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Counter-regulation of alpha- and beta-synuclein expression at the transcriptional level

Josephine A. Wright, Patrick C. McHugh, Siyi Pan, Adam Cunningham, David R. Brown *

Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

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ABSTRACT

Alpha-synuclein is a cytosolic protein associated with a range of diseases including Parkinson's disease. In these diseases alpha-synuclein aggregates and this is believed to play a causative role in disease progression. Alpha-synuclein aggregation has been suggested to be caused by increased expression levels and has also been suggested to be countered by increased beta-synuclein expression. In this regard, strategies to counter-regulate the expression of the synucleins by increasing beta-synuclein expression relative to alpha-synuclein may be beneficial in preventing disease progression. We therefore studied the regulation of alpha-synuclein to try to identify pathways that might counter-regulate the synucleins. We identified members of the ZSCAN family of transcription factors as specific repressors of alpha-synuclein. In particular ZSCAN21 was found to both repress alpha-synuclein and increase beta-synuclein expression. These findings support the notion that a single pathway in the cell can counter-regulate the expression of the synucleins. Support for this came from experiments that showed that ZSCAN21 expression decreases alpha-synuclein aggregation in the cells.

Introduction

The synucleins are a family of proteins whose functions remain to be fully determined (Goedert, 2001). Alpha-synuclein (α-syn) is associated with a group of diseases including Parkinson's disease (PD), multiple system atrophy and dementia with Lewy bodies (DLB) (Burke, 2004). Point mutations in the α-syn gene (SNCA) are associated with inherited forms of PD (Adams et al., 1991; Kruger et al., 1998; Proukakis et al., 2013; Zarranz et al., 2004). An aggregated form of the protein is the main component of Lewy bodies. α-syn is a metal binding protein (Paik et al., 1999; Rasia et al., 2005), a fact it shares with other members of the synuclein family including beta-synuclein (β-syn) (Davies et al., 2010). Binding of metals to α-syn is associated with an increase propensity to aggregate and form toxic oligomers (Uversky et al., 2001; Wright et al., 2009). However, it has also been suggested that metal binding might also be necessary for a possible function of the protein as a ferrireductase (Davies et al., 2011). There is no evidence that β-syn has a similar function.

α-Syn and β-syn are both expressed in the brain and it has been suggested that β-syn may play a role preventing α-syn aggregation. A variety of in vivo and in vitro experiments support this (Hashimoto et al., 2001; Park and Lansbury, 2003; Uversky et al., 2002). Expression patterns and levels of α-syn and β-syn most closely overlap with the highest levels throughout the brain (Jakes et al., 1994). While β-syn is not detected in Lewy bodies nor does it form fibrils like α-syn (Biere et al., 2000), β-syn mutants have been identified in DLB patients (Ohtake et al., 2004). β-Syn protein is abundant in neurofibrillary lesions of patients with AD (Galvin et al., 1999). Additionally, in both the mouse brain and the human substantia nigra, α-syn mRNA decreases and β-syn mRNA increases with age (Li et al., 2004). In contrast to control patients, there is a dramatic increase in α-syn and decrease in β-syn mRNA levels in the substantia nigra of PD, Diffuse Lewy Body Disease and a Lewy body variant of AD (Rockenstein et al., 2001) as well as the cortex in DLB (Beyer et al., 2010). While there has been some disagreement with these findings, such changes in expression have been confirmed in the majority of studies (McLean et al., 2012; Neystat et al., 1999). This concentration reversal of synuclein transcript levels with disease suggests that the balance of α-syn and β-syn expression may be important, which is supported by several studies (Mori et al., 2003; Shen et al., 2006). This suggests that β-syn may be a natural negative regulator of α-syn expression and activity. Thus strategies that alter expression levels of the synuclein in favour of beta-synuclein might have some value in developing potential therapies of synuclein associated diseases.

Despite the potential antagonistic role of α-syn and β-syn, little is known about the regulation of their expression or whether one of the proteins can influence the level of expression of the other. There has been some study of the regulation of α-syn expression through analysis of the promoter (SNCA). Polymorphisms of the dinucleotide repeat complex NACP-Rep1 (10.7 kb upstream of the translational start site (TSS)) are associated with AD and PD (Kruger et al., 1999; Xia et al., 1996). Different NACP-REP1 alleles have varying repressive effects on

* Corresponding author at: Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom. Fax: +44 1225 386779.
E-mail address: bssdrb@bath.ac.uk (D.R. Brown).

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α-syn promoter driven reporter activity in SH-SY5Y cells (Chiba-Falek and Nussbaum, 2001). Assessment of regions of the α-syn promoter suggests the presence of activator sites between −1.5 kb and −1.9 kb and repressor sites between −6.2 kb and −9.8 kb upstream of the TSS (Chiba-Falek and Nussbaum, 2001). Also, α-syn translation has been shown to be strongly influenced by the mRNA 5′ UTR region which can form unique stem loop structures typical of iron-responsive elements seen for other proteins (Mikkilineni et al., 2012; Rogers et al., 2011). Despite the interest in SNCA there has been almost no study of the β-syn promoter (SNCB). As far as is known the two genes are structurally similar, with two exons and an intron in the non coding 5′ UTR and 5–6 exons forming the coding domain (Lavedan et al., 1998; Xia et al., 2001). Both genes generate multiple splice variants (Beyer et al., 2012; McLean et al., 2012). We recently investigated the regulation of β-syn expression through its promoter and identified metal transcription factor-1 (MTF-1) as a major regulator of β-syn expression (McHugh et al., 2011). However, this remains currently the only published study of its kind making further comparison of the two genes difficult.

The current study is aimed at reassessing the regulation of α-syn to determine the possible interplay between α-syn and β-syn. We have also assessed a range of known transcription factors to identify any possible commonality in the regulation of expression of the synucleins. We have identified that levels of expression of one synuclein alter the expression of the other and that expression of one known transcription factor counter-regulated the expression of both proteins in favour of β-syn.

Results

Basal α-syn promoter activity

The basal expression level of α-syn in SH-SY5Y cells was assessed using a luciferase reporter system driven by fragments of the SNCA promoter. Sixteen different fragments from the known 10 kb sequence were clone and assessed. The sixteen fragments were as shown in Fig. 1. The purpose of the analysis was to verify that the protein is expressed in these cells and to identify potential repressor sites within the sequence. A previous study (Chiba-Falek and Nussbaum, 2001) demonstrated that the full length promoter has little activity in these cells. Our study confirmed this. The largest fragment with any significant activity (Student's t test, $p < 0.05$) was the 6.1 kb-ATG fragment (fragment 4 in Fig. 1). Fragments 5′ to this also demonstrated no activity on their own. Further, deletions of 5′ increased the measured luciferase activity. The fragment with the highest activity measured was a 200 bp fragment (−1.5/−1.3 or fragment 10) containing the region directly 5′ to the first exon. This is the likely binding site for the transcriptional machinery. The next most active fragment (fragment 13) was −1.9/ATG and contained the 5′ untranslated domains and intron 1. However, smaller fragments containing these regions (fragments 14–16) showed dramatically less activity, indicating that these regions play only a small role in the basal expression levels of α-syn. These domains may contain other sites that alter regulation of expression under non-basal conditions. The −1.9/−1.3 fragment showed less activity than the −1.5/−1.3 fragment indicating the presence of a possible repressor region between −1.5 kb and −1.9 kb. The slightly large fragment of −2.3/−1.5 had further diminished activity. Additionally, the −2.3/ATG fragment had quite significantly less activity than the −1.9/ATG. Together this finding suggests the presence of a strong repressor domain in the vicinity of −2.3 to −1.5 kb in the α-syn promoter. This apparent repression is lost in the larger fragment −4.1/ATG. The higher activity of this fragment when compared to −2.3/ATG suggests the presence of transcription factor binding sites that increase activity of the promoter between −4.1 and −2.3 kb. Fragments containing the domain between −6.1 and −4.1 seem to have little impact on the activity in the reporter system. This suggests that this domain of the promoter has little importance to the basal expression. Lastly, when comparing the fragments −6.1/−1.3 to −6.1/ATG, the shorter fragment has higher activity. This again suggests the presence of a repressor either in the 5′ untranslated region or intron-1. Fragment 6 (−6.1/−3.7) has no activity due to the absence of the −1.5/−1.3 domain.

Increased expression of a synuclein due to overexpression of another synuclein

As increased β-syn expression relative to α-syn requires either decreasing α-syn expression or enhancing β-syn above α-syn levels, it is important to identify ways to either repress α-syn expression or enhance β-syn transcription. An exciting possibility would be to identify a single factor that co-regulates both proteins. However, in order to do this it is important to first identify the impact of increased protein expression of either protein on the other. To assess this we used the

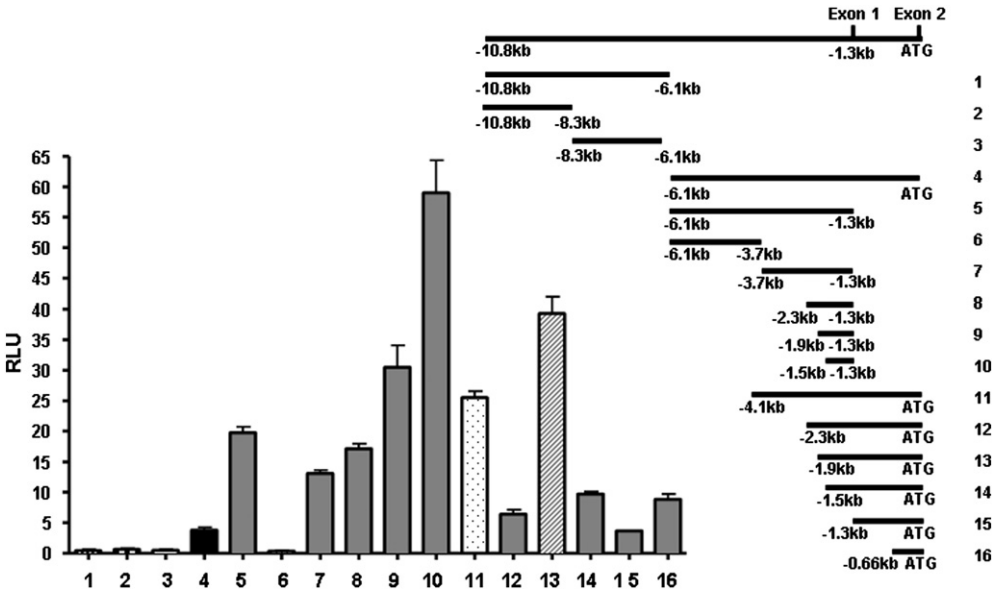

Fig. 1. SNCA genomic structure and basal promoter activity. The basic α-syn promoter (SNCA) scheme is shown top right, including the 5′ untranslated region up to the start site (ATG). We generated a large battery of 16 fragments of the promoter. The relation of these fragments to the full promoter is shown on the right. The fragments were cloned into the pGLBasic luciferase reporter vector. Analysis of the relative activity of these fragments in SH-SY5Y cells was carried out using a Dual-Luciferase reporter system. The relative activity of all the fragments is shown in the graph. RLU = relative luciferase units.