

**Za Luciju, Sofiju i Vilima**

# **Prion Protein Gene and Its Shadow**

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**A thesis submitted for the degree of Doctor of Philosophy of The Australian  
National University.**

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**September, 2004**

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## Abstract

Prion protein (PrP) is best known for its involvement in prion diseases. A normal, dynamic isoform of prion protein (PrP<sup>C</sup>) transforms into a pathogenic, compact isoform (PrP<sup>Sc</sup>) during prion disease pathogenesis. The PrP<sup>Sc</sup>, acting as a template upon which PrP<sup>C</sup> molecules are refolded into a likeness of itself, accumulates in the brain neurones and causes disease. It is the only known component of prions, proteinaceous infectious particles. Both prion protein isoforms have the same primary amino acid structure and are encoded by the same prion protein gene (*PRNP*). *PRNP* determines susceptibility/disposition to prion diseases and their phenotypes.

The normal function of *PRNP* is elusive. The *Prnp* knock-out mice with disrupted ORF show only very subtle phenotype. A number of hypotheses were proposed on the function of mammalian *PRNP*. The extracellular, GPI-anchored, glycosylated mammalian PrP<sup>C</sup> expressed in a heterogenous set of cells could: transport copper from extracellular to intracellular milieu, buffer copper from synapse, contribute to redox signalling, act neuroprotectively, mediate cell-cell contacts, affect lymphocyte activation, participate in nucleic acid metabolism, be a memory molecule, and be a signal-transduction protein.

Experimental evidence demonstrated a redundancy between the *PRNP* and another, unknown gene. The critical issue therefore is to discover new genes homologous with *PRNP*, candidates for this redundancy. Using unpublished data, a sequence of zebrafish cDNA sequenced by Prof. Tatjana Simonic's group (University of Milan, Italy), I discovered a new paralogue of *PRNP*. By searching manually, and in a targeted fashion, data deposited in public biological databases, I compiled support for the new human gene Shadow of prion protein (*SPRN*) including the direct evidence, homology-based evidence and *ab initio* gene prediction. The protein product called Shadoo (shadow in Japanese) is an extracellular, potentially glycosylated and GPI-anchored protein of a mature size of 100-odd amino acids. It is conserved from fish (zebrafish, *Fugu*, *Tetraodon*) to mammals (human, mouse, rat), and exhibits similarity of overall protein

features with PrP. Most remarkably, the Sho is the first human/mammalian protein apart from PrP that contains the middle hydrophobic region that is essential for both normal and pathogenic properties of PrP. As this region is critical for heterodimerization of PrP, Sho may have potential to interact with PrP and is a likely candidate for the Protein X. Mammalian *SPRN* could be predominantly expressed in brain (Tatjana Simonic Lab, University of Milan, Italy).

Using the same approach to search public databases, I found, in addition, a fish duplicate of *SPRN* called *SPRNB*, and defined a new vertebrate *SPRN* gene family. Further, I also expanded a number of known fish genes from the *PRNP* gene family. The total number of the new genes that I discovered is 11. With the representatives of two vertebrate gene family datasets in hand, I conducted comparative genomic analysis in order to determine evolutionary trajectories of the *SPRN* and *PRNP* genes. This analysis, complemented with phylogenetic studies (Dr. Lars Jermiin, University of Sydney, Australia), demonstrated conservative evolution of the mammalian *SPRN* gene, and more relaxed evolutionary constraints acting on the mammalian *PRNP* gene. This evolutionary dialectic challenges widely adopted view on the “highly conserved vertebrate” *PRNP* and indicates that the *SPRN* gene may have more prominent function. More conserved *Sprn* could therefore substitute for the loss of less conserved, dispensable *Prnp* in the *Prnp* knock-out mice. Furthermore, the pathogenic potential of *PRNP* may be a consequence of relaxed evolutionary constraints.

Depth of comparative genomic analysis, strategy to understand biological function, depends on the number of species in comparison and their relative evolutionary distance. To understand better evolution and function of mammalian *PRNP*, I isolated and characterized the *PRNP* gene from Australian model marsupial tammar wallaby (*Macropus eugenii*). Marsupials are mammals separated from their eutherian relatives by roughly 180 million years. Comparison of the tammar wallaby and Brazilian opossum PrP with other vertebrate PrPs indicated patterns of evolution of the PrP regions. Whereas the repeat region is conserved within lineages but differs between lineages, the hydrophobic region is invariably conserved in all the PrPs. Conservation of PrP between marsupials and eutherians suggests that marsupial PrP could have the same

pathogenic potential as eutherian PrPs. Using the marsupial *PRNP* gene in comparison with the *PRNP* genes from eutherian species in which prion diseases occur naturally (human, bovine, ovine) or experimentally (mouse), I defined gene regions that are conserved mammalian-wide and showed the utility of the marsupial genomic sequence for cross-species comparisons. These regions are potential regulatory elements that could govern gene expression and posttranscriptional control of mRNA activity. These findings shed new light on the normal function of mammalian *PRNP* supporting best the signal-transduction hypothesis. The normal function of *PRNP* may be triggering of signalling cascades which contribute to cell-cell interactions and may act anti-apoptotically. Yet, in the heterogenous set of cells expressing PrP<sup>C</sup> these pathways will contribute to a number of cell-specific phenotypes, such as the synaptic plasticity and activation of lymphoid cells.



## Statement of Authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere. No other person's work has been used without due acknowledgement in the main text of the thesis.

Computational work was performed in the Computational Proteomics and Drug Design Group (The John Curtin School of Medical Research). Laboratory equipment, materials and resources for experimental work were provided in the Comparative Genomics Group (Research School of Biological Sciences).

Prof. Tatjana Simonic and her collaborators Dr. Lorenzo Sangiorgio and Dr. Bice Strumbo (University of Milan, Italy) made available unpublished cDNA sequence from zebrafish as part of exchange of results. Prof. Simonic's Group also performed the RT-PCR analysis of mammalian *SPRN* expression.

Dr. Lars Jermiin (University of Sydney, Australia) conducted phylogenetic analysis of the PrP- and Sho-protein families.

Dr. Jill Gready and Prof. Jenny Graves constructed a model to rationalize evolution of vertebrate *PRNP*- and *SPRN*-gene families.

Products of cycle sequencing reactions were run on DNA sequencers in Biomolecular Resource Facility (The John Curtin School of Medical Research).

The 67 kb tammar wallaby BAC harbouring *PRNP* was sequenced at The Australian Genome Research Facility (Brisbane, Australia).

This thesis has not been submitted for award of any other degree or diploma at any other tertiary institution.

Marko Premzl,

14 September 2004

Typographical errors on pages 55, 66 and 153 and figure captions on pages 143b and 143c were corrected upon a reviewer's request.

Marko Premzl

25 January 2005

**Publication List**

**Premzl, M.,** Delbridge, M., Gready, J.E., Wilson, P., Johnson, M., Kuczek, E., Graves, J.A.M. (2004) The prion protein gene: identifying regulatory signals using marsupial sequence. *Gene* (submitted).

**Premzl, M.,** Gready, J.E., Jermiin, L., Simonic, T., Graves, J.A.M. (2004) Evolution of vertebrate genes related to Prion and Shadoo proteins using comparative genomic analysis. *Molecular Biology and Evolution* (in press).

**Premzl, M.,** Sangiorgio, L., Strumbo, B., Graves, J.A.M., Simonic, T., Gready, J.E. (2003) Shadoo, a new protein highly conserved from fish to mammals and with similarity to prion protein. *Gene* 314: 89-102.

## **Acknowledgements**

To my supervisors Dr. Jill Gready (The John Curtin School of Medical Research) and Prof. Jenny Graves (Research School of Biological Sciences) I thank for giving me a chance to study in the prion field. I thank all members of their labs for their supervision, both on campus and outside ANU.

I am indebted to Prof. Tatjana Simonic (University of Milan, Italy) for her kind gift, unpublished sequence that was critical for success of my study.

Valuable discussions of Prof. Miroslav Radman (Faculté de Médecine Necker-Enfants Malades, Université Paris V, Paris, France) and Dr. Frances Shannon (The John Curtin School of Medical Research) enabled me to properly interpret results of my work.

I am very grateful to Prof. Judith Whitworth (The John Curtin School of Medical Research) for her kind understanding and support.

To Profs. Frank Fenner, Gordon Ada (The John Curtin School of Medical Research) and Peter Doherty (University of Melbourne) I thank for inspiration and their support.

I thank my referees Dr. Vera Gamulin (Rudjer Boskovic Institute, Zagreb, Croatia), Prof. Ivo Karadjole (Veterinary Faculty, University of Zagreb, Croatia) and Prof. Nikola Ljubescic (Rudjer Boskovic Institute, Zagreb, Croatia) for supporting my application to study at the ANU.

Finally, I am grateful to many other nice people who have helped me to find Australia that I was looking for.

## Preface

“I, however, believe that there is at least one philosophical problem in which all thinking men are interested. It is the problem of cosmology: the problem of understanding the world—including ourselves, and our knowledge, as part of the world. All science is cosmology, I believe, and for me interest in philosophy, no less than that in science, lies solely in the contributions which it has made to it. For me, at any rate, both philosophy and science would lose all their attraction if they were to give up that pursuit.”

**K. Popper**, *The logic of scientific discovery*, Preface to the first English edition, 1959.

“[Socrates] And now, I said, let me show in a figure how far our nature is enlightened or unenlightened: --Behold! human beings living in a underground cave, which has a mouth open towards the light and reaching all along the cave; here they have been from their childhood, and have their legs and necks chained so that they cannot move, and can only see before them, being prevented by the chains from turning round their heads. Above and behind them a fire is blazing at a distance, and between the fire and the prisoners there is a raised way; and you will see, if you look, a low wall built along the way, like the screen which marionette players have in front of them, over which they show the puppets.

[Glaucou] I see.

[Socrates] And do you see, I said, men passing along the wall carrying all sorts of vessels, and statues and figures of animals made of wood and stone and various materials, which appear over the wall? Some of them are talking, others silent.

[Glaucou] You have shown me a strange image, and they are strange prisoners.

[Socrates] Like ourselves, I replied; and they see only their own shadows, or the shadows of one another, which the fire throws on the opposite wall of the cave?

[Glaucou] True, he said; how could they see anything but the shadows if they were never allowed to move their heads?

[Socrates] And of the objects which are being carried in like manner they would only see the shadows?

[Glaucou] Yes, he said.

[Socrates] And if they were able to converse with one another, would they not suppose that they were naming what was actually before them?

[Glaucou] Very true.

[Socrates] And suppose further that the prison had an echo which came from the other side, would they not be sure to fancy when one of the passers-by spoke that the voice which they heard came from the passing shadow?

[Glaucou] No question, he replied.

[Socrates] To them, I said, the truth would be literally nothing but the shadows of the images.”

**Plato** (427-347 B.C.), *The Republic*, The allegory of the cave.