nucleosome, something that is achieved by the presence of one, two or three methyl groups[8]. Because much of the genome must be blocked (the effects are general rather than local) a distributive mechanism is appropriate, and, as the modification has to be frequent, then di- and trimethylated isoforms will arise through rebinding of the enzyme to already methylated nucleosomes. In this case, the degree of methylation is of no significance, and the code, if we can call it that, is a simple binary switch in which H3K79 methylation prevents Sir3 binding and lack of methylation allows it[3]. Unfortunately, this elegantly simple model may prove to be the exception, not the rule. The identification of two Dot1 homologs in *Trypanosoma*, each selective for either di- or trimethylation[20], suggests that Dot1-mediated functions may be less straightforward in higher eukaryotes.

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Escaping amyloid fate

Blake E Roberts & James Shorter

Small molecules that safely antagonize amyloidogenesis are desperately needed for many devastating disorders that plague humankind, including Alzheimer's and Parkinson's diseases. New work brings important mechanistic insights into how one promising candidate, (–)-epigallocatechin-3-gallate (EGCG), diverts amyloid-β and α-synuclein down innocuous folding trajectories at the expense of the deleterious states populated during amyloidogenesis.

Successful protein folding is key to all life. Yet, a significant portion of eukaryotic proteomes, perhaps even 30%, comprises proteins that are either entirely unfolded or contain large regions (~40 amino acids or more) of intrinsic disorder[1]. Such proteins are often the culprits behind debilitating and increasingly prevalent neurodegenerative diseases[2,3]. For example, tau and amyloid-β (Aβ) in Alzheimer's disease (AD) and α-synuclein (α-syn) in Parkinson's disease (PD) all seem to transition from a natively unfolded state through heterogeneous oligomers to the generic 'cross-β' form of amyloid fibers[2,3]. There are no effective treatments for any of these conditions. However, hope remains in the vastness of unexplored chemical space[4], which may harbor small molecules able to derail amyloidogenesis. Indeed, promising small-molecule candidates are beginning to emerge[5–7], although the mechanisms by which they inhibit the amyloidogenesis of initially unstructured proteins remain largely obscure. Elegant work by Ehrnhoefer *et al.*[8] provides mechanistic insight into the largely uncharted territory of how small molecules might preclude amyloid formation and propagation by natively unfolded proteins.

The challenges facing potential small-molecule antagonists of amyloidogenesis are daunting to say the least. First, it is inherently difficult for small molecules of limited steric bulk to prevent protein-protein interactions where the binding energy is distributed among dozens of amino acids and thousands of square angstroms of contact area[9]. Second, contact sites are often relatively flat, providing few opportunities for small-molecule insertion, and frequently have a high degree of plasticity that can accommodate a small molecule and remain unperturbed[9]. For amyloids, these issues are exacerbated by the exceptional stability of their intermolecular contacts, which generally require boiling in SDS or high denaturant concentrations (for example, 8 M urea) to be disrupted[2]. Furthermore, once initiated, amyloidogenesis can cascade out of control because amyloid fibers self-template their own 'cross-β' structure by recruiting nonamyloid copies of the same protein to fiber ends and converting them to the amyloid form[2]. Even more problematic is the ability of amyloidogenic proteins to access multiple, structurally distinct amyloid forms[10,11], which in some cases are distinguished by distinct sets of intermolecular contacts[10]. An effective small molecule would need to target all of these. Additionally, *en route* to fiber formation, amyloidogenic proteins often populate an ensemble of diverse oligomeric states, many of which seem to be highly toxic[3,12]. Thus, not only must small-molecule antagonists prevent fiber formation, they must do so in a manner that prevents the accumulation of toxic preamyloid conformers. Finally, if the foregoing was not enough, for the neurodegenerative amyloidoses, there is the accompanying conundrum of traversing the 'blood-brain barrier', which sharply limits the size and nature of the small molecule[13].

Despite these challenges, one small molecule, (–)-epigallocatechin-3-gallate (EGCG; **Fig. 1a**,**b**), may surprise us in its ability to safely prevent amyloidogenesis. The major polyphenol in green tea, EGCG has risen to fame for its antioxidant and potential antitumor activities[14], but more recently has begun to enter the spotlight of the amyloid world. EGCG has emerged as a potent inhibitor of tau, Aβ, α-syn and polyglutamine fibrillization *in vitro*[5,6]. Furthermore, EGCG antagonizes polyglutamine aggregation and toxicity in both yeast and fly models of Huntington's disease[5].

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In this issue, Ehrnhoefer *et al.*[8] probe the mechanisms by which EGCG inhibits Aβ and α-syn fibrillization. At concentrations equimolar with Aβ and α-syn, EGCG effectively inhibits spontaneous fibrillization and has significant inhibitory activity at substoichiometric concentrations. Remarkably, EGCG seems to interact with natively unfolded forms of Aβ and α-syn and divert them into stable oligomeric forms that resist solubilization by SDS. Using a clever method originally developed to detect quinoproteins, Ehrnhoefer *et al.*[8] show that EGCG binds extremely tightly to Aβ and α-syn, as well as to other unfolded proteins. The interaction is tight enough to survive SDS-PAGE and does not seem to reflect a covalent modification of the polypeptide[8]. The minimal number of Aβ and α-syn monomers that must be bound by EGCG to inhibit fibrillization remains unclear. However, EGCG clearly traps Aβ and α-syn in monomeric and oligomeric forms with diminished ability to participate in amyloidogenesis (**Fig. 1c**). By stabilizing these assembly-incompetent forms, EGCG effectively precludes fiber assembly and neatly sidesteps many of the apparent challenges facing small-molecule antagonists of amyloidogenesis. This is reminiscent of how small molecules that stabilize the native tetrameric structure of transthyretin potently inhibit amyloidogenesis[15].

In their natively unfolded state, α-syn monomers are highly dynamic and rapidly sample an ensemble of distinct transient conformations[16]. A subset of these involve an interaction between the C-terminal domain (residues 110–130) and the C-terminal part of the hydrophobic NAC region (residues 85–95)[16]. This interaction may be autoinhibitory for fiber assembly, as it could obstruct the NAC region (residues 61–95) from entering the solvent-inaccessible cross-β core (residues 39–101) of mature α-syn fibers[17]. CD analysis revealed that EGCG maintained α-syn in predominantly unstructured forms[8]. Thus, EGCG probably stabilizes natural α-syn conformations that are autoinhibitory for fiber assembly. Moreover, NMR data revealed a progressive broadening of particular resonances with increasing EGCG concentrations[8]. As EGCG induced formation of SDS-resistant α-syn oligomers, up to 50% of the resonances disappeared from the spectra[8]. Notably, resonances in the NAC region remained visible, implying that this region does not drive EGCG-induced oligomerization[8]. Resonances for four residues in the C-terminal domain disappeared at lower EGCG concentrations, indicating that EGCG might cause C-terminal domains to cluster and oligomerize[8]. Nucleation of α-syn and Aβ fibers probably occurs in structurally malleable oligomers that reorganize to establish the intermolecular contacts that spark assembly[18–20] (**Fig. 1c**). By cogently locking α-syn and Aβ into extremely stable, alternative oligomeric forms, EGCG probably prevents the conformational rearrangements within oligomers required to nucleate assembly (**Fig. 1c**).

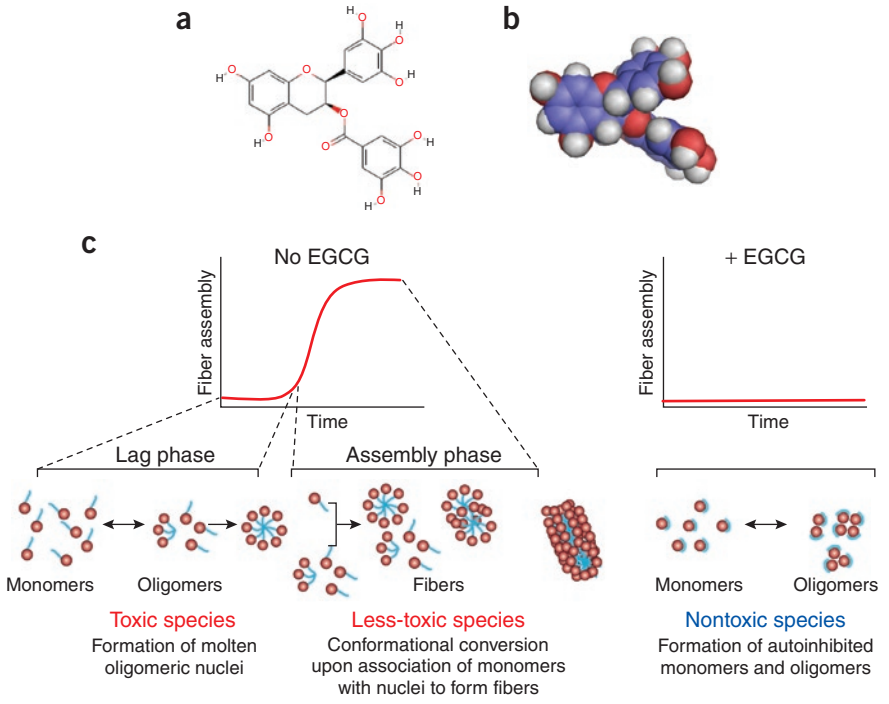

Figure 1 Structure and mode of action of EGCG. (**a**) Chemical structure of EGCG. (**b**) Space-filling model of EGCG reveals a nonplanar structure. (**c**) In the absence of EGCG, α-syn or Aβ amyloid fibers assemble after a lag phase during which a dynamic ensemble of natively unfolded monomeric and molten oligomeric species form. The intermolecular contacts that nucleate fiber assembly are probably established within molten oligomers. Once formed, fibers stimulate their own assembly by recruiting and converting monomers at their ends. EGCG rapidly converts natively unfolded monomers and oligomers to autoinhibited forms that are nontoxic and unable to participate in fiber formation.

Importantly, the unstructured oligomers induced by EGCG did not seed fiber assembly, reinforcing the idea that they are the product of an alternative pathway that proceeds more rapidly than fiber formation[8]. The exploitation of alternative pathways is a successful strategy used by molecular chaperones and protein-remodeling factors to antagonize amyloidogenesis. For example, the chaperonin TRiC promotes the formation of alternative, nontoxic polyglutamine oligomers that are assembly incompetent[21], and the protein-remodeling factor Hsp104 converts Sup35 prions to noninfectious amyloid-like forms[22]. An interesting possibility is that EGCG-induced oligomers are more susceptible to disassembly by molecular chaperones or protein-remodeling factors than toxic oligomers. The collaboration and potential synergy between small molecules and molecular chaperones[23], which is virtually unexplored, may prove to be a powerful adversary that is able to counter diverse neurodegenerative disorders.

Crucially, the oligomeric forms of Aβ and α-syn induced by EGCG were not toxic to PC12 cells in culture, in contrast to Aβ and α-syn fibers[8]. However, whether EGCG can mitigate Aβ and α-syn toxicity in a more disease-relevant setting remains unclear. Yet, there is reason to be hopeful. A conformation-specific antibody, A11, which recognizes a transient, highly toxic oligomeric state common to many amyloidogenic proteins[12], did not detect EGCG-induced oligomers. Indeed, EGCG promoted the remodeling of preformed A11-reactive oligomers of Aβ and α-syn, indicating an ability to eliminate perhaps the most toxic species that accumulate during Aβ and α-syn amyloidogenesis[12]. Equally promising is the observation that EGCG inhibits Aβ or α-syn assembly seeded by preformed fibers. Thus, EGCG-induced conformers are not substrates for conformational conversion. Furthermore, the ability to block seeded polymerization is of great importance from a therapeutic standpoint because considerable amounts of amyloid fibers are likely to have accumulated by the time a disease is diagnosed. Intriguingly, although not an approved therapeutic option,

anecdotal evidence of the ability of green tea to remedy various amyloid diseases, such as light-chain amyloidosis, have begun to appear[24].

It remains unclear whether EGCG can remodel mature amyloid fibers in a similar way to other small molecules[7]. However, once again there is reason to be optimistic. EGCG may take advantage of Le Châtelier's principle to disassemble amyloids and prevent conformational conversion by shifting the equilibrium dramatically toward nonamyloid conformers. Amyloid fibers seem to dynamically exchange monomers from their ends by the spontaneous dissociation and reassociation of monomers over a biologically relevant timeframe (days)[25]. Given that EGCG inhibits seeded polymerization, it is possible, perhaps even probable, that it might inhibit the reassociation of dissociated monomers with fiber ends and drive the equilibrium toward soluble forms.

One must keep in mind, however, that EGCG binds to unfolded proteins in a nonselective fashion[8], which may have unanticipated pleiotropic consequences *in vivo*. Indeed, this property might explain the diverse activities attributed to EGCG[14]. EGCG might inhibit the amyloidogenesis of many polypeptides that begin in a natively unfolded state. However, such broad specificity may be undesirable, because mounting evidence suggests that amyloids and prions have also been captured during evolution for beneficial purposes[26]. Pmel17 amyloids mediate melanosome biogenesis and particular CPEB prions might promote synaptic changes associated with memory[26]. Nonetheless, the studies by Ehrnhoefer *et al.*[8] provide an important foundation to understand the interactions between small molecules and natively unfolded proteins, and may facilitate the design of more potent and selective compounds with activity against exclusively deleterious amyloids.

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Endo-siRNAs: yet another layer of complexity in RNA silencing

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Organisms possessing RNA-dependent RNA polymerase activity are known to produce endogenous small interfering RNAs (esiRNAs). It had been thought that organisms such as flies and mammals lacking this activity would not produce esiRNAs. However, it has now been shown that a functional esiRNA pathway is present in such animals; the esiRNAs are derived from a variety of endogenous double-stranded RNA substrates.

The world of small-RNA silencing phenomena has just become markedly larger with the recent publication of seven complementary papers (five in *Nature*[1–5], one in *Science*[6] and one in this issue of *Nature Structural & Molecular Biology*[7]) that collectively document an additional silencing pathway in *Drosophila melanogaster* and mammals. This pathway involves the synthesis and processing of endogenous double-stranded RNAs (dsRNAs) to yield functional small interfering RNAs (siRNAs) that serve to silence transposable elements in both germ cells and somatic tissues, and some specific mRNAs. It is also possible that these endo-siRNAs, esiRNAs, have a role in heterochromatin formation analogous to the function of some endogenous siRNAs in plants and fission yeast.

To date, esiRNAs have been detected only in organisms that possess RNA-dependent RNA polymerases (RDRPs): plants, *Caenorhabditis elegans* and fission yeast. Because these polymerases transcribe single-stranded RNA (ssRNA) to make dsRNA and are essential for the production of esiRNAs, it was thought that organisms that did not have RDRPs would not use an esiRNA pathway owing to the lack of endogenous dsRNA. However, it is well known that there are other sources of dsRNAs besides those generated by RDRPs (**Fig. 1**). These include long hairpin structures generated by the transcription of palindromic sequences and dsRNAs generated by the annealing of complementary RNAs synthesized by convergent transcription units. Indeed, these dsRNAs have now been shown to be the source of esiRNAs in both *D. melanogaster* and mice[1–7].

In both organisms, extensive studies in many laboratories have revealed three distinct RNA silencing pathways, each using distinct small RNAs and a distinct set of protein factors. The Piwi-interacting RNA (piRNA) pathway is involved in silencing transposons in the germ line, and piRNAs are bound to the Piwi class of argonaute proteins; it is not yet clear how these RNAs and proteins exert their silencing

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