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The prion protein gene: Identifying regulatory signals using marsupial sequence

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Abstract

The function of the prion protein gene (*PRNP*) and its normal product PrP^C is elusive. We used comparative genomics as a strategy to understand the normal function of *PRNP*. As the reliability of comparisons increases with the number of species and increased evolutionary distance, we isolated and sequenced a 66.5 kb BAC containing the *PRNP* gene from a distantly related mammal, the model Australian marsupial *Macropus eugenii* (tammar wallaby). Marsupials are separated from eutherians such as human and mouse by roughly 180 million years of independent evolution. We found that tammar *PRNP*, like human *PRNP*, has two exons. Prion proteins encoded by the tammar wallaby and a distantly related marsupial, *Monodelphis domestica* (Brazilian opossum) *PRNP* contain proximal PrP repeats with a distinct, marsupial-specific composition and a variable number. Comparisons of tammar wallaby *PRNP* with *PRNP*s from human, mouse, bovine and ovine allowed us to identify non-coding gene regions conserved across the marsupial–eutherian evolutionary distance, which are candidates for regulatory regions. In the *PRNP* 3′ UTR we found a conserved signal for nuclear-specific polyadenylation and the putative cytoplasmic polyadenylation element (CPE), indicating that post-transcriptional control of *PRNP* mRNA activity is important. Phylogenetic footprinting revealed conserved potential binding sites for the MZF-1 transcription factor in both upstream promoter and intron/intron 1, and for the MEF2, MyT1, Oct-1 and NFAT transcription factors in the intron(s). The presence of a conserved NFAT-binding site and CPE indicates involvement of PrP^C in signal transduction and synaptic plasticity.

Keywords: Prion protein gene; Prion protein; Comparative genomics; 3′ untranslated region; Phylogenetic footprinting; Transcription factors; Signal transduction; Synaptic plasticity

Abbreviations: PRNP, prion protein gene; PrP, prion protein; PrP^C, normal isoform of prion protein; PrP^{Sc}, pathogenic isoform of prion protein; *SPRN*, Shadow of prion protein gene; Sho, Shadow protein; ORF, open reading frame; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; MY, million years; BAC, bacterial artificial chromosome; FISH, fluorescent in situ hybridisation; UTR, untranslated region; CPE, cytoplasmic polyadenylation element; CPEB, cytoplasmic polyadenylation element binding protein; MZF-1, Myeloid zinc finger-1; Oct-1, Octamer 1; MEF2, Myocite enhancer factor-2; MyT1, Myelin transcription factor 1; NFAT, Nuclear factor of activated T-cells.

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1. Introduction

The prion protein gene *PRNP* is best known for its role in prion diseases, but its normal function remains elusive. Its product, prion protein (PrP), has the ability to fold into a dynamic, physiological conformation (PrP^{C}), and into a compact, pathogenic conformation (PrP^{Sc}) that causes prion diseases (Prusiner, 1998).

There are several hypotheses about the normal role of PrP^{C} consistent with its localization on the cell membrane. Among other alternatives, PrP^{C} could be a signal transduction protein, as its activation in vitro triggers a signalling pathway for which the terminal targets in both neuronal and non-neuronal cells are the MAP kinases ERK1/2 (Schneider et al., 2003).

Mammalian *PRNP* is a housekeeping gene. It has been characterized in several eutherian species: hamster (Li and Bolton, 1997), human, sheep, mouse (Lee et al., 1998) and bovine (Hills et al., 2001). These analyses have identified, as conserved features of eutherian *PRNP* promoters, their GC richness and a lack of TATA box.

However, there are some differences in gene structure and regulation of gene expression among species. *PRNP* genes contain three exons, with exons 1 and 2 encoding the 5′ UTR region of mRNA in mouse, sheep (Lee et al., 1998), rat (Saeki et al., 1996) and bovine (Hills et al., 2001), but only two exons in human, the first of which encodes the 5′ UTR region (Lee et al., 1998). Two or three exons are transcribed alternatively in the mRNA encoded by Syrian hamster *PRNP* (Li and Bolton, 1997). Complete ORF and 3′ UTR region are encoded by the 3′ terminal *PRNP* exon. There is a single transcription start site in all eutherian *PRNP*s known except for rodent species (mouse, rat, hamster), which have multiple transcription start sites.

Next, regulatory signals controlling expression of the mammalian *PRNP* gene have not yet been resolved by *PRNP* gene expression studies (Table S1). Experiments on rat, mouse, bovine and human *PRNP* have variously ascribed regulatory roles for the elements in promoter and intron/intron 1. The problems are that functional regions differ between species and between the cell lines used in different experiments. Of note here is that the regulatory elements that determine physiological expression of *Prnp* reside in the upstream promoter and both introns in mouse (Fischer et al., 1996), but no regulatory elements were described in the intron 2 by present.

Comparative genomics is a powerful tool for understanding biological function. Such analysis is particularly useful for detecting conserved regulatory sequences (e.g. transcription factor-binding sites), a process known as phylogenetic footprinting (Blanchette and Tompa, 2002). However, availability of genomic DNA sequence from representatives of major mammal and vertebrate lineages limit such studies.

Reliability of the cross-species analysis increases with number of species compared and the optimal evolutionary distance between species depends on the biological question addressed. We therefore isolated and characterized the *PRNP* gene from a distantly related mammal in order to identify features of *PRNP* gene structure and discover potential regulatory elements. Marsupial mammals diverged from eutherian ("placental") mammals about 180 million years ago, so provide important middle ground (~180 MY) between intra-eutherian (~80 MY) and mammal-bird (~310 MY) comparisons (Wakefield and Graves, 2003). Comparisons of marsupial and eutherian genes and chromosomes have provided many important and unexpected insights, for instance in identifying the mammalian testis—determining gene and understanding sex chromosome evolution, and have also lead to discovery of new human genes (reviewed by Graves and Westerman, 2002).

We used the model Australian marsupial *Macropus eugenii* (tammar wallaby). Here we report the isolation and characterization of a BAC harbouring the tammar *PRNP* gene, and compare it with the *PRNP* genes from species in which prion diseases occur naturally (human, bovine, ovine) or experimentally (mouse). This comparison across the eutherian–marsupial distance enabled us to infer the evolution and dynamics of the mammalian *PRNP* gene and identify conserved non-coding gene segments representing potential regulatory elements.

2. Materials and methods

2.1. Cloning of tammar wallaby PRNP cDNA

Using genomic DNA as a template we performed PCRs in a total volume of 50 μl containing 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$; Roche), 200 μM dNTPs (Roche), 1–2 U of the Taq polymerase (Roche), 200 ng of template, and 200 pmol of degenerate primers G-Forward and G-Reverse, respectively (Table S2). After 2 min of denaturation at 94 °C, we ran 35 amplification cycles using a touch-down protocol as follows: 1 min of denaturation at 94 °C, 1 min of annealing with temperatures ranging from 59 °C to 52 °C, and 1 min of extension at 72 °C. Finally, we extended the PCR products further during 10 min at 72 °C. The 214 bp PCR product was cloned using the pGEM-T Easy (Promega) cloning kit. The plasmids harbouring cloned fragment were templates for sequencing reactions using the standard primers T3 and T7 and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). Products of the sequencing reaction were run on an ABI3730 DNA sequencer (Applied Biosystems).

Next, we screened a random primed pouch young tammar wallaby cDNA library as template using PCR. This library was made previously using the ZAPII vector (Clontech) and a female (day 0) and a male (day 0) tammar wallaby pouch young as mRNA sources. Its titre was 10^9 pfu/ml. After two extension steps in the reverse direction, and after four extension steps in the forward direction, we