is that "... if pollution of the gene pool were all that was involved, genes that tended to cause women to continue having babies despite the risk involved would certainly outreproduce those that influenced women to stop having babies"[9].

Alternatively, menopause may have evolved as a counter-strategy to senescence. According to this 'good mother' theory, "... if the mother has more babies (with associated dangers) even as the ravages of age become severe, she is having children she may not be able to care for, and she is risking the future success of her existing children. If, instead, she stops having children and devotes herself to helping those she already has, she may have more total offspring who grow up to reproduce themselves"[7]. This hypothesis interprets early reproductive cessation as an adaptive response to prolonged infant dependency. In support, there are some wild species in which females often live well beyond their last pregnancy, including Japanese macaques[10], chimpanzees[10], elephants[11], and pilot and killer whales[2,12]. In all of these species, offspring require extended maternal care.

Good mother theorists have debated which targets of nepotism favour menopause. Is it the last-born offspring[5], grown daughters and their children[6,8,13], or an individual's entire genetic clan[9]? Much attention has been paid to grown daughters and their young, with quantitative models of traditional societies both disputing[14,15] and affirming[13] that the fitness benefits of helping to rear grandchildren can exceed the costs of reproductive cessation. Now, Packer *et al.*[1] introduce a comparative perspective to the good mother debate.

The Gombe baboons and Serengeti lions have been studied continuously for over 30 years, beginning with Jane Goodall and George Schaller, respectively. Two to ten times each month, a census is made of recognizable animals, and births and deaths are recorded. The data reveal that female baboons live up to 27 years and female lions up to 17 years. However, fertility drops precipitously when baboons are about 20 years old and the lionesses around 13. Because juvenile baboons require at least two years of maternal care and lion cubs at least one, in both species the mothers can live long enough after ceasing reproduction to rear their last-born young. Thus, lion-cub survival does not decline with maternal age, and, in baboons, survival of infants from old mothers (19–24 years) is just slightly less than those from younger mothers.

Packer *et al.* found that in neither species do post-reproductive females consistently improve the reproductive performance of grown daughters. Whether a female baboon's mother was alive did not affect her age at puberty, interbirth interval, rate of successful pregnancy or survival of her infants to their first birthday. In lions, a female's litter size and first-year survival of her cubs was also the same regardless of whether her mother was dead, or alive and post-reproductive. Cub survival was improved by the presence of a reproducing grandmother, however, due to better nourishment (females nurse their grandcubs).

The authors interpret their data as support for the senescence theory. They argue that those few lions and baboons that live to old age make a negligible contribution to fitness. Therefore, selection cannot stop their reproductive machinery from deteriorating. Packer *et al.* believe that senescence also accounts for the timing of menopause — by extrapolating from the observed relationship between mortality and maternity in baboons and lions, and assuming a childhood dependency of 10 years in humans, they calculate that the expected lifespan of women who have reached 40 years (when reproduction starts to decline) would be 58–65 years.

Packer *et al.* argue that reproductive cessation in female mammals is a non-adaptive by-product of life-history patterns, and herein lies my only disagreement. Senescence is something that natural selection cannot prevent. Although senescence is non-adaptive, menopause is not. Senescence is inevitable, so in species with prolonged infant dependency, females have evolved a precisely choreographed, adaptive counter-strategy. They cease ovulating abruptly, when they are still young enough to assist kin that would not survive or successfully reproduce without their mother[5–7,9]. Males are usually not essential to the survival of offspring, so their fertility deteriorates gradually, solely due to senescence.

Female lions and olive baboons survive only a few years after ceasing to reproduce — apparently, their main targets of maternal nepotism are last-born offspring. But women routinely live to nearly twice the average age at which menopause occurs, implying that children are dependent for a greater proportion of their lives than juveniles in the other species (that is, longer than 10 years). This also suggests that a broad array of relatives benefit from the accumulated wisdom, status and resources of post-reproductive women. □

Paul W. Sherman is in the Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853, USA.

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Protein folding

Matching speed and locality

Hue Sun Chan

How does a protein fold? What is the folding code, and how do interactions between amino-acid residues determine the native structure of a protein? Some theoreticians have attempted to tackle these complex problems of molecular recognition and self-assembly by simulations using simple lattice models (Fig. 1, overleaf) — proteins are modelled as chains configured on regular lattices, and, because they lack high-resolution atomic representations, these are models for 'generic proteins'. They try to rationalize general trends in real proteins, but they do not address properties specific to, say, lysozyme or cytochrome *c*. So, theoreticians and experimentalists in the folding field are dealing with different objects — generic versus specific proteins. How can the two be reconciled?

The experimentally observed folding times for different proteins can differ by more than nine orders of magnitude — from tens of microseconds to hours. Can these be rationalized by models for generic proteins? Unfortunately, theoreticians have not yet reached a consensus on the main determining factor that gives rise to this tremendous diversity in folding rates. In this month's *Journal of Molecular Biology*, Plaxco, Simons and Baker[1] provide much-needed systematic comparisons between theory and experiment. By statistical analysis of the correlations between measured folding rates and the various determinants proposed by theoreticians, they have identified a main structural factor that governs folding speed for a set of non-homologous, single-domain proteins that fold with essentially two-state kinetics. Remarkably, they find this to be the average sequence separation between contacting residues in the native state, normalized by the length of the protein chain — an

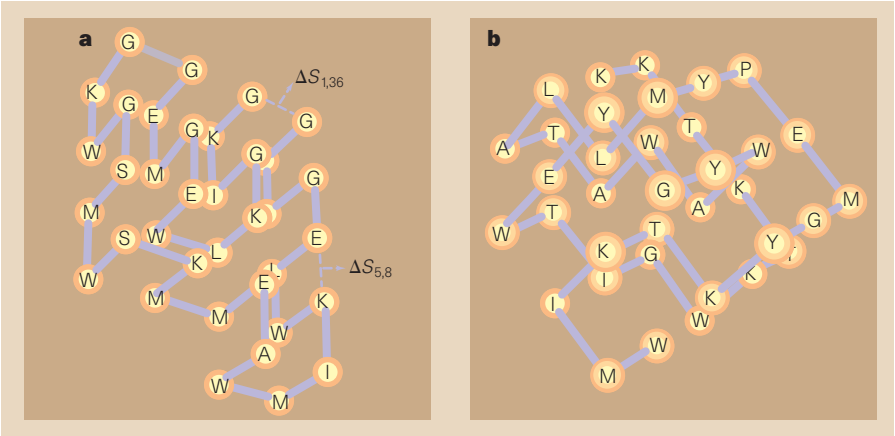

Figure 1 **Theoretical proteins, adapted from the cubic lattice models of Abkevich *et al.*[8]. Each model sequence consists of 36 residues, given by the one-letter codes for the amino acids. The model native structures have 37** (a) **and 36** (b) **nearest-neighbour contacts between residues not adjacent along the sequence. Dotted lines in** a **represent one non-local (high-order) contact (sequence separation, $\Delta S_{1,36}$ = 35) and one local (low-order) contact ($\Delta S_{5,8}$ = 3). The average contact order — sequence separation between contacting residues in the native state — is 10.0 for** a **and 15.8 for** b**. Normalized by chain length, the relative contact order is 27.9% for** a **and 44.0% for** b**. Abkevich *et al.* reported that under conditions in which both model native structures are stable, the sequence with mostly non-local contacts** (b) **folds two orders of magnitude faster than the one with mostly local contacts** (a)**. (Figure courtesy of Kaizhi Yue.)**

extremely simple measure that they call relative contact order.

Plaxco *et al.* use their own folding kinetics data, as well as results from other groups. Figure 2 shows the native structures of two proteins in their data set, cytochrome *c* and acyl phosphatase. Although they are about the same size, the folding rates of these two proteins under similar external conditions differ by more than four orders of magnitude. Plaxco *et al.* suggest that the key to this difference in rate is chain topology — the relatively simple topology of cytochrome *c* (with many local contacts) can be formed much more rapidly than the complex topology of acyl phosphatase, which involves the many non-local contacts that make up its β-sheet. For a data set of 12 proteins (which were selected from a larger set to exclude all traces of statistical correlation produced by sequence similarity), Plaxco *et al.* find a significant correlation between relative contact order and folding speed. Also interesting is that native stability and chain length, which have been thought to be important for folding kinetics, fail to show significant correlations with folding rates.

This statistical study confirms a general picture that has been emerging from recent experiments — that stronger local interactions, especially those conducive to helix formation, tend to lead to faster folding[2]. Nanosecond laser temperature-jump experiments on short peptides show that folding of a β-hairpin at room temperature is about 30 times slower than formation of an α-helix[3]. Moreover, in a set of protein engineering experiments[4], folding rate decreased with increasing length of an inserted polyglycine loop. Local guidance seems to be critical in reducing the time that is needed for a protein to find its native structure.

Many theoreticians believe in a step-by-step approach to solving the protein-folding problem. They begin by trying to understand the mechanisms by which protein thermodynamics and folding rates are related to low-resolution properties such as overall chain topology, percentage of hydrophobic residues, strength of intrachain interactions, amount of helices and sheets, and so on. These studies are expected to generate increasingly refined questions and experiments. Indeed, valuable insights have been gained by these approaches, especially with regard to how protein-like behaviours can arise from general properties of chain molecules.

But there is no general agreement among theoreticians on the question of how folding speed is affected by chain topology. In 1978, Gō and Taketomi, two pioneers of protein lattice models, concluded[5] that proteins tend to fold faster if (local) interactions among residues that are close to one another along the sequence are stronger than (non-local) interactions between residues that are far apart. Based on another lattice model, however, Šali *et al.* proposed[6] that the native state being a "pronounced global minimum" is the "necessary and sufficient" condition for rapid folding. Moreover, they reported that the relative number of local versus non-local contacts in the native state does not determine whether a sequence is fast-folding or very slow-folding (that is, practically non-folding). Because these predictions were made about generic proteins, it is hard to tell whether they can be verified or falsified by experiments on specific proteins, yet these hypotheses must somehow be matched up with real data to be relevant.

Theoreticians have much to learn from the work of Plaxco *et al.*[1], because their statistical analysis comes close to meeting the generic protein on theoreticians' ground. On the face of it, the new study vindicates theories which predict that local interactions increase the speed of folding[5,7], and casts doubt on theories predicting the opposite[8,9] (Fig. 1). But on closer examination the relation between different theories, and the correspondence between theory and experiment, are more complex. For example, owing to different modelling assumptions, different conclusions have been reached by studies based on the same lattice model[6,7]. In another instance, two theories[5,8] agree that non-local interactions increase the thermodynamic stability of the native structure, but come to opposite conclusions about whether local interactions speed up or slow down folding.

Many models predict that non-local interactions increase stability. As a corollary, theories that emphasize a positive correlation between stability and folding speed[8,9] predict that non-local interactions should increase the speed of folding. But, for the data set analysed by Plaxco *et al.*, stability turns out to be only a secondary determi-

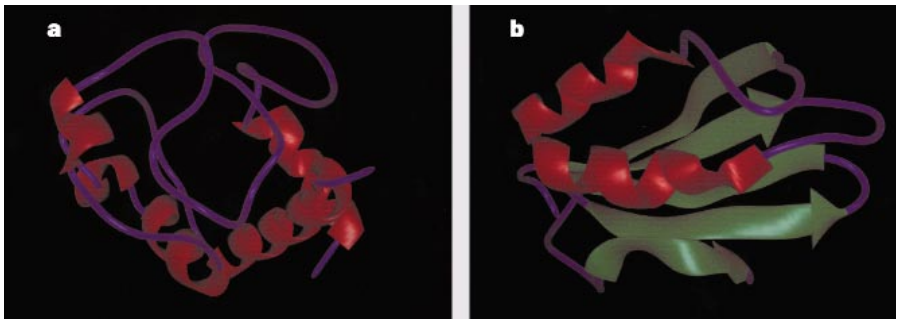

Figure 2 **Two proteins in the data set analysed by Plaxco *et al.*[1]. Experiments show that single-domain proteins whose native structures have mostly local contacts fold faster.** a**, Equine cytochrome *c* (length, 104) has mostly local contacts (relative contact order, 11.2%), and folds quickly at a rate[14] of 6,400 s^{-1} at 23 °C.** b**, Muscle acyl phosphatase (length, 98) has more non-local contacts (relative contact order, 21.2%), and folds at the much slower rate of 0.23 s^{-1} at 28 °C. (Data of F. Chiti, N. A. J. van Nuland, N. Taddei, F. Magherini, M. Stefani, G. Ramponi & C. M. Dobson.)**

nant of folding rate — a far less significant factor than contact order. Furthermore, the expected correlation between stability and relative contact order is lacking, leading one to wonder whether any of the theories is directly comparable to the data set of Plaxco *et al.*

The problem seems to be that most of the model sequences studied by theoreticians have a higher tendency to get stuck — kinetically trapped in non-native conformations during folding[10–12] — than the real, single-domain proteins of Plaxco *et al.* For many model sequences, the key to rapid folding is to overcome kinetic traps. Higher native stability would allow folding to occur at a higher simulation temperature, making it easier to escape from traps. This is the basis for the predictions[8,9] that non-local interactions cause fast folding, because non-local contacts tend to increase native stability in these models and higher temperatures can be used to simulate folding of more stable sequences with more non-local contacts (Fig. 1)[8]. However, the proteins studied by Plaxco *et al.* do not have much problem with kinetic traps[12], so they should be described by models that minimize the effects of traps. Future modelling will need to capture this generic feature of single-domain, two-state proteins.

It goes without saying that the new statistical analysis does not account for all of the factors that may contribute to folding speed. For example, mutated proteins sharing a common topology (and, thus, the same relative contact order) can fold at different rates: in one case[13], the variations cover a range of about one order of magnitude. However, if the general picture suggested by the analysis is correct, these other effects should also support the idea that stronger local interactions produce faster folding. This should be testable by further experiments. In the meantime, the work of Plaxco *et al.* is one of many signs that, in the community of protein folders, an increasing constructive feedback between theory and experiment is under way. □

Hue Sun Chan is in the Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-1204, USA.
e-mail: chan@maxwell.ucsf.edu

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Spongiform encephalopathies

The prion's perplexing persistence

Adriano Aguzzi and Charles Weissmann

Since prion diseases were identified, we have come to consider such infections as an irrevocable death sentence preceded by severe clinical disease. Take, for example, the cases of iatrogenic Creutzfeldt–Jakob disease elicited by minute traces of the infectious agent, administered through contaminated surgical instruments, hormone preparations or tissue transplants[1]. On page 770 of this issue, however, Race and Chesebro[2] report that infectivity can persist long-term in brain and spleen tissue, without causing the development of clinical symptoms.

There were reasons to believe that exposure to infectious prions may not necessarily lead to clinical disease. For example, mice lacking *Prnp* (the gene that encodes the normal prion protein, PrP^C) are completely immune to clinical disease. They cannot replicate prions, and the traces of infectivity that were occasionally found in the brain several months after injection[3,4] have been attributed to persistence in the absence of replication[4]. Moreover, mice that contain half the normal level of PrP^C take a very long time to develop symptoms after intracerebral inoculation[5]. Finally, if immunodeficient mice are inoculated peripherally with the scrapie agent, they do not fall ill, despite the occasional presence of large prion loads in their brains[6].

Another example in which exposure to infectivity does not always lead to disease is the epidemiology of bovine spongiform encephalopathy (BSE). Typically, only single animals from affected farms develop disease, even though the genetic make-up of herds is probably similar, and each cow is likely to have been exposed to similar amounts of prion infectivity. Moreover, although the BSE agent is quite likely the origin of 'new variant' Creutzfeldt–Jakob disease[7,8], and although many people in the United Kingdom and Europe may have ingested the infectious agent, only a small minority has developed overt disease to date. Why?

One reason could be that the infectious agent fails to penetrate the organism — perhaps because predisposing factors, such as lesions in the digestive tract, are required. Alternatively, prions may not transfer from the site of entry to the central nervous system. Or, they may not replicate to the extent

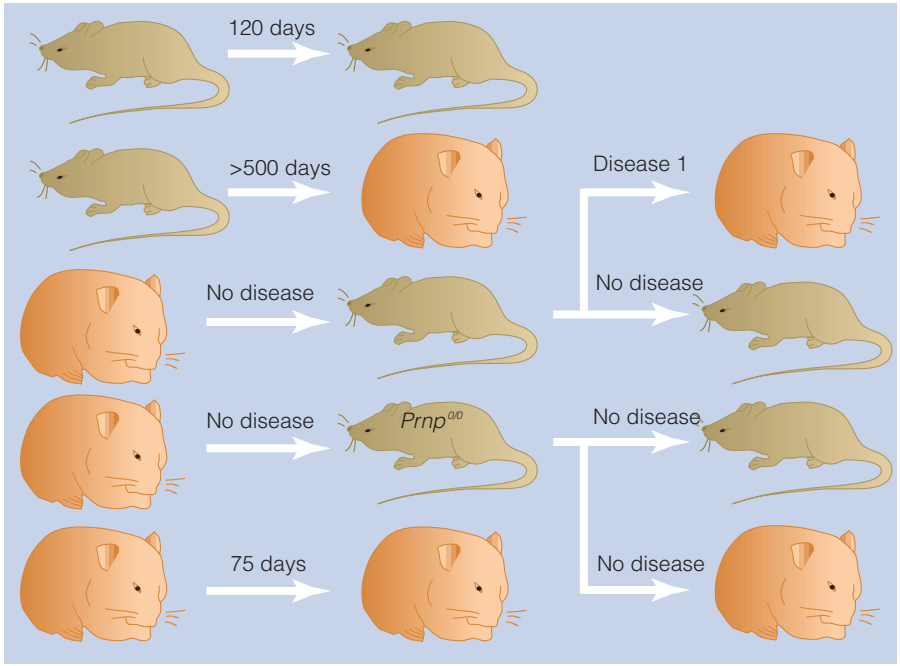

Figure 1 **Due to the species barrier between mice and hamsters, prions originating from one species produce spongiform encephalopathy rather quickly when transferred to a further animal of the same species, yet only produce disease — if any — with much longer latency when passed to the other species. This barrier can be abolished by expressing appropriate transgenic prion proteins in the recipient animals[12,13].**