concepts of solid state physics in action to unravel mysteries of astronomical objects.

(ii) The organizers had conducted a 'session on diamonds' late in the evening preceding dinner on the beaches of Mamallapuram. The session was compered by Govinda Rajan and included a brief talk by Michael Bonke of Aditi Diamonds (P) Ltd of Pondicherry and Germany. Bonke discussed various aspects of choice of proper diamonds for high pressure diamond anvil cell (DAC) processes involved in cleaving along required planes, cuts on diamond, checking the perfect parallelism between the culet and the table of the diamond anvil, etc. His team from Pondicherry had put together several tools for demonstration of cutting, polishing, etc. Aditi Diamonds have been providing specially prepared and characterized diamonds for the DAC based on the technology developed at IGCAR. The DAC is available for sale. This session, I must say, was a rather unusual one, the type of which one does not come across in conferences. The audience participation in the question–answer session was quite good and informative.

(iii) Another important highlight of this conference was inclusion of nearly a dozen papers dealing with mineral science, geophysics, geochemistry and planetary sciences in the proceedings. These papers covered a variety of topics wherein high pressure plays an important role in various phenomena: behaviour of chromium as a fluorescing element, variation of coordination of silicon, phase transformation of minerals, high pressure and high temperature metamorphization of various types of rocks, etc. Facilities available for synthesis of minerals at Mysore University had been made use of in preparing many synthetic minerals with and without H_2O. This effort of the organizers to bring together geologists and solid state physicists is commendable as such, inter-disciplinary approaches provide an opportunity for appreciating each other's points of view and promote collaboration to further studies in mineral physics, Earth's structure and planetary sciences.

K. R. Rao, 'Gokula' 29/2, 11th Cross, 3rd Main, Malleswaram, Bangalore 560 003, India

RESEARCH NEWS

1997 Nobel Prize for Medicine: Prions as proteinaceous infectious particles

The term 'prion' was introduced in 1982 by Stanley B. Prusiner of the University of California (San Francisco), who has been awarded the 1997 Nobel Prize for Medicine. Earlier, he also won the prestigious Lasker Award in 1994. The term 'prion' was used by him to characterize the proteinaceous infectious particles causing a variety of mammalian neurodegenerative diseases which are generally fatal and are referred to as transmissible spongiform encephalopathies (TSEs)[1]. These diseases included scrapie in sheep and CJD (Creutzfeldt–Jakob Disease), FFI (fatal familial insomnia), GSS (Gerstmann–Straussler–Scheinker disease) and kuru in humans. Kuru is a fatal disease, which occurred among cannabilistic tribals of Papua New Guinea. Aberrant infectious proteins are also believed to cause the 'mad cow disease' or 'bovine spongiform encephalopathy' (BSE) in cattle, which in 1996 created a panic in Britain following reports of human cases that were suspected to be caused due to eating BSE-tainted beef. Two prion-like determinants {[PSI], [URE3]} have also been described in yeast.

Despite being infectious, prions differ from viruses, viroids, virusoides or satellite RNAs and retroviruses in having no nucleic acid components whatsoever (viruses have their own DNA/RNA and proteins; viroids are RNA molecules not encapsidated; virusoids are nucleic acid molecules encapsulated by protein coat derived from other viruses, and the retroviruses carry cellular oncogenes). The absence of nucleic acids and presence of only proteins in these infectious particles were initially proved, when it was observed that UV and ionizing radiations (which damage nucleic acids) did not cause any loss of infectivity[2], while treatments involving modification or hydrolysis of proteins caused loss of infectivity[3]. Prion-like elements of yeast are also discussed as cases of 'protein conformation-based inheritance', thus challenging the paradigm that nucleic acids are the sole hereditary determinants in all living organisms. However, there are workers, who still believe that some undiscovered viruses and/or nucleic acids may be associated with the above prion diseases and the prion-like elements of yeast.

Earlier in 1994 and more recently, in 1997 (see ref. 15), experiments have been reported, where a change in one protein due to another protein has been successfully achieved in the test tube, thus giving further strong support to the 'prion hypothesis'. However, it could not be proved beyond any doubt, that prion proteins can cause these diseases without another 'helper' molecule (e.g. a lipid molecule, a sulphated glycosaminoglycan, or a chaperone protein). Such a final proof would actually require designing of an experiment involving the following steps: (i) *in vitro* synthesis of prion protein under conditions guaranteed to be free of viral nucleic acid; (ii) change of the protein synthesized *in vitro*, into its 'rogue state', and (iii) the ability of this modified 'rogue state' of protein to cause disease on infection. Although success

for the first two steps of this exercise has been achieved in recent experiments, there is no successful attempt yet to demonstrate the step (iii). In view of this, it is inferred by many that although there is strong support in favour, and no evidence against the prion hypothesis, the final positive evidence in favour of prion hypothesis is still lacking. Since the idea inherent in prion hypothesis attacks the very basis of genetics and molecular biology, its final acceptance is viewed with very high level of skepticism and scrutiny. The present status of research in this area is briefly summarized in this article.

Prion's protease-resistant protein (PrP)

The prion protein is designated as PrP (protease-resistant protein). Since the protease-resistant core of PrP has a molecular weight of 27–30 kDa, it is also known as PrP 27–30 (ref. 4). PrP occurs both in the normal and the infected hamsters. Therefore, it cannot be the causal agent for the disease. This PrP can actually exist as one of the two chemically indistinguishable isomers, PrP^C and PrP^{Sc}. PrP^C has been shown to be a part of a larger protein PrP^{Sc} with a molecular weight of 33–35 kDa (superscript Sc stands for protein causing scrapie), which causes infection[5]. PrP^C is a subset of PrP, which is protease-sensitive and works as a substrate for PrP^{Sc}, the essential component of the infectious prion particle. Physically, PrP^{Sc} differs from PrP^C by its insolubility in detergents and its relative resistance to proteolysis[6]. While PrP^C is rich in α helices, PrP^{Sc} is rich in β sheets[7] (Figure 1). Further, unlike PrP^C, PrP^{Sc} tends to form insoluble aggregates and fibrils in brain cells (Figure 2). It is also known that the different strains of prions differ in incubation period and protease cleavage pattern, which are reproducible and represent different stable conformations of the PrP polypeptide[8].

It has been shown that PrP^{Sc} is produced slowly through a post-translational process in contrast to PrP^C which is synthesized due to information encoded in a nuclear gene, and degraded rapidly. Both these isoforms (PrP^{Sc} and PrP^C) appear to pass through Golgi apparatus, where their Asn-linked oligosaccharides are modified and sialylated. PrP^C destined to become PrP^{Sc} is transported through secretory vesicles to the external cell surface where it is anchored by a glycosyl phosphatidyl inositol (GPI) moiety. It returns from the cell surface to the cell interior through caveolae to form PrP^{Sc}, which accumulates within brain cells in cytoplasmic vesicles, many of which appear as secondary lysosomes[9].

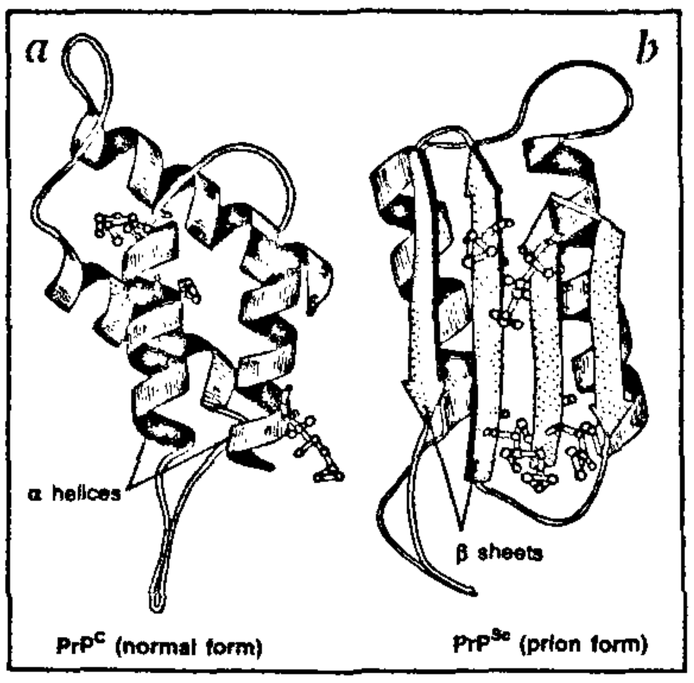

Figure 1. Proposed structures of the two alternative forms of PrP protein, showing differences in the contents of α-helix and β-sheets (modified from reference 12).

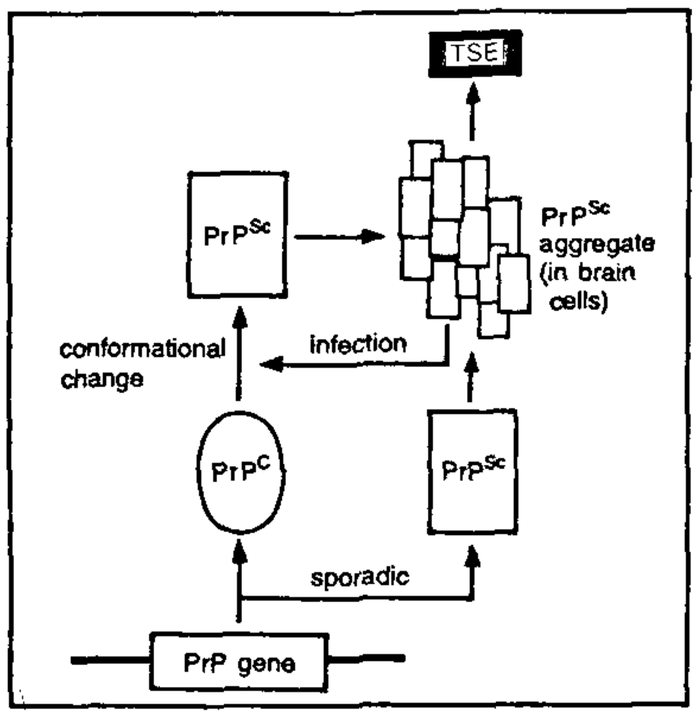

Figure 2. Prion cycle showing PrP^{Sc}-induced conversion of PrP^c into PrP^{Sc} and its aggregation in brain cells causing the disease (modified from reference 13).

Genetics of prion diseases

The prion diseases have also been shown to be hereditary, since the protein PrP has been shown to be encoded in the gene *Prn-P* located on chromosome 20 in humans and chromosome 2 in mouse[10,11]. Several prion diseases are actually caused due to mutations in the PrP gene (*Prn-P*). The following are examples of some of these mutations: (i) an insertion of 144 bp containing six octarepeats at codon 53 caused CJD; (ii) substitution of a proline (P) for leucine (L) at codon 102 caused GSS; (iii) following point mutations in *Prn-P* also caused CJD: aspartic acid (D) → asparagine (N) substitution at codon 178; glutamate (E) → leucine (K) substitution at codon 200; valine (V) → isoleucine (I) substitution at codon 210. Prion diseases were also caused by point mutations at codons 105, 117, 145, 180, 198, 217 and 232. Transgenic mice-carrying Pr-P mutant genes were also shown to develop the disease, confirming that mutations can cause these diseases[10]. The PrP gene (*Pm-P*) in Syrian hamster has two exons, while that of mouse has three exons. However, the open reading frame (ORF) of all the known PrP genes is present in a single exon in each case, so that PrP^{Sc} cannot result due to alternative RNA splicing, but should only result due to post-translational modification of PrP^C.

Genetic nature of prion diseases was also demonstrated by using transgenic mice carrying *Prn-P* from Syrian Hamster (SHaPrP) and also by ablation of the *Prn-P* in mice (*Prn-P*$^{0/0}$). In both these cases, it was shown that, when injected with prion protein, the synthesis of prion is species-specific or transgene-specific, thus further confirming that these diseases have a genetic control (for review, see ref. 1).

Propagation of prions

Since there is no evidence available for the presence of scrapie-specific nucleic acid, and since proteins are incapable of undergoing self-replication, alternative mechanisms for prion propagation have been proposed. It has been shown that post-translational conversion of PrP^C (encoded in gene, *Prn-P*) into PrP^{Sc} is obligatory for development of prion disease. This conversion can be achieved in one of the following ways (Figure 3)[12]: (i) PrP^{Sc} combines with

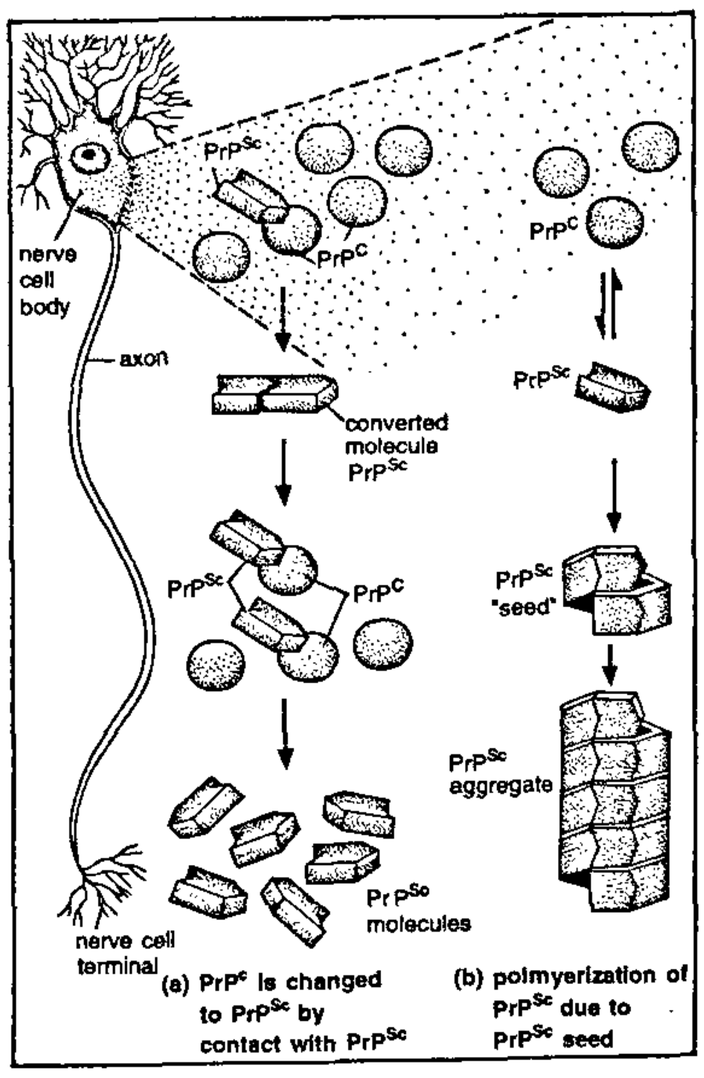

Figure 3. Two models for the propagation of PrP^{Sc} causing prion diseases in mammals (modified from reference 12).

PrP^{C} to give PrP^{C}–PrP^{Sc} complex which changes to PrP^{Sc}–PrP^{Sc}; the two molecules of PrP^{Sc} in this homodimer separate and form two heterodimers (PrP^{C}–PrP^{Sc}), which again change to two molecules of homodimers (PrP^{Sc}–PrP^{Sc}), thus forming four molecules of PrP^{Sc} and so on; (ii) In the presence of PrP^{Sc}, a part of PrP^{C} changes to PrP^{Sc} seeds through conformational change from α-helices to β-sheets, which then polymerizes. It is also believed that the PrP^{C} molecule occasionally flips into PrP^{Sc}, even in the absence of PrP^{Sc}. This implies that the presence of PrP^{C} is necessary for development of TSEs. *In vitro* systems have also been recently developed, which can induce PrP^{C} to acquire PrP^{Sc} characteristics (see later for details).

'Knockout' mice lacking PrP^{C}

In 1993, a team led by Charles Weismann and Adriano Aguzzi, both of the University of Zurich in Switzerland, produced what they described as 'knockout' mice lacking gene, *Prn-P*. They found that these mice did not get sick when injected with scrapie protein. Subsequently, in 1996, they reported that they had grafted neurons, which made Prp, into brains of these knockout mice lacking the gene, *Prn-P*. When these mice were infected with scrapie, the grafted tissue became degenerated, and the rest of the brain remained healthy. In still another set of transgenic mice engineered to make varying levels of PrP^{C}, it was observed that the mice making more PrP^{C} got sick faster, when inoculated with scrapie (for a review see ref. 12).

[PSI] and [URE3] as prion-like determinants in yeast

In yeast, the two prion-like determinants[13,14], [PSI] and [URE3], were first identified some 25 to 30 years ago. The [PSI] determinant is the protein Sup35p, which causes t-RNA-mediated suppression of nonsense codons (proof reading) and is encoded by the gene, *SUP35* (also called *SUP2* by some workers). Similarly, [URE3] determinant is the protein Ure2P, which is a regulator of nitrogen metabolism, and is encoded by the gene, *URE2*. While [PSI] is assayed in the laboratory by the efficiency of t-RNA-mediated suppression of nonsense codons in auxotrophs *ade 2-1* and *his-5-2*, [URE3] is characterized by its ability to take up uredosuccinate even in the presence of good nitrogen source (e.g. ammonia or glutamate). [PSI^{+}] is genetically dominant over [psi], because diploids derived from the cross [PSI^{+}] × [psi^{-}] are [PSI^{+}]. Further, all the products of meiosis are [PSI^{+}] suggesting cytoplasmic inheritance. Deletions for this gene are lethal, suggesting that the gene encodes a protein performing a vital function, but point mutations give [PSI^{+}] phenotype. Sup35p has also been shown to be evenly distributed in [psi^{-}], but clumped together in [PSI^{+}]. This protein has also been shown to be eRF3, a subunit of release factor eRF. Similarly, [URE3] is dominant over [ure3] and is cytoplasmically inherited like [PSI^{+}]. Unlike the gene *SUP35*, deletions for *URE2* are not lethal, but are unable to maintain the [URE3] determinant Ure2P. The protein Ure2P inhibits positive transcription regulator encoded by the *GLN3* gene, thereby preventing expression of genes needed for utilizing poor nitrogen sources.

Evidence has become available during the last three years which suggested that Sup35p and Ure2p are prion-like proteins. Despite best efforts, association of these two yeast determinants with nucleic acids (e.g., mtDNA, 2μDNA plasmids and viruses) could not be shown, so that the inheritance of these determinants was consistent with prion-like mechanisms. Although no amino acid similarity exists either between Sup35p and Ure2p or between these two proteins and the PrPs, the N-terminal regions (which determine prion-like properties) of these two proteins resemble PrPs in the following two features: (i) They have an unusual amino acid composition leading to high degree of flexibility in assuming secondary structures. (ii) PrP and Sup35P (but not Ure2p) both have short peptide repeats in the N-terminal regions. Therefore it was recognized that these two proteins can be used to elucidate the molecular mechanisms underlying *protein conformation-based inheritance*. This was particularly true, because yeast provides a much simpler and easy-to-manipulate system for detailed studies using classical as well as molecular strategies.

Following unusual genetic attributes of the above two traits in yeast have been attributed to protein conformation-based inheritance: (i) Both states of each of the two determinants {(e.g. [PSI^{+}] and [psi^{-}] in case of [PSI]} are metastable, changing into one another, albeit at a low frequency. (ii) The dominant states can be cured by growing cells in the presence of compounds, which do not induce gene mutations, but bring about denaturation of proteins. For instance, 5 mM GuHCl (guanidine hydrochloride) causes 100% conversion of [PSI^{+}] cells into [psi^{-}] cells within 7–8 generations of growth. It is possible that GuHCl induces expression of a protein chaperone involved in the inheritance of yeast prions. (iii) A transient overexpression of Sup35 and Ure2p genes increases the appearance of [PSI^{+}] and [URE3] states respectively, which can later be maintained even in the absence of such overexpression. However, the propagation of these states depends on the presence of wild type genes (*SUP35, URE2*), which unlike PrP encoding gene (*Prn-P*), encode proteins of known function. The functional element, however, can be sepa-

rated from prion-determining element located at the N-terminus of the protein. Deletion of this N-terminus (like deletion of PrP gene), leads to irreversible curing of *[PSI⁺] and [URE3], thus* eliminating their susceptibility to the prion. In contrast to the above, a transient overexpression of a chaperone protein (e.g. *Hsp104*) converts a cell from [PSI⁺] to [psi⁻], a conversion which is also heritable. (iv) Mutant forms of *SUP35* and *URE2* genes can give [PSI⁺] and [URE3] phenotypes, but unlike mutant forms, these are inherited in a normal Mendelian manner.

Molecular chaperones and prions

It has been suggested that the conversion of PrP^{C} to PrP^{Sc} might require one or more *trans*-acting molecular chaperones. The following observations suggested such an involvement of molecular chaperones in mammalian prion diseases: (i) In scrapie-infected cultured cells, inducibility and distribution of heat shock proteins undergo changes; (ii) In transgenic mice, development of prion disease may involve a second factor, designated as protein X.

Evidence for the involvement of a chaperone in yeast's prion-like elements is even stronger, as evident from the fact that the appearance of [PSI⁺] phenotype is facilitated quite efficiently by Hsp104 (a protein of chaperonin family), and less efficiently by Hsp70. Absence and overexpression of Hsp104, however, lead to disappearance of [PSI⁺] state. This implies that a precise level of Hsp104 is needed to induce [PSI⁺] state. Even a transient overexpression of Hsp104 is sufficient to cure cells of [PSI⁺], so that once induced, such cured cells maintain their [psi⁻] phenotype, even when transferred back to non-inducing medium containing no Hsp104. It is thus interesting to note that, while transient overexpression of the Sup35p protein converts a cell from [psi⁻] to [PSI⁺], transient overexpression of a chaperone protein (e.g., Hsp104) can convert a cell from [PSI⁺] to [psi⁻]. According to one model, Hsp104 is assumed to facilitate the formation of [PSI⁺] prion family of Sup35p protein from newly-synthesized Sup35p (eRF3) molecules, through the production of an intermediate transition state (Figure 4).

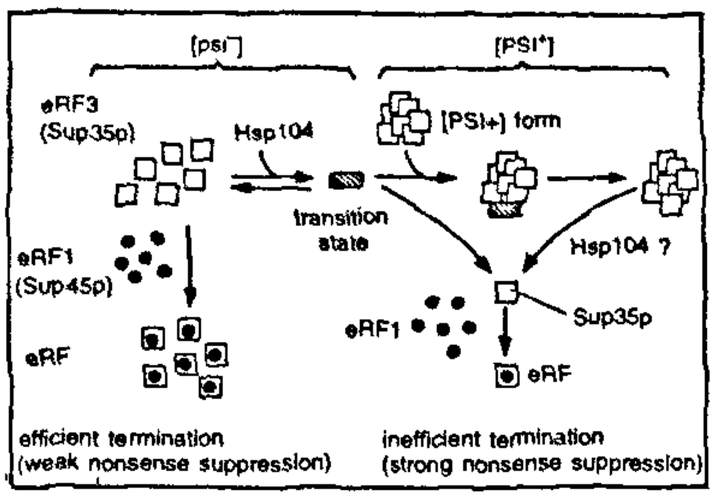

Figure 4. A hypothetical model showing the role of Hsp104 in conversion of yeast's Sup35 protein from normal [psi⁻] state to the [PSI⁺] state causing the prion phenotype (modified from reference 13).

Hsp104, though dispensable for normal growth, is crucial for survival after exposure to extreme protein denaturing stresses, such as high temperature or high concentration of ethanol. It is believed that the chaperone does not prevent stress-induced denaturation of proteins, but actually allows reactivation of the proteins, when cells are returned to normal condition. This attribute of chaperone fits well with the prion model. Susan Lindquist of the University of Chicago, in collaboration with Stanley Prusiner and B. Caughey, was reported to be conducting experiments, where she had plans to introduce the gene for PrP^{C} into yeast cells and then manipulate yeast's chaperone's level to study the role of chaperones in changing protein conformation leading to acquisition of prion characteristics[12].

Prion-like process in test tube

In 1994, B. Caughey (now at Hamilton, Montana, USA), while earlier working with P. Lansbury at Harvard, reported that they mixed purified PrP^{Sc} with normal PrP^{C} in a test tube, and could successfully obtain new PrP^{Sc} molecules. However, several questions were raised about these experiments (see ref. 12 for a review). More recently, in July 1997, Ter-Avanesyan and his coworkers from the Institute of Experimental Cardiology in Moscow reported results of more convincing experiments, where in the test tube a purified protein could cause changes in other protein molecules, so as to convert them into aggregates of proteins like those seen in the yeast cells affected by [PSI] or [URE3] (refs. 14, 15). This has been compared with the processes, by which prions are believed to cause diseases in mammals. They combined cell extracts from normal [psi] cells in which Sup35 is soluble, with cell extracts from [PSI⁺] cells in which Sup35 is insoluble. The mixture was spun in a centrifuge to separate the soluble and insoluble proteins. It was shown that within two hours, all soluble proteins became insoluble and clumped together to form an insoluble pellet. A piece of this newly-formed pellet was also shown to convert a fresh solution of normal Sup35 into an insoluble pellet again. Even when only the fragments carrying the sticky ends of Sup35 were used, clumping was caused.

In May 1997, Susan L. Lindquist from Chicago also reported that Sup35, produced in genetically-engineered *E. coli*, could spontaneously form long fibrils (like those found in the brains of CJD patients and BSE-infected cows). In a freshly-prepared solution, these fibrils were capable of quickly converting normal proteins into new fibrils[15]. The formation of such fibrils in test tubes could also be induced by just the sticky ends of Sup35. These fibrils were also shown to contain β-sheets. However none of these purified altered forms of prion like-proteins could be shown to cause the [PSI⁺] trait.

Conclusions

The above discussion shows the power and the significance of the prion hypothesis, which is likely to change our basic concepts in the field of genetics and molecular biology. Significant advances in this area have been made through *in vitro* experiments conducted and reported during 1997 (ref. 15). However, as pointed out earlier, a crucial experiment is yet to be conducted, where a purified protein modified *in vitro*, is shown to cause a prion disease on infection. Once it is done, we will have to believe in 'protein conformation-based inheritance', in addition to all pervading 'nucleic-acid based inheritance'. This will also provide a basis for development of new drugs for the prion diseases. The yeast model systems discussed in this article will be extensively used in future for further advancement of our understanding in this area.

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P. K. Gupta is in the Department of Agricultural Botany, Ch. Charan Singh University, Meerut 250 005, India.

OPINION

Decade of the brain: Reflections on some Indian concerns in cognitive neuroscience*

T. Ramakrishna

'Of all the wonders nothing is more wonderful than man, who has learned the arts of speech, of windswift thought, and of living in neighbourliness.'

– Sophocles

Brain, the organ of reason: The early search and the outcome

Nearly two hundred years of search for a physiological basis of behaviour more precisely for the organ of reason which was thought to be the heart by Aristotle, ended in the hippocratic proclamation[1], 'And men should know that from nothing else but from the brain come joys, delight, laughter and jests and sorrows and griefs, despondency and lamentations and by this in an especial manner, we acquire wisdom and knowledge and see and hear and know what are foul and what are fair, what sweet and what unsavoury... and by the same organ we become mad and delirious and fears and terrors assail us, some by night and some by day, and dreams and untimely wanderings, and cares that are not suitable and ignorance of present circumstances and unskilfulness. All these things we endure from the brain, when it is not healthy, but is more hot, more cold, more moist or more dry than natural, or when it suffers any other preternatural and unusual affliction.'

Since then biological disciplines have made remarkable progress towards understanding the structure and function of human brain and the mechanisms which underlie behaviour. However as Seymour Kety[2] wrote, '... but in the area of information, content, and experience, stored as it is in the complex interreletionships of 13 billion neurons, biology is extremely pretentious if it thinks that it can unravel them by means of its tools. There will no doubt be a biochemistry or a biophysics of memory – but not of *memories*' (emphasis mine). It is significant that nearly three decades later, *Science*, the journal which published Kety's article brought out a special issue on cognitive neuroscience[3] dealing with the question: How do we think?

The decade of the brain: Advances in cognitive neuroscience

It is also significant that the American senate declared that this decade will be a decade of the brain, giving the necessary impetus and thrust to researches towards understanding the human brain. Several other countries followed this example. The number of interrelated disciplines engaged in understanding the mechanisms underlying normal and abnormal mental processing are numerous and include anatomic and computer studies of neural circuits, animal and human lesion studies, neurophysiology, neuroimaging, neuropharmacology and experimental cognitive psychology. While the approaches to understanding schizophrenia and Alzheimer's disease as a dysfunction of specific cognitive systems may lead to amelioration of disease, understanding the normal working memory and consciousness will bring numerous rewards because of its relevance to our daily lives in terms of language as a means of communication, memory and planning. The intensity and the excitement of such recent global endeavours in brain research are palpable. As a result of these efforts, it is becoming clear that the processing of information that leads to complex behaviours such as learning and memory involves multiple brain regions that must operate in an interactive synchrony.

The advent of two spectacular techniques namely, measured brain activity

*This article is based on the lecture, 'Hemispheric Mechanisms of Language Processing' delivered at the Institute of Nuclear Medicine and Allied Sciences (INMAS), Defence Research and Development Organization, New Delhi on 4 June 1997.