REVIEW ARTICLE

MECHANISMS OF DISEASE

# Molecular Mechanisms of Amyloidosis

Giampaolo Merlini, M.D., and Vittorio Bellotti, M.D., Ph.D.

THE AMYLOIDOSES CONSTITUTE A LARGE GROUP OF DISEASES IN WHICH misfolding of extracellular protein has a prominent role. This dynamic process, which occurs in parallel with or as an alternative to physiologic folding, generates insoluble, toxic protein aggregates that are deposited in tissues in bundles of $\beta$-sheet fibrillar protein (Fig. 1). (A $\beta$-sheet consists of strands of polypeptides in zigzag formation, as shown in Fig. 2.) These amyloid deposits are identified on the basis of their apple-green birefringence under a polarized light microscope after staining with Congo red and the presence of rigid, nonbranching fibrils 7.5 to 10 nm in diameter, on electron microscopy (Fig. 2).[1] Amyloid deposits are the basis of several conditions that have an enormous social and medical impact as well as the cause of rare conditions that challenge the physician's diagnostic capability (Table 1).

The deposition of amyloid in brain tissue underlies Alzheimer's disease,[2,3] which affects more than 12 million people worldwide. The central nervous system is also the target of prion proteins, the cause of a group of rare hereditary or acquired neurodegenerative conditions.[4] The approximately 1 million patients who are receiving dialysis worldwide are at risk for symptomatic amyloidosis.[5] The two most common forms of systemic amyloidosis are light-chain (AL) amyloidosis, with an incidence of approximately 1 case per 100,000 person-years in Western countries,[6] and reactive amyloidosis due to chronic inflammatory diseases (e.g., rheumatoid arthritis and chronic infections). Hereditary amyloidosis is an ever-expanding group of disorders that pose difficult diagnostic problems.[7] The clinical features of systemic amyloidosis were reviewed in the *Journal* in 1997.[8]

From the Amyloid Center, Biotechnology Research Laboratory, University Hospital IRCCS Policlinico San Matteo; and the Department of Biochemistry, University of Pavia — both in Pavia, Italy. Address reprint requests to Dr. Merlini at the Amyloid Center, Biotechnology Research Laboratory, Padiglione Forlanini, University Hospital IRCCS Policlinico San Matteo, Piazzale Golgi, 2, 27100 Pavia, Italy, or at gmerlini@smatteo.pv.it.
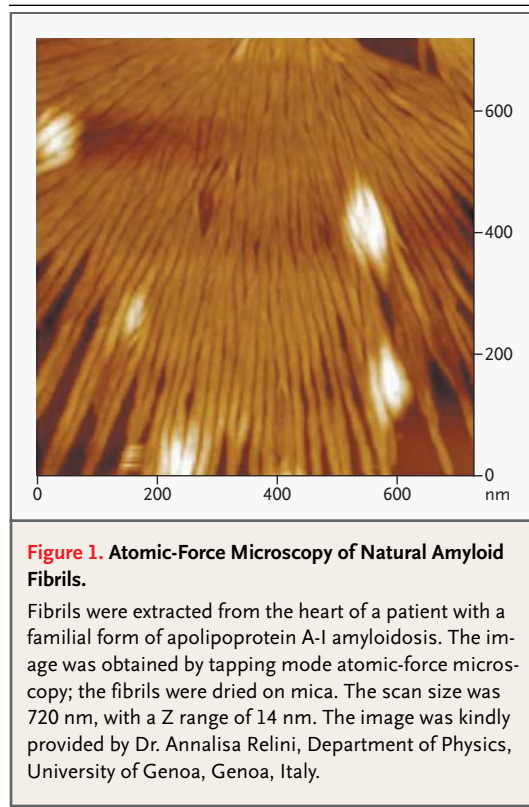
## MOLECULAR MECHANISMS

### BIOCHEMICAL CHARACTERISTICS OF AMYLOIDOGENIC PROTEINS

Isolation of the protein components of natural amyloid and the chemical characterization of these components are indispensable investigative tools.[9] To date, at least 21 different proteins have been recognized as causative agents of amyloid diseases.[1] Despite having heterogeneous structures and functions, all these proteins can generate morphologically indistinguishable amyloid fibrils (Fig. 2).[10] The generic fibrillar form of proteins can be regarded as a primordial structure dominated by hydrogen bonding between the amide and the carbonyl groups of the main chain, rather than by specific interactions of the side chains, which dictate the structure of functional globular proteins.[11] The essence of amyloidosis lies in the capacity of these proteins to acquire more than one conformation, a feature that has earned them the sobriquet of chameleon proteins.[12]

### A DYNAMIC VIEW OF THE PATHOGENIC PROCESS

The conversion of the structure of the native protein into a predominantly antiparallel $\beta$-sheet secondary structure (in which the N- and C-terminals are oriented in opposite directions) is a pathologic process closely related to physiologic protein folding. The folding of a newly synthesized polypeptide occurs in a rapid sequence of conformation-

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**Figure 1. Atomic-Force Microscopy of Natural Amyloid Fibrils.**

Fibrils were extracted from the heart of a patient with a familial form of apolipoprotein A-I amyloidosis. The image was obtained by tapping mode atomic-force microscopy; the fibrils were dried on mica. The scan size was 720 nm, with a Z range of 14 nm. The image was kindly provided by Dr. Annalisa Relini, Department of Physics, University of Genoa, Genoa, Italy.

al modifications in the cytoplasm. According to the "folding energy landscape theory," the process follows a funnel-like pathway (Fig. 3) in which the conformational intermediates progressively merge into a final species.[13] In addition, at a minimum of energy similar to that reached by the native protein, the polypeptide can acquire an alternative and relatively stable "misfolded state,"[14] which is prone to aggregation. Once the folding process has been completed and the native protein secreted (Fig. 4), many proteins are in dynamic equilibrium with a partially folded conformation, and in this state, they retrace the final part of the folding pathway, ultimately forming either a native or misfolded protein.

In amyloid disease, potentially pathogenic misfolded proteins can form in different ways. The protein may have an intrinsic propensity to assume a pathologic conformation, which becomes evident with aging (e.g., normal transthyretin in patients with senile systemic amyloidosis)[15] or at persistently high concentrations in serum (e.g., beta$_2$-microglobulin in patients undergoing long-term hemodialysis).[16] Another mechanism is the replacement of a single amino acid in the protein, as occurs in hereditary amyloidosis.[17] A third mechanism is prote-

olytic remodeling of the protein precursor, as in the case of $\beta$-amyloid precursor protein (APP) in Alzheimer's disease.[2] These mechanisms can act independently or in association with one another. In addition to the intrinsic amyloidogenic potential of the pathogenic protein, other factors may act synergistically in amyloid deposition. For example, the protein precursor must reach a critical local concentration to trigger fibril formation, a process enhanced by local environmental factors and by interactions with extracellular matrixes.[18]

## MUTATIONS AND THE MOLECULAR MECHANISM OF AMYLOID FORMATION
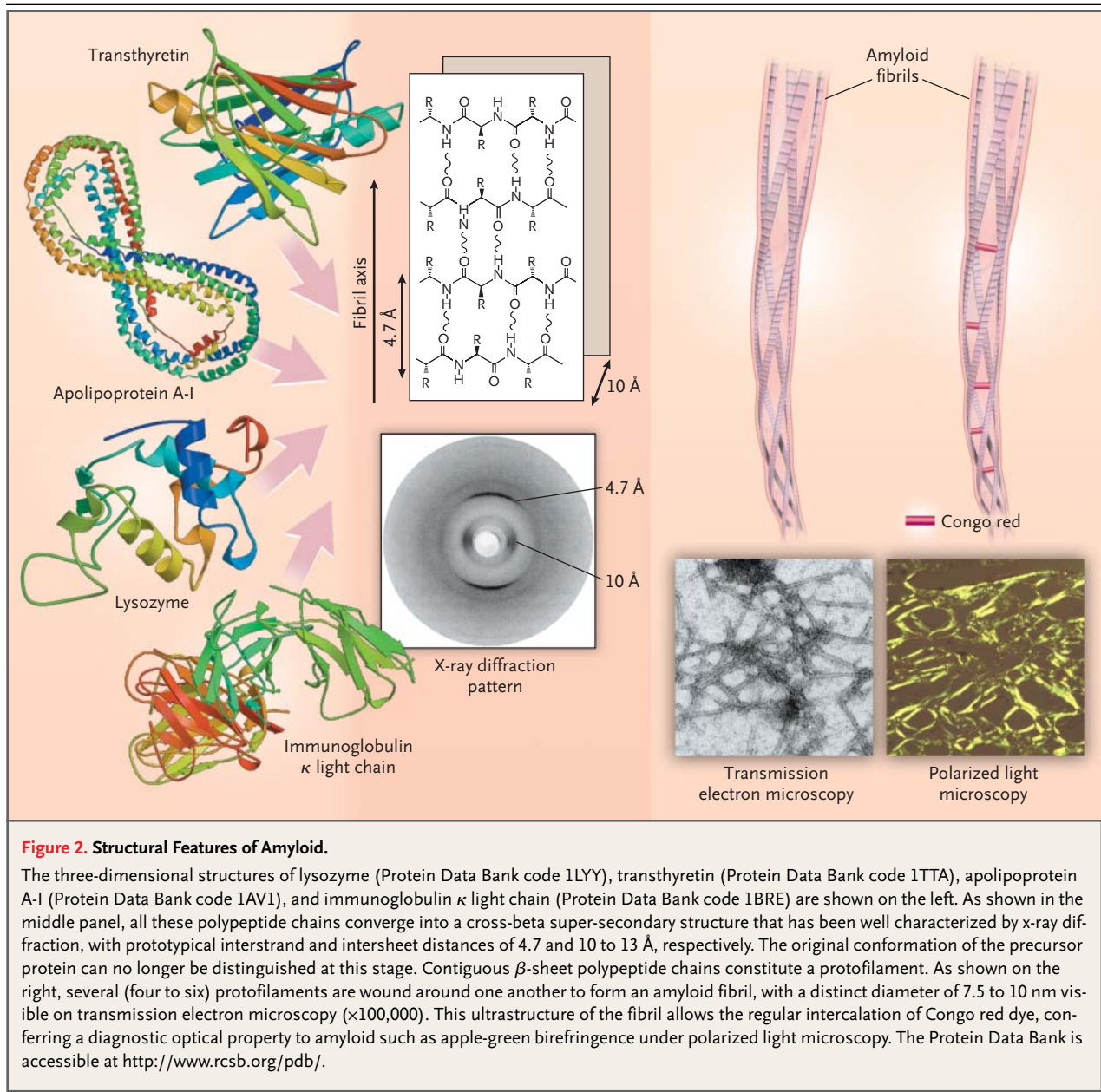
### Immunoglobulin Light Chains
Only a small proportion of immunoglobulin light chains are amyloidogenic; for example, AL amyloidosis occurs in only 12 to 15 percent of patients with myeloma. Certain structural features are related to amyloidogenicity: the $\lambda$ isotype and the $V_{\lambda VI}$ variability subgroup (a homologous family of light-chain variable regions).[19] Two $V_\lambda$ gene segments — 6a and 3r — contribute equally to the encoding of 42 percent of amyloidogenic $\lambda$ chains.[20] The variable domains of light chains V(L), including the amyloidogenic chains,[21] mutate during the immune response. Some of these physiologic mutations can affect critical structural sites, destabilizing the domain and favoring the generation of an aggregation-prone state.[22,23]

### Familial Amyloidosis
In the familial amyloidoses, the substitution of a single amino acid transforms a normal protein into an amyloidogenic one; prototypical proteins are transthyretin[24] and lysozyme.[25] Transthyretin is a homotetrameric protein with a prominent $\beta$-sheet secondary structure, whereas lysozyme consists of a single polypeptide with a predominantly helical structure (Fig. 2). Approximately 80 different mutations in transthyretin have been reported[26]; a few mutations are not associated with amyloidosis, and a couple are thought to protect against the deposition of amyloid. Four pathogenic variants of lysozyme have been reported[27,28]; a fifth variant, Thr70Asn,[29] is apparently not pathogenic.

### The Role of Instability
The property shared by these amyloidogenic variants and confirmed in studies of cystatin C,[30] immunoglobulin light chains,[31] and gelsolin[32] is a native conformation that is thermodynamically less

**Figure 2. Structural Features of Amyloid.**

The three-dimensional structures of lysozyme (Protein Data Bank code 1LYY), transthyretin (Protein Data Bank code 1TTA), apolipoprotein A-I (Protein Data Bank code 1AV1), and immunoglobulin κ light chain (Protein Data Bank code 1BRE) are shown on the left. As shown in the middle panel, all these polypeptide chains converge into a cross-beta super-secondary structure that has been well characterized by x-ray diffraction, with prototypical interstrand and intersheet distances of 4.7 and 10 to 13 Å, respectively. The original conformation of the precursor protein can no longer be distinguished at this stage. Contiguous β-sheet polypeptide chains constitute a protofilament. As shown on the right, several (four to six) protofilaments are wound around one another to form an amyloid fibril, with a distinct diameter of 7.5 to 10 nm visible on transmission electron microscopy (×100,000). This ultrastructure of the fibril allows the regular intercalation of Congo red dye, conferring a diagnostic optical property to amyloid such as apple-green birefringence under polarized light microscopy. The Protein Data Bank is accessible at http://www.rcsb.org/pdb/.

stable than that of the normal counterpart. A reduction in the stability of transthyretin should make it easier for the tetramer to dissociate into monomers,[15] whereas lysozyme mutations destabilize the tertiary structure and thus give rise to partially folded conformers[33] (alternative spatial arrangements of the same polypeptide). Monomers of transthyretin and partially folded conformers of lysozyme have a strong propensity to self-aggregate and assemble into fibrils.

The role of protein stability in the formation of fibrils in vivo has been clarified by studies of natural, nonamyloidogenic variants of both transthyretin and lysozyme. The Thr119Met variant of transthyretin has a thermodynamic stabilizing effect on transthyretin tetramers in association with both the wild-type polypeptide (wild type/Met119 genotype) and the amyloidogenic variant Val30Met.[34] Persons with transthyretin tetramers reflecting the Met30/Met119 genotype are protected from the disease that

**Table 1.** Amyloid Proteins and Their Precursors.*

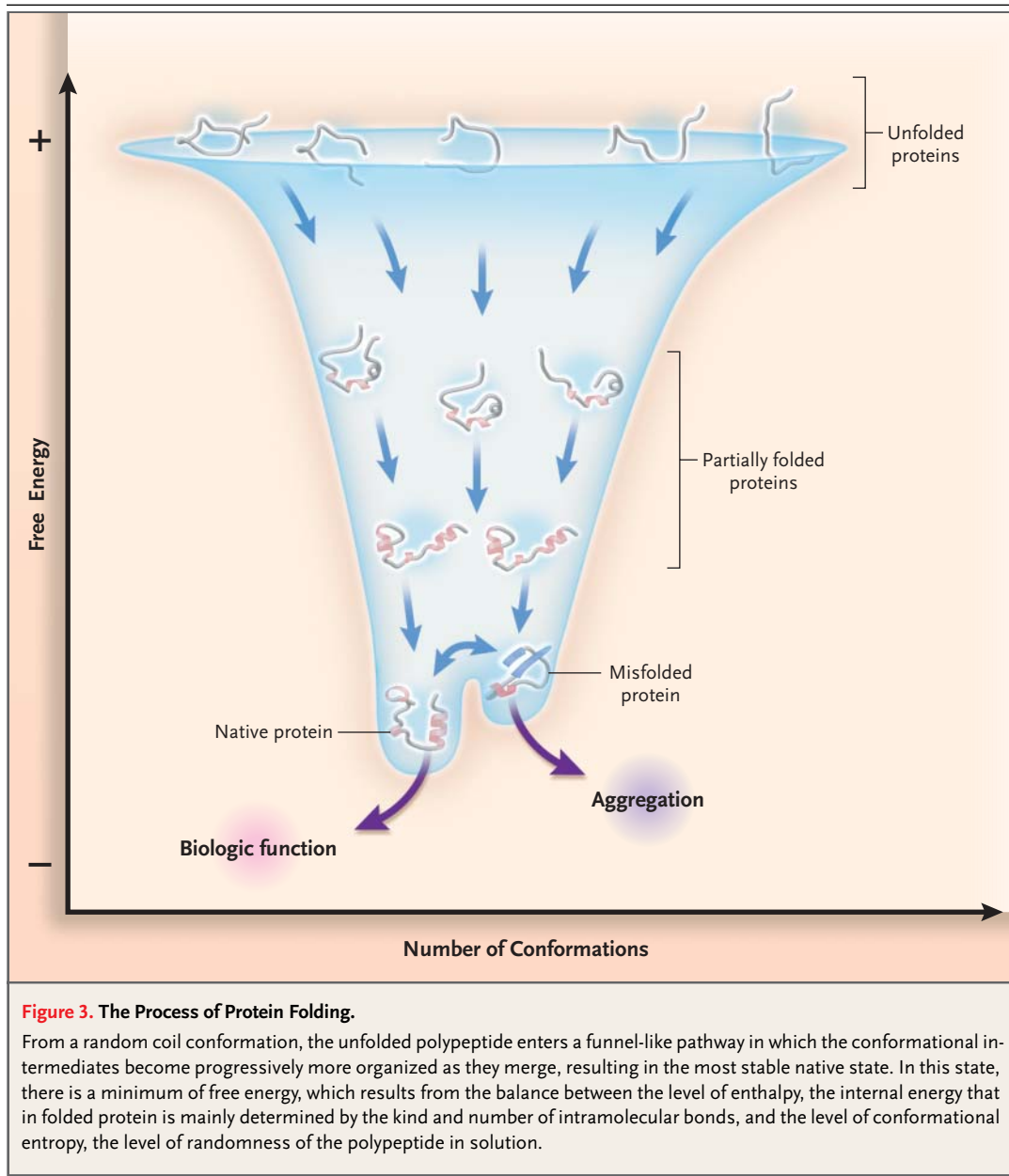| Amyloid Protein | Precursor | Distribution | Type | Syndrome or Involved Tissues |
|---|---|---|---|---|
| A$\beta$ | A$\beta$ protein precursor | Localized<br>Localized | Acquired<br>Hereditary | Sporadic Alzheimer's disease, aging<br>Prototypical hereditary cerebral amyloid angiopathy, Dutch type |
| APrP | Prion protein | Localized<br><br>Localized | Acquired<br><br>Hereditary | Sporadic (iatrogenic) CJD, new variant CJD (alimentary?)<br>Familial CJD, GSSD, FFI |
| ABri | ABri protein precursor | Localized or systemic? | Hereditary | British familial dementia |
| ACys | Cystatin C | Systemic | Hereditary | Icelandic hereditary cerebral amyloid angiopathy |
| A$\beta$2M | Beta$_2$-microglobulin | Systemic | Acquired | Chronic hemodialysis |
| AL | Immunoglobulin light chain | Systemic or localized | Acquired | Primary amyloidosis, myeloma-associated |
| AA | Serum amyloid A | Systemic | Acquired | Secondary amyloidosis, reactive to chronic infection or inflammation including hereditary periodic fever (FMF, TRAPS, HIDS, FCU, and MWS) |
| ATTR | Transthyretin | Systemic<br>Systemic | Hereditary<br>Acquired | Prototypical FAP<br>Senile heart, vessels |
| AApoAI | Apolipoprotein A-I | Systemic | Hereditary | Liver, kidney, heart |
| AApoAII | Apolipoprotein A-II | Systemic | Hereditary | Kidney, heart |
| AGel | Gelsolin | Systemic | Hereditary | Finnish hereditary amyloidosis |
| ALys | Lysozyme | Systemic | Hereditary | Kidney, liver, spleen |
| AFib | Fibrinogen A$\alpha$ chain | Systemic | Hereditary | Kidney |

* Data were adapted from Westermark et al.[1] The following proteins may also cause amyloidosis: immunoglobulin heavy chain, calcitonin, islet-amyloid polypeptide, atrial natriuretic factor, prolactin, insulin, lactadherin, keratoepithelin, and Danish amyloid protein (which comes from the same gene as ABri and has an identical N-terminal sequence). CJD denotes Creutzfeldt–Jakob disease, GSSD Gerstmann–Sträussler–Scheinker disease, FFI fatal familial insomnia, FMF familial Mediterranean fever, TRAPS tumor necrosis factor receptor–associated periodic syndrome, HIDS hyper-IgD syndrome, FCU familial cold urticaria, MWS Muckle–Wells syndrome, and FAP familial amyloidotic polyneuropathy.

occurs in those with the Met30/wild-type genotype. The folding stability of the newly identified Thr70Asn variant of lysozyme is between that of normal and amyloidogenic species.[35] However, although destabilized, this molecule is not amyloidogenic, which suggests that certain proteins have a marginal degree of protection against amyloidogenesis, even when their thermodynamic stability is less than that of wild-type protein.

Destabilization is necessary but probably not sufficient to confer an amyloidogenic propensity on a protein; other structural features are required for the formation of fibrils. Recently, the role of charged residues in modulating the aggregation process, acting as structural gatekeepers by means of repulsive forces, has been highlighted.[36] In certain proteins, such as gelsolin,[37] the partial unfolding caused by the mutation renders the protein susceptible to the attack of proteases, thus provoking the release of highly amyloidogenic polypeptides.

These findings provide support for the mechanism shown in Figure 4. Amyloidogenic and normal counterparts are synthesized and secreted as native proteins, but the system of intracellular quality control appears to be incapable of recognizing and removing dangerous mutants.[38] Outside the cell, the amyloidogenic variants ultimately reach a state of equilibrium between fully folded and partially folded forms, but there is a much greater fluctuation in the concentrations of the two forms than would be expected. All factors that perturb the three-dimensional structure — such as a low pH, oxidation, in-
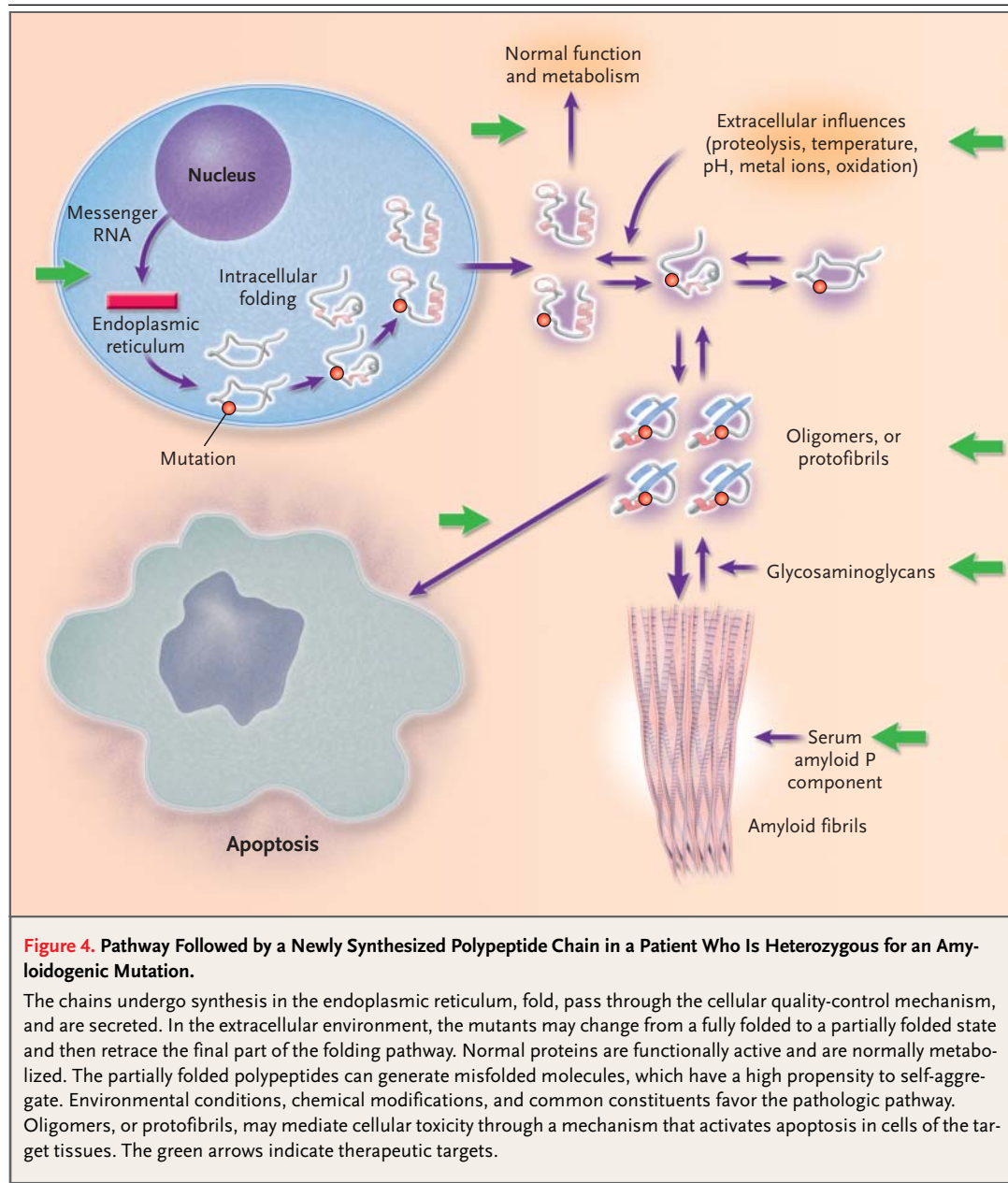
**Figure 3. The Process of Protein Folding.**

From a random coil conformation, the unfolded polypeptide enters a funnel-like pathway in which the conformational intermediates become progressively more organized as they merge, resulting in the most stable native state. In this state, there is a minimum of free energy, which results from the balance between the level of enthalpy, the internal energy that in folded protein is mainly determined by the kind and number of intramolecular bonds, and the level of conformational entropy, the level of randomness of the polypeptide in solution.

creased temperature, limited proteolysis, metal ions, and osmolytes — can shift the equilibrium toward the partially folded amyloidogenic state. For example, urea at the concentrations present in the inner renal medulla enhances the formation of fibrils by reducing the time required for a nucleus to form, which in turn initiates rapid growth of the fibril.[39] In addition, local microenvironmental conditions affect the ultrastructural organization of protein deposits. For example, pH influences the processing of immunoglobulin light chains, causing them to form either fibrillar amyloid aggregates or amorphous aggregates characteristic of light-chain–deposition disease.[40] Common components of amyloid deposits, such as glycosaminoglycans and serum amyloid P (SAP) component, may exert identical effects by hastening the integration of a soluble polypeptide into a more stable fibril.

*Proteolysis*

In certain amyloidoses, only a limited portion of the amyloid protein precursor forms the fibril; the pro-

**Figure 4. Pathway Followed by a Newly Synthesized Polypeptide Chain in a Patient Who Is Heterozygous for an Amyloidogenic Mutation.**

The chains undergo synthesis in the endoplasmic reticulum, fold, pass through the cellular quality-control mechanism, and are secreted. In the extracellular environment, the mutants may change from a fully folded to a partially folded state and then retrace the final part of the folding pathway. Normal proteins are functionally active and are normally metabolized. The partially folded polypeptides can generate misfolded molecules, which have a high propensity to self-aggregate. Environmental conditions, chemical modifications, and common constituents favor the pathologic pathway. Oligomers, or protofibrils, may mediate cellular toxicity through a mechanism that activates apoptosis in cells of the target tissues. The green arrows indicate therapeutic targets.

totypical example is Alzheimer's disease, in which the fibrils consist of proteolytic fragments of 39 to 43 residues derived from the 753-residue APP. In lysozyme amyloidosis,[25] the full-length protein is detectable in natural fibrils. There is a wide gradation of proteolytic remodeling of the protein precursor in all types of amyloidosis, but the remodeling of light chains in AL amyloidosis can be considered the archetype of the heterogeneity of this process.[41] Proteolysis is generally ascribed to extracellular, or pericellular, enzymes, such as those that cleave se-

rum amyloid A.[42] However, in the cases involving gelsolin[43] or the amyloid ABri protein,[44] the proteases act in the Golgi apparatus. The structural flexibility of the target protein allows limited proteolysis and therefore the release of polypeptides that cannot display conformational plasticity in the constrained structure of the original protein.[31]

### AMYLOIDOSIS AS A CONFORMATIONAL DISEASE

Amyloidosis properly belongs to the category of conformational diseases because pathologic protein

aggregation is largely due to reduced folding stability and a strong propensity to acquire more than one conformation.[45] The risk of protein aggregation, which poses toxic threats to the cell, is minimized by protein sequences that confer the properties of high stability and fast folding kinetics, both of which minimize the concentration of easily aggregating, partially folded proteins. Certain proteins, however, seem to require a high degree of structural disorder in their native states to fulfill their function. For instance, the structural plasticity of the emerging class of "natively unfolded proteins"[46] favors their interaction with ligands. These proteins represent an intriguing gamble of molecular evolution in which the subtle border between risky self-aggregation and sophisticated function is easily crossed. Amyloidogenic lipoproteins are examples. On the basis of its amino acid composition, lipid-free apolipoprotein A-I should behave as a natively unfolded protein; this state guarantees the plasticity of the protein, which partially unfolds when lipids are released and refolds when lipids are taken up. These properties are particularly evident in the N-terminal domain of apolipoprotein A-I, the major constituent of apolipoprotein A-I amyloid fibrils.[47] Other lipoproteins, such as apolipoprotein A-II,[48] apolipoprotein E,[49] and serum amyloid A,[50] also form amyloid or are implicated in amyloidogenesis and thus constitute a unique group of proteins. They have structural similarities that confer the conformational plasticity necessary for their function and at the same time favor the formation of amyloid.

### IS AMYLOIDOSIS A PROTEIN-MEDIATED TRANSMISSIBLE DISEASE?

Certain aspects of animal models of the amyloidosis caused by serum amyloid A[51] and apolipoprotein A-II[52] have introduced the possibility that amyloidosis is transmissible. In mice, amyloid protein A amyloidosis (AA) is caused by an inflammatory reaction that results in overproduction of the acute-phase protein serum amyloid A. Injection or oral administration of amyloid-enhancing factor, a crude homogenate of natural amyloid fibrils, accelerates the deposition of amyloid during the inflammatory process.[51,53] These findings are consistent with the capacity of fibrillar seeds to catalyze conformational changes in the soluble protein. The capacity of preformed fibrils to trigger fibrillogenesis has been demonstrated in vitro for amyloid $\beta$ (A$\beta$) peptides,[54] lysozyme,[55] and beta$_2$-microglobulin[56] (Fig. 5).

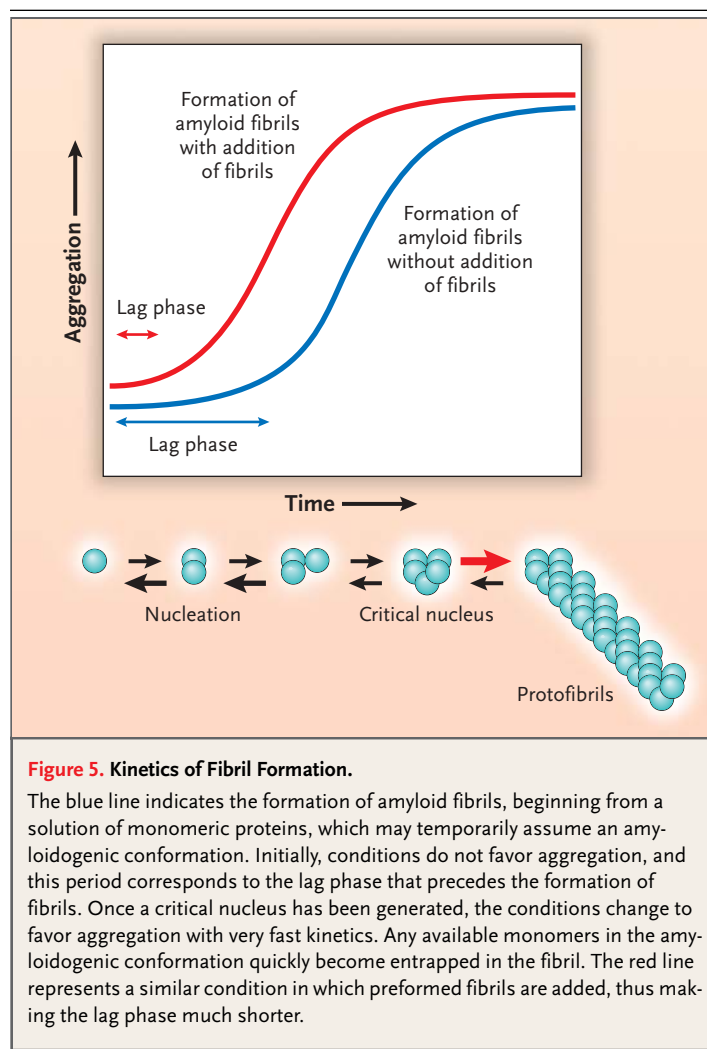With the exception of prion diseases, there is no



**Figure 5. Kinetics of Fibril Formation.**

The blue line indicates the formation of amyloid fibrils, beginning from a solution of monomeric proteins, which may temporarily assume an amyloidogenic conformation. Initially, conditions do not favor aggregation, and this period corresponds to the lag phase that precedes the formation of fibrils. Once a critical nucleus has been generated, the conditions change to favor aggregation with very fast kinetics. Any available monomers in the amyloidogenic conformation quickly become entrapped in the fibril. The red line represents a similar condition in which preformed fibrils are added, thus making the lag phase much shorter.

evidence that amyloidosis is transmissible in humans. However, the formation of amyloid can be accelerated by the presence of fibril nuclei in tissues. A pertinent example is the patient with transthyretin variants who has cardiac involvement and receives a liver transplant. The transplanted liver minimizes the production of amyloidogenic transthyretin but does not halt the progression of amyloid deposition in the heart.[57] Wild-type instead of mutant transthyretin continues to accumulate in the heart.[58] This finding is reminiscent of the capacity of the pathologic prion protein (PrP$^{sc}$) to convert its normal counterpart (PrP$^c$) into a pathologic conformation.[4] The main difference is that the dominant negative effect, in the case of transthyretin, is due entirely to fibrils within the patient and is not transmitted from one person to another, as in prion disease.

### THE COMMON CONSTITUENTS OF AMYLOID

All amyloid deposits contain SAP, a glycoprotein that belongs to the pentraxin family and binds amyloid independently of the protein of origin. It has a specific binding motif for the common conformation of amyloid fibrils.[59] This property makes radiolabeled SAP a diagnostic tool for the imaging of amyloid deposits.[60] SAP is highly protected against proteolysis and thus makes amyloid fibrils resistant to degradation.[61] Proteoglycans are also common in amyloid deposits and contribute extensively to the carbohydrate composition of natural amyloid.[62] Heparan sulfate proteoglycans, in particular, have kinetics of deposition in tissue similar to that of fibrillar proteins[63] and localize with constitutive elements of the extracellular matrix, such as perlecan, laminin, entactin, and collagen IV. These molecules can constitute a scaffold, facilitating the initial phases of fibril nucleation,[18] and could have a targeting role in the localization of amyloid deposits in tissue. For example, apolipoprotein E is reportedly a common constituent of amyloid deposits,[64] and epidemiologic studies have shown an increased risk of Alzheimer's disease among white persons carrying the $\epsilon$4 allele of apolipoprotein E.[65] However, the role of apolipoprotein E in systemic amyloidoses is less clear.

## CLINICAL IMPLICATIONS

### TISSUE SPECIFICITY OF AMYLOID DEPOSITION

The remarkable diversity in the organ distribution of amyloid deposits remains one of the most important unsolved problems in amyloid research. Specific proteins aggregate predominantly in defined target organs: beta$_2$-microglobulin in joints, the fibrinogen A$\alpha$ chain in the kidney, and the transthyretin Met30 variant in peripheral nerves. In light-chain amyloidosis, the deposits can involve virtually any organ (Fig. 6). One quarter of patients present with clinical involvement of a single organ, and the organ affected establishes the prognosis. Localized deposition of proteins that are normally deposited systemically can also occur, such as in localized AL amyloidosis, an intriguing condition characterized by localized growth of monoclonal plasma cells and the restriction of amyloid deposits to sites adjacent to the synthesis of the precursor.

The site of deposition may depend on the concurrence of several factors favoring the formation of fibrils, such as a high local protein concentration, a low pH, the occurrence of proteolytic processing,
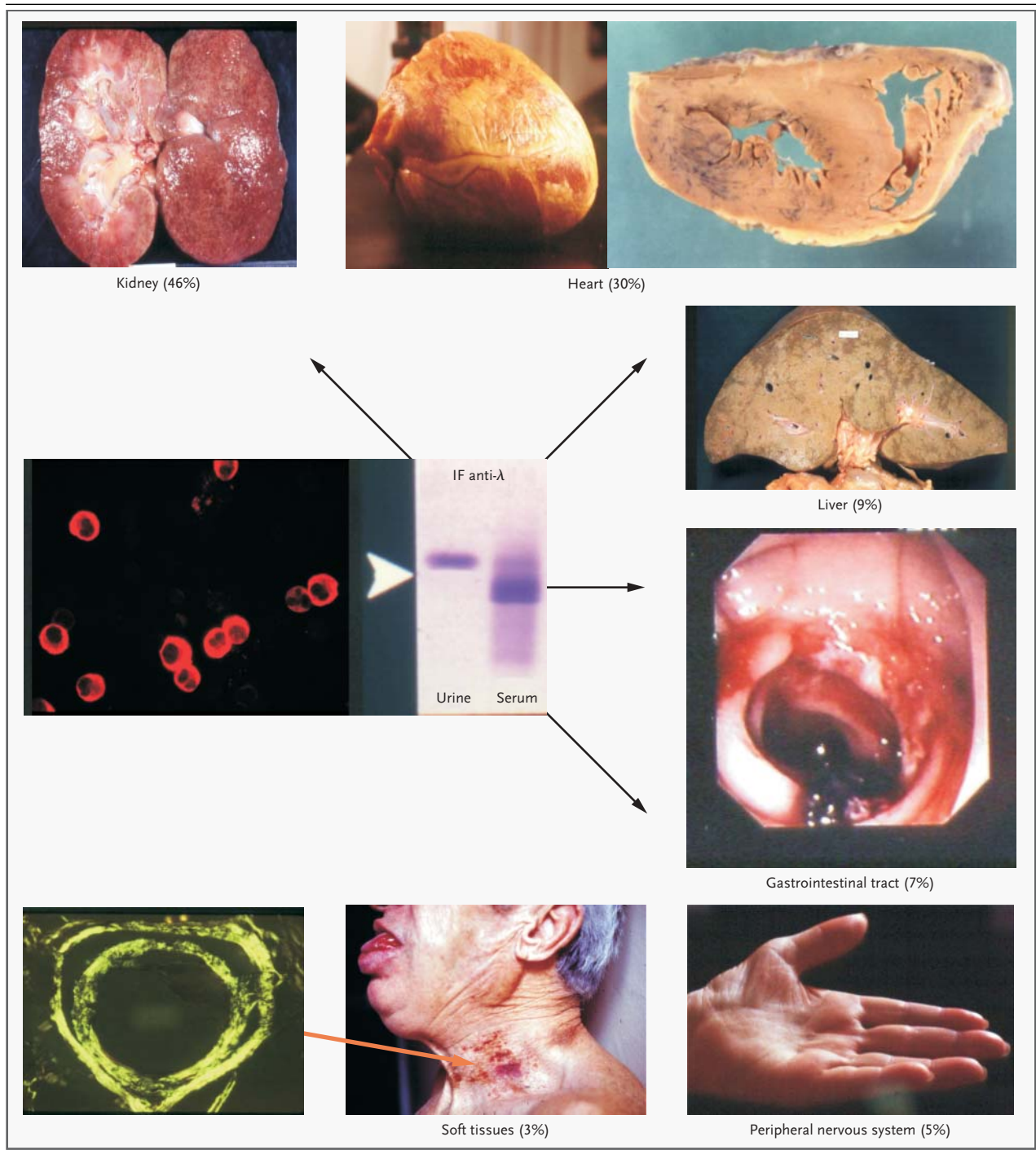
**Figure 6 (facing page). Distribution of Organ Involvement in 445 Patients with Light-Chain Amyloidosis.**

The light chains are predominantly $\lambda$ (ratio of $\lambda$ to $\kappa$ light chains, 4 to 1). Usually, a bone marrow plasma-cell clone, identified here by means of an immunofluorescence assay with rhodamine-labeled anti-lambda antibody (anti-$\lambda$), synthesizes monoclonal light chains, which are best identified by immunofixation (IF) of serum and urine. The light chains can produce amyloid deposits in virtually every organ, with the exception of parenchymal brain tissue. Percentages indicate the frequency of dominant organ involvement. Only one quarter of the patients had involvement of a single organ at presentation; the remaining patients had involvement of two organs (36 percent) or three or more organs (39 percent). The heart mass can be markedly increased, and the interventricular septum is thickened. Involvement of the gastrointestinal tract may cause life-threatening bleeding. Shown is one example of peripheral nervous system involvement: infiltration of the carpal tunnel by amyloid, which may damage the median nerve, with consequent marked hypotrophy of the hand muscles. Soft tissues that may be infiltrated by amyloid include the tongue and the submandibular regions; bruising is typical and is caused by vascular fragility resulting from the infiltration of the vessel walls by amyloid (as indicated by the apple-green birefringence on polarized light microscopy).

and the presence of fibril seeds. Specific interactions with tissue glycosaminoglycans[66] or cell-surface receptors such as the receptor for advanced glycation end-products (RAGE) may be important.[67] In AL amyloidosis, recognition of particular tissue constituents (i.e., collagen)[68] by amyloidogenic light chains may determine the specificity of tissue deposition. A specific kidney tropism of the $\lambda$ light chains derived from the 6a germ-line gene has been demonstrated[20,69]; the tropism may occur because of the interaction of these proteins with mesangial cells.[69]

### MECHANISM OF TISSUE DAMAGE

There is lively debate about the mechanism by which aggregation causes tissue damage and organ dysfunction. The deposition of large amounts of fibrillar material can subvert the tissue architecture and consequently cause organ dysfunction. Amyloid fibrils may also cause organ dysfunction by interacting with local receptors, such as RAGE.[67,70] In Alzheimer's disease, an inflammatory response in the cerebral cortex elicited by the progressive accumulation of A$\beta$ contributes to the pathogenesis of the disease.[71] In A$\beta$ and transthyretin amyloidosis, soluble oligomeric intermediates of fibril assembly are cytotoxic in vitro[72-75] and in vivo.[76] Soluble fibril

Kidney (46%)

Heart (30%)

Liver (9%)

IF anti-λ

Urine    Serum

Gastrointestinal tract (7%)

Soft tissues (3%)

Peripheral nervous system (5%)

precursors are likely to be the quaternary structures that mediate cellular toxicity through a mechanism that causes oxidative stress and activates the apoptotic pathway. According to this hypothesis, mature amyloid fibrillary deposits are inactive proteinaceous reservoirs that are in equilibrium with small-er, putatively toxic assemblies[2] (ordered aggregates). Several clinical clues suggest that in AL amyloidosis as well, soluble oligomers are cytotoxic and contribute to organ dysfunction. For example, peripheral neuropathy and renal and cardiac function improve dramatically after chemotherapy has

halted the production of amyloidogenic light chains but before the expected resolution of amyloid deposits.[77,78] The ongoing elucidation of the mechanism of tissue damage by other amyloid proteins may ultimately redirect therapeutic efforts.

### DIAGNOSTIC PROBLEMS AND PITFALLS

The number of recognized amyloidogenic proteins is ever expanding, posing increasing difficulties in formulating a correct diagnosis. Unequivocal identification of the deposited amyloidogenic protein is essential in order to avoid misdiagnosis and inappropriate treatment, to assess the prognosis, and to offer genetic counseling when appropriate.[26] The diagnostic approach is multidisciplinary and demands careful clinical evaluation combined with refined histochemical studies,[79] biochemical tests,[80] genetic analyses,[7,26] and possibly functional imaging studies (for cerebral amyloidosis).[81] The presence of a family history is important in the diagnosis of hereditary systemic amyloidoses. However, the clinical onset of these autosomal dominant diseases can be modulated by genetic or environmental factors or both. Furthermore, a low penetrance in carriers of the mutation, the involvement of organs usually affected in acquired (AL and AA) amyloidoses (the heart, liver, kidneys, and peripheral nervous system), and failure of immunohistochemical typing to identify the mutant protein can lead to the misdiagnosis of hereditary systemic amyloidosis as an acquired syndrome.[7,26] These factors are important because early diagnosis is the key to effective treatment.[82]

### MOLECULAR TARGETS AND THERAPEUTIC STRATEGIES

Amyloid deposits can be reabsorbed and organ dysfunction reversed if the synthesis of the amyloidogenic protein is shut down. There seems to be a fine balance between the rate at which amyloid is formed and its clearance. It may therefore be possible to promote the resorption of amyloid by reducing the concentration of the amyloidogenic protein to a level below a critical threshold and not necessarily eliminating the precursor. This principle is supported by the excellent outcome of liver transplantation in patients with hereditary apolipoprotein A-I amyloidosis, which can decrease the supply of the amyloid fibril precursor by 50 percent.[83] The same principle also applies to the new formation of amyloid: to halt the pathogenic process, it may be sufficient to reduce the concentration of the pre-

cursor to a level below the threshold at which oligomers form. Further studies are necessary to define these thresholds in order to exploit them in clinical settings.

The mechanism of resorption is still unknown, although recent data obtained in studies of immunotherapy for Aβ amyloidosis indicate that phagocytosis has a substantial role.[84] Knowledge of the amyloidogenic pathway (Fig. 4) is the basis of some recently proposed therapeutic strategies.

### EFFECTIVE THERAPIES

At present, the most effective approach to the treatment of the systemic amyloidoses involves shutting down or substantially reducing the synthesis of the amyloid precursor. In AL amyloidosis, reduction or elimination of the amyloidogenic clone by chemotherapy almost invariably improves the function of the affected organs.[77,78,85] In reactive amyloidosis (AA), control of the underlying inflammatory disorders can result in regression of the disease. In familial Mediterranean fever, which belongs to the expanding group of hereditary periodic fever syndromes, colchicine controls the febrile attacks and the synthesis of the acute-phase protein SAA, preventing amyloid from forming and reversing amyloid-induced organ dysfunction.[86] Similarly, the recent identification of the genetic defects in other syndromes of periodic fever has led to several breakthroughs in the understanding of the molecular basis of the inflammatory response[87,88] and has opened the way to tailored therapies for these syndromes.[89] In transthyretin amyloidosis, elimination of the circulating pathogenic protein by means of liver transplantation arrests the progression of the neurologic symptoms,[90] although wild-type protein may continue to be added to the existing deposits in the myocardium.[58] This finding suggests that liver transplantation should be performed early, before amyloid nuclei have been deposited in the heart.

### FUTURE PERSPECTIVES

The concentration of amyloid protein could be reduced by interfering with the expression of the corresponding gene by means of antisense oligonucleotides and small interfering RNA. These strategies have proved successful experimentally in reducing the synthesis of amyloidogenic light chains[91] and of a neurotoxic polyglutamine disease protein that forms nonamyloid intracellular aggregates.[92] However, technical difficulties related to targeting specific messenger RNA and to modulating the intracel-

lular concentration of small interfering RNA need to be overcome before clinical applications become feasible.

The conversion of native, fully folded protein into a highly amyloidogenic, partially folded conformer can be blocked by stabilizing native proteins with a specific ligand. In vitro studies have shown that stabilization of the transthyretin tetramer with the natural ligand thyroxin inhibits the formation of fibrils.[93] These results have led to structure-based designs, which in turn have yielded several structurally distinct families of small ligands that stabilize the transthyretin tetramer and inhibit the formation of amyloid fibrils.[94] Problems persist in vivo with regard to the specificity with which these small molecules bind to transthyretin and to the high dose required to saturate circulating transthyretin. In principle, this approach is applicable to the numerous other amyloidogenic proteins for which a stabilizing ligand can be identified.

The inhibition of proteases that generate amyloidogenic fragments is another approach. In Alzheimer's disease, the inhibition of $\beta$- and $\gamma$-secretases that generate the amyloidogenic peptide is a leading therapeutic strategy,[95] although the toxicity of $\gamma$-secretase and the selectivity of $\beta$-secretase inhibitors are major unresolved issues. Epidemiologic studies indicate that statins (cholesterol-lowering drugs) might prevent Alzheimer's disease,[96] probably by modulating the ability of secretases to cleave the amyloid precursor. Also, certain antiinflammatory drugs used in the treatment of Alzheimer's disease may have direct effects on secretases.[97] As other proteases involved in amyloidogenesis are identified (e.g., furin for gelsolin[43] and the amyloid ABri protein[44]), they will become targets for specific inhibitors.

All the compounds that inhibit the formation of fibril nuclei could prove important, both to prevent the deposition of amyloid and to avoid the cytotoxicity of the soluble oligomers. Synthetic peptides that bind natural amyloidogenic peptides and prevent further polymerization have already been designed[98,99] and used successfully in vitro[99] and in transgenic mice with amyloidosis.[100] The effect of chelation of metal ions (copper and zinc) in modulating the aggregation of $A\beta$ is also being studied.[101] Amyloid-induced cytotoxicity can be mitigated by compounds that inhibit the toxic mediators, such as free radical scavengers and antioxidants,[2] and by inhibitors and antagonists of cell-surface receptors.[67]

Another approach works directly on amyloid deposits targeting the common fibrillar architecture and common protective elements. Molecules capable of clearing SAP from amyloid deposits[102] or of inhibiting their interaction with glycosaminoglycans[103] have been designed and tested in animal models and phase 2 clinical trials. Several small ligands avidly bind the common ultrastructure of amyloid fibrils; iodinated anthracycline (4′-iodo-4′-deoxydoxorubicin) is a prototype of these molecules. It binds specifically and with high affinity to all the natural amyloid fibrils and promotes the disaggregation of fibrils both in vitro[104,105] and in vivo.[106] However, its clinical efficacy remains to be determined.[107,108]

An innovative approach is immunization against fibrillar proteins. The immune response to these proteins increases the clearance of amyloid deposits. In transgenic mouse models of Alzheimer's disease, immunization with the $A\beta$ peptide attenuates existing abnormalities and prevents neurodegeneration.[109,110] However, phase 2 clinical trials with an $A\beta$ vaccine were stopped when a central nervous system inflammatory reaction occurred in some patients.[111] Manipulation of the immunization protocols might circumvent this problem.[112] This approach also seems viable as an intervention in prion disease[113] and in light-chain amyloidosis.[114]

The increase in our knowledge of the structure and abnormal metabolism of the proteins involved in amyloidosis is closely paralleled by the search for remedies for this disease. On both fronts, the rate of progress is rapidly accelerating, hence legitimizing the hope that effective therapy, exploiting integrated strategies, will soon become a reality.

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