Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: Cross-seeding as a disease mechanism

Katarzyna Lundmark*, Gunilla T. Westermark†, Arne Olsén‡, and Per Westermark§¶

*Division of Pathology, Karolinska University Hospital, SE-141 86 Huddinge, Sweden; †Division of Cell Biology, Linköping University, SE-581 85 Linköping, Sweden; ‡Department of Clinical Immunology, Göteborg University, SE-413 46 Göteborg, Sweden; and §Department of Genetics and Pathology, Uppsala University, SE-751 85 Uppsala, Sweden

Communicated by D. Carleton Gajdusek, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France, March 4, 2005 (received for review December 15, 2004)

Secondary, or amyloid protein A (AA), amyloidosis is a complication of chronic inflammatory diseases, both infectious and noninfectious. AA constitutes the insoluble fibrils, which are deposited in different organs, and is a major N-terminal part of the acute phase protein serum AA. It is not known why only some patients with chronic inflammation develop AA amyloidosis. Nucleation is a widely accepted mechanism in amyloidogenesis. Preformed amyloid-like fibrils act as nuclei in amyloid fibril formation *in vitro*, and AA amyloid fibrils and synthetic amyloid-like fibrils also may serve as seed for fibril formation *in vivo*. In addition to amyloid fibrils, there is a variety of similar nonmammalian protein fibrils with β-pleated structure in nature. We studied three such naturally occurring protein fibrils: silk from *Bombyx mori*, Sup35 from *Saccharomyces cerevisiae*, and curli from *Escherichia coli*. Our results show that these protein fibrils exert amyloid-accelerating properties in the murine experimental AA amyloidosis, suggesting that such environment factors may be important risk factors in amyloidogenesis.

amyloid | seeding | protein misfolding | aggregation | prion

Amyloidosis is a heterogeneous group of protein conformational diseases, characterized by accumulation of insoluble protein fibrils with β-pleated structure in extracellular spaces of different organs and tissues. Secondary amyloidosis, one of the most common forms of clinically important systemic amyloidosis, is a result of chronic inflammatory diseases such as rheumatoid arthritis (1–3) and chronic infections such as malaria, tuberculosis, and leprosy (4–6). Amyloid protein A (AA) is the amyloid fibril protein deposited in secondary, or AA, amyloidosis. The precursor of protein AA is serum amyloid A (SAA), a family of major acute-phase proteins produced by the liver under the influence of cytokines IL-1-β, TNF-α, and IL-6 (7). SAA is normally degraded, but in some patients, AA amyloid fibrils containing 44- to 100-aa N-terminal fragments of SAA are deposited in different tissues. Because there is not one definitive amyloidogenic form of SAA identified in humans, it is unknown why only a fraction of patients with longstanding inflammation and persistently high SAA plasma levels develop amyloidosis.

AA amyloidosis can easily be induced in mice provoked with an inflammatory challenge and, in many strains, all animals develop the disease. Only SAA type 1 serves as a precursor for amyloid fibrils in mice (8, 9). Typically, amyloidosis develops after a lag phase of $\geq$1 month. The time for development of amyloidosis is dramatically shortened, to $<$1 week, when inflammation-stimulated animals receive injections of cells, homogenates, or extract of an amyloid-containing tissue (10–13). The active component in these different preparations, named amyloid-enhancing factor (AEF), is known to serve as a seed for fibril formation (14). In addition to murine AA amyloidosis, murine apolipoprotein A-II amyloidosis also is transmittable (15, 16), pointing to a general mechanism in the pathogenesis of systemic amyloidosis. Characterization of AEF, derived from animals with AA amyloidosis, demonstrated that it contained small AA-fibril fragments, and it was proposed that its amyloid-enhancing activity was related to these fragments (17). Studies with synthetic amyloid-like fibrils made from short peptides of different amyloid proteins (18, 19) confirmed that fibrils without other constituents accelerate AA-amyloid deposition and thus might represent the biologically active component in AEF. Similar results were obtained in studies with silk-derived fibrils as AEF (20).

In the present study, we further investigated seeding properties of amyloid-like protein fibrils derived from naturally occurring nonamyloidogenic proteins in a murine model of AA amyloidosis. In addition to silk fibroin from fibers of the silkworm *Bombyx mori*, two other such proteins have been studied: the prion-like protein Sup35 from *Saccharomyces cerevisiae* (21, 22) and the fibrillary surface organelle curli from *Escherichia coli* (23–25). Both of these proteins have important functions in host organisms. Sup35 is an omnipotent suppressor of nonsense mutations, and curli facilitate bacterial adaptation to different ecological niches resulting from mediation of binding to fibronectin (23), plasminogen (26), and major histocompatibility complex class I molecules (27). Interestingly, polymerization of curli and Sup35 are both nucleation-dependent processes similar to prion (scrapie) protein fibril formation (21, 22, 28, 29). We found that all three fibril preparations studied were sufficient to shorten the initial lag phase in amyloid deposition. The fact that AA amyloidosis can be accelerated by fibrils derived from unrelated normally nonpathogenic proteins indicates that environmental factors may be important risk factors for amyloidogenesis.

Materials and Methods

Animals. Five- to 10-week-old female Naval Medical Research Institute (NMRI) mice obtained from B & K Universal (Sollentuna, Sweden) and age-matched FVB mice of both sexes, bred at the Linköping University Hospital animal facility, were used in the experiments. The mice were kept in cages with steel-wire lids under 12-h dark/12-h light cycles and had free access to water and pellets (type R36, Lactamin, Vadstena, Sweden). The local animal ethics committee in Linköping, Sweden, approved the experimental protocols.

Silk Fibrils. Silk fibrils from the cocoon of *B. mori* (a gift from Fjärilhuset, Stockholm) were prepared as described (30). Briefly, silk was boiled extensively for 2 h in 2% Na_2CO_3, rinsed, and dried at 60°C. Ten milligrams of treated silk was dissolved in 700

Abbreviations: AA, amyloid protein A; SAA, serum AA; AEF, amyloid-enhancing factor; NMRI, Naval Medical Research Institute.

¶To whom correspondence should be addressed. E-mail: per.westermark@genpat.uu.se.

μl of 65% aqueous LiSCN brought to 1% 2-mercaptoethanol. The solution was dialyzed overnight against double-distilled water and brought to 10 ml with double-distilled water (1 mg of protein per ml). The salt concentration was adjusted to 0.9% by using solid NaCl, and the solution was left at 4°C for 4 weeks for fibril formation. The fibril suspension was thoroughly sonicated on ice with Soniprep 150 (Crawley, Sussex, U.K.) before use in animal experiments.

Sup35 Fibrils. Fibrils from the recombinant wild-type prion-determining N-terminal and middle (NM) region of Sup35 and its variant, R_2E_2 NM (31), were kindly provided by Susan Lindquist (Massachusetts Institute of Technology, Cambridge). The fibrils were diluted to a concentration of 15 μg/ml with 0.9% NaCl and sonicated as described before use.

Curli Fibrils. A curli-proficient *E. coli* K12 strain, YMel, was grown on colonization factor antigen (CFA)–agar plates for 48 h at 28°C, assayed for fibronectin binding (23), heat-inactivated, and suspended to a concentration 7.7×10^8 colony-forming units per ml. Two-hundred-microliter samples of YMel were diluted 10× with 0.9% NaCl and were thoroughly sonicated as described before use in the experiments. Then, curli were purified as described (32). Briefly, bacteria grown as above were treated with RNase A and DNase 1, incubated with lysozyme, and brought to 1% SDS buffer. The material was washed and pelleted by centrifugation. The RNase A, DNase 1, and lysozyme treatment was repeated, and curli were pelleted by centrifugation. The pellet was resuspended in 2 ml of SDS/PAGE sample buffer, boiled, and subjected to electrophoresis for 5 h on an SDS/12% PAGE gel. The curli protein remaining in the wells after electrophoresis (≈25 μg) was recovered, washed, and resuspended in 4 ml of double-distilled water. Then, 500 μl of purified curli was diluted with equal volume of 0.9% NaCl and sonicated as above before use in animal experiments.

Actin Fibrils. Lyophilized actin from rabbit muscle was obtained from Sigma. Actin was dissolved (1 mg/ml) in a G-buffer (33), pH 7.8, containing 5 mM Tris·HCl, 0.1 mM $CaCl_2$, 0.2 mM DTT, 0.01% sodium azide, and 0.2 mM ATP (Amersham Pharmacia), and stored on ice. Polymerization was started by the addition of 1 mM $MgCl_2$ to the actin solution at 24°C (33). Actin fibrils (F-actin) were diluted with an equal volume of G-buffer and immediately used in the experiments.

Induction of Amyloid with Silver Nitrate. In the first series of experiments, the time for induction of amyloidosis was estimated for both female and male FVB mice. All mice were injected with 0.5 ml of 1% silver nitrate at the start of the experiments and received injections of 0.1 ml of 1% silver nitrate once a week until the end of the experiments. The mice were divided into groups of three to six and were killed 7, 14, 17, 19, 21, 25, 28, or 58 days after the first silver nitrate injection. Spleens from all mice were collected and, for the demonstration of amyloid, one half of each spleen was crushed between two glass slides, smeared homogeneously over both of the slides, and air-dried in room temperature overnight. The other half was fixed in 4% buffered neutral formaldehyde solution and embedded in paraffin. The time for induction of amyloidosis by repeated s.c. injections of silver nitrate in NMRI mice was estimated in ref. 18.

Enhancement of Amyloid Induction. Amyloid was induced as described elsewhere (18, 19) by i.v. injections of 0.1 ml (100 μg) of silk fibrils, 0.1 ml (1.5 μg) of Sup35 fibrils or its variant, 0.1 ml of sonicated heat-inactivated YMel *E. coli*, or 0.1 ml of purified curli (≈0.3 μg), followed by s.c. injections of 0.5 ml of 1% silver nitrate. Control groups received i.v. injections of 0.1 ml of 0.15 M NaCl, F-actin (50 μg), or G-buffer, followed by 0.5 ml of 1% silver nitrate exactly as in the experimental groups. Injections of 0.1 ml of 1% silver nitrate were repeated after 7 and 14 days, and the animals in both experimental and control groups were killed 2 days after the last treatment by cervical dislocation in ether narcosis. The spleens were collected, divided into two halves, and treated as described above. In another experiment, one half of spleen was frozen for amyloid protein purification, and the other half was divided in two parts. One of them was smeared between two slides, and the other one was fixed in formaldehyde as described above.

Light Microscopy. Samples from silk, Sup35 variants, purified curli fibril suspensions, YMel *E. coli* bacteria, and F-actin were dried on glass slides and stained with alkaline Congo red (34). Smears and 10-μm-thick deparaffinized spleen sections were stained with alkaline Congo red and hematoxylin/eosin. The slides were examined blindly in a polarization microscope (BX51, Olympus, Melville, NY) for bright green birefringence, typical of amyloid. Amyloid grading was performed on spleen sections for each mouse separately, as described in ref. 14.

Electron Microscopy. Samples of silk fibrils, Sup35 fibrils, YMel *E. coli* bacteria, and purified curli were prepared directly after sonication by applying small drops of the suspensions on Formvar-coated nickel grids. Samples of F-actin were prepared immediately after polymerization. The grids were negatively contrasted with 1% uranyl acetate, air-dried, and viewed in a JEOL 1230 electron microscope at 100 kV.

Characterization of Amyloid Deposits. Sections from formalin-fixed spleen samples were labeled with rabbit antiserum (1:400) against mouse protein AA (18, 19). A mouse monoclonal antibody to protein AA (S. Nyström and G.T.W., unpublished data) also was used. Frozen spleen samples from two mice treated with Sup35, three mice treated with silk fibrils, and two mice treated with purified curli were homogenized separately, and amyloid fibrils were extracted as described (18, 19). Fibrils were dissolved in 6 M guanidine·HCl/0.1 M Tris·HCl (pH 8.0) and subjected to size-exclusion chromatography on a Superose 12 HR column (Amersham Pharmacia). Protein material was eluted with 10% formic acid at a flow rate of 0.25 ml/min and monitored at 280 nm. The retarded main protein peaks were dried in a Speed Vac (Savant) and analyzed by MALDI-TOF MS. For this procedure, material was dissolved in 70% acetonitrile/0.3% trifluoroacetic acid and heated to 95°C for 5 min. Equal volumes of sample and α-cyano-4-hydroxycinnamic acid, saturated with 70% acetonitrile/0.3% trifluoroacetic acid, were mixed, and 2-μl aliquots were spotted onto the target plate and analyzed. Reflector mass spectra were recorded by using the instrument setting recommended by the manufacturer. The analysis was carried out by using a mass spectrometer (Voyager-DE Pro, Applied Biosystems).

In further experiments, material from the main retarded protein AA peak from mice that received silk fibrils was dissolved in 0.1 M ammonium carbonate and digested with trypsin (sequencing grade, substrate:enzyme 50:1, Promega), and the material was subjected to MALDI-TOF MS.

Statistical Analysis. For statistical comparison between groups, Fisher's exact test was used with INSTAT 2.01 software (GraphPad, San Diego). $P < 0.05$ was considered statistically significant.

Results

Fibrils. Modified silk, studied directly after the preparation, did not bind Congo red and contained only amorphous material in the electron microscope. Modified silk that had been incubated for 4 weeks, Sup35, and purified curli, all bound Congo red and exhibited green birefringence in the polarization microscope.

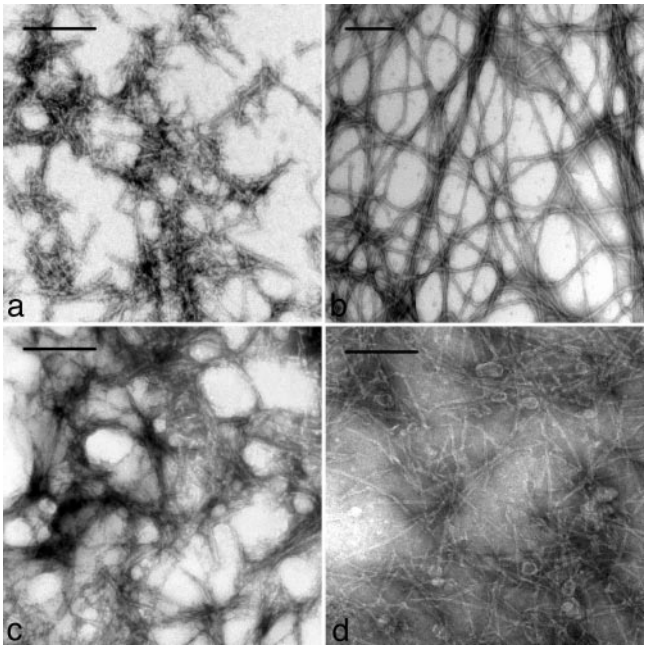

Fig. 1. Fibrils formed from silk (*a*), Sup35 (*b*), curli (*c*), and native mouse AA (*d*). All specimens negatively contrasted with uranyl acetate. (Scale bar, 0.2 μm.)

Curli-forming *E. coli* bacteria bound Congo red dye, but no green birefringence was detected in polarized light. Actin fibrils showed no affinity for Congo red and no green birefringence. Electron microscopy revealed that all these preparations contained fine, nonbranching, twisted fibrils of various lengths (Fig. 1). Curli-forming *E. coli* bacteria were surrounded by a large network of fibrils with the same morphology as purified curli fibrils. In the sonicated samples, this network was disrupted into small aggregates of fibrils.

Development of Amyloidosis After Inflammation. Amyloid deposits did not develop in any female FVB mice within 28 days of treatment or in any male FVB mice within 58 days of treatment with repeated s.c. injections of 1% silver nitrate. From this finding, we choose 16 days for the study of effects of the different fibril preparations, which is the same amount of time that was used in previous experiments with NMRI mice (18).

Effects of Modified Silk. Of the seven FVB mice treated with silver nitrate and modified silk used 4 weeks after its preparation, five animals had splenic amyloid deposits on day 16. No amyloid was detected in controls treated with 0.9% NaCl and silver nitrate ($P < 0.01$; Table 1).

Effects of YMel *E. coli* Bacteria and Purified Curli. Amyloid was detected in the spleens of four of seven FVB mice ($P < 0.03$) treated with silver nitrate and bacteria, and in three of eight FVB mice ($P < 0.04$) treated with silver nitrate and purified curli. No amyloid was detected in controls treated with 0.9% NaCl and silver nitrate (Table 1).

Effects of Sup35 Fibrils. Amyloid deposits were detected in the spleens of 10 of the 12 FVB mice treated with Sup35, whereas 2 of 11 mice in a control group treated with 0.9% NaCl developed minimal amyloid deposits ($P < 0.005$; Table 1). In a separate study, performed in NMRI mice, we wanted to explore possible differences in AEFs with a Sup35 variant with enhanced propensity for fibril formation (31). No significant differences were found (amyloid occurred in three of nine mice treated with wild-type NM and one of eight mice treated with R2E2 NM). No amyloid was detected in nine NMRI controls treated with 0.9% NaCl.

Effects of Actin Fibrils. No amyloid was detected in 13 (9 NMRI and 4 FVB) mice treated with actin fibrils or in 14 (9 NMRI and 5 FVB) mice treated with G-buffer.

Characterization of the Amyloid Induced with Different Enhancers. The morphology of the splenic amyloid depositions was the same in all animals, regardless of the type of enhancer used (Fig. 2 *a* and *b*). Immunohistochemistry verified that the amyloid was type AA (Fig. 2 *c* and *d*). Purified amyloid proteins from animals with AA amyloidosis induced with silk and Sup35 were further characterized by using MALDI-TOF MS. The amount of protein extracted from mice treated with curli was too small for further purification. The analysis of protein AA extracted from mice injected with Sup35 showed that it had a molecular mass of ≈9,222 Da. A molecular mass of 9.2 kDa corresponds most closely to protein AA subspecies containing 80 or 81 amino acid residues. The analysis of protein AA extracted from mice injected with silk showed that the sample contained two protein AA subspecies, one with a molecular mass of 9,192 Da and the other with a molecular mass of ≈9,543 Da. A molecular mass of 9.5 kDa corresponds to a protein AA subspecies containing 84 amino acid residues. These findings correspond well to a previous study of the composition of mouse protein AA induced with silver nitrate and native AEF (14) but differ from some earlier studies of murine protein AA (35).

MALDI-TOF MS of trypsin-treated peak material revealed masses of 2,022.7 Da (expected 2,022.9), 1,680.0 Da (expected 1,679.8), and 1,146.3 Da (expected 1,146.5), corresponding to

Table 1. Occurrence of amyloid deposits in the spleen of mice treated with one i.v. injection of different amyloid-like fibrils, followed by s.c. injections of $AgNO_3$ repeated once weekly for 16 days, compared with controls treated with 0.15 M NaCl i.v. and $AgNO_3$ s.c.

Treatment	Dose per mouse	Mice with amyloid	Amyloid grade	*P*
Silk fibrils	100 μg	5/7	1–3	<0.01
Vehicle		0/8		
YMel *E. coli*	7.7×10^6 cfu	4/7	2–4	<0.03
Purified curli	≈0.3 μg	3/8	1–3	<0.04
Vehicle		0/15	–	
Sup35	1.5 μg	10/12	2–4	<0.005
Vehicle		2/11	1	

cfu, colony-forming units. In the Amyloid grade column, the following codes are used: –, no amyloid found; 1, trace amount of amyloid; 2, small amyloid deposits in perifollicular spaces; 3, moderate amount of amyloid around follicles; and 4, extensive amyloid deposits around follicles and in the interfollicular spaces.

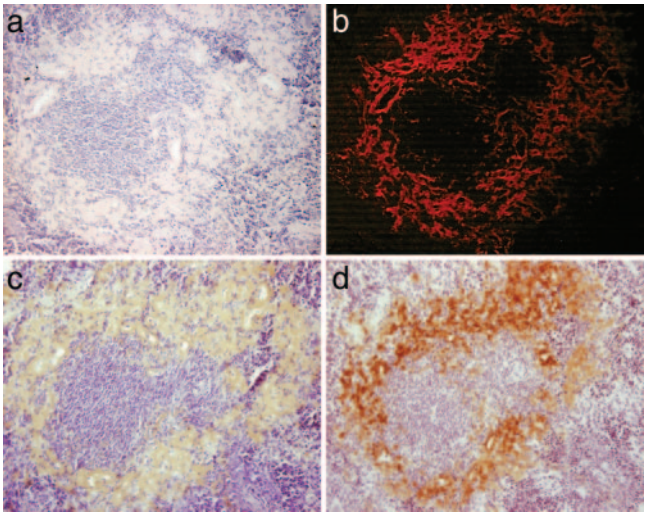

Fig. 2. Murine perifollicular splenic amyloid induced by one i.v. injection of different amyloid-like fibrils, followed by s.c. injections of $AgNO_3$, repeated once weekly. Controls were treated with 0.15 M NaCl i.v. and $AgNO_3$ s.c. The duration of the experiments was 16 days. Shown are sections stained with Congo red and viewed in ordinary light (*a*) and fluorescence (*b*). Adjacent sections show that the amyloid was labeled specifically with a polyclonal antiserum (*c*) and mouse monoclonal antibody (*d*) to mouse protein AA.

mouse SAA type 1 positions 1–18, 25–38, and 62–70. One mass of 2,388.8 Da corresponds to positions 19–38 and 39–61, both of which have expected masses of 2,389.1 Da.

Discussion

In this study, we used several different fibril preparations: prion-like protein Sup35 fibrils from the yeast *S. cerevisiae* (22), amyloid-like fibrils from *B. mori* silk (20), *E. coli* K12 strain with fibril-forming polymers at the bacterial surface (23), curli purified from these bacteria, and actin fibrils. Silk fibrils, different variants of Sup35 fibrils, and purified curli preparations all showed green birefringence in polarized light after staining with Congo red dye, indicating amyloid fibril-like organization of the protein molecules. Actin fibrils had no affinity to Congo red and were consequently not amyloid-like. Electron microscopy studies of fibril samples revealed that all of the Sup35, silk, and curli preparations contained small aggregates of fibrils similar to the native AA fibrils (Fig. 1).

All amyloid-like fibril preparations and *E. coli* bacteria were sufficient to accelerate AA amyloidosis induced with silver nitrate in experimental animals and thus possessed an AEF activity, whereas non-amyloid-like actin fibrils had no such effect. These results give further support to the theory that experimental AA amyloidosis is a nucleation-dependent process, and that amyloid fibrils themselves serve as a template for further fibril formation *in vivo* by means of a mechanism of interaction between fibrils used as seeds and SAA, similar to the prion protein (PrP) conversion (36). The MS analyses showed that SAA is C-terminally trimmed in the amyloid fibrils. Whether precleavage is necessary for recruitment of SAA to the fibril template or it occurs after its association to the amyloid is not known.

Occurrence of cross-seeding, although with lower efficiency than native AEF (18, 19), is of great interest, suggesting the lack of definitive conformational barriers in this protein-conformational conversion phenomenon. This mechanism may be of great importance for the understanding the pathogenesis of human AA amyloidosis and, perhaps, other forms of amyloidosis. Exposure (by ingestion or inhalation) to naturally occurring amyloid-like protein fibrils like silk, Sup35, or curli may bring seeds that start a nucleation process in predisposed individuals with persistently high SAA production. Such a process would offer an explanation as to why only some patients with long-standing inflammation develop systemic AA-amyloid deposits. Environmental factors also may be important risk factors for other kinds of amyloidosis. Interestingly, in an epidemiological study of possible environmental elements important in the precipitation of Swedish familial transthyretin amyloidosis, which is a dominantly hereditary disease with low penetrance, only two factors were significant, one of which was the occupation of dressmaking (37).

We thank Marie-Louise Eskilsson for skilled technical support, Karin Edsparr (Division of Cell Biology, Linköping University), for purification of curli, Alexander Vener (Division of Cell Biology, Linköping University) for the MS analyses, and Susan Lindquist for Sup35 proteins. This work was supported by Swedish Medical Research Council Projects 5981 and 14272, the Swedish Association Against Rheumatism, the Swedish Society for Medical Research, Östergötlands Läns Landsting Project 7/2002, and the Lions Research Foundation.

1. Kobayashi, H., Tada, S., Fuchigami, T., Okuda, Y., Takasugi, K., Matsumoto, T., Iida, M., Aoyagi, K., Iwashita, A., Daimaru, Y. & Fujishima, M. (1996) *Br. J. Rheumatol.* **35,** 44–49.
2. Bély, M. & Apáthy, A. (1999) in *Amyloid and Amyloidosis 1998*, eds. Kyle, R. A. & Gertz, M. A. (Parthenon, Rochester, MN), pp. 408–410.
3. Myllykangas-Luosujarvi, R., Aho, K., Kautiainen, H. & Hakala, M. (1999) *Rheumatology* **38,** 499–503.
4. McAdam, K. P. W. J., Anders, R. F., Smith, S. R., Russell, D. A. & Price, M. A. (1975) *Lancet* **ii,** 572–576.
5. McAdam, K. P. W. J., Westermark, P., Anders, R. F. & Voller, A. (1980) in *Amyloid and Amyloidosis*, eds. Glenner, G. G., Costa, P. P. & de Freitas, F. (Excerpta Medica, Amsterdam), pp. 207–210.
6. McAdam, K. P. W. J., Raynes, J. G., Alpers, M. P., Westermark, G. T. & Westermark, P. (1996) *P. N. G. Med. J.* **39,** 284–296.
7. Husby, G., Marhaug, G., Dowton, B., Sletten, K. & Sipe, J.D. (1994) *Amyloid* **1,** 119–137.
8. de Beer, M. C., de Beer, F. C., McCubbin, W. D., Kay, C. M. & Kindy, M. S. (1993) *J. Biol. Chem.* **268,** 20606–20612.
9. Hoffman, J. S., Ericsson, L. H., Eriksen, N., Walsh, K. A. & Benditt, E. P. (1984) *J. Exp. Med.* **159,** 641–646.
10. Werdelin, O. & Ranløv, P. (1966) *Acta Pathol. Microbiol. Scand.* **68,** 1–18.
11. Hardt, F. & Ranløv, P. (1968) *Acta Pathol. Microbiol. Scand.* **73,** 549–558.
12. Shirahama, T., Lawless, O. J. & Cohen, A. S. (1969) *Proc. Soc. Exp. Biol. Med.* **130,** 516–519.
13. Kisilevsky, R. & Boudreau, L. (1983) *Lab. Invest.* **48,** 53–59.
14. Lundmark, K., Westermark, G. T., Nyström, S., Murphy, C. L., Solomon, A. & Westermark, P. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 6979–6984.
15. Korenaga, T., Fu, X., Xing, Y., Matsusita, T., Kuramoto, K., Syumiya, S., Hasegawa, K., Naiki, H., Ueno, M., Ishihara, T., *et al.* (2004) *Am. J. Pathol.* **164,** 1597–1606.
16. Fu, X., Korenaga, T., Fu, L., Xing, Y., Guo, Z., Matsushita, T., Hosokawa, M., Naiki, H., Baba, S., Kawata, Y., *et al.* (2004) *FEBS Lett.* **563,** 179–184.
17. Niewold, T. A., Hol, P. R., van Andel, A. C. J., Lutz, E. T. G. & Gruys, E. (1987) *Lab. Invest.* **56,** 544–549.
18. Ganowiak, K., Hultman, P., Engström, U., Gustavsson, Å. & Westermark, P. (1994) *Biochem. Biophys. Res. Commun.* **199,** 306–312.
19. Johan, K., Westermark, G., Engström, U., Gustavsson, Å., Hultman, P. & Westermark, P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2558–2563.
20. Kisilevsky, R., Lemieux, L., Boudreau, L., Yang, D.-S. & Fraser, P. (1999) *Amyloid* **6,** 98–106.
21. King, C.-Y., Tittmann, P., Gross, H., Gebert, R., Aebi, M. & Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6618–6622.
22. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J. & Lindquist, S. (1997) *Cell* **89,** 811–819.
23. Olsén, A., Jonsson, A. & Normark, S. (1989) *Nature* **338,** 652–655.
24. Olsén, A., Arnqvist, A., Hammar, M., Sukupolvi, S. & Normark, S. (1993) *Mol. Microbiol.* **7,** 523–536.
25. Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S. & Hultgren, S. J. (2002) *Science* **295,** 851–855.
26. Sjöbring, U., Pohl, G. & Olsén, A. (1994) *Mol. Microbiol.* **14,** 443–452.

27. Olsén, A., Wick, M. J., Mörgelin, M. & Björck, L. (1998) *Infect. Immun.* **66,** 944–949.
28. Hammar, M., Bian, Z. & Normark, S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 6562–6566.
29. Serio, T. T., Cashikar, A. G., Kowal, A. S., Sawicki, G. J., Moslehi, J. J., Serpell, L., Amsdorf, M. F. & Lindquist, S. L. (2000) *Science* **289,** 1317–1321.
30. Kisilevsky, R., Lemieux, L. & Bourdreau, L. (1999) in *Amyloid and Amyloidosis 1998*, eds. Kyle, R. & Gertz, M. (Parthenon, Rochester, MN), pp. 423–425.
31. Liu, J.-J & Lindquist, S. (1999) *Nature* **400,** 573–576.
32. Collinson, S. K., Emödy, L., Muller, K.-H., Trust, T. J. & Kay, W. W. (1991) *J. Bacteriol.* **173,** 4773–4781.
33. Carlier, M.-F., Pantaloni, D. & Korn, E. D. (1985) *J. Biol. Chem.* **260,** 6565–6571.
34. Puchtler, H., Sweat, F. & Levine, M. (1962) *J. Histochem. Cytochem.* **10,** 355–364.
35. Dwulet, F. E. & Benson, M. D. (1987) *J. Lab. Clin. Med.* **110,** 322–329.
36. Cohen, F. E. & Prusiner, S. B. (1998) *Annu. Rev. Biochem.* **67,** 793–819.
37. Hardell, L. Holmgren, G., Steen, L., Fredrikson, M. & Axelson, O. (1995) *Epidemiology* **6,** 598–601.