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Viral capsid assembly as a model for protein aggregation diseases: Active processes catalyzed by cellular assembly machines comprising novel drug targets

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ABSTRACT

Viruses can be conceptualized as self-replicating multiprotein assemblies, containing coding nucleic acids. Viruses have evolved to exploit host cellular components including enzymes to ensure their replicative life cycle. New findings indicate that also viral capsid proteins recruit host factors to accelerate their assembly. These assembly machines are RNA-containing multiprotein complexes whose composition is governed by allosteric sites. In the event of viral infection, the assembly machines are recruited to support the virus over the host and are modified to achieve that goal. Stress granules and processing bodies may represent collections of such assembly machines, readily visible by microscopy but biochemically labile and difficult to isolate by fractionation.

We hypothesize that the assembly of protein multimers such as encountered in neurodegenerative or other protein conformational diseases, is also catalyzed by assembly machines. In the case of viral infection, the assembly machines have been modified by the virus to meet the virus' need for rapid capsid assembly rather than host homeostasis. In the case of the neurodegenerative diseases, it is the monomers and/or low n oligomers of the so-called aggregated proteins that are substrates of assembly machines. Examples for substrates are amyloid β peptide (Aβ) and tau in Alzheimer's disease, α-synuclein in Parkinson's disease, prions in the prion diseases, Disrupted-in-schizophrenia 1 (DISC1) in subsets of chronic mental illnesses, and others. A likely continuum between virus capsid assembly and cell-to-cell transmissibility of aggregated proteins is remarkable. Protein aggregation diseases may represent dysfunction and dysregulation of these assembly machines analogous to the aberrations induced by viral infection in which cellular homeostasis is pathologically reprogrammed. In this view, as for viral infection, reset of assembly machines to normal homeostasis should be the goal of protein aggregation therapeutics.

A key basis for the commonality between viral and neurodegenerative disease aggregation is a broader definition of *assembly* as more than just simple aggregation, particularly suited for the crowded cytoplasm. The assembly machines are collections of proteins that catalytically accelerate an assembly reaction that would occur spontaneously but too slowly to be relevant *in vivo*. Being an enzyme complex with a functional allosteric site, appropriated for a non-physiological purpose (e.g. viral infection or conformational disease), these assembly machines present a superior pharmacological target because inhibition of their active site will amplify an effect on their substrate reaction. Here, we present this hypothesis based on recent proof-of-principle studies against Aβ assembly relevant in Alzheimer's disease.

Proteins have evolved to execute multiple functions, amongst them the prominent function of catalysis, i.e. the acceleration of a chemical reaction without changing its final equilibrium. Biochemical reactions occur spontaneously if given enough time, but their kinetic facilitation by enzymes greatly accelerates evolvability of biological processes themselves which is why, ultimately, they were selected by evolution (Kirschner and Gerhart, 1998).

Proteins are generated in the cell through translating mRNA according to the genetic code on multiprotein assemblies containing RNAs, called ribosomes, followed by posttranslational

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modifications by virtue of a remarkable diversity of more or less complex energy-requiring processes (Xue and Barna, 2012). Complementing Anfinsen's paradigm of sequence-encoded spontaneous (Anfinsen, 1973), and unassisted folding of a protein notwithstanding, two critical dimensions of this process require enzymic catalysis to be fully explained. The first is how these processes proceed on the timescale observed in living cells, and the second is how they can occur in the crowded environment within the cell, where interacting proteins cannot find themselves as readily as it would occur for purified proteins in isolation (Fulton, 1982; Alberts, 1998; Hartl et al., 2011).

Full functionality of a protein is achieved after a series of posttranslational modifications that occur simultaneously with or after protein biogenesis. These can include covalent modifications like glycosylation, sumoylation and others, but also folding into different conformers or multimerization. For posttranslational modifications such as glycosylation, the host glycosyl transferases that catalyze the addition of carbohydrate moieties to defined amino acids within a recognition motif (Dennis et al., 1999) are known. However, host factors regulating the folding of a protein into different conformations (Lingappa et al., 2002) or multimers (Fink, 1999) are less well understood. One reason for this could be that the enzymatic activity for protein multimerization is not performed by a single protein molecule as for glycosylation but by a transient and labile complex of proteins that are difficult to detect as such. Put another way, if the ribosome, itself a multiprotein complex RNA-containing covalent assembly machine, were as unstable as hypothesized here for non-covalent assembly machines, we might still not understand how proteins are made.

In the course of our studies we have noted a striking similarity between new findings in virus capsid assembly (Gay and Neuman, 2013; Lingappa et al., 2013a,b) and endogenous protein aggregation. This leads us to speculate that much of what has been conventionally viewed as spontaneous protein *aggregation* may in fact be catalyzed protein *assembly*. By analogy to viral capsid formation, long viewed as self-assembly, and only more recently recognized as the result of host catalysis through the action of labile multiprotein complexes, perhaps diseases of protein aggregation are initiated by action of aberrant assembly machines and their disordered or dysregulated assembly intermediate products. The novel notion that viral capsid assembly is an active process executed by host factors that may be similar, overlapping or completely different from host factors promoting assembly of other endogenous host proteins, including those involved key events in neurodegenerative diseases, has consequences for drug discovery strategies – it may greatly accelerate drug discovery by directing research in a completely new direction from the current dominant focus. What we should be asking then, is what are the catalysts that bring this about, how are their functions different from the catalysis that occurs under physiological, homeostatic conditions, and how can that dysfunctional non-homeostatsis catalysis be normalized.

1. Virus capsid assembly

Viruses can be considered as dynamic molecular assemblies, containing a nucleic acid genome that is enclosed in a protein capsid shell. In many cases a lipid envelope is also observed. Typically, the envelope also contains the protein(s) that bind to cell surface receptors to target the virus. Some viral families (e.g. the *Picornaviridae* and *Enteroviridae*) have no envelope: for them, the capsid *is* the virus.

Through evolutionary selection pressure favoring the fastest generation cycles, virus components have evolved to manipulate host cellular machinery for rapid and optimal replication (Dimmock et al., 2007; Prasad and Schmid, 2012). By virtue of their fast replication cycles they have, in effect, discovered all cellular "niches" that can be exploited to their advantages. That diverse viral families appear to have chosen distinctive pathways of host-catalyzed capsid assembly is remarkable (Lingappa et al., 2013b): it suggests that such reprogramming of assembly machines is highly profitable for viruses and therefore may be an inherent weakness of metazoan biology–the price we pay for such complex organ systems such as the central nervous system (CNS) in constant need of repair and attention, and therefore at constant risk of subversion. And since protein machines are likely themselves built by protein machines, the potential for "prion-like" epigenetic propagation of dysfunctional assembly may exist.

Viral replication and propagation is a process of alternating cycles with viral invasion of suitable cells, release of virus genome into the cytosol where encoded viral components are replicated and, finally, assembly of new infectious particles before their release from the host cell, subsequently possibly infecting other host cells and re-initiating the replication cycle (Mateu, 2013b). The viral capsid consists of a protein shell that serves as a protection of the virus genome and encodes invasion and release signals.

Several different stages in virus morphogenesis can be distinguished such as capsid assembly, nucleic acid packaging, and virus particle maturation. Here, the focus will be on capsid assembly. The size and shape of capsid structure is in general regular like symmetric oligomers made by assembling of capsid protein subunits (Klug, 1999; Prasad and Schmid, 2012). The individual protein subunits are asymmetrical, but they are assembled to form symmetrical structures. Different viruses can build their capsids with a different number of capsid protein subunits. The most abundant type of capsids throughout virus families are helical and icosahedral capsids. Theoretically, the structure of helical capsid is extremely simple, since the number of capsid protein subunits required is extendable, depending only the length of nucleic acid genome that needs to be encapsulated. On the other hand, the small icosahedral capsids are made exactly with 60 capsid protein subunits that limit the size of nucleic acid genome that can be enclosed. The larger icosahedral capsids can be made using 60 multiples of subunits of a capsid protein (Amos and Finch, 2004; Carter and Saunders, 2007). The intracellular compartments where this process occurs are denominated "viral factories" (Novoa et al., 2005) (see below).

So far, capsid assembly has mainly been viewed as a spontaneously occurring process of self-assembly (Zlotnick, 2005), dependent only on the presence of capsid protein subunits themselves (Johnson et al., 2005; Mateu, 2013a; Prevelige, 1998). Thermodynamically, viral capsid assembly is described in two states, the dissociated state where monomers of the capsid protein are found, and the associated state where the capsid is formed (Fig. 1a). Energetically, this assembly process follows a sigmoidal curve with a lag phase where the concentration of the proteins that compose the capsid determines the speed of the reaction rate. Off-pathway reactions can occur leading to aberrant capsid formation where the quaternary structure is not native (Endres and Zlotnick, 2002; Zlotnick, 2003). In the self-assembly of complex icosahedral capsids, different thermodynamic kinetic models have been proposed consisting of a lag phase, an equilibrium phase, and an elongation phase with the formation of new virus capsid nuclei, that initiate viral capsid assembly (Endres and Zlotnick, 2002; Zlotnick, 2005). *In vitro* experiments are useful to understand the self-assembly of capsids, but do not take into account the interaction with scaffolding proteins or viral nucleic acids that are present *in vivo*. More specifically, because something can happen spontaneously does not mean that *in vivo* it is not accelerated by catalysis.

Recently, findings comprising various viral families (Klein et al., 2004, 2011; Lingappa et al., 2006, 2013a,b; Zimmerman et al., 2002) suggest that the cellular pathway to capsid assembly may be