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Nrf-2 regulation of prion protein expression is independent of oxidative stress

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ABSTRACT

Cellular expression of host prion protein (PrP) is essential to infection with prion disease. Understanding the mechanisms that regulate prion protein expression at both the transcriptional and translational levels is therefore an important goal. The cellular prion protein has been associated with resistance to oxidative, and its expression is also increased by oxidative stress. The transcription factor Nrf-2 is associated with cellular responses to oxidative stress and is known to induce upregulation of antioxidant defense mechanisms. We have identified an Nrf-2 binding site in the prion protein promoter (*Prnp*) and shown that Nrf-2 downregulated PrP expression. However, this effect is independent of oxidative stress as oxidative stress can up-regulate PrP expression regardless of the level of Nrf-2 expression. Furthermore, Nrf-2 has no impact on PrP expression when cells are infected with scrapie. These findings highlight that Nrf-2 can regulate PrP expression, but that this regulation becomes uncoupled during cellular stress.

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1. Introduction

The prion protein (PrP^c) is a normal cellular glycoprotein (Prusiner, 1998) expressed highly in neurons and other cells (Brown, 2004; Brown et al., 1998; Kretzschmar et al., 1986). It is a cell surface protein and binds copper (Brown et al., 1997a), which it may utilize for a function in cell protection (Brown et al., 1999; Jouvin-Marche et al., 2006; Stanczak and Kozlowski, 2007; Treiber et al., 2007; Zocche Soprana et al., 2011). However, the prion protein is better known for its association with a family of neurodegenerative diseases that include Creutzfeldt–Jakob disease, scrapie and bovine spongiform encephalopathy (Collinge, 2001). In these diseases the protein is converted to an abnormal isoform (PrP^{Sc}), which is associated with both disease transmission and toxicity (Legname et al., 2004; Muller et al., 1993). In this form it is more associated with cell damage than with cell protection. Alterations in the expression of PrP^c causes changes in the expression of other proteins associated with cellular protection from oxidative stress, supporting the theory that the main cellular role of PrP^c involves defense against oxidative stress (Brown et al., 1997c; Klamt et al., 2001; Kralovicova et al., 2009; Rachidi et al., 2003; White et al., 1999).

Prion protein knockout mice are protected from both infection with the prion agent and the neurodegenerative effects of PrP^{Sc} (Brandner et al., 1996; Bueler et al., 1993). Increased expression of PrP^c results in increased susceptibility to prion infection, a shorter disease incubation time and decreased cellular resistance to the toxicity of PrP^{Sc} (Brown, 1998; Fischer et al., 1996). Studies of conditional knockout mice have shown that during prion disease, inactivation of PrP^c expression causes halting of disease progression (Mallucci et al., 2003). These findings universally demonstrate that understanding the regulation of PrP^c expression is important to determining the mechanism behind neurodegeneration in prion disease. While the promoter of the *Prnp* gene has been studied there is evidence that intron-1 plays a strong role in the regulation of PrP^c expression and even includes its own TATA box (Baybutt and Manson, 1997; Haigh et al., 2007; Wright et al., 2009). Various studies have shown that different transcription factors can alter PrP^c expression. Metal transcription factor-1 (MTF-1), Sp1, Sp3, p53 and Atox-1 have all been shown to increase expression (Bellingham et al., 2009; Vincent et al., 2009; Wright et al., 2009). Two repressors of *Prnp* have been identified and these are Yin Yang-1 (YY1) and Hes-1 (Burgess et al., 2009; Wright et al., 2009). A minor effect has also been reported for repression of PrP by Forkhead Box O3a (Foxo3a) (Liu et al., 2013). However, understanding of what regulates PrP expression remains incomplete.

Nuclear Factor–Erythroid2-related factor-2 (Nrf-2) is a transcription factor associated with rapid cellular response to oxidative stress (Johnson et al., 2008). Nrf-2 is associated with the activation of proteins whose genes contain an ARE (antioxidant response element). Studies of Nrf-2 both in vivo and in vitro have confirmed that increase in its expression diminished many neurotoxic changes from lipid peroxidation (Ansari et al., 2011), excitotoxicity (Li et al., 2007), calcium metabolism (Lee et al., 2003) and mitochondrial changes (Ludtmann et al., 2014). In particular there has been a lot of interest in the role of Nrf-2 in the

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protective mechanisms of astrocytes and how this can be modulated to combat neurodegeneration in various animal models (Joshi and Johnson, 2012; van Muiswinkel and Kuiperij, 2005). Modulation of Nrf-2 has been suggested to be of benefit for many diseases including Alzheimer's and Parkinson's disease as well as Amyotrophic Lateral Sclerosis (Gan et al., 2012; Kanninen et al., 2009).

Currently, there is no evidence linking Nrf-2 to either the expression of PrP^c or a protective role in prion disease. Our initial studies suggested that the Prnp gene has a binding site for Nrf-2 in intron-1, but that increased expression of Nrf-2 did not alter luciferase reported activity. However, in the current study we discovered a different Nrf-2 binding site on the Prnp promoter. We found that Nrf-2 decreases PrP expression through this site. Curiously, in cells where PrP expression has been repressed by Nrf-2, oxidative stress causes a much greater increase in PrP expression. These finding suggest that Nrf-2 acts as a regulator of PrP expression under healthy conditions, but oxidative stress and prion infection override this regulation.

2. Results

2.1. A conserved Nrf-2 binding site

We sought to identify whether the *Prnp* promoter contained a conserved binding site for Nrf-2. We compared 4000 bp prior to the translation start site of both the human and bovine (*Bos taurus*) *Prnp* promoter sequences. The sequences were obtained from the USCS genome browser (human: NM_183079, bovine: NM_181015.2). A search was then made to identify conserved transcription factor binding sites using rVista (http://rvista.dcode.org/). Using the Nrf-2 recognition sequence (CTTCCTGT) a single conserved site was found in both bovine (−3.13 kb) and human (−2.5 kb) sequences.

2.2. Transcriptional Regulation of PrP Expression by Nrf-2

We have previously used luciferase reporter constructs to study the regulation expression of PrP by its promoter (Wright et al., 2009). Using the promoter reporter construct from that study we made a mutation at −3.0 kb to delete the sequence 5′ to this point that included the Nrf-2 recognition sequence described above (PromoM). Using a dual luciferase reporter system we looked at the expression driven by the Prnp promoter in N2A cells transfected to overexpress either Nrf-2 or transfected with the empty vector (pCDNA3.1). Compared to pCDNA3.1, Nrf-2 caused a significant reduction in Prnp promoter reported activity (Fig. 1). In comparison the deletion mutant showed no significant difference to the control when Nrf-2 was overexpressed. This data supports the notion that Nrf-2 decreases Prnp activity through the identified Nrf-2 binding site in the promoter.

Electrophoresis mobility shift assays were also performed to confirm Nrf-2 binding to the predicted sequence in the Prnp promoter. Extracts from Nrf-2 overexpressing and pCDNA3.1 transfected cells were prepared and exposed to the labeled DNA probe. A shift in labeled DNA was observed in the Nrf-2 overexpressing cell extracts and not in the pCNDA3.1 extracts. This interaction could be quenched with unlabeled DNA probe (Fig. 2).

2.3. Nrf-2 and PrP expression

We have identified that Nrf-2 repressed Prnp activity at the transcription level. The expression of PrP was also studied using Western blot and immunodetection. Protein extracts were prepared from two independently derived cell lines both overexpressing Nrf-2. The level of expression of Nrf-2, PrP and tubulin were detected with specific antibodies and the level of expression in these cell lines compared to the expression in N2A cells transfected with the empty vector (Fig. 3). Nrf-2 expression was increased in both transfected cell lines and in comparison, PrP was significantly decreased. These results confirm that Nrf-2 suppresses PrP expression.

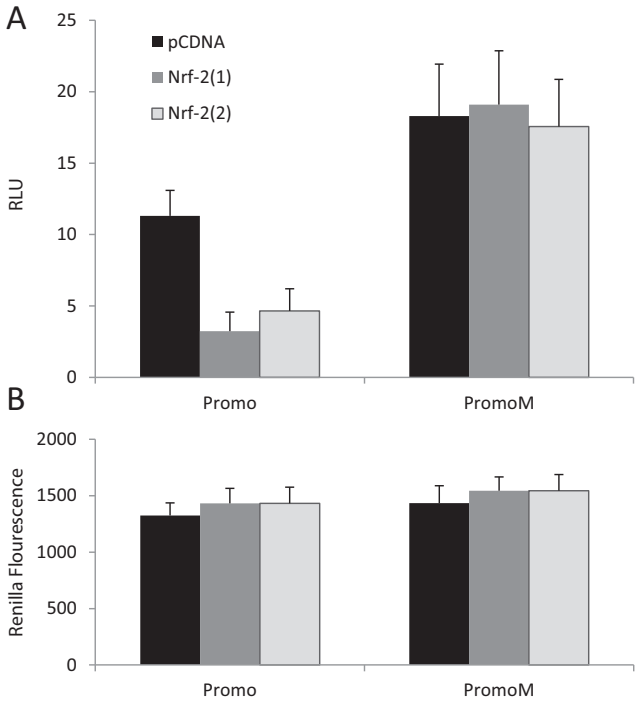

Fig. 1. Promoter analysis. A. N2A stable cell lines stabley transfected either with pCDNA-Nrf-2 (Nrf-2(1) and Nrf-2(2)) or the empty vector (pCDNA) were transiently transfected with luciferase based Prnp reporter vectors. The vectors were either pGL3Basic with the full length bovine Prnp promoter (Promo) or 3 kb fragment (PromoM). A luciferase based reporter assay was carried out. Values are presented as relative luficerase units (RLU, a ratio of the luciferase values to pTK renilla reporter cotransfected) and are the mean and s.e. of four experiments in triplicate. B. Shown are the raw pTK renilla values for the above experiments. The data is provided as evidence of the equivalent transfection efficiency between experiments.

2.4. Oxidative stress and PrP expression

The now classic role of Nrf-2 is as a response factor under conditions of oxidative stress. Data has also been published indicating that there is an increased expression of PrP in response to oxidative stress (Brown et al., 1997b). We therefore looked at the level of PrP expression in cells that were exposed to 1 μM hydrogen peroxide for 3 and 6 h. Cells were either transfected with pCDNA3.1 (empty vector) or the vector containing Nrf-2. Control cells showed an increase in expression of Nrf-2 and PrP (Fig. 4). In cells overexpressing Nrf-2 there is also an increase in PrP expression in response to oxidative stress. However, the response is much greater. These results suggest that under conditions of oxidative stress Nrf-2 is not able to suppress PrP expression and changes to PrP expression in oxidative stress conditions is due to other factors and thus independent of Nrf-2.

2.5. Nrf-2 and PrP expression in astrocytes

Nrf-2 responses in the brain are associated with astrocytes and it is through changes in astrocytes that Nrf-2 is thought to have its protective effects against oxidative stress. In astrocytes, PrP expression is quite low compared to neurons (Brown, 1999). However, PrP expression is increased when astrocytes are exposed to oxidative stress (Miele et al., 2003). It is possible that PrP's low expression level in astrocytes is a response to the higher levels of Nrf-2 expression. SFhERRβ (short form estrogen related receptor beta) has been shown to

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