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Research Report

Prion protein mRNA expression in *Xenopus laevis*: No induction during melanotrope cell activation

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ARTICLE INFO

Article history:
Accepted 15 December 2005
Available online 8 February 2006

Keywords:
Background adaptation
Embryogenesis
Intermediate pituitary
mRNA expression
Prion protein
Xenopus laevis

Abbreviations:
AL, anterior lobe
GAPDH, glyceraldehyde-3-phosphate dehydrogenase
GFP, green fluorescent protein
GPI, glycosylphosphatidylinositol
NIL, neurointermediate lobe
POMC, proopiomelanocortin
PrP^C, cellular prion protein

ABSTRACT

In mammals, the prion protein (PrP) is expressed in most tissues, but predominantly in neuronal tissues. Here, we investigated the temporal and spatial mRNA expression of PrP in the non-mammalian South African claw-toed frog *Xenopus laevis*. PrP transcripts were maternally expressed and detected throughout embryonic development, most strongly from neurulation onwards and including the tadpole stage. Microinjection of PrP mRNA into fertilized *Xenopus* eggs did not affect early embryonic development. In adult frogs, PrP mRNA expression was observed in all tissues examined, with high expression in brain, pituitary and testis. In *Xenopus*, the intermediate pituitary melanotrope cells are involved in background adaptation of the animal and produce high levels of the prohormone proopiomelanocortin (POMC) when the melanotrope cells are active (i.e. when the animal is black-adapted). Remarkably and in contrast to most secretory pathway components, PrP was not upregulated in the melanotropes of black-adapted animals, arguing against a direct role of this protein in POMC biosynthesis.

1. Introduction

Transmissible spongiform encephalopathies (TSEs or prion diseases) form a biologically unique group of infectious fatal neurodegenerative disorders, which are caused by changes in the three-dimensional conformation of the normal cellular prion protein (PrP^C) that lead to the formation of the abnormal, protease-resistant, disease-associated prion protein (PrP^{Sc}) (Bolton et al., 1982). Mature PrP^C is a glycosylphosphatidylinositol (GPI)-anchored sialoglycoprotein, which is expressed in nearly all tissues, but highest levels are found in the central nervous system (Caughey et al., 1988; Collinge et al., 1994; Tichopad et al., 2003; Vey et al., 1996). Despite sequence variations among different species,

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doi:10.1016/j.brainres.2005.12.105

PrP from turtle to human retains certain structural elements such as a signal peptide, tandem repeats, a hydrophobic region, two glycosylation sites, a disulfide bridge and a GPI-anchor site (Simonic et al., 2000; Strumbo et al., 2001; Suzuki et al., 2002; Wopfner et al., 1999). The isolation and characterization of the full-length coding sequence have shown that *Xenopus* PrP harbors considerable conservation but lacks the repeats found in all known PrPs (Strumbo et al., 2001). Thus far, no studies on PrP expression in this primitive vertebrate have been performed.

In this study, we aimed to characterize *Xenopus* PrP during development by examining the temporal and spatial expression patterns of PrP transcripts in *Xenopus* eggs, embryos and tadpoles. Furthermore, we examined PrP expression in a number of tissues in the adult animal, including in the neuroendocrine melanotrope cells of the intermediate pituitary. In *Xenopus*, the process of background adaptation provides the opportunity to manipulate the activity of the melanotrope cells in vivo. In animals adapted to a black background, the melanotrope cells are very active and produce vast amounts of the prohormone proopiomelanocortin (POMC), which is processed to a number of bioactive peptides, including α-melanophore-stimulating hormone. This hormone causes pigment dispersion in skin melanophores, giving the animal a black appearance. In white-background-adapted animals, the activity of the melanotrope cells is inhibited by neurons originating from the hypothalamic suprachiasmatic nucleus, which make direct synaptic contacts with the melanotrope cells (Jenks et al., 1977). Placing the amphibian on a white or black background thus allows physiological manipulation of the biosynthetic and secretory activity of the melanotrope cell. Here, we explored the developmental expression and egg microinjection of *Xenopus* PrP mRNA as well as its expression in the adult frog, in particular, in the pituitary gland.

2. Results and discussion

2.1. *Temporal and spatial distribution of PrP mRNA in Xenopus*

We analyzed the temporal expression profile of *Xenopus* PrP transcripts by RT-PCR, using GAPDH as internal standard, and ensured that the measurements were performed in the exponential phase of PCR by determining the number of PCR cycles necessary for a quantitative amplification. *Xenopus* PrP mRNA was maternally expressed, and zygotic expression was detected at a low level from stage 1 onwards and markedly increased up to stage 13 after which the level of expression remained stable during further development (Fig. 1). We next investigated the tissue distribution of PrP mRNA in adult *Xenopus*. RT-PCR revealed that PrP mRNA was expressed in every tissue examined, but most abundantly in the central nervous system (brain and pituitary) and testis (Fig. 2). This expression pattern is in line with that found in mammalian tissues, including human and mouse, in which PrP is also expressed mainly in the central nervous system (Bendheim et al., 1992; Brown et al., 1990; Horiuchi et al., 1995; McLennan et al., 2001).

2.2. *Microinjection of Xenopus PrP mRNA into in vitro fertilized Xenopus eggs*

To investigate the function of PrP^C in *Xenopus* development, we microinjected mRNA encoding *Xenopus* PrP that was fused to the C-terminus of GFP (GFP-PrP) into the animal pole of undivided fertilized *Xenopus* eggs. In parallel, we injected 0.1× MR (not shown) and control mRNA encoding GFP-GPI (in which the GPI-anchor signal sequence of PrP was fused to the C-terminus of GFP). Expression of the GFP-PrP (Figs. 3A–F) and GFP-GPI (Figs. 3G–L) fusion proteins was followed in time under a fluorescence microscope. For both GFP-PrP- and GFP-GPI-microinjected embryos, GFP fluorescence and thus fusion protein expression (Fig. 3M) were detected already at stage 9 and eventually diminished after reaching the tadpole stage (staging according to Nieuwkoop and Faber (1967)). No phenotype was observed, and the microinjected embryos developed normally.

2.3. *PrP mRNA expression in the pituitary of black- and white-adapted Xenopus*

The *Xenopus* pituitary consists of the neurointermediate lobe (NIL) and the anterior lobe (AL). The melanotrope cells of the NIL constitute a homogeneous population of strictly regulated neuroendocrine secretory cells that are involved in the process of background adaptation (Jenks et al., 1977). POMC is the major cargo protein in the melanotropes, and, during adaptation to a black background, the amount of POMC mRNA is highly induced, and cell activity and cell size increase enormously (reviewed by Roubos, 1997). The AL of the pituitary of *Xenopus* consists of corticotrope cells, and its activity is not affected by background adaptation. To investigate whether PrP was also upregulated in the NIL of black-adapted *Xenopus*, we examined PrP mRNA expression in the NIL and the AL of the pituitary of black- and white-adapted animals by real-time quantitative RT-PCR (Fig. 4). PrP mRNA was detected in both lobes of the pituitary. As expected, the levels of PrP mRNA expression were not significantly different in the ALs of black- and white-adapted animals. Remarkably, PrP mRNA expression was also similar in the NILs of black- and white-adapted animals (Fig. 4A). In contrast, POMC mRNA levels showed an ~10-fold increase in the NIL of black-adapted animals, while its mRNA expression in the AL showed no significant differences (Fig. 4B). Moreover, it has been reported previously that mRNA levels of Ac45, prohormone convertase PC2, carboxypeptidase H (Holthuis et al., 1995), amyloid-β precursor protein APP (Collin et al., 2005), p24 proteins (Rotter et al., 2002), secretogranin II and III (Holthuis and Martens, 1996) and neuroserpin (de Groot and Martens, 2005) are increased 3- to 10-fold in the biosynthetically active *Xenopus* melanotrope cells.

Taken together, the results presented here show that *Xenopus* PrP mRNA is expressed maternally and throughout whole embryonic development. In addition, like in mammals, *Xenopus* PrP mRNA is expressed predominantly in neuronal tissues, including the pituitary. Overexpression of PrP mRNA did not result in a visible phenotype during *Xenopus* development. Of special interest was the finding that PrP mRNA