Molecular Determinants of Accurate Translation Initiation

How do ribosomes identify the correct translation initiation codons in mRNAs?

Hinnebusch Lab (NICHD)
Lorsch Lab (NIGMS/NICHD)
Ramakrishnan Lab (MRC, U.K.)
Translation initiation by the scanning mechanism
PIC attachment to mRNA

scanning →

43S PIC assembly
Scanning favors initiation at 5′-proximal AUGs
...and near-cognate triplets in good context can be used instead
Translation initiation defects in human disease

- Mutations adding or removing upstream AUGs or changing AUG context: melanoma, breast cancer, thalassemia, thrombocytopenia, hereditary pancreatitis, familial hypercholesterolemia

- Overexpression of eIFs: malignant transformation.

- Mutations affecting eIF2B, the GEF for eIF2: leukoencephalopathy with vanishing white matter.

- eIF2\(\gamma\) mutation: intellectual disability

- eIF1A mutations: uveal melanoma (UM) and thyroid carcinomas
eIF1 and eIF1A promote “open” conformation of the 40S
eIF1 and eIF1A promote “open” conformation of the 40S conducive to TC loading and scanning...
eIF1 and eIF1A promote “open” conformation of the 40S conducive to TC loading and scanning...
...but eIF1 must be ejected to allow Pi release and stabilize TC binding in $P_{IN}$ state
eIF1 promotes $P_{OUT}$ for scanning and blocks $P_{IN}$ at non-AUG codons…

…requiring eIF1 release for AUG selection
Prediction: eIF1 mutations that weaken 40S binding should reduce TC binding to open complex in \( P_{OUT} \) state...
Prediction: eIF1 mutations that weaken 40S binding should reduce TC binding to open complex in $P_{OUT}$ state...

...but allow transition to $P_{IN}$ at UUG codons
Translational Control of *GCN4* by phosphorylation of eIF2
Translational Control of *GCN4* by phosphorylation of eIF2

Amino acid starvation

\[ \text{GCN2} \]

\[ \text{eIF2}^{(\alpha)-\text{P}} \]

\[ \text{eIF2-GTP} \]

\[ \text{tRNA}^\text{Met} \]

(High levels)

TC

1

4

GCN4

OFF

Amino acid biosynthetic genes

AUG

AUG

AUG

(Low levels)

TC

1

4

GCN4

ON

AUG

AUG

AUG

1

4

AUG

AUG

AUG

TC
Integrated Stress Response by phosphorylation of eIF2

- Amino Acid Limitation
  - GCN2
- Heme Deprivation
  - HRI
- Viral dsRNA
  - PKR
- Unfolded proteins
  - PERK

\[ eIF2(\alpha) - \text{Phospho} \]

- General Translation
- uORF-regulated Transcription Factors
  - ATF4, ATF5, CHOP
- Stress Response
- Synaptic Plasticity/Learning
GCN4 translation: *in vivo* reporter of defective TC loading on 40S subunits

Rapid binding

Slow binding

Amino acid biosynthetic genes

ON

GCN4-lacZ expression

(Gcd−)
**GCN4 translation: in vivo reporter of defective TC loading on 40S subunits**

Kinas of TC loading

- **Rapid binding**
- **Slow binding**

**eIF* mutant**

**Amino acid biosynthetic genes**

TC\(\rightarrow\)40S: **GCN4-lacZ expression**\(\uparrow\)(Gcd\(^{-}\))

**Gcd\(^{-}\) mutations:**
- eIF1 (sui1)
- eIF1A (tif11)
- 18S rRNA
- tRNA\(_i\)

**Lorsch et al**
Sui− and Ssu− mutations alter accuracy of start codon selection.
Sui− and Ssu− mutations alter accuracy of start codon selection

<table>
<thead>
<tr>
<th></th>
<th>AUG</th>
<th>UUG</th>
<th>-His</th>
<th>UUG: AUG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIS4</strong></td>
<td></td>
<td></td>
<td>His+</td>
<td>low</td>
</tr>
<tr>
<td><strong>his4-301</strong></td>
<td>ACG</td>
<td>UUG</td>
<td>His−</td>
<td>low</td>
</tr>
<tr>
<td><strong>his4-301 sui−</strong></td>
<td>ACG</td>
<td>UUG</td>
<td>His+, Sui−</td>
<td>increased</td>
</tr>
<tr>
<td><strong>his4-301 sui− ssu−</strong></td>
<td>ACG</td>
<td>UUG</td>
<td>His−, Ssu−</td>
<td>reduced</td>
</tr>
</tbody>
</table>

Quantify UUG/AUG initiation ratio:

- **UUGHIS4-lacZ**
- **AUGHIS4-lacZ**
Prediction: eIF1 mutations that weaken 40S binding should reduce TC loading rate (Gcd− phenotype)...

...and elevate UUG initiation (Sui− phenotype)
eIF1 affinity for 40S dictates TC loading and initiation accuracy
eIF1 affinity for 40S dictates TC loading and initiation accuracy

Pilar Martin-Marcos & Jagpreet Nanda

40S-eIF1 crystal structure
eIF1 affinity for 40S dictates TC loading and initiation accuracy

GDP $\rightarrow$ Pi

Gcd$^-$: TC loading
Sui$^-$: UUG initiation

K60E

eIF1 $\rightarrow$ Ssu$^-$

TC loading
UUG initiation

K60E, Q84H
K60E, D61G

Fraction of eIF1 bound

[40S] nM

0 0.2 0.4 0.6 0.8 1

0 100 200 300 400 500

WT

K60E

K60E, Q84H

K60E, D61G

K60E
eIF1 affinity for 40S subunit is finely tuned for optimum initiation accuracy
eIF1 blocks transition to $P_{IN}$ at non-AUG codons...
Cryo-EM structures of yeast PICs at 4.0 Å

- Assembled using Sui- mutants of tRNA_i and eIF2 characterized at NIH

Hussain & Llacer et al (Ramakrishnan)
Cryo-EM structures of yeast PICs at 4.0 Å

- Assembled using Sui- mutants of tRNA_i and eIF2 characterized at NIH

Hussain & Llacer et al (Ramakrishnan)
Transition to $P_{IN}$ alters eIF1 location to alleviate clash with tRNA$_i$

- likely facilitates eIF1's dissociation for AUG selection
Transition to $P_{IN}$ alters eIF1 location to alleviate clash with tRNA$_i$

tRNA$_i$ ($P_{IN}$)

tRNA$_i$ ($P_{OUT}$): Hashem et al. (Frank)

eIF1 in 40S•eIF1•eIF1A

eIF1 in 48S PIC ($P_{IN}$)

• Anil Thakur: mutations in eIF1 loops that should diminish the clash stabilize $P_{IN}$ at UUG codons (Sui$^-$)
Tails of eIF1A regulate transition from open to closed conformation

“toggling factor”
eIF1A

Scanning Enhancer (SE)

Scanning Inhibitor (SI)

“Open” Scanning

“Closed” Arrested

GTP

“P_{OUT}”
eIF1

eIF1A

“Open”

SE

CTT

PI

GDP

“P_{IN}”

AUG

NTT
Mutating SE elements in eIF1A CTT decreases accuracy and impairs TC loading.

Saini et al Genes Dev
Mutating SI elements in eIF1A NTT restores accuracy and rapid TC loading

Saini et al. *Genes Dev*
eIF1A NTT promotes the $P_{\text{IN}}$ state
eIF1A NTT interacts with AUG-anticodon helix

- Ssu- mutations in the eIF1A NTT impede start codon recognition

Hussain & Llacer et al (Ramakrishnan)
eIF1A NTT interacts with AUG-anticodon helix

Exome sequencing identifies recurrent somatic mutations in *EIF1AX* and *SF3B1* in uveal melanoma with disomy 3

Marcel Martin¹,², Lars Maßhöfer³, Petra Temming⁴, Sven Rahmann¹, Claudia Metz⁵, Norbert Bornfeld⁵, Johannes van de Nes⁶, Ludger Klein-Hitpass⁷, Alan G Hinnebusch⁸, Bernhard Horsthemke³, Dietmar R Lohmann³,⁹ & Michael Zeschnigk³,⁹
Conserved bases in tRNA\textsubscript{i} play distinct roles in the accuracy of AUG selection
Disruption of C3-G70 confers Sui− and Gcd− phenotypes co-suppressed by eIF1A NTT mutation
G70A mutation decreases rate of TC binding in vitro...

Kinetics of TC loading

Tony Munoz (Lorsch lab)
...in a manner reversed by eIF1A NTT mutation 17-21
Base-pair substitutions of G31-C39 confer Sui\textsuperscript{−} but not Gcd\textsuperscript{−} phenotypes

\[ \text{Anticodon: } A \quad U \quad U \quad A \quad C \quad A \quad U \quad A \quad C \quad A \quad U \quad A \quad C \quad A \quad U \]
Hypothesis: U31:A39 substitution in ASL removes barrier to \( P_{IN} \)
TC is less tightly bound to the PIC at UUG codons

\[ k_{\text{off}}: \text{low} \]

\[ k_{\text{off}}: \sim \text{high} \]

<table>
<thead>
<tr>
<th>WT</th>
<th>AUG</th>
<th>UUG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>
U31:A39 replacement stabilizes $P_{IN}$ at UUG codons

$K_{off}: 1.2/hr$

$K_{off}: <0.01/hr$

Tony Munoz (Lorsch lab)
G31:C39 impedes $P_{IN}$ state & demands perfect AUG-anticodon duplex
tRNA$_i$ anticodon stem is distorted in $P_{IN}$ state
Evidence for 40S conformational changes was lacking
Structural probing of PICs by free-radical cleavage directed by eIF1A

Fan Zhang & Adesh Saini
Structural probing of PICs by free-radical cleavage directed by eIF1A

Fan Zhang & Adesh Saini
Greater cleavage of P-site residues in "open" (AUC) versus "closed" (AUG) complex

AUG recognition evokes closure of P site ($P_{IN}$)
Cleavages in P-site and mRNA binding cleft suppressed in AUG vs AUC complex

- AUG recognition constricts mRNA binding cleft and closes the P site
Open PIC conformation at AUC shows upward movement of 40S head

- py48S-open: (AUC)mRNA
- py48S-closed: (AUG)mRNA

Conducive for mRNA recruitment & scanning

Llacer et al (Ramakrishnan)
Open PIC conformation at AUC shows widened P-site

- Compatible with triplet sampling by tRNA$_i$ during scanning

Llacer et al (Ramakrishnan)
eIF2β contacts tRNAᵢ, eIF1, and eIF1A in open complex

Ramakrishnan et al. (Ramakrishnan)
eIF2β contacts eIF1 exclusively in open complex

open (AUC): scanning

Closed (AUG) initiation
eIF2β contacts with eIF1 promote scanning and impede UUG initiation

Laura Marler
Anil Thakur

UUG HIS4-lacZ
AUG HIS4-lacZ

eIF2β mutations
eIF1 mutations
Rps5 hairpin substitutions suppress UUG initiation

Jyothsna Visweswaraiah
Yvette Pittman (Dever lab)
rps5-E144R impairs AUG recognition by the scanning PIC

1\textsuperscript{st} AUG blocks initiation downstream

Bypass of 1\textsuperscript{st} AUG enables initiation downstream

Jyothsna Visweswaraiah
Yvette Pittman (Dever lab)
rps5-E144R impairs AUG recognition by destabilizing P\textsubscript{IN} state

1\textsuperscript{st} AUG blocks initiation downstream

Bypass of 1\textsuperscript{st} AUG enables initiation downstream

• Rps5 is on par with eIFs in controlling AUG recognition
Conformational rearrangements in transition from scanning to AUG selection

- Downward head movement constricts mRNA cleft
  - P site closes around tRNA_i
- eIF1A NTT interacts with codon:anticodon duplex
  - eIF1 displaced by tRNA_i from P site
  - eIF1 dissociates to allow P_i release from eIF2
MRC Laboratory of Molecular Biology, University of Cambridge, UK

Not shown: Israel Fernandez

Venki Ramakrishnan

Tanweer Hussain

Jose Llácer
Lorsch Lab

Shardul  Colin  Jon  Paul  Sarah  Jagpreet

Not shown: Tony Munoz & Fujun Zhou

Funding: NIH
Alan's Lab

Dong, Yashpal, Neelam, David, Neha, Laura, Hongfang, Quira, Jyothsna

Not shown: Suna Gulay, Pilar Martin-Marcos, Adesh Saini
Alan's Lab

Not shown: Suna Gulay, Pilar Martin-Marcos, Adesh Saini