treated with caution, and that studies should therefore ensure that overall numbers are adequate, populations specifically defined, and appropriate controls included that exactly match the patient population. A difficulty with studies of sepsis is that accepted definitions of the syndrome are very broad — perhaps too broad. This has become evident from the many clinical trials of the past decades, which have generally failed to obtain consistent results from similar studies of patients with severe sepsis. Indeed, the current framework for designing clinical trials in this area seems fundamentally flawed. In such trials, patients with infections caused by different microbes, and affecting different organs, have been lumped together under the same diagnosis of severe sepsis. The situation is almost akin to taking patients who have symptoms in the hip caused by osteomyelitis and patients with similar symptoms that are secondary to osteosarcoma, and entering them in the same clinical trial.

What can be done? There is abundant evidence that numerous molecules, as well as an individual's genetic background, influence both protective innate immunity and the development of severe sepsis. Saleh and colleagues' work[3] provides a good example of this. The next step might be to select patients and controls for these clinical trials on the basis of clinical, molecular and genomic data. This would allow disease criteria to be defined that are specific to a single molecular derangement, and to be linked to a treatment specific to that derangement. ■

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Cell biology

Designer prions

Daniel C. Masison

Prions are clumps of misshapen proteins that can be passed between cells without the need for genetic intermediaries. The parts of the proteins that account for such infectivity are now being dissected.

Alzheimer's disease, type II diabetes and prion diseases — mad cow disease being the most notorious — are all characterized by the accumulation of misshapen proteins into aggregates in various parts of the body. Of these disorders, however, only prion diseases are infectious. Clumps of prion proteins alone are thought to be the infectious agent in such diseases, implying that infectivity is a special property of these proteins. But, despite extensive studies, researchers have discovered little more than this about the basis for the transmissibility of prions. Now, by swapping portions of one yeast protein with those of another, Osherovich *et al.*[1] have found hints to a possible mechanism, as they discuss in *Public Library of Science Biology*.

Yeast prions are known to be transmitted between yeast strains, along with the cellular cytoplasm, during cell fusion, and from mother to daughter yeast cells during cell division. Two events are necessary to ensure that these protein clumps continue to be transmitted. First, they must grow, by causing normal prion proteins to take on a warped shape that favours their aggregation. And second, they must divide, generating new prion particles that can be passed to a new cell. This division, or replication, is thought to involve small clumps breaking off from the main mass, with the help of a 'chaperone' molecule[2,3]. Transmission then probably occurs by diffusion. Thus, transmission efficiency is related to the efficiency of prion replication.

Most proteins do not replicate in this way, so what enables a prion protein to do so? To find out, Osherovich *et al.*[1] looked at [*PSI*$^+$] — the name given to the prion form of the yeast protein Sup35, which normally functions in the synthesis of other proteins. The

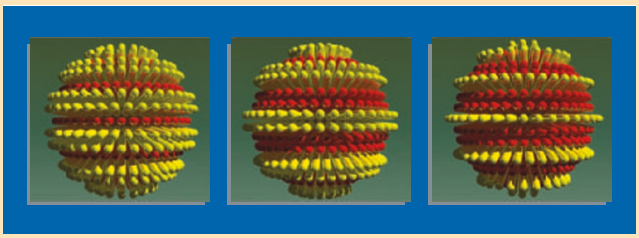
Materials science

Variations on a golden core

Alicia M. Jackson *et al.* have looked at nanotechnology in the round, and describe what they believe to be a promising new class of structured material (*Nature Mater.* **3,** 330–336; 2004). They have examined nanoparticles that consist of a metal centre surrounded by a mixed-ligand shell. Using scanning tunnelling microscopy backed up by X-ray diffraction, they show that the shell is separated into ordered phases at the unprecedented scale of fractions of nanometres.

The particles were produced in a one-step synthesis involving the spontaneous assembly of ligands on nanosized gold cores. The ligand shell — a mixture of 1-octanethiol and mercaptopropionic acid — separates into chemically distinct domains that are as small as 0.5 nm. These computer-generated representations show how the domains can form ripples that encircle the nanoparticles, and reveal the different ordering that stems from making the ligand shell with different molecular proportions of 1-octanethiol (yellow lobes) and mercaptopropionic acid (red). The nanoparticle diameter is 3.7 nm.

The principle works with cores of silver as well as gold. From this and other evidence, Jackson *et al.* argue that the phase separation that produces the different domains is not controlled by the characteristics of the core. Rather, after experimenting with ligand assembly on small gold



hemispheres of different diameters, they conclude that the ordering is primarily driven by surface curvature — how is not clear. But production of coated nanoparticles with tailored characteristics could involve varying the core curvature as well as the ligand type and mixture.

In looking into the properties of their nanoparticles, Jackson and colleagues tested the binding of three proteins — cytochrome *c*, lysozyme and fibrinogen — to the ligand shell. The proteins didn't bind at all, and the authors consider that this failure to stick is probably down to the unique size and patterns of hydrophobic and hydrophilic areas on the shell. This 'nonspecific protein resistance' could be a major virtue in bioengineering applications. **Tim Lincoln**

ability of Sup35 to form a prion depends on a region at one end of the protein, the amino-terminal end (Fig. 1). This well-studied 'prion domain' is rich in glutamine and asparagine amino acids — a property shared by all three confirmed yeast prion proteins. It also contains five-and-a-half repeats of a sequence of nine amino acids, which resemble five repeats that are seen in the only known mammalian prion protein, PrP. The glutamine/asparagine-rich region contributes to species specificity: the prion domains of Sup35 from different yeast species, which have different glutamine/asparagine-rich sequences, can aggregate in the same cell, but do so independently of one another[4]. The role of the repeats has been less clear, although they are involved in [*PSI*$^+$] propagation.

Osherovich *et al.*[1] uncovered a role for the repeats while studying the prion-related properties of a region in New1, a putative prion protein that they had previously identified[4] while searching the yeast genome for asparagine-rich sequences. In addition to an asparagine-rich region, New1 has one-and-a-half Sup35-like repeats. As the protein has no known function, it is difficult to show that it can behave as a prion (such a demonstration requires a protein's normal function to be disrupted by the prion form). But the authors did obtain some evidence for this when they created a new prion[4] — which they named [*NU*$^+$] — by replacing the entire prion domain of Sup35 with that of New1.

Osherovich *et al.* now show that the asparagine-rich stretch alone of [*NU*$^+$] causes aggregation — but that stable transmission of this prion also requires the repeats (Fig. 1). Deleting the repeats from Sup35 showed that they are also required for transmission of [*PSI*$^+$]. Surprisingly, however, propagation of [*PSI*$^+$] seemed to require all five-and-a-half Sup35 repeats; four were not enough. That suggests that the New1 repeats might be better at supporting prion transmission (because just one-and-a-half such repeats do the trick).

The authors then showed that the repeats are interchangeable. They created a functional hybrid prion domain by fusing the asparagine-rich region of New1 to the Sup35 repeats (Fig. 1). This hybrid domain was 'species-specific' in that it interacted with [*NU*$^+$] but not [*PSI*$^+$] — confirming that the asparagine-rich region determines the specificity of aggregation, and showing that the repeats promiscuously confer transmissibility.

As a further demonstration of this promiscuity, Osherovich *et al.* designed another prion, based on the aggregation properties of tracts of glutamine amino acids. Tracts containing fewer than 35 consecutive glutamines do not aggregate readily, but those with longer stretches have a propensity to clump together in a manner that is proportional to the number of glutamines. This characteristic underlies the build-up of proteins seen in disorders such as Huntington's disease[5]. Osherovich *et al.* found that peptides containing 22 glutamines, with or without repeats, neither aggregated nor supported prion propagation. Peptides containing 62 glutamines readily aggregated — but required adjacent repeats from Sup35 to be transmissible. So the repeats did not cause aggregation but were necessary for transmission; aggregation was also a prerequisite for propagation.

In line with the view that the efficiency of transmission of yeast prions reflects prion replication, Osherovich *et al.* propose that the peptide repeats promote transmission by facilitating the chaperone-mediated division of aggregates. They suggest that the repeats either act as sites of interaction with chaperones or alter the conformation of aggregates in a way that increases their accessibility to chaperones. This scenario is simple, and provides a testable model for addressing questions on the properties of the repeats and the ability of prions that lack them to propagate stably. For example, do prions without repeats have other sequences that enable them to interact with chaperones? Or can they replicate on their own, as suggested by the discovery of a prion that apparently forms more fragile filaments, and so replicates without chaperones[6]? Yeast prions should prove useful for exploring such questions.

But can experiments with yeast provide insight into the infectivity of mammalian prions? The peptide repeats of mammalian PrP are not essential for infectivity[7]. Intriguingly, however, a PrP repeat can functionally replace a Sup35 repeat to allow [*PSI*$^+$] propagation[8]. And there are further parallels worth mentioning. PrP that contains more repeats than normal is associated with some familial prion diseases and with increased infectivity in mice[6]; extra repeats in Sup35 increase its propensity to become [*PSI*$^+$][9]. Conversely, deleting repeats reduces both the infectivity of PrP and the appearance and transmissibility of [*PSI*$^+$][7–9]. As Osherovich *et al.*[1] suggest, designing artificial yeast prions based on the aggregation properties of these proteins may provide a good model for studying aggregate–chaperone interactions in multicellular organisms. ■

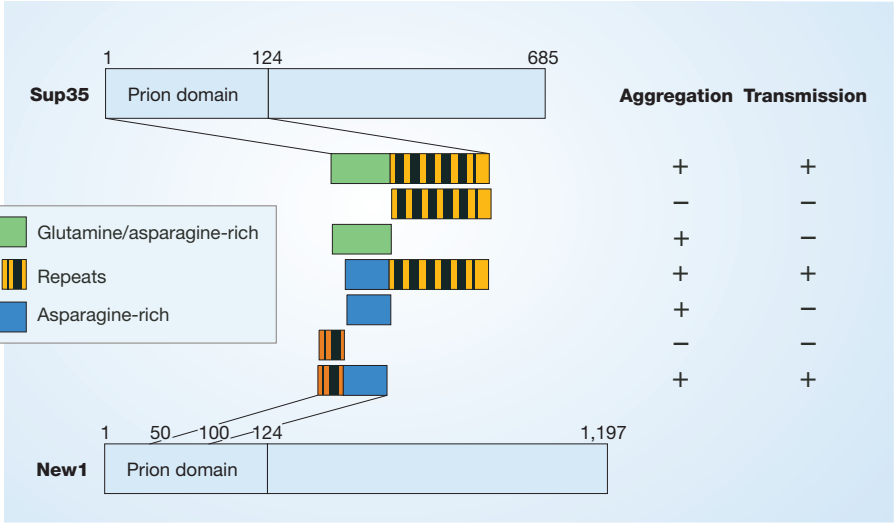


Figure 1 **The modular properties of prion domains. The prion domains of the yeast Sup35 and New1 proteins contain several repeats of a particular amino-acid sequence (five-and-a-half repeats in Sup35, one-and-a-half in New1), along with either an asparagine-rich stretch or a tract that is rich in both asparagine and glutamine. Osherovich *et al.*[1] studied the effects of deleting one or other of these regions, and the results are shown here. Prion aggregation was measured by fusing the relevant domains to green fluorescent protein and looking for fluorescent foci. Transmission was tested by fusing the domains to the region of Sup35 that is involved in regulating protein synthesis, and assessing the stable propagation of prions among cells of a yeast population (as indicated by reduced termination of protein synthesis). The authors conclude that the asparagine- or glutamine/asparagine-rich region is needed for aggregation, and that both this region and the repeats are needed for transmission.**

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