Repeat Associated Non-ATG (RAN) translation in SCA8

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Discovery of RAN translation

- Story about an Elephant:

Ed Young: Seven Blind Mice
Central Dogma

DNA

 transcribes

RNA

 translates

Protein

Adenine = A (DNA & RNA)
Guanine = G (DNA & RNA)
Cytosine = C (DNA & RNA)
Thymine = T (DNA)
Uracil = U (RNA)
Central Dogma

DNA → RNA → Protein

- DNA transcription
- RNA processing
- Reverse transcriptase

RNA modifications:
- rRNA, tRNA
- snRNA
- miRNA
- lncRNA

Translation:
- Protein

Translational mechanisms?
DNA → RNA CODONS → Protein

Genomic DNA  THYBIGRYDDOGRANOUT
Messenger RNA  THEBIGREDDOGRANOUT
Translated Message  THE BIG RED DOG RAN OUT

The code is read three letters at a time and the ATG sets the reading frame
Eukaryotic Translation: Scanning and Initiation

<table>
<thead>
<tr>
<th>Position</th>
<th>Nucleotide Sequence</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 20</td>
<td>ATGCTCCTGGCTTTTTGTACTGCCTGCTGTGGAGTTTCCAGACCTCCGCTGGCCATTTC</td>
<td>MetLeuLeuAlaValLeuTyrCysLeuLeuTrpSerPheGlnThrSerAlaGlyHisPhe</td>
</tr>
<tr>
<td>21 - 40</td>
<td>CCTAGAGCCTGTGCTCTCTCTTAAGAACCTGATGGAGAAGGAATGCTGTCCACCGTGGAGC</td>
<td>ProArgAlaCysValSerSerLysAsnLeuMetGluLysGluCysCysProProTrpSer</td>
</tr>
<tr>
<td>41 - 60</td>
<td>GGGGACAGGAGTCCTGTGGCCAGCTTTTCAGGCAGGGTTCTGTCCAGAATATCTCTTCTG</td>
<td></td>
</tr>
<tr>
<td>61 - 80</td>
<td>GGGGACAGGAGTCCCTGTGGCCAGCTTTTCAGGCAGGGTTCTGTCCAGAATATCTCTTCTG</td>
<td></td>
</tr>
<tr>
<td>81 - 100</td>
<td>TCCAATGCAACACTTGGGCTCAATTTCCTCAACAGGGGTGGAATGACCGGGAGTCGTGG</td>
<td>SerAsnAlaProLeuGlyProGlnPheProPheThrGlyValAspAspArgGluSerTrp</td>
</tr>
<tr>
<td>101 - 120</td>
<td>TCCAATGCAACACTTGGGCTCAATTTCCTCAACAGGGGTGGAATGACCGGGAGTCGTGG</td>
<td>SerAsnAlaProLeuGlyProGlnPheProPheThrGlyValAspAspArgGluSerTrp</td>
</tr>
<tr>
<td>121 - 140</td>
<td>GGAAACTGCAAGTTTGGCTTTTGGGGACCAAACTGCACAGAGAGACGACTCTTGGTGAGA</td>
<td>GlyAsnCysLysPheGlyPheTrpGlyProAsnCysThrGluArgArgLeuLeuValArg</td>
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<tr>
<td>141 - 160</td>
<td>AGAAACATCTTCGATTTGAGTGCCCCAGAGAAGGACAAATTTTTTGCCTACCTCACTTAA</td>
<td>ArgAsnIlePheAspLeuSerAlaProGluLysAspLysPhePheAlaTyrLeuThr***</td>
</tr>
</tbody>
</table>
Microsatellite Expansion Diseases

Fragile X Syndrome

- Protein Loss
- Pre-mutant/mutant (60 → >1,000)
- Normal (6 → 52)
- FMR-1

Huntington Disease

- Protein Gain
- Pre-mutant/mutant (35 → 121)
- Normal (6 → 39)
- ATG

Myotonic Dystrophy

- RNA Gain
- Pre-mutant/mutant (50 → >2,000)
- Normal (5 → 37)
- DMPK
Protein coding and non-coding expansion disorders ~2005
Spinocerebellar Ataxia Type 8
SCA8
Clinical Features of SCA8

- Limb and gait incoordination
- Eye movement abnormalities
- Slurred speech
- Babinski signs
- Slowly progressive
- Cerebellar/Purkinje cell degeneration
- Reduced penetrance
SCA8 transgenic mice

Moseley et al. *Nature Genetics* 2006

Nuclear polyGln inclusions in humans and mice detected with 1C2 antibody
Bidirectional Expression: SCA8

Toxic RNA Mechanism
Daughters et al. 2009
_PLoS Genetics_

\[ TAAAAA\]

\[ ATG\ (CAG)_{103}\ CGG\ (CAG)_{7}\ CGG\ (CAG)_{5}\ TAGTAGTAGTAAACG \]

\[ \text{M(Q)_{103}R(Q)_{7}R(Q)_{5}} \]

\[ 5' \text{Exon B} \hspace{1cm} \text{Exon A} \text{3'} \]

\[ \text{CUG}_{\text{EXP}} \]

\[ (\text{CTG})_{n} \]

\[ \text{ATXN8OS} \]
Unexpected results . . .
Translation of polyGln protein w/o ATG

Additional experiments show polyQ made in a variety of 5’ sequence contexts

If ATG not required for polyGln are proteins also made in other frames?
Are Proteins made in other Frames?

### Table

<table>
<thead>
<tr>
<th>Frame</th>
<th>Codon</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAG</td>
<td>Glutamine(Q)</td>
</tr>
<tr>
<td>2</td>
<td>AGC</td>
<td>Serine(S)</td>
</tr>
<tr>
<td>3</td>
<td>GCA</td>
<td>Alanine(A)</td>
</tr>
</tbody>
</table>

### DNA Sequences

- CAGCAGCAGCAGCAGCAGCAGCAGCAG
- (CAG)\textsubscript{118}
- 6X STOP

### Proteins

- FLAG
- HA
- HIS/Myc
- PolyQ
- PolyS?
- PolyA?
Translation in Three Frames

- PolyQ migrates at one or more distinct positions
- PolyAla migrates as a smear
- PolyS at the top of the gel
- Other controls

Repeat Associated Non-ATG translation (RAN translation)

Mass spectrometry of polyAla protein

Zu et al. PNAS PNAS 2011

<table>
<thead>
<tr>
<th>CMV</th>
<th>6xStop TAG</th>
<th>AATTCA(GCA)17GCGCGC(GCA)69</th>
<th>3T</th>
</tr>
</thead>
</table>

*NSAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA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RAN translation is length dependent

- PolyGln 42-107 repeats (but not 20 repeats)
- PolyAla 73-107 repeats
- PolySer 58-107 repeats

Longer repeats express proteins in three frames

Hairpin structures favor RAN translation

Suggests hairpin structure is important

Brian Gibbens
RAN translation occurs across an existing open reading frame
RAN translation differs by cell type
RAN translation occurs in SCA8

α-SCA8 poly-GCA-Ala

Antibody Validation

Mouse Cerebellum

WT

50μm

10μm

SCA8

α-SCA8 polyAla

Noelle Doty
C9ORF72 FTD/ALS

caused by GGGGCC expansion mutation

Renton et al., Neuron (2011) 72(2):257-68
De Jesus Hernandez et al., Neuron (2011) 72(2):245-56
GGGGGCC RAN translation products

Our Current Understanding

– RNA gain of function
  • spliceopathy
  • DM1, DM2, SCA8 etc
  • C9ORF72 ALS/FTD?

– RAN Translation
  • Proteins expressed w/o ATG
  • Sequence context highly permissive
  • Translation in 3 frames
  • RAN favored by
    – Hairpins
    – Long repeats
    – Cellular factors
  • RAN translation in SCA8, DM1, C9ORF72 ALS/FTD
Additional basic questions

• How and why are RAN proteins expressed?

• Are unexpected proteins expressed in many of expansion disorders and throughout the genome?

• What is their role in disease and normal biology?
New view of C9-ALS/FTD and other expansion disorders

Framework for understanding disease mutations needs revision.

Bidirectional expression is common
Repeats express unexpected proteins
Other RNAs expressed at these loci - microRNAs
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