Prion replication and secondary nucleation

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The infectious agents responsible for the prion diseases appear to be aggregates of a modified form of the prion protein, PrP^{Sc}, that grow at the expense of the normal form, PrP^{C}. The mechanism of the transformation of PrP^{C} to PrP^{Sc} is the subject of intense research interest. The way in which PrP^{Sc} aggregates generate new nuclei also deserves attention.

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The infectious agent responsible for scrapie appears to be the prion protein, which is capable of self-replication by some means [1–3]. While the details of the process that converts the normal form of the prion protein, PrP^{C}, to the infectious form, PrP^{Sc}, are still controversial [3], many investigators suggest that a phenomenon related to nucleation is involved [2,4,5]. PrP^{Sc}, they believe, forms insoluble, protease-resistant aggregates that grow by recruiting the soluble, protease-sensitive form [6].

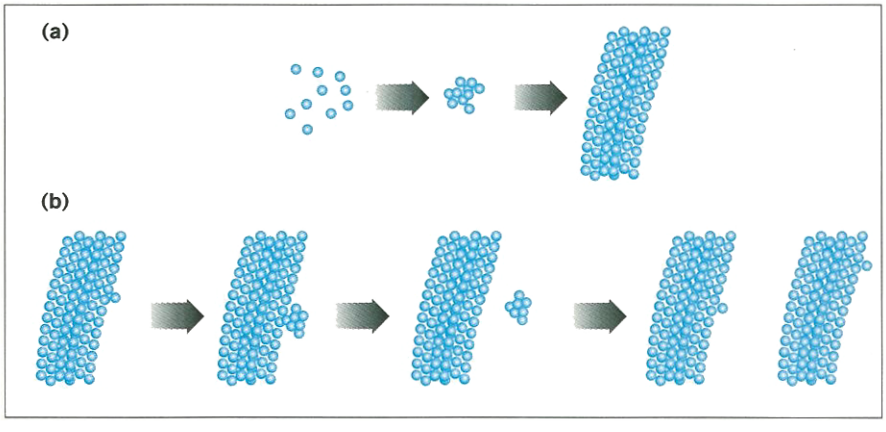
If nucleation is important in prion infections, a full understanding of the kinetics of infection will require, in addition to knowledge of the mechanisms of nucleation and growth, knowledge of the mechanisms of secondary (heterogeneous) nucleation that enable one nucleus to generate daughter nuclei. Although, in the case of the prion diseases, mechanisms of replication attract most attention, secondary nucleation may be of considerable importance. A loose analogy may be drawn with metastasis in cancer. Carcinogenesis attracts the attention of the majority of researchers, but metastases account for the majority of cancer deaths.

The phenomenon of secondary nucleation is illustrated in a striking fashion by a supersaturated aqueous solution of an inorganic salt, sodium chlorate [7]. If the solution is allowed to stand undisturbed in a beaker it deposits a mixture of enantiomorphic D and L crystals in roughly equal number. However, if the solution is stirred vigorously while it is crystallizing, all of the crystals formed in any one experiment have the same handedness, and they are equally likely to be all-L or all-D isomers. Vigorous stirring presumably fragments the first primary nucleus formed. The secondary nuclei produced in this way grow and are, in turn, fragmented. Before a second primary nucleus has time to form, the beaker fills with crystals 'cloned' from the first nucleus. Since D-nuclei and L-nuclei 'breed true', one might have been inclined to call the D and L enantiomorphs 'strains' if they were not simple inorganic structures.

A secondary (heterogeneous) nucleation model has already been developed in detail to explain unusual features of the kinetics of the polymerization of sickle-cell haemoglobin [8]. The autocatalytic nature of the reaction is explained by the ever-increasing area of surface on which new nuclei can form. This model, in a somewhat modified form (Fig. 1), illustrates one of the many ways in which a prion aggregate could replicate and spread.

The efficiency of secondary nucleation of prion aggregates is likely to be one of the factors that determines the details of the pathology of the prion diseases. If secondary nucleation does not occur, each nucleus introduced from

Figure 1



Primary and secondary nucleation. **(a)** Primary nucleation occurs when several monomers aggregate. **(b)** Secondary nucleation is a result of buds forming on and then detaching from pre-existing aggregates. The increase in the number of aggregates gives the reaction an autocatalytic nature. The geometric details should not be taken literally.

outside or formed endogenously grows to form a single localized plaque. Would this cause disease? If secondary nucleation is very efficient, no localized plaques might form even at a late stage in the disease process. The severity of disease might be determined by the number of nuclei, many of which could be invisible, rather than by the total amount of nucleate. The mechanisms controlling the rate of secondary nucleation need to be investigated.

First, it is necessary to confirm that secondary nucleation is a fact rather than a fantasy. As infectious scrapie can be transmitted serially to successive generations of animals, the total amount of scrapie agent and the total number of plaques can certainly grow exponentially. But does the number of plaques increase in the brain or in the test-tube? It is possible, in theory, that the increase in the number of nuclei is achieved by fragmentation during handling of the infectious agent. This seems unlikely, but the possibility should be excluded.

If the relevance of secondary nucleation is firmly established, it will be important to discover the mechanism that leads to the formation of secondary nuclei. Does the production of secondary nuclei involve enzymes, such as proteases, or can it be explained by budding and related phenomena familiar to colloid chemists? Does secondary nucleation occur in the cytoplasm, in lysosomes, in association with membranes, or in the space between cells?

To sustain an infectious process, newly formed nuclei must migrate to and become fixed at sites where they can, in turn, grow and throw off new daughter nuclei. Much is already known about the movement of infectious material along axons [9] and about the site-specificity of the different scrapie strains [10]. Does diffusion in the space between cells also contribute to the spread? What proportion of new nuclei are degraded or lost in other ways before they can attach at productive sites?

I do not see how to apply the methods of molecular biology to these problems. Microscopy seems the most promising approach. The way in which the distribution of plaque size and the number of observable plaques changes with time after infection might distinguish between simple growth of a fixed number of primary sites and an exponentially increasing production of new sites. Microscopy (or electron microscopy) might reveal incipient nuclei, and indicate the favored sites for their formation and the mechanism by which they are generated. Are there any relevant data in the literature that could be interpreted to throw light on secondary nucleation?

Finally, are there non-infectious degenerative conditions in which amyloid plaques grow as efficiently and by essentially the same autocatalytic mechanism as in the scrapie-like diseases, but fail to generate new plaques? The critical difference between infectious and non-infectious agents may sometimes depend on the efficiency or inefficiency of secondary nucleation, rather than on the rates of autocatalytic growth of primary nuclei.

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