A novel extended form of alpha-synuclein 3′UTR in the human brain

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Abstract

Alpha-synuclein (α-SYN) is one of the key contributors in Parkinson’s disease (PD) pathogenesis. Despite the fact that increased α-SYN levels are considered one of the key contributors in developing PD, the molecular mechanisms underlying the regulation of α-SYN still needs to be elucidated. Since the 3′ untranslated regions (3′UTRs) of messenger RNAs (mRNAs) play important roles in translation, localization, and stability of mRNAs through RNA binding proteins (RBPs) and microRNAs (miRNAs), it is important to identify the exact length of 3′UTRs of transcripts in order to understand the precise regulation of gene expression. Currently annotated human α-SYN mRNA has a relatively long 3′UTR (2529 nucleotides [nt]) with several isoforms. RNA-sequencing and epigenomics data have suggested, however, the possible existence of even longer transcripts which extend beyond the annotated α-SYN 3′UTR sequence. Here, we have discovered the novel extended form of α-SYN 3′UTR (3775 nt) in the substantia nigra of human postmortem brain samples, induced pluripotent stem cell (iPSC)-derived dopaminergic neurons, and other human neuronal cell lines. Interestingly, the longer variant reduced α-SYN translation. The extended α-SYN 3′UTR was significantly lower in iPSC-derived dopaminergic neurons from sporadic PD patients than controls. On the other hand, α-SYN protein levels were much higher in PD cases, showing the strong negative correlation with the extended 3′UTR. These suggest that dysregulation of the extended α-SYN 3′UTR might contribute to the pathogenesis of PD.

Keywords: Parkinson’s disease, Alpha-synuclein, 3′ untranslated region (3′UTR), mRNA

Introduction

α-synuclein (α-SYN) is the major component of Lewy bodies (LBs) and Lewy neurites (LN)s, the pathological hallmarks of Parkinson’s disease (PD) [1]. Mutations and multiplication of SNCA gene coding for α-SYN protein have been strongly implicated in familial forms of PD [2–4]. Furthermore, in sporadic PD, the significant increase in α-SYN expression has been reported [5, 6]. However, the molecular mechanisms underlying the regulation of α-SYN expression that leads to the pathogenesis of PD remain unclear.

The 3′ untranslated regions (3′UTRs) of messenger RNAs (mRNAs) play important roles in translation, localization, and stability of mRNAs through providing binding sites for RNA binding proteins (RBPs) and microRNAs (miRNAs) [7]. Different lengths of the 3′UTRs are generated through alternative polyadenylation, and 3′ UTR isoforms vary across tissue types [8–11]. It is noteworthy that neurons usually have transcripts with much longer 3′UTRs, suggesting a more complicated regulation of protein expression in this highly polarized cell [12–14]. Therefore, it is important to identify the 3′ ends of transcripts to better understand regulatory mechanisms conferred by the 3′UTR and their roles in pathological conditions.

A recent study demonstrated that α-SYN transcripts have at least five different lengths of 3′UTR ranged from 290 to 2520 nucleotides [nt] and there are correlations between lengths of α-SYN 3′UTR and PD [15, 16]. Some of the single nucleotide polymorphisms (SNPs) that are located in the 3′UTR of α-SYN have been shown to be associated with sporadic PD [17, 18]. Together, the 3′UTR of α-SYN plays important roles in regulating α-SYN expression and eventually PD pathogenesis.

Recent accomplishment of the ENCODE (Encyclopedia of DNA Elements) provides comprehensive information on tissue-specific gene regulations. Data suggest that the last exon of SNCA might be much longer than the annotated length, generating α-SYN mRNA containing the...
extended 3′UTR. In this study, we sought to identify this extended α-SYN transcript in human postmortem brain tissues and various human neuronal cell lines and its role in translational regulation of α-SYN.

**Methods**

**Post-mortem human brain samples**

The use of post-mortem brain tissue was approved by the University of Central Florida Institutional Review Board. In the present study, 8 post-mortem brain samples without any neurodegenerative disease were used. The substantia nigra (SN) region containing brain tissues were obtained from the NIH Neurobiobank consortium. Ages ranged from 54 to 89 years and the post-mortem interval (PMI) varied from 10 to 30.25 h.

**Cell culture**

**iPSCs (induced pluripotent stem cells)**

Four iPSC lines were generated from the skin fibroblast of control (SC1014, SC1015), and sporadic PD (ND35302, ND35322) obtained from Coriell Institute for Medical Research (Additional file 1: Table S1). Each iPSC line had 2–5 clones from different batches of reprogramming. Using CytoTune™ iPS 2.0 Sendai reprogramming protocol (Thermo Scientific), we reprogrammed fibroblasts into transgene-free iPSC. Fibroblasts were followed reprogrammed into transgene-free iPSC as we described previously [19]. The iPSC were cultured on cell cycle arrested mouse embryonic stem cell media containing DMEM/F12, 20% knockout serum replacement (KSR), basic fibroblast growth factor (bFGF, 4 ng/ml), glutamine (2 mM), non-essential amino acids (NEAA, 0.1 mM) and β-mercaptoethanol (0.1 mM). The iPSC lines were differentiated into dopaminergic neurons following our previous protocol [19].

**ReNcell VM (Human ventral mesencephalic neuronal progenitor cells)**

Cells were cultured on laminin-coated (20 µg/ml) dishes in maintenance medium containing DMEM/F-12 with B27 supplement, glutamax, heparin (10 U/ml), gentamicin (50 µg/ml), bFGF (20 ng/ml) and epidermal growth factor (EGF, 20 ng/ml).

**SH-SY5Y cells**

Cells were grown in DMEM/F12 medium containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (50 µg/ml).

**RT-PCR**

Total RNAs were extracted from SN tissues using TRIzol and then they were treated with DNasel (DNA-free™ Kit, Ambion) to remove contaminating genomic DNA. DNA-free RNAs were converted to cDNA using the amfiRivert cDNA Synthesis Kit (GenDepot) according to the manufacturer’s protocol. For negative RT reaction, nuclease-free water was added instead of RT enzyme. Synthesized cDNA samples were subject to PCR amplification of α-SYN using different sets of primers: F1 + R1, F2 + R2, F3 + R3, and β-actin. The following are primer sequence information: Human α-SYN Forward (F) 1; 5′-GTGGCTGCTGCTGAGAAAAAC, Reverse (R) 1; 5′-CACCCACTGCTCTCTCAACT, F2; 5′-CTCCCGAGACATTCTACCTGCG, R2; 5′-TCTTGGTTAAGCCGACCGTG, F3; 5′-ACAGAAGCTATGAGTAACATGAGG, R3; 5′-TACACCTACCTCAACACACTCAA, and β-actin F; 5′-GGAGTCCTGTGGCATCCACG, R; 5′-CAGAGCATTGGC GGTGGA.

Total RNAs from neuronal cell lines (ReNcell VM and SH-SY5 cells) were similarly extracted described above and RNAs from iPSCs derived dopaminergic neurons were extracted using the RNeasy Plus Mini Kit (Qiagen). Total RNAs from LUHMES cells were kindly given by Dr. Coetzee (Van Andel Research Institute). Received RNAs were treated with DNasel and cDNA was generated as above.

**3′Rapid Amplification of cDNA Ends (3′-RACE)**

3 µg of total RNA were used for 3′-RACE reaction. First strand cDNA was synthesized using SuperScript™ II Reverse Transcriptase (ThermoFisher Scientific) with a QT primer containing a 17 nucleotide oligo-(dT) sequence at the 3′ end followed by a 35 nucleotide sequence. Then, first round amplification for α-SYN ends was done using α-SYN 3′ RACE F1 and Q2 primer set. The first round product was diluted to 1:20 in a Tris-EDTA solution and used for the second round amplification using α-SYN 3′ RACE F2 and Q1 primer set. Third round amplification was done as described above using a α-SYN 3′ RACE F3 and Q1 primer set. Final PCR product was confirmed using gel electrophoresis (Fig. 3a) and sent out for sequencing analysis. The followings are primer sequence information: QT; 5′-CCAGTGGAGCAGAGTGACCGACT CGAGCTCAAGCTTTTTTTTTTTTTTTTTT, Q2; 5′-CCAGTGGAGCAGAGTGACCG ACT CGAGCTCAAGCTTTTTTTTTTTTTTTTTT, Q1; 5′-GAGGACTCG AGCTCAAGCT, α-SYN 3′ RACE F1; 5′-ACCGAGAAAG GTCAAGCGCATTTCCACGAG, α-SYN 3′ RACE F2; 5′-GTCTGTGATATGACACTCAATTTTATCAACCT, α-SYN 3′ RACE F3; 5′-CAGAGCATTGAGTAACATGAGG, α-SYN 3′ RACE F4.

Newly found sequence, the extended α-SYN 3′UTR, was reported to DDBJ/ENA/GenBank Databases. Nucleotide sequence data reported are available in the third party annotation (TPA) Section of the DDBJ/ENA/GenBank databases under the accession number TPA: BK010481.

**Luciferase reporter constructs**

To generate luciferase constructs carrying the annotated (2.5 kb) or extended (3.8 kb) α-SYN 3′UTR, 2.5 kb or
Fig. 1 (See legend on next page.)
3.8 kb of human α-SYN 3’ UTR with terminal MluI and Pmel restriction sites were PCR-amplified from genomic DNA using a Q5 high-fidelity DNA polymerase (NEB). Then enzyme digested PCR products were inserted into a pMIR-reporter luciferase vector (Ambion). Constructs were confirmed by sequencing before using.

**Luciferase assay**

For the luciferase assay, SH-SY5Y cells were co-transfected with firefly luciferase constructs containing either 2.5 or 3.8 kb α-SYN 3’ UTR along with Renilla luciferase in 24-well plates using jetPRIME (Polyplus Transfection). Cells were collected after 36 h post-transfection and dual luciferase assay was performed according to the manufacture’s protocol (Promega). Relative luciferase activity was calculated by normalizing activity obtained for firefly to Renilla. Experiment was repeated three independent times.

**Statistical analysis**

Statistical analysis was performed with GraphPad Prism v.7.04 (GraphPad Software). Data are presented as mean ± S.E.M of each experimental condition. Two-tailed unpaired t test was performed in each experimental condition. To determine the correlation between extended α-SYN 3’ UTR with α-SYN protein expression, two-tailed Pearson’s correlation was used for the groups followed by linear regression analysis. Values of $p < 0.05$ were considered significant.

**Results**

RNA-sequencing (RNA-seq) alignments on a genetic locus around the last exon of SNCA in NCBI Homo sapiens Annotation Release 109 shows the existence of RNA-seq reads on the region after the end of annotated last exon (Fig. 1a). In this same region of SNCA, peaks of trimethylated histone H3 at lysine 36 (H3K36me3) are also enriched (Fig. 1b). H3K36me3 has been shown to indicate actively transcribed regions of the gene body [23, 24]. Therefore, together with RNA-seq coverage data and the continuous H3K36me3 on a genomic locus beyond the annotated last exon of SNCA implies existence of the extended 3’UTRs of α-SYN. Based on this analysis (Fig. 1a and b), we roughly estimated the length of the extended α-SYN 3’ UTR to be about 1500 nt.

In order to identify this extended α-SYN transcript, we designed three different sets of reverse transcriptase-PCR primers to amplify regions beyond the annotated last exon of SNCA. The resulting PCR products were subcloned into a plasmid and sequenced.

Prediction of miRNAs targeting the extended α-SYN 3’ UTR sequence was done through miRDB with custom prediction (http://www.mirdb.org). miRNAs with the highest target prediction scores (>70) obtained by MirTarget algorithm were depicted in Fig. 3b. Their expression levels in human brain tissue were checked using the human miRNA expression database (miRmine) [21].

PD implicated SNPs in extended 3’UTR were examined in regulome SNP database (http://regulomedb.org), as well as in the UCSC genome. Eight SNPs were found and four of them (rs7675290, rs8180214, rs8180209, rs17016071) were shown to be in tight linkage disequilibrium ($r^2 ≥ 0.95$; in several populations) with a previously reported lead SNP (rs11931074) described in a large Japanese cohort using genome wide association studies with PD (http://regulomedb.org/GWAS/rs11931074_r2thr0.8_all.html) [22].
(RT-PCR) primers targeting the protein coding region, the distal region of the known 3′ UTR, and the predicted extended 3′ UTR; F1 + R1, F2 + R2, and F3 + R3, respectively (Fig. 2a). RT-PCR was performed on the SN tissue of postmortem brains, human iPSCs-derived dopaminergic neurons, ReNcell VM (human ventral mesencephalic neuronal progenitor cells), SH-SY5Y cells (human neuroblastoma cells), and LUHMES cells (immortalized human dopaminergic neuronal precursor cells). Regardless of the types of cell lines or brain tissue, the extended 3′ UTR was successfully amplified (Fig. 2b). To exclude the possibility of genomic DNA amplification, the same sets of RNA primers were used to amplify the 3′ UTR region of α-SYN mRNA in various cell lines and brain tissues. Regardless of the cell lines or brain tissue, the extended 3′ UTR was successfully amplified (Fig. 2b). To exclude the possibility of genomic DNA amplification, the same sets of RNA primers were used to amplify the 3′ UTR region of α-SYN mRNA in various cell lines and brain tissues.
Fig. 3 The effect of the extended α-SYN 3' UTR on α-SYN translation and their level changes in iPSC-derived dopaminergic neurons. 

a) Firefly luciferase reporter constructs containing the annotated (2.5 kb) or extended (3.8 kb) form of α-SYN 3' UTR. 
b) Luciferase activity from SH-SY5Y cells co-transfected with firefly luciferase containing either 2.5 or 3.8 kb α-SYN 3' UTR and Renilla luciferase. The firefly luciferase values were normalized to Renilla luciferase activity. 
c) RT-PCR for total α-SYN transcripts, the extended α-SYN 3' UTR and β-actin from iPSC-derived dopaminergic neurons (DIV 60). RNA samples from total 12 iPSC lines; three iPSC clones from each patient (two control; CTRL1 and 2, two sporadic PD; sPD1 and 2), were used. β-actin was used as an internal control. “+” or “-” RT; with or without RT reaction. 
d) Quantitative analysis of total α-SYN mRNA expression after normalization by β-actin. 
e) Quantitative analysis of the extended α-SYN 3' UTR expression after normalization by β-actin. 
f) Western blotting for α-SYN, tyrosine hydroxylase (TH), and β-actin from iPSC-derived dopaminergic neurons (DIV 60). β-actin was used as an internal control. 
g) Quantitative analysis of α-SYN protein expression after normalization by β-actin. 
h) Quantitative analysis of TH protein expression after normalization by β-actin. 
i) Reverse correlation between the extended α-SYN 3' UTR and α-SYN protein levels. The Pearson's correlation coefficient = −0.6688. Error bars denote mean ± S.E.M. n.s (not significant), **P < 0.01, ***P < 0.001, ****P < 0.0001 by unpaired two-tailed t test in d, e, g, h.
samples without RT reactions (shown as “- RT”) were included, confirming no genomic DNA contamination. Moreover, we compared the expression levels of the extended 3′ UTR before and after differentiation of LUHMES cells. Interestingly, α-SYN transcript containing the extended 3′ UTR was proportionally increased as LUHMES cells were differentiated (Fig. 2c).

Next, to find the last sequence of the extended α-SYN 3′ UTR region, 3′-Rapid Amplification of cDNA Ends (3′-RACE) was performed (Fig. 2d). With DNA sequencing, we confirmed that the extended 3′ UTR contains an additional 1246 nt after the longest known annotated α-SYN 3′ UTR (2529 nt) (Fig. 2e). Our findings extend the end of the last exon of SNCA by 1246 bp that can generate human α-SYN mRNA having the maximum 3775 nt-length 3′ UTR.

To explore whether the extended α-SYN 3′ UTR affects translation of α-SYN, we generated luciferase reporter constructs carrying the annotated (2.5 kb) or extended (3.8 kb) α-SYN 3′ UTR (Fig. 3a). Firefly luciferase constructs containing either 2.5 or 3.8 kb α-SYN 3′ UTR along with Renilla luciferase were transfected into SH-SY5Y cells. Luciferase activity was significantly lower in cells transfected with the extended α-SYN 3′ UTR compared to the 2.5 kb α-SYN 3′ UTR (Fig. 3b), suggesting that additional cis-elements in the extended α-SYN 3′ UTR negatively regulate translation of α-SYN. Next, we investigated the expression levels of extended α-SYN 3′ UTR from iPSC-derived dopaminergic neurons from sporadic PD patients and control subjects. Surprisingly, level of the extended α-SYN 3′ UTR from sporadic PD iPSC-derived dopaminergic neurons (sPD) was significantly lower than one from control dopaminergic neurons (CTRL) (Fig. 3c and e), even though total α-SYN mRNA levels were higher in sPD (Fig. 3c and d). On the other hand, α-SYN protein levels were significantly increased in sPD without changing tyrosine hydroxylase levels (Fig. 3f, g and h). The strong negative correlation between the expression of the extended α-SYN 3′ UTR and α-SYN protein levels was found in these iPSC-derived dopaminergic neurons (Fig. 3i).

The length of the 3′ UTR affects its translation, localization, and stability through providing binding sites for RBPs and miRNAs [7]. Altered expression of RBPs may affect the 3′ UTR length. Therefore, it is worth to explore the potential regulatory cis-elements and cognate trans-factors, RBPs, present in the extended 3′ UTR. Predicted RBPs using RBPmap [25] were ranked according to their brain-specific expression levels and known roles in the brain. The 13 highest-ranked RBPs which are feasible in regulating α-SYN in the brain are listed in Table 1. Next, we investigated the number of miRNA binding sites in this region using the MirTarget algorithm (miRDB) [26, 27]. Ten miRNAs showing the highest target prediction score (> 70) are depicted with their binding sites (Fig. 2e). Among them, has-miR-708-5p and has-miR-28-5p have high expression in human brain tissues [21]. We also have looked for SNPs in this extended region from the SNP database (RegulomeDB and UCSC genome) in search of PD association. We found four SNPs: rs17016071, rs8180209, and rs8180214, and rs11931074. These four SNPs are in strong linkage disequilibrium (r² ≥ 0.95) with a downstream disease implicated SNP (rs11931074) that is strongly associated with PD in all HapMap 2 populations as found in the genome-wide association study by Satake et al., 2009 (Fig. 2e) [22].

Table 1 Potential RNA binding proteins for the extended α-SYN 3′UTR. RBPs highly expressed in the human brain with their binding motifs in the extended α-SYN 3′UTR.

| Protein name | Full protein name | Target RNA motifs |
|--------------|-------------------|-------------------|
| CELF4;BRUNOL4 | CUGBP Elav-like family member 4 | kgugukk |
| CELF5;BRUNOL5 | CUGBP Elav-like family member 5 | uguugg |
| CELF6;BRUNOL6 | CUGBP Elav-like family member 6 | uguagk |
| CNOT4 | CCR4-NOT transcription complex subunit 4 | gacaga |
| FXR1 | FMR1 autosomal homolog 1 | aayacr |
| HuR;ELAVL1 | ELAV like RNA binding protein 1 | ukuuruu |
| MATR3 | matrin 3 | maucuur |
| MSI1 | musashi RNA binding protein 1 | uaguvrg |
| NOVA1 | NOVA alternative splicing regulator 1 | ycay |
| PUM2 | pumilio RNA binding family member 2 | uguanaua |
| QKI | QKI, KH domain containing RNA binding | acuaay |
| RBFOX1 | RNA binding protein, fox-1 homolog 1 | wguauigm |
| RBM28 | RNA binding motif protein 28 | gwguguad |
Discussion

The current reference sequence length of the longest human α-SYN 3’UTR is 2529 nt. In the current study, we identified α-SYN transcript having a much longer 3’UTR—an additional 1246 nt, in fact—in postmortem human brain samples and iPSCs-derived dopaminergic neurons, as well as various human neuronal cell lines, further extending the last exon of SNCA.

Recent studies have demonstrated that mRNAs containing the longer 3’UTRs, on average, were exclusively observed in the nervous system when compared to other tissues, suggesting neuron-specific functions of the extended form of 3’UTR [12–14]. The longer extended form of α-SYN 3’UTR identified in this study confers an additional intricate regulation that might be important for specific neuronal functions or pathological conditions. In fact, we found that the longer extended α-SYN 3’UTR is expressed more in differentiated LUHMES cells when compared to undifferentiated conditions. The extended form of α-SYN 3’UTR, however, was decreased in sporadic PD iPSC-derived dopaminergic neurons in contrast to increased α-SYN protein levels. These suggest that aberrant expression of the extended α-SYN 3’UTR may influence PD pathogenesis.

Changes in RBPs may affect the production of 3’UTR with different lengths. We found 13 potential RBPs that can bind to the extended 3’UTR of α-SYN based on high expression levels in the brain. In addition, 10 miRNAs were predicted to bind to this region. Further studies are necessary to determine the mechanisms underlying regulation of the extended α-SYN 3’UTR, which might bring new therapeutic targets for human diseases especially in PD.

Finally, several annotated SNPs were found in the newly identified 3’UTR region. Four SNPs, although not evaluated in PD, are shown in significant linkage disequilibrium (r² ≥ 0.95) with another lead SNP (rs11931074) downstream of the gene. This SNP was significantly associated with PD in the Japanese population in a large GWAS study [22]. Moreover, these four SNPs were originally described as being located in the “intergenic region” on chromosome 4. With our discovery of the extended 3’UTR of SNCA, these variants will now be included within the SNCA 3’UTR region.

In summary, the present study identified the longer extended form of human α-SYN 3’UTR and its negative correlation with α-SYN translation. These findings would bring forth a better understanding not only how α-SYN expression is regulated but also how the length of α-SYN 3’UTR is involved in PD pathogenesis.

Abbreviations

3’-RACE: 3’ Rapid Amplification of cDNA Ends; 3’UTRs: 3’ untranslated regions; H3K36me3: Trimethylated histone H3 at lysine 36; iPSC: Induced pluripotent stem cell; LbN: Lewy bodies; lNs: Lewy neurites; miRNAs: MicroRNAs; miRNAs: Messenger RNAs; nt: Nucleotides; PD: Parkinson’s disease; RBPs: RNA binding proteins; SN: Substantia nigra; SNPs: Single nucleotide polymorphisms; TH: Tyrosine hydroxylase; TPA: Third Party Annotation; α-SYN: Alpha-synuclein

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Availability of data and materials

All data generated or analyzed during this study are included in this published article. Nucleotide sequence data reported are available in the Third Party Annotation Section of the DDBJ/ENA/GenBank databases under the accession number TPA: BK010481.

Authors’ contributions

GJ and YSK designed the study, GJ and SPY performed experiments and analyzed the data. SGT was involved in the bioinformatic analysis. GJ wrote the manuscript. GJ, SGT, HSK and YSK edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakos R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839–40.
2. Ibanez P, Bonnet AM, Debarges B, Lohmann E, Tison F, Pollak P, Agid Y, Durr A, Brice A. Causal relation between alpha-synuclein gene duplication and familial Parkinson’s disease. Lancet. 2004;364(9440):1169–71.
3. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson’s disease. Science. 1997;276(5321):2045–7.
4. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, et al. alpha-Synuclein locus triplication causes Parkinson’s disease. Science. 2003;302(5646):841.
5. Vila M, Vyukovics S, Jackson-Lewis V, Neyst M, Jakowec M, Przedborski S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. J Neurochem. 2000;74(2):721–9.

Additional file

Additional file 1: Table S1. The information of six iPS cell lines from Coriell Institute for Medical Research. (DOCX 16 kb)
6. Grundemann J, Schlaudraff F, Haedel O, Liss B. Elevated alpha-synuclein mRNA levels in individual UV-laser-microdissected dopaminergic substantia nigra neurons in idiopathic Parkinson’s disease. Nucleic Acids Res. 2008;36(7):e38.

7. Shyu AB, Wilkinson MF, van Hoof A. Messenger RNA regulation: to translate or to degrade. EMBO J. 2008;27(3):471–81.

8. Derti A, Garrett-Engele P, Macisaac KD, Stevens RC, Sriram S, Chen R, Rohr CA, Johnson JM, Babak T. A quantitative atlas of polyadenylation in five mammals. Genome Res. 2012;22(6):1173–83.

9. Oszolak F, Kapranov P, Foissac S, Kim SW, Fishilevich E, Monaghan AP, John B, Milos PM. Comprehensive polyadenylation site maps in yeast and human reveal pervasive alternative polyadenylation. Cell. 2010;143(6):1018–29.

10. Sandberg R, Neilson JR, Samra A, Sharp PA, Burge CB. Proliferating cells express mRNAs with shortened 3′ untranslated regions and fewer microRNA target sites. Science. 2008;320(5883):1643–7.

11. Hughes TA. Regulation of gene expression by alternative untranslated regions. Trends Genet. 2006;22(3):119–22.

12. Zhang H, Lee JT, Tian B. Biased alternative polyadenylation in human tissues. Genome Biol. 2005;6(12):R100.

13. Hilgert V, Perry MW, Hendrix Q, Stark A, Levine M, Haley B. Neural-specific elongation of 3′ UTRs during Drosophila development. Proc Natl Acad Sci U S A. 2011;108(38):15864–9.

14. Muira P, Shenker S, Andreu-Agullo C, Westholm JO, Lai EC. Widespread and extensive lengthening of 3′ UTRs in the mammalian brain. Genome Res. 2013;23(5):812–25.

15. Wedeking T, Lochte S, Richter CP, Bhagawati M, Piehler J, You C. Single cell GFP-trap reveals stoichiometry and dynamics of cytosolic protein complexes. Nano Lett. 2015;15(5):3610–5.

16. Rhinn H, Qiang L, Yamashita T, Rhee D, Zolin A, Vanti W, Abeliovich A. Alternative alpha-synuclein transcript usage as a convergent mechanism in Parkinson’s disease pathology. Nat Commun. 2012;3:1084.

17. Sotiriou S, Gibney G, Baxevanis AD, Nussbaum RL. A single nucleotide polymorphism in the 3′UTR of the SNCA gene encoding alpha-synuclein is a new potential susceptibility locus for Parkinson’s disease. Neurosci Lett. 2009;461(2):196–201.