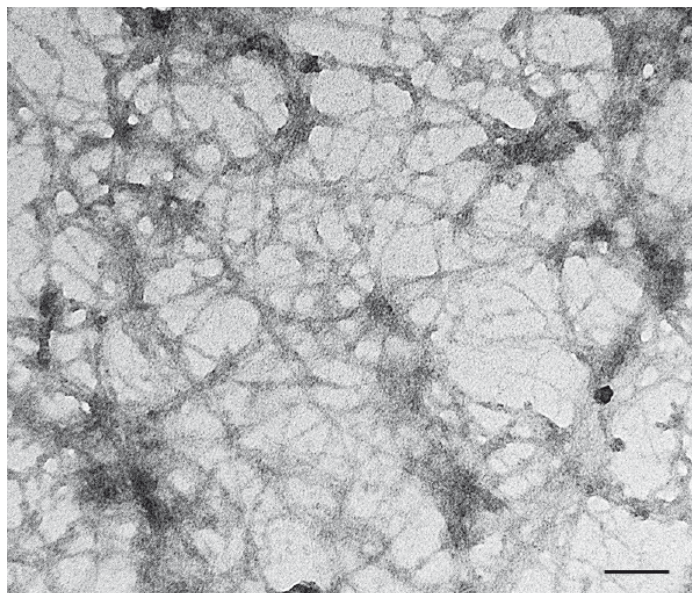
The discovery that amyloid proteins can carry out important physiological functions in bacteria has forced the re-evaluation of the concept that amyloidogenesis is always an erratic process of protein aggregation. As we describe in this section,

rather than being an uncontrolled process, it is now clear that in bacteria, and probably in other organisms, sophisticated machineries have evolved that direct the polymerization of these amyloid fibers outside the cell, thus avoiding toxicity yet providing the structural robustness to produce very stable organelles [36].

Curli: a paradigm for bacterial amyloidogenesis.

Amyloid proteins have the intrinsic propensity to polymerize from the native monomeric state to the ordered and insoluble amyloid fibers [50]. During the formation of the fibers, amyloids go through diverse stages of aggregation with variable biochemical and morphological features. The kinetics of amyloid polymerization follow a typical sigmoidal curve with an initial lag phase, followed by an exponential phase of growth and a final plateau, where the fibers saturate and do not grow any further [25]. What allows the members of this family of proteins to form fibers? The study of curli in *E. coli* has clarified much of the molecular mechanisms that direct amyloidogenesis. The first clear difference with pathogenic amyloids is that curli formation is a highly regulated process. The protein products of two divergent operons are directly involved in the formation of curli fibers: *csgABC* and *csgDEFG*. The products of *csgA* and *csgB* are the main components of the fibers and the other proteins participate in regulating gene expression or control the proper secretion and polymerization of the fibers. CsgD regulates the expression of *csgABC*. CsgG forms a pore-like structure in the outer membrane, and allows the translocation of CsgA and CsgB from the periplasm to the outside of the cell. CsgF helps expose CsgB to the surfaces, and CsgE is thought to facilitate the access of CsgA to the secretion complex formed by CsgG. Finally, CsgC, which also has oxidoreductase activity, is thought to control the formation of the CsgG pore-like structure. The fibrillation of curli follows a nucleation-polymerization model, which means that the mixture of single mutants *csgA* and *csgB* complement each other extracellularly.

This is a fascinating result indicating that there is no need to produce both subunits in the same cell. The detailed analysis of the curli subunits CsgA and CsgB demonstrated the existence of an amyloidogenic core, composed of four imperfect repeats within the proteins' amino acid sequence. Thus, when CsgB encounters CsgA, it induces a conformational change towards the amyloidogenic state making its polymerization into fibers possible. The reaction between the two subunits is mediated by CsgC [4]. All this knowledge on curli biogenesis has served to establish an elegant *E. coli* cell-based methodology to evaluate the potential amyloidogenic proper-



Int Microbiol

Fig. 2. Fibers of TasA purified from *Bacillus subtilis* as seen in electron microscopy and negative staining. Bar equals 100 nm.

ties of proteins [43]. In this study, human or yeast amyloid proteins were shown to be targeted to the envelop of *E. coli* cells via the curli system, where they propagated fibers with amyloid properties.

How conserved is the mechanism of polymerization among bacterial amyloids is a question that needs intensive investigation. In the closely related bacterium *Salmonella typhimurium*, homologs to each curli gene have been identified and thus the polymerization is hypothesized to follow a similar scheme [58]. Consistent with the above idea, a recent study demonstrated that homologs of CsgA found in *E. coli* and *S. typhimurium*, among other Gram-negative bacteria, can cross seed fiber formation in vitro. As stated by the authors, this observation leads to the idea that these heterogeneous curli fibers may be produced in mixed-species biofilms in natural settings [57]. In the case of the amyloid protein FapC of *Pseudomonas* species, although no similarity in sequence with curli genes is observed, the presence of genes that could play similar roles to each component of the machinery dedicated to curli in *E. coli* has been demonstrated [12]. Studies on other bacterial filaments described as pilli and fimbriae have demonstrated differences in the specific way that they are formed in Gram-positive and Gram-negative bacteria which should come as no surprise given the dramatic differences in the cell envelopes of these two general classes of bacteria [18].

Amyloid fibers in Gram-positive bacteria: TasA in *Bacillus subtilis*. A good model for the study of amyloid proteins of Gram-positive bacteria is TasA in *B. subtilis*.

The story of TasA is quite intriguing. TasA was first described in the late 1990s, by two separate groups, as a protein that was both secreted into the medium during stationary phase and as a constituent of the spores [38,46]. The absence of TasA did not affect the viability of the spores but the spores appeared morphologically altered. In addition, Stover and Driks [46] reported an intriguing result for TasA; when overexpressed in *E. coli*, it had broad-spectrum of antimicrobial activity. The name of TasA reflects the findings of this protein as a translocation-dependent antimicrobial spore component. Later, TasA was shown to be a major component of the extracellular matrix of *B. subtilis* biofilms and also required for the formation of biofilms [6]. This functionality was further described as being related to the amyloid-like nature of TasA (Fig. 1) [31]. Note that although not demonstrated or even predicted, the observation of TasA's antimicrobial activity when produced in *E. coli* pointed towards one of the putative features of TasA as an amyloid protein: when produced in large quantities and in the absence of the additional elements necessary for the assembly in fibers, TasA may form toxic aggregates that like other amyloids, may cause cell death [46].

As introduced earlier, it can be proposed that *B. subtilis* uses TasA to produce amyloid fibers outside the cell for two purposes: (i) to detoxify the possible accumulation of toxic aggregates of this protein in the cytoplasm, and (ii) to form the protein-scaffold that supports the assembly of the extracellular matrix, the network of molecules necessary for the formation of bacterial biofilms. Thus, the formation of such fibers needs to be highly regulated. There is a complex regula-

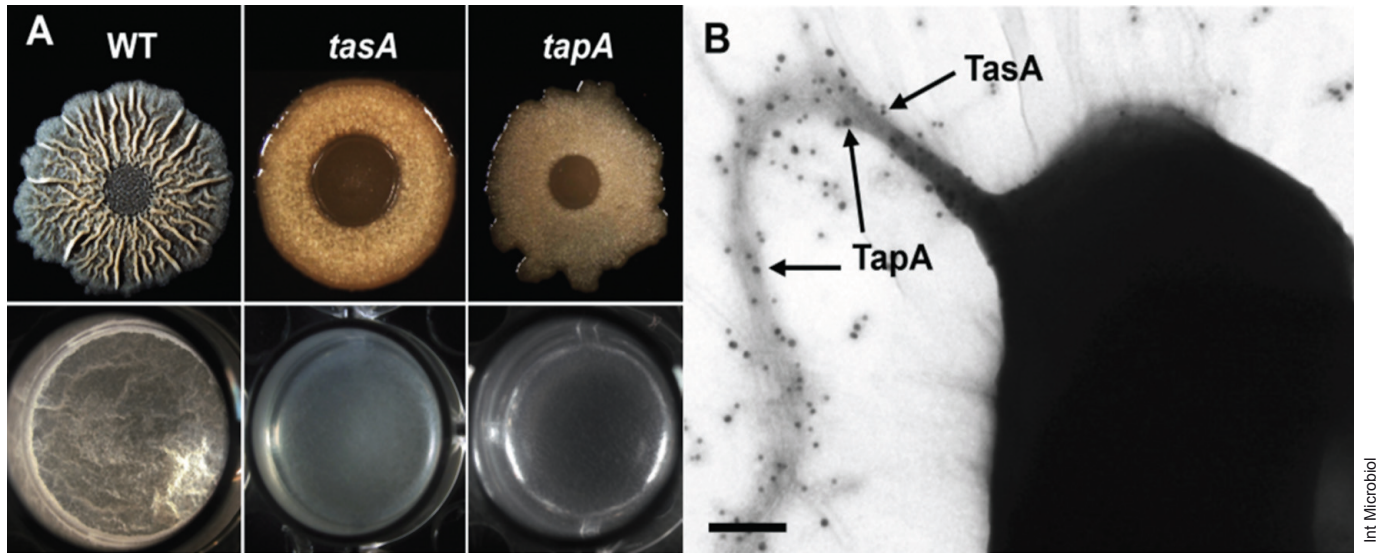


Fig. 3. The formation of biofilms of *Bacillus subtilis* and TasA amyloid-like fibers in *B. subtilis* depend on the proteins TasA and TapA. (A) Top row: Highly wrinkly colony of wild type and featureless colony of *tasA* or *tapA* mutants after 72 h of growth at 30 °C in MSgg agar plates. Bottom row: wrinkly pellicles of wild type and absence of pellicle of *tasA* and *tapA* mutants grown in MSgg broth for 24 h at 30 °C. (B) Transmission electron microscopy and co-immunolabeling with anti-TapA (15 nm gold particles) and anti-TasA (10 nm gold particles) antibodies of TasA fibers in *B. subtilis* biofilms. Bar equals 100 nm.

tory network that controls and connects the expression of TasA with other bacterial factors [54]. To summarize, the master regulator SinR directly represses the expression of *tasA* until conditions are propitious for biofilm development [17]. One operon containing three genes, *tapA-sipW-tasA*, is necessary for the formation of the amyloid fibers [31]. This is markedly different from the chaplin amyloid fibers in the Gram-positive bacterium *Streptomyces coelicolor*, where eight chaplin genes have been described [13]. *Streptomyces coelicolor* shows a complex developmental program and it is thought that the diversity of chaplins play important roles in the different stages of the *S. coelicolor* life cycle [5].

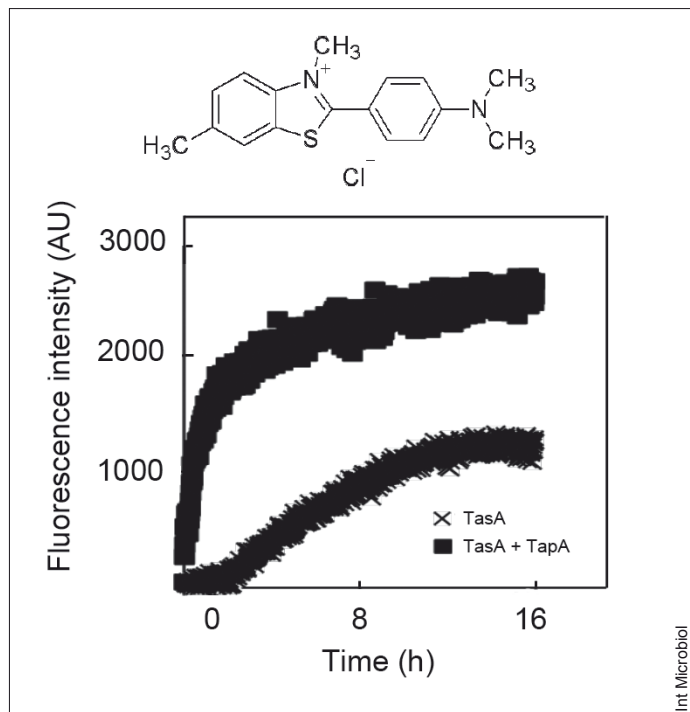
TasA is the main component of the *B. subtilis* amyloid fibers. When purified directly from *B. subtilis*, TasA retains amyloid properties, such as self-aggregation into insoluble fibers (Fig. 2). TasA fibers can be depolymerized with aggressive acid treatments and then they can be observed to repolymerize. Such a property has been useful to study the kinetics of polymerization of TasA, which reflects a dynamic transition through different stages of aggregation. In agreement with its amyloid nature, some of these intermediate aggregates, but not the fiber nor the monomers, reacts with an antibody that specifically detects the toxic aggregates of the amyloid protein A β associated with Alzheimer's disease [31]. However, TasA is not purified from *B. subtilis* in its monomeric form. Rather, we obtain a homogenous suspension of stable oligo-

mers, which could aggregate as different morphotypes depending on the physical properties of the medium: (i) fibers form on hydrophobic surfaces but (ii) plaques form under acidic conditions. This is an appealing discovery that could reflect a way in which bacteria can adapt to different habitats, modifying the state of aggregation of an external protein and probably the final arrangement of the extracellular matrix depending on environmental cues such as pH and surface hydrophobicity [8].

Bacterial cell surfaces are hydrophobic, and we have seen that this physical feature promotes the formation of fibers [8]. However, additional factors must be dedicated to increase the efficiency of the fiber polymerization process outside the cells. To build the curli fibers, *E. coli* uses CsgA and the nucleator protein CsgB [3]. As stated above, *tasA* is part of a three gene operon that encodes another protein, TapA, which is also necessary for the assembly of biofilms (Fig. 3A) [6,34]. The failure of a *tapA* deletion mutant to form biofilms has been related to the absence of TasA fibers and even attachment of TasA to cell surfaces [34]. Note that, differently from what was reported for curli, TapA and TasA have to be produced in the same cell in order to form the fibers, a process similar to the formation of pili in *B. cereus* [7].

Immunolocalization studies proved that TapA localizes, as foci, at certain regions of the cell wall, where presumably it directs the polymerization of TasA into fibers. In addition,

Fig. 4. A feature of TasA amyloid-like fibers is the binding of the amyloid specific fluorescent dye thioflavin T (structure on top). Thioflavin T fluorescence is used to follow the kinetics of polymerization of amyloid proteins. The increase of the fluorescence intensity indicates an increase in the β -sheet content of the protein, typical in amyloid fibers. Purified TasA follows a typical amyloid kinetics of polymerization (x), and the addition of TapA accelerates the polymerization of TasA into amyloid-like fibers (■).



TapA appears distributed along the TasA fibers (Fig. 3B), although at much less abundance (the ratio of TasA:TapA is 100:1) [34]. Based on these findings, we proposed that TapA is an accessory protein that promotes the efficient polymerization of TasA at the cell envelope and contributes to the organization of the growing fibers. In addition, the localization of TapA at the base of the TasA fibers, and its close association to the cell wall led to the idea that TapA is the connector of the fibers to the cell envelope [34].

Consistent with this conclusion, the absence of TapA causes a decrease in the amount of detectable extracellular TasA, which in addition appears as small and disorganized fibrils that are disconnected from the cell envelope. The name TapA was coined to refer to these functions: **TasA anchoring and assembly protein** [34].

In a recent publication we showed that indeed TapA accelerates the polymerization of TasA in vitro (Fig. 4) [35]. How it is that TapA facilitates the polymerization of TasA into amyloid fibers is still unclear. What we do know is that a mutant with a *tapA* allele lacking an eight-amino-acid sequence in the N-terminal half of the protein failed to form biofilms and failed to promote TasA fiber formation [35]. By comparison with curli in *E. coli*, we propose that this domain in the N-terminal half of TapA can either promote the processing of TasA or induce a conformational change of TasA toward its amyloidogenic state.

The knowledge of the polymerization of pili in other Gram-positive bacteria can be compared and contrasted with TasA fiber formation. The formation of pili in *B. cereus* needs two subunits, BcpA and BcpB. The major subunit, BcpA is processed by a specific sortase, and the product is linked to the minor subunit BcpB [7]. However, a hypothetical processing of TasA and TapA would have to involve other proteins than the putative sortases identified in *B. subtilis* because mutants in those sortases had no defect in biofilm formation [34]. The anchoring of TasA fibers mediated by TapA could be thought to be similar to other pili in Gram-positive bacteria. However, two lines of evidence discard this hypothesis: (i) The C-terminal region of TapA does not contain a canonical sorting signal for anchoring to the peptidoglycan, and (ii) the deletion of the two putative sortases, the enzymes that recognize the sorting signals, did not alter biofilm formation [34]. These findings open the possibility that there exist diverse mechanisms of attachment of fibers to the cell wall of Gram-positive bacteria.

Finally, the formation of TasA fibers depends on a third protein, SipW, also encoded in the same operon. This is a signal peptidase that processes TasA and TapA to their mature form before sorting to the cell envelope [45,46]. SipW is an atypical signal peptidase, given that it resembles more those found in the endoplasmic reticulum of eukaryotic cells than typical bacterial signal peptidases [48]. An additional role for

SipW has been recently proposed. A version of this protein impaired in protease activity was shown to stimulate the expression of the *tapA-sipW-tasA* operon during the formation of biofilms on solid surfaces, thus introducing a new level of control and complexity to the formation of amyloids [47].

Final notes

The studies of bacterial amyloids have revolutionized our perception of this family of proteins: from their pathological implications and erratic processing to the possibility that they sometimes arise as the result of a well-defined program that has evolved to maintain the homeostasis of the organism and to interact with the environment [36]. To the increasing number of functional amyloids, we have added a new member, the TasA amyloid-like fiber in the Gram-positive bacterium *B. subtilis*. A number of reasons make the TasA amyloid-like fibers an attractive model for the molecular study of amyloidogenesis. (i) *B. subtilis* is a model microbe for the study of developmental programs such as biofilm formation and sporulation, two features mediating the interaction of the bacterium with its environment. The study of TasA fiber formation will contribute not only to our understanding of the mechanisms of amyloid fiber formation, but also its implications in bacterial adaptation to the habitat. The fact that TasA is purified to homogeneity directly from *B. subtilis* offers the outstanding capability to investigate which other factors, physical-chemical or intrinsic to bacterial cells, are involved in fiber formation. (ii) Amyloid fibers represent interesting targets for the search of new therapies that contribute to the fight of bacterial biofilms and the biochemical problems they may cause [32]. We have previously demonstrated that *B. subtilis* biofilms represent a formidable tool for the search of molecules that can be exploited in two directions: anti-biofilm and anti-amyloid [33].

Acknowledgements. The authors thank Hera Vlamakis for critical reading and suggestions during the writing of the manuscript. DR is funded by the program Ramón y Cajal (RyC-2011-080605) and grant AGL-2012-31968 from the Plan Nacional de I+D+I from Ministerio de Economía y Competitividad (Spain) and co-financed by FEDER funds (European Union).

Competing interest. None declared.

References

1. Alteri CJ, Xicohtencatl-Cortes J, Hess S, Caballero-Olín G, Girón JA, Friedman RL (2007) *Mycobacterium tuberculosis* produces pili during human infection. Proc Natl Acad Sci USA 104:5145-5150

2. Arranz R, Mercado G, Martin-Benito J, Giraldo R, Monasterio O, Lagos R, Valpuesta JM (2012) Structural characterization of microcin E492 amyloid formation: Identification of the precursors. J Struct Biol 178: 54-60
3. Bian Z, Normark S (1997) Nucleator function of CsgB for the assembly of adhesive surface organelles in *Escherichia coli*. EMBO J 16:5827-5836
4. Blanco LP, Evans ML, Smith DR, Badtke MP, Chapman MR (2012) Diversity, biogenesis and function of microbial amyloids. Trends Microbiol 20:66-73
5. Bokhove M, Claessen D, de Jong W, Dijkhuizen L, Boekema EJ, Oostergetel GT (2013) Chaplins of *Streptomyces coelicolor* self-assemble into two distinct functional amyloids. J Struct Biol 184:301-309
6. Branda SS, Chu F, Kearns DB, Losick R, Kolter R (2006) A major protein component of the *Bacillus subtilis* biofilm matrix. Mol Microbiol 59: 1229-1238
7. Budzik JM, Marraffini LA, Souda P, Whitelegge JP, Faull KF, Schneewind O (2008) Amide bonds assemble pili on the surface of bacilli. Proc Natl Acad Sci USA 105:10215-10220
8. Chai L, Romero D, Kayatekin C, Akabayov B, Vlamakis H, Losick R, Kolter R (2013) Isolation, characterization, and aggregation of a structured bacterial matrix precursor. J Biol Chem 288:17559-17568
9. Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science 295:851-855
10. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333-366
11. de Jong W, Wosten HA, Dijkhuizen L, Claessen D (2009) Attachment of *Streptomyces coelicolor* is mediated by amyloid fimbriae that are anchored to the cell surface via cellulose. Mol Microbiol 73:1128-1140
12. Dueholm MS, Petersen SV, Sonderkaer M, Larsen P, Christiansen G, Hein KL, Enghild JJ, Nielsen JL, Nielsen KL, Nielsen PH, Otzen DE (2010) Functional amyloid in *Pseudomonas*. Mol Microbiol 77:1009-1020
13. Elliot MA, Karoonuthaisiri N, Huang J, Bibb MJ, Cohen SN, Kao CM, Buttner MJ (2003) The chaplins: a family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. Genes Dev 17:1727-1740
14. Fowler DM, Koulov AV, Balch WE, Kelly JW (2007) Functional amyloid--from bacteria to humans. Trends Biochem Sci 32:217-224
15. Gibson DL, White AP, Rajotte CM, Kay WW (2007) AgfC and AgfE facilitate extracellular thin aggregative fimbriae synthesis in *Salmonella enteritidis*. Microbiology 153:1131-1140
16. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486-489
17. Kearns DB, Chu F, Branda SS, Kolter R, Losick R (2005) A master regulator for biofilm formation by *Bacillus subtilis*. Mol Microbiol 55:739-749
18. Kline KA, Dodson KW, Caparon MG, Hultgren SJ (2010) A tale of two pili: assembly and function of pili in bacteria. Trends Microbiol 18: 224-232
19. Kummer MP, Maruyama H, Huelsmann C, Baches S, Weggen S, Koo EH (2009) Formation of Pmel17 amyloid is regulated by juxtamembrane metalloproteinase cleavage, and the resulting C-terminal fragment is a substrate for gamma-secretase. J Biol Chem 284:2296-2306
20. Kyle RA (2001) Amyloidosis: a convoluted story. Br J Haematol 114: 529-538
21. Lagos R, Tello M, Mercado G, Garcia V, Monasterio O (2009) Antibacterial and antitumorigenic properties of microcin E492, a pore-forming bacteriocin. Curr Pharm Biotechnol 10:74-85

22. Larsen P, Nielsen JL, Dueholm MS, Wetzel R, Otzen D, Nielsen PH (2007) Amyloid adhesins are abundant in natural biofilms. *Environ Microbiol* 9:3077-3090
23. Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, Rissman RA, Singru PS, Nilsson KP, Simon R, Schubert D, Eisenberg D, Rivier J, Sawchenko P, Vale W, Riek R (2009) Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science* 325: 328-332
24. Marcoleta A, Marin M, Mercado G, Valpuesta JM, Monasterio O, Lagos R (2013) Microcin E492 amyloid formation is retarded by posttranslational modification. *J Bacteriol* 195:3995-4004
25. Naiki H, Gejyo F (1999) Kinetic analysis of amyloid fibril formation. *Methods Enzymol* 309:305-318
26. Nair P (2013) Nonpathogenic prions. *Proc Natl Acad Sci USA* 110:6612 (Core concepts)
27. Newby GA, Lindquist S (2013) Blessing in disguise: Biological benefits of prion-like mechanisms. *Trends Cell Biol* 23:251-259
28. Oh J, Kim JG, Jeon E, Yoo CH, Moon JS, Rhee S, Hwang I (2007) Amyloidogenesis of type III-dependent harpins from plant pathogenic bacteria. *J Biol Chem* 282:13601-13609
29. Oli MW, Otoo HN, Crowley PJ, Heim KP, Nascimento MM, Ramscook CB, Lipke PN, Brady LJ (2012) Functional amyloid formation by *Streptococcus mutans*. *Microbiology* 158:2903-2916
30. Olsen A, Jonsson A, Normark S (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* 338:652-5
31. Romero D, Aguilar C, Losick R, Kolter R (2010) Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc Natl Acad Sci USA* 107:2230-2234
32. Romero D, Kolter R (2011.) Will biofilm disassembly agents make it to market? *Trends Microbiol* 19:304-306
33. Romero D, Sanabria-Valentin E, Vlamakis H, Kolter R (2013) Biofilm inhibitors that target amyloid proteins. *Chem Biol* 20:102-110
34. Romero D, Vlamakis H, Losick R, Kolter R (2011) An accessory protein required for anchoring and assembly of amyloid fibres in *B. subtilis* biofilms. *Mol Microbiol* 80:1155-1168
35. Romero D, Vlamakis H, Losick R, Kolter R (2014) Functional analysis of the accessory protein tapA in *Bacillus subtilis* amyloid fiber assembly. *J Bacteriol* 196:1505-1513
36. Sawyer EB, Claessen D, Gras SL, Perrett S (2012) Exploiting amyloid: how and why bacteria use cross-beta fibrils. *Biochem Soc Trans* 40: 728-734
37. Schwartz K, Syed AK, Stephenson RE, Rickard AH, Boles BR (2012) Functional amyloids composed of phenol soluble modulins stabilize *Staphylococcus aureus* biofilms. *PLoS Pathog* 8:e1002744
38. Serrano M, Zilhão R, Ricca E, Ozin AJ, Moran CP Jr, Henriques AO (1999) A *Bacillus subtilis* secreted protein with a role in endospore coat assembly and function. *J Bacteriol* 181:3632-3643
39. Sgro GG, Ficarra FA, Dunger G, Scarpeci TE, Valle EM, Cortadi A, Orellano EG, Gottig N, Ottado J (2012) Contribution of a harpin protein from *Xanthomonas axonopodis* pv. *citri* to pathogen virulence. *Mol Plant Pathol* 13:1047-1059
40. Shahnawaz M, Soto C (2012) Microcin amyloid fibrils A are reservoir of toxic oligomeric species. *J Biol Chem* 287:11665-11676
41. Sipe JD, Cohen AS (2000) Review: history of the amyloid fibril. *J Struct Biol* 130:88-98
42. Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER (2010) Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. *Cell* 140:421-425
43. Sivanathan H, Hochschild A (2010) Generating extracellular amyloid aggregates using *E. coli* cells. *Genes Dev* 26:2659-2667
44. Stevens FJ (2004) Amyloid formation: an emulation of matrix protein assembly? *Amyloid* 11:232-244
45. Stover AG, Driks A (1999) Control of synthesis and secretion of the *Bacillus subtilis* protein YqxM. *J Bacteriol* 181:7065-7069
46. Stover AG, Driks A (1999) Secretion, localization, and antibacterial activity of TasA, a *Bacillus subtilis* spore-associated protein. *J Bacteriol* 181:1664-1672
47. Terra R, Stanley-Wall NR, Cao G, Lazazzera BA (2012) Identification of *Bacillus subtilis* SipW as a bifunctional signal peptidase that controls surface-adhered biofilm formation. *J Bacteriol* 194:2781-2790
48. Tjalsma H, Stover AG, Driks A, Venema G, Bron S, van Dijk JM (2000) Conserved serine and histidine residues are critical for activity of the ER-type signal peptidase SipW of *Bacillus subtilis*. *J Biol Chem* 275: 25102-25108
49. Uptain SM, Lindquist S (2002) Prions as protein-based genetic elements. *Annu Rev Microbiol* 56:703-741
50. Uversky VN, Fink AL (2004) Conformational constraints for amyloid fibrillation: the importance of being unfolded. *Biochim Biophys Acta* 1698:131-153
51. Valincius G, Heinrich F, Budvytyte R, Vanderah DJ, McGillivray DJ, Sokolov Y, Hall JE, Losche M (2008) Soluble amyloid beta-oligomers affect dielectric membrane properties by bilayer insertion and domain formation: implications for cell toxicity. *Biophys J* 95:4845-4861
52. Villar-Piqué A, Espargaro A, Sabaté R, de Groot NS, Ventura S (2012) Using bacterial inclusion bodies to screen for amyloid aggregation inhibitors. *Microb Cell Fact* 11:55
53. Villaverde A (2012) Bacterial inclusion bodies: an emerging platform for drug delivery and cell therapy. *Nanomedicine* 7:1277-1279
54. Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R (2013) Sticking together: building a biofilm the *Bacillus subtilis* way. *Nat Rev Microbiol* 11:157-168
55. Wickner RB, Edskes HK, Bateman DA, Kelly AC, Gorkovskiy A, Dayani Y, Zhou A (2013) Amyloids and yeast prion biology. *Biochemistry* 52:1514-1527
56. Wosten HA, de Vocht ML (2000) Hydrophobins, the fungal coat unravelled. *Biochim Biophys Acta* 1469:79-86
57. Zhou Y, Smith D, Leong BJ, Brannstrom K, Almqvist F, Chapman MR (2012) Promiscuous cross-seeding between bacterial amyloids promotes interspecies biofilms. *J Biol Chem* 287:35092-35103
58. Zogaj X, Nimtz M, Rohde M, Bokranz W, Romling U (2001) The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. *Mol Microbiol* 39:1452-1463