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PrP^{Sc} AS A PRODUCT OF MEMBRANE FOLDING

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Summary. *The features of the course of transmissible spongiform encephalopathies have been correlated with the regularities of protein structure formation and their interaction with cell membranes. Evidence is given about the mechanism of formation of the pathogenic isoform of the prion protein as a consequence of membrane folding of the protein with incomplete structure formation. Thus, the reproduction of the pathogenic form of the prion protein takes on the character of a cellular process, and not an intermolecular process, as is commonly believed.*
Keywords: *prions, protein folding, chaperones.*

Among the immense variety of infectious agents, prion proteins occupy a special place. First of all, this is due to their unusual protein-only nature. Unlike various bacteria, viruses, fungi, unicellular and multicellular parasites, prion protein does not contain nucleic acids. Moreover, the infectious form of the prion protein (PrP^{Sc}) is a structural isomer of the cellular protein (PrP^{C}) [1]. The latter is intensively synthesized and degraded by cells of the central nervous system of all mammals [2]. It is the cancellation of the tertiary structure that gives the labile cellular protein high stabilization, resistance to the action of proteolytic enzymes and the ability to cause irreversible and progressive damage of the tissues of the central nervous system (Fig. 1).

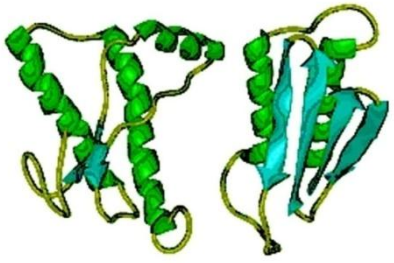

Fig. 1. Normal (left) and pathogenic (right) prion protein isoforms [3].

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Diseases caused by prion infection form a group of transmissible spongiform encephalopathies (TSEs), which reveal characteristic tissue damage of the central nervous system and are capable of transferring infections between organisms of the same species (Fig. 2)..

Creutzfeldt–Jakob disease (CJD)	human
New version CJD (vCJD, nvCJD)	human
Gerstmann–Sträussler–Scheinker syndrome	human
Fatal familial insomnia	human
Kuru	human
Scrapie	sheep and goats
Bovine spongiform encephalopathy	cows
Transmissible minks encephalopathy	minks
Chronic wasting disease (CWD)	deer and moose
Feline spongiform encephalopathy (FSE)	cats

Fig. 2. The main forms of TSEs and their carriers.

By origin, sporadic, infectious and genetically determined forms of TSEs are distinguished. The most common form is sporadic, that is, of unknown origin [4]. Mass dying of cells of the central nervous system leads to the formation of a peculiar spongy structure. In most cases, it is accompanied by the deposition of protein aggregates (amyloids) (Fig. 3)

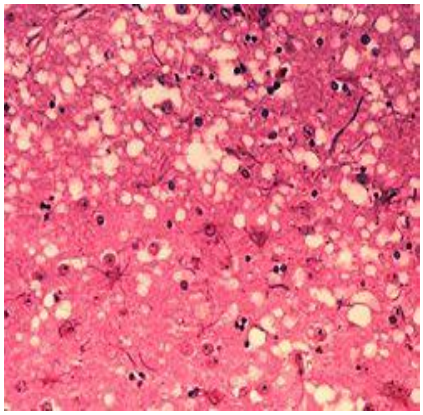

Fig.3. A typical lesion of the tissue of the central nervous system by TSE [4].

That is, TSE are typical proteinopathies, that is, diseases associated with the accumulation of insoluble protein aggregates. The most famous proteinopathies are Alzheimer's, Parkinson's and Huntington's diseases [4].

The oldest known form of TSEs is scrapie, which has been known since ancient times. Its first mention in scientific literature dates back to 1732. The ability of this disease to be horizontally transmitted has long been known, which unequivocally testified to its infectious nature. The nature of the infectious agent caused the crisis of biological science, which continues to some extent in our time. The long period of the latent stage of the disease and the ability of the pathogen to pass through bacterial filters caused a false conclusion about its viral nature. The name "slow viral diseases" is still often found. It quickly became clear that this infectious agent has a

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number of properties that are incompatible with the presence of nucleic acids in its composition (Fig. 4).

chemical processing	viruses	?????
Et_2PC	(-)	+
NH_2OH	+	-
Psoralen	+	-
Phenol	-	+
SDS	-	+
Zn^{2+}	+	-
Urea	-	+
Alkali	(-)	+
KSCN	-	+
enzymatic processing	**viruses**	?????
RNAse A	+	-
DNAse	-	-
Proteinase K	-	+
Trypsin	-	+

Fig. 4. The influence of various reagents on the inactivation (+) or preservation (-) of the infectious properties of the TSEs pathogen.

Instead, the TSE infectious agent was found to be susceptible to substances that are typical protein denaturants. This clearly proved the purely protein nature of the TSE pathogen. The final point in clarifying its nature was put by the work of Stanley Prusiner's group, who discovered and identified the pathological form of the prion protein (Fig.1, left).

However, the question of how it all works remains unanswered. The most common explanation for PrP^{Sc}'s action is its ability to associatively rearrange the healthy form of the cellular protein PrP^{C}. Such an assumption well explains a number of features of the pathogenesis of TSEs. The need for own biosynthesis of the prion protein for the development of the disease is becoming clear [5]. Experimental animals with a knocked-out prion protein gene naturally become invulnerable to prion infection. The lack of dependence of the latent period of the disease on the size of the initial infectious dose and its high dependence on the intensity of the prion protein's own biosynthesis is also explained. However, the assumption of associative rearrangement of the cellular isoform to the pathogenic one does not explain the existence of a minimal infectious dose of the prion protein [6]. Theoretically, one bacterium or virus can trigger the relevant pathological processes, but PrPSc becomes infectious only at a dose of no less than 10^5 molecules [7]. Direct incubation of cellular and pathogenic forms of prion protein under in vitro conditions does not lead to an increase in the amount of infectious material [8]. There is no explanation for the existence of different strains of the disease that are capable of transitioning from one to another during repeated infectious episodes [9,10]. And, of course, the existence of an inter-species barrier that limits or excludes the ability of the prion protein to infect foreign organisms needs to be explained.

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In order to explain these properties, an assumption has been formulated about the participation in the process of restructuring of such a protein X [1,11]. It is assumed that this protein, together with the pathogenic form of the prion protein, ensures the restructuring of the cellular form of the prion protein into the pathogenic form. True, the unsuccessful search for protein X has been going on for half a century. This makes one agree with Deng Xiaoping's opinion about the difficulty of catching a black cat in a dark room in its absence. It is not worth following the example of William Occam to grab the razor, but is it not possible to explain the specifics of the pathogenesis of TSEs without involving intangible components? A certain systematization of data on the mechanisms of protein structure formation allows us to answer this question positively.

Similarly to any protein, the formation of the structure of the native molecule (folding) of PrP^{C} is a complex multistage process that occurs by an ATP-dependent mechanism with the participation of chaperone proteins [12]. In addition to forming the structure of newly synthesized proteins, chaperone proteins are involved in the corrected structures of damaged or to some extent denatured proteins. Therefore, the pathogenic form of the prion is perceived by the corresponding chaperones as its own denatured one, which is subject to correction. But PrP^{Sc} is characterized by an exceptionally high level of structure stabilization [3]. This, together with the ability of PrP^{Sc} to embed and pass through cell membranes, gives it the ability to effectively block the corresponding components of the chaperone system and, as a result, the folding of de-novo synthesized prion molecules. Given the intensity of the synthesis of the latter, this is guaranteed to lead to the formation and accumulation of significant amounts of prion protein with incomplete structure formation. Is there an alternative to native holding? And this question can be answered in the affirmative. The interaction of unstructured and denatured proteins with the outer membrane of cells occurs according to certain rules (membrane folding) [13]. This ensures the formation of structures that are significantly different from molecules that have undergone native folding. Similar structures are characterized by a reduced content of β-stacked structures, increased surface hydrophobicity, and the ability to penetrate cell membranes. By its structure, PrP^{Sc} is a typical membrane-bound protein [14]. This point of view is supported by the acquisition of protein resistance and infectious properties by the prion protein only after interaction with the outer membrane of the cell [15]. The formation of a pathogenic isoform in the absence of an initial infectious dose as a result of ultrasonic sonification of neuroblastoma cells is no less indicative [16]. The appearance of significant amounts of denatured proteins will suppress the ability of the chaperone system to fold de-novoy synthesized proteins and, as a result, ensure their entry into the membrane folding. The above considerations are far from exhaustive of the consequences of the disruption of the native structure formation of prion proteins and the properties of alternative folding. From the above considerations, it can be concluded that the pathological form of the prion protein is not formed as a result of the rearrangement of the mature cellular form, but is a product of membrane folding of the prion protein with incomplete structure formation.

In other words, the reproduction of the pathogenic isoform is not intermolecular process, but is the cellular one [17]. This explains a whole series of

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complications in the course of TSEs. The existence of a minimum infectious dose, an interspecies barrier, and distinct disease lineages become an obvious and inevitable consequence of such a process. But if you meet someones who continue to search for protein X, let them search. For by God, they deserve it.

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