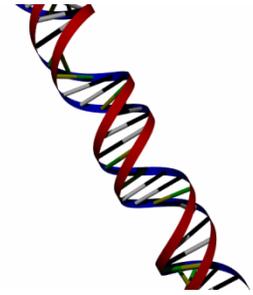
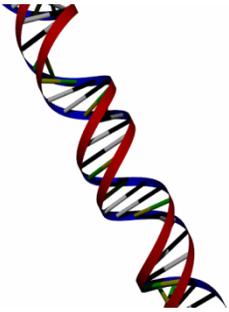


Evolution of the Genetic Code



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KITP, Santa Barbara – Feb. 8, 2010

Major Transitions in Evolution

(Maynard-Smith & Szathmary)

■ Replicating molecules → Populations of molecules in compartments.

■ Independent replicators → Chromosomes.

➔ RNA as genes and enzymes → DNA & proteins – Development of the Genetic Code and its subsequent evolution.

■ Prokaryotes → Eukaryotes (chromosomes enclosed within nucleus)

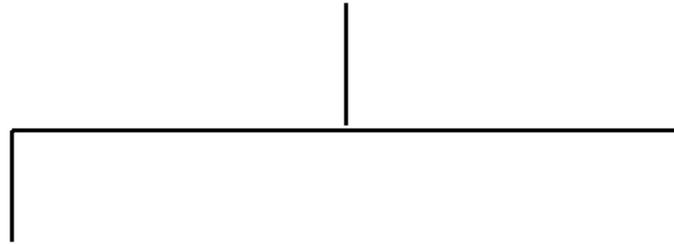
■ Asexual clones → Sexual populations

■ Single celled eukaryotes → Multicellular organisms (fungi, plants, animals)

■ Solitary individuals → colonies (Division of labour and origin of cooperation)

■ Primate societies → Human societies (evolution of the nervous system & origin of language) .

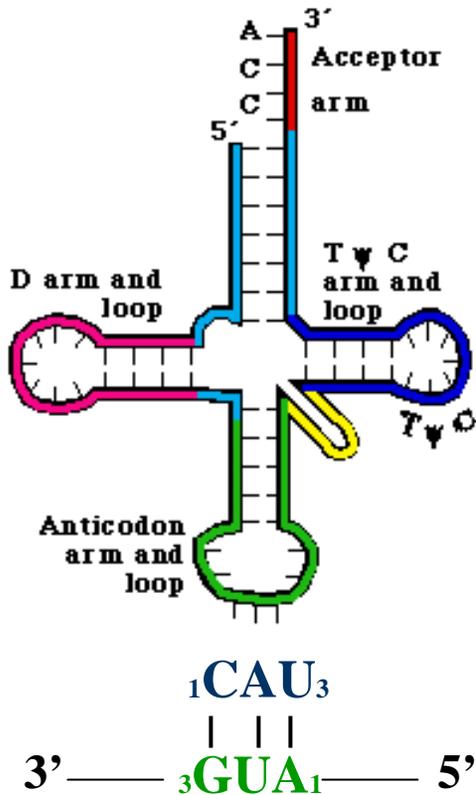
Genetic Code Evolution



Pre-LUCA Phase

Post-LUCA Phase

- The Genetic Code provides a recipe for synthesis of proteins from DNA sequences.



Source : National Human Genome Research Institute

Wobble Pairing : Pairing between 1st anti-codon position and 3rd codon position.

➔ Allows greater pairing flexibility compared to pairing at other codon/anti-codon positions which strictly follow Watson-crick rules.

		Second base						
		U	C	A	G			
First base	U	UUU } Phenyl- UUC } alanine UUA } Leucine UUG }	UCU } Serine UCC } UCA } UCG }	UAU } Tyrosine UAC } UAA } Stop codon UAG } Stop codon	UGU } Cysteine UGC } UGA } Stop codon UGG } Tryptophan	U	C	
	C	CUU } Leucine CUC } CUA } CUG }	CCU } Proline CCC } CCA } CCG }	CAU } Histidine CAC } CAA } Glutamine CAG }	CGU } Arginine CGC } CGA } CGG }	U	C	A
	A	AUU } Isoleucine AUC } AUA } AUG } Methionine start codon	ACU } Threonine ACC } ACA } ACG }	AAU } Asparagine AAC } AAA } Lysine AAG }	AGU } Serine AGC } AGA } Arginine AGG }	U	C	A
	G	GUU } Valine GUC } GUA } GUG }	GCU } Alanine GCC } GCA } GCG }	GAU } Aspartic GAC } acid GAA } Glutamic GAG } acid	GGU } Glycine GGC } GGA } GGG }	U	C	A
						G	A	G

Khorana, Holley, Nirenberg (Nobel 1968), Matthei, Crick

Number of **Synonymous** changes = 137

Number of **Non-Synonymous** Changes = 392

The code is **not** universal.

Reassignments of one or more codons to a different amino acid have occurred in many separate groups of organisms.

Codon reassignments → Alternative Genetic Codes

AUU AUC : Ile AUA			
AUG : Met			

AUA: Ile → Met

AUU AUC : Ile			
AUA AUG : Met			

Total number of observed reassignments = **44** (mitochondrial + nuclear + bacterial)

11 *distinct* codon reassignments - **mitochondrial** genome.

4 *distinct* codon reassignments - **nuclear** genome.

1 *distinct* codon reassignment - **bacteria**

Implications of codon reassignment

mRNA sequence: A U G **AGG** U C A **AGG** C U **UAGG** A U **CAGGAGG** U G
A

Protein *before* reassignment : M R S R L R I R R Stop

AGG : Arg → Serine – observed in starfish and some insects

Protein *after* reassignment : M S S S L S I S S Stop

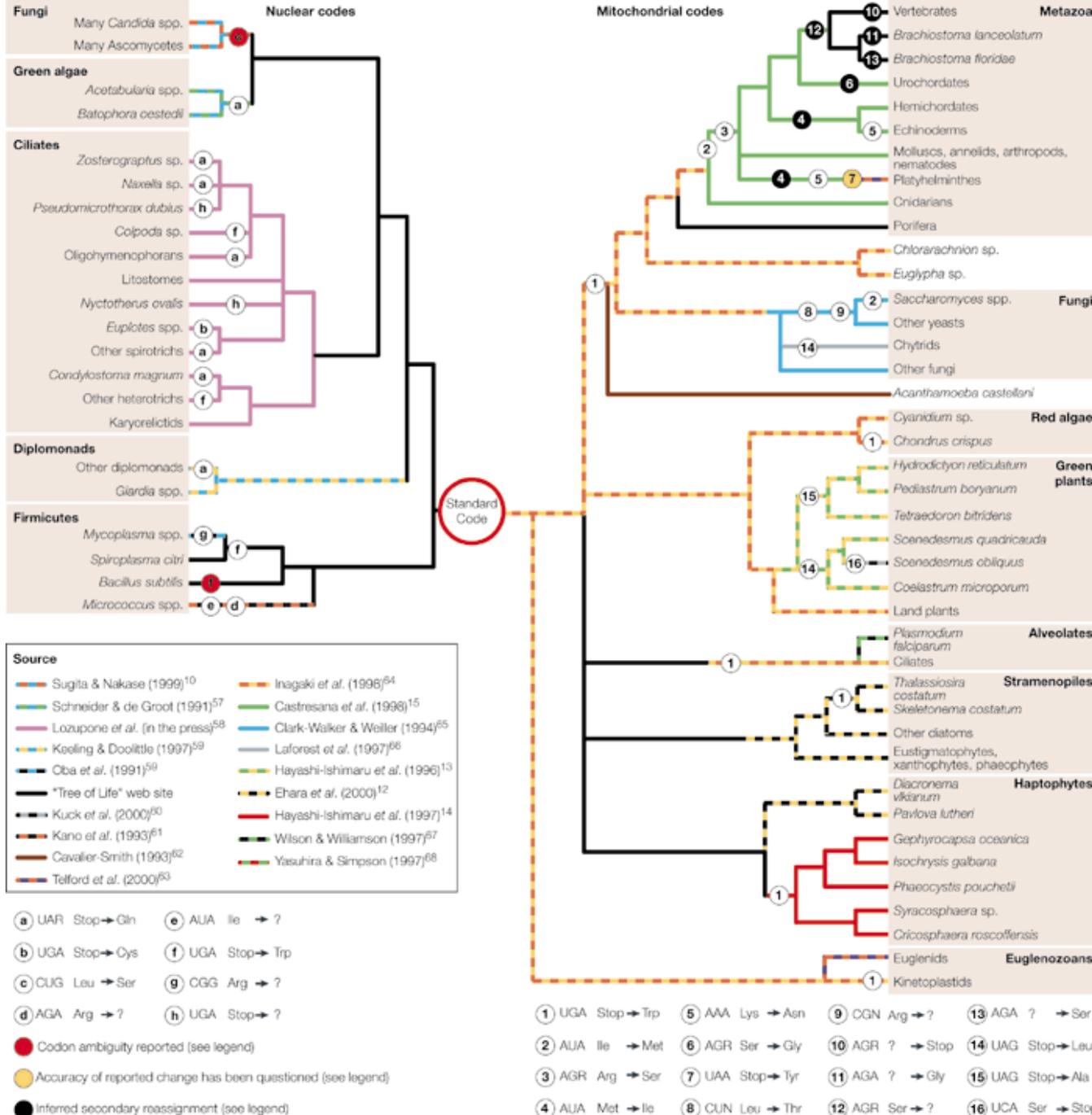
mRNA sequence: A U G C C A **UCA** A U U C U U **UCA** A A A A G G **UCA** G G G U G A

Protein *before* reassignment : M P S I L S K R S G Stop

UCA : Ser → Stop – observed in some green algae

Protein *after* reassignment : M P Stop I L Stop K R Stop G Stop

Codon reassignment changes the amino acid sequence of *every* protein which uses the codon.

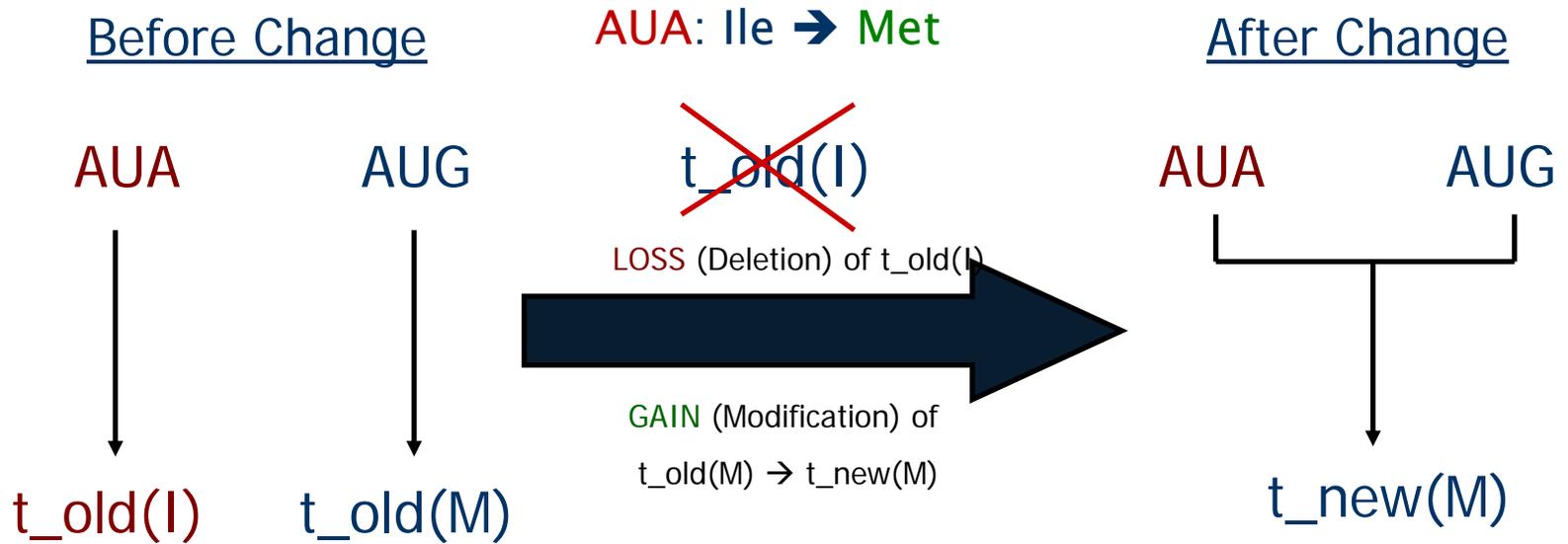


Knight et al. (2001)
Nature Reviews Genetics

Molecular Basis of Codon Reassignment

Example : AUA was reassigned from **Ile** to **Met** during the early evolution of the mitochondrial genome

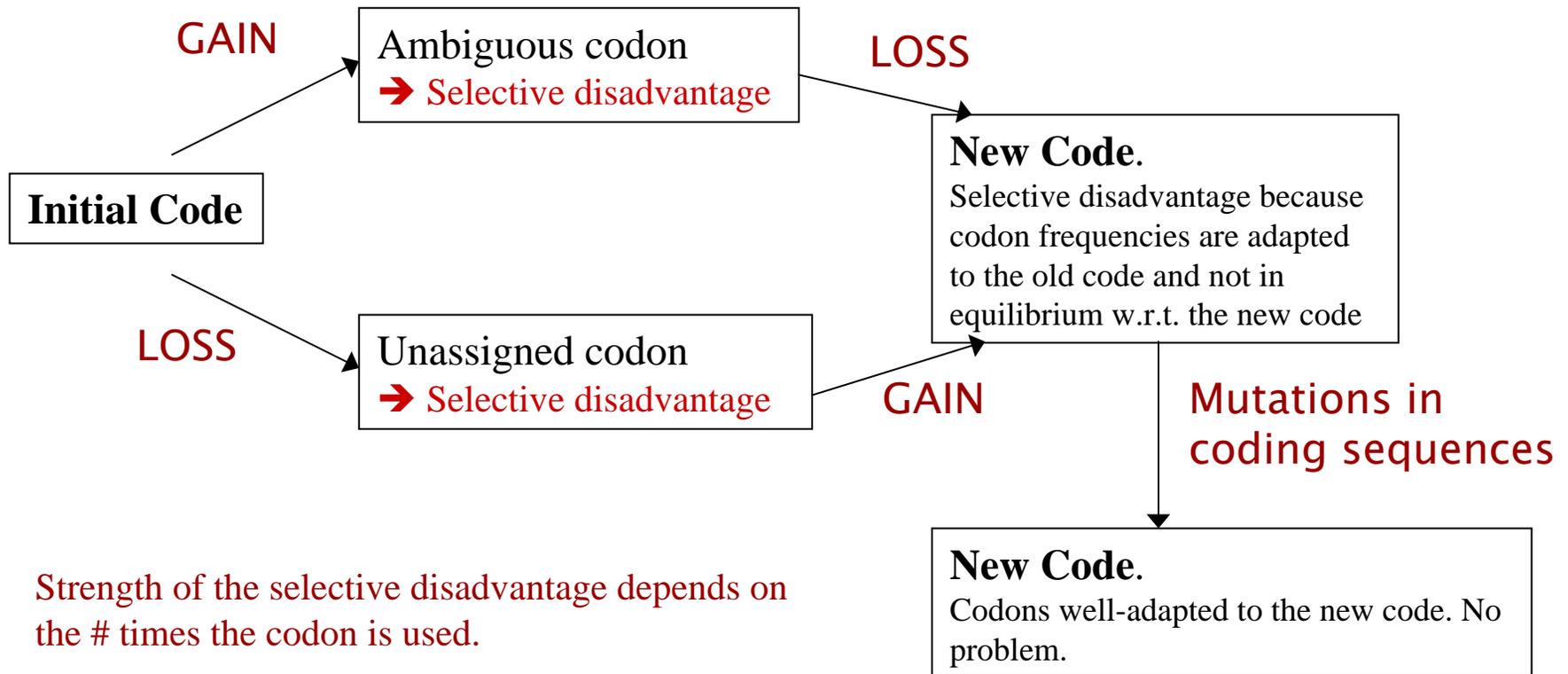
	Before Change	After Change
Amino Acid	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Ile</p> <div style="border-top: 1px solid red; width: 100%;"></div> </div> <div style="text-align: center;"> <p>Met</p> <div style="border-top: 1px solid blue; width: 100%;"></div> </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Ile</p> <div style="border-top: 1px solid red; width: 100%;"></div> </div> <div style="text-align: center;"> <p>Met</p> <div style="border-top: 1px solid blue; width: 100%;"></div> </div> </div>
Codon	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>¹AUU₃</p> <p> </p> </div> <div style="text-align: center;"> <p>AUC</p> <p> </p> </div> <div style="text-align: center; border: 2px solid blue; padding: 2px;"> <p>AUA</p> <p> </p> </div> <div style="text-align: center;"> <p>AUG</p> <p> </p> </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>¹AUU₃</p> <p> </p> </div> <div style="text-align: center;"> <p>AUC</p> <p> </p> </div> <div style="text-align: center;"> <p>AUA</p> <p> </p> </div> <div style="text-align: center;"> <p>AUG</p> <p> </p> </div> </div>
Anti-codon	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>₃UAG₁</p> </div> <div style="text-align: center;"> <p>UAG</p> </div> <div style="text-align: center; border: 2px solid blue; padding: 2px;"> <p>UA^{k2}C</p> </div> <div style="text-align: center;"> <p>UAC</p> </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>₃UAG₁</p> </div> <div style="text-align: center;"> <p>UAG</p> </div> <div style="text-align: center;"> <p>UA^{f5}C,U</p> </div> <div style="text-align: center;"> <p>UA^{f5}C,U</p> </div> </div>
Effect	<p>Proteobacteria and some protist mitochondria possess an extra tRNA-Ile (^{k2}CAU) with a modified base that translates AUA only.</p>	<p>Loss of the <i>special</i> tRNA-Ile(^{k2}CAU) and</p> <p>Gain of function of the tRNA-Met so that it translates both AUA and AUG.</p> <p>UAU (Sea Squirts), ^{f5}CAU (insects).</p>



Temporal Ordering of occurrence of GAIN/LOSS events determine mechanism of change

LOSS before **GAIN** ≠ **GAIN** before **LOSS**

Proposition: Codon reassignments involve a LOSS (i.e. deletion or loss of function of a tRNA or RF) and a GAIN (i.e. gain of a new tRNA or a gain of function of an existing one).



Strength of the selective disadvantage depends on the # times the codon is used.

NO selective disadvantage if the codon disappears.

The Gain-Loss framework allows for **4** *distinct* mechanisms of codon reassignment.

Codon Disappearance (CD) - Osawa & Jukes

Ambiguous Intermediate (AI) - Schultz & Yarus

Unassigned Intermediate (UI)

Compensatory Change (CC) – Code change occurs *without fixation* of the AI or UI state

Objectives of Modeling

- Does the codon disappear from the genome during the intermediate stages?
- In the *absence* of codon disappearance, does the population pass through the **ambiguous** state or the **unassigned** state?
- Does the intermediate **ambiguous** or **unassigned** state get *fixed* in the population ?

Computational Model of a Population Undergoing Codon Reassignment

Part 1 – Coding sequence evolution

A population of N individuals.

Each individual has a genome that evolves with mutation rate u per site per generation.

AUGAUUCUCAAAAGUCAUAGGGUAUAGAUGA t=0

AUGAUU**CCC**AAAGUCAUAGGGUAUAGAUGA t=1

AUGAUU**CCC**AAAG**GUA**AUAGGGUAUAGAUGA t=2

AUGAUU**CCC**AAAG**GUA**AUAGGGUAUAGA**CGA** t=3

CCC is **P** not L
→ (1-s)

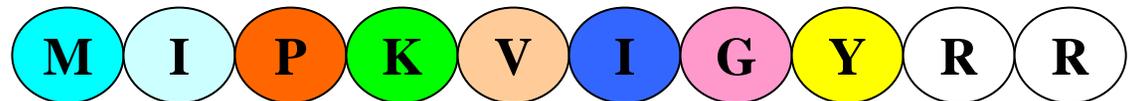
Synonymous Mutation

CGA is **R** not Stop
→ (1-s)

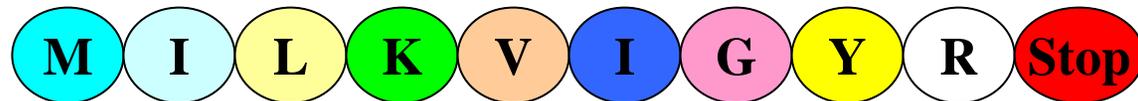
Base sequence at t=3

TRANSLATE ↓

Evolved amino acid sequence



Optimum amino acid sequence



Calculating the fitness of an individual sequence in the population

- Ascertain the genetic code used by the individual sequence.
- Compare the translated mRNA sequence to the optimal protein sequence.
- Assign factor (1-s) whenever there is a ‘wrong’ amino acid.

Eg.:

AUGAUUCCCAAAGUAUAGGGUAUAGACGA

$$\text{Fitness} = (1-s)(1-s)$$

Individuals reproduce in proportion to their fitness

Computer Model of a Population Undergoing Codon Reassignment

Part 2 – tRNA genes controlling translation

Each individual has a LOSS gene and a GAIN gene with two alleles, 0 or 1. These genes represent tRNAs associated with one codon (e.g. AUA in the Ile → Met case).

LOSS = 0 - the original tRNA is still present.

LOSS = 1 - the original tRNA has been deleted or is non-functional.

GAIN = 0 - the new tRNA is not present.

GAIN = 1 - the new tRNA has been acquired.

Block	Before	After
AUN Ile → Met	AUU > GAU {Ile}	AUU > GAU {Ile}
	AUC > GAU {Ile}	AUC > GAU {Ile}
	AUA — K2CAU {Ile}	AUA > f5C, U AU {Met}
	AUG — CAU {Met}	AUG > f5C, U AU {Met}

There are 4 **types** of individuals defined by the GAIN-LOSS combination.

- Type 00 (old code) - fitness's calculated as in Part 1.
- Type 11 (new code) - translation follows the modified genetic code.
- Type 01 (unassigned codon) - Specify fitness factor $(1-s_{\text{unas}})$ wherever this codon appears.
- Type 10 (ambiguous codon) - This will be wrongly translated half the time. Specify fitness $(1-s)^{1/2}$ when it appears at either the old or the new amino acid position.

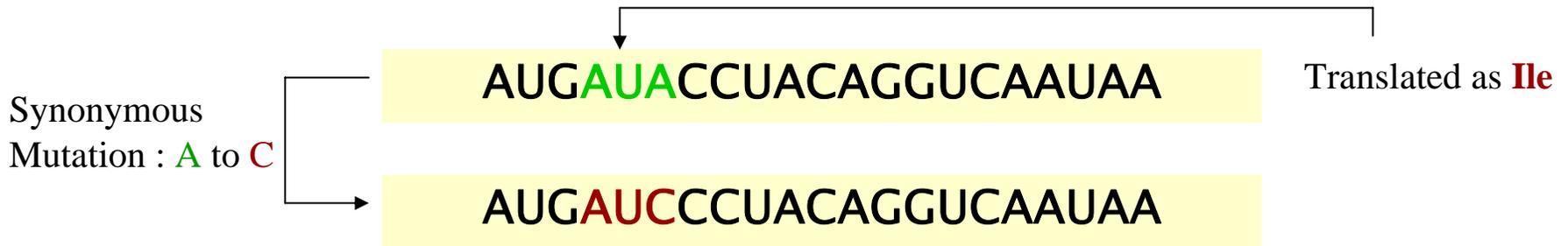
Possible distribution of tRNA-types and individual sequences in a generation

<u>Sequence</u>	<u>Gain-Loss</u>	<u>Code Used</u>	<u>Fitness Assignment for AUA</u>
Seq-1	Type 00	Old Code	1 (Ile); (1-s) - otherwise
Seq-2	Type 01	Old Code	(1-s_unas)
Seq-3	Type 01	Old Code	(1-s_unas)
Seq-4	Type 10	Old Code	(1-s) ^{1/2}
Seq-5	Type 11	New Code	1 (Met); (1-s) - otherwise
Seq-6	Type 01	Old Code	(1-s_unas)

- Individuals begin in state 00. Alleles mutate from state 0 to 1 with a probability U_{gl} per individual per generation.
- Simulations begin with all type 00 and end with all type 11. Type 01 and/or type 10 are present at intermediate stages.

Codon Disappearance (CD) [Osawa & Jukes]

- Codon undergoing reassignment vanishes during a **transient intermediate** stage.

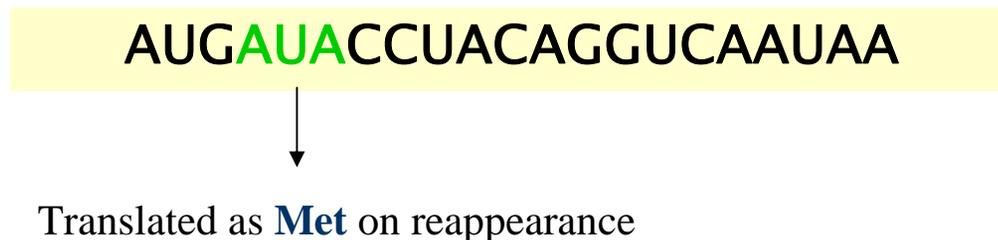


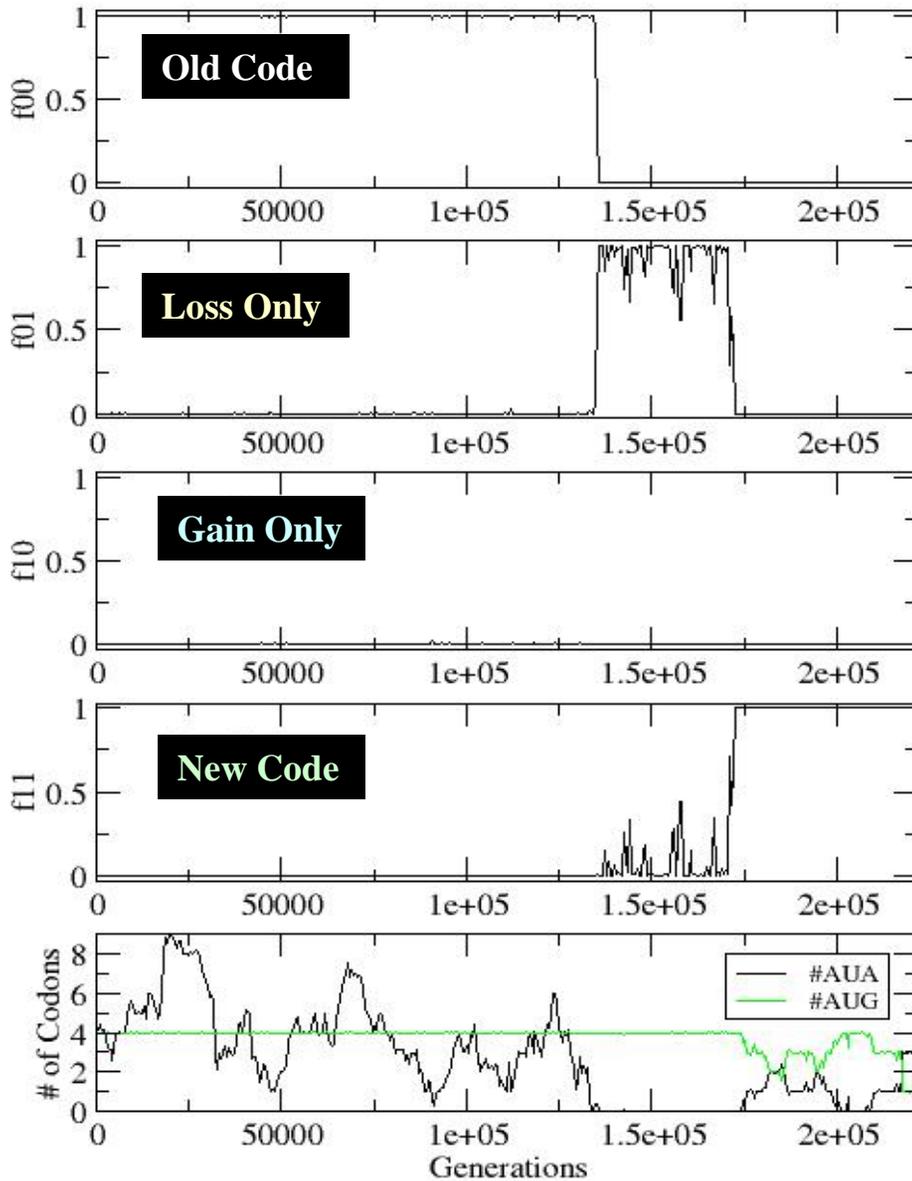
- A new tRNA acquires the ability to decode the codon

E.g: **Gain** of function of tRNA-Met(**UAU** or ^{f5}**CAU**)

Loss (deletion) of tRNA-Ile (^{k2}**CAU**)

- On subsequent reappearance ,the AUA codon has a **new** meaning (amino acid) associated with it.





Evidence of codon reassignment via the **Codon Disappearance** mechanism.

f_{ij} is the *frequency* of individuals with tRNA type ij .

Parameters

$N=1000$; $u=0.0001$;
 $s=s_{\text{stop}}=s_{\text{unas}}=0.05$; #AUA=4.
#AUG=4

Unassigned Intermediate Mechanism (UI)

tRNA decoding a codon is lost (**Loss** of function), codon becomes **unassigned** during a **transient** intermediate stage.

Eg.: **Loss** (deletion) of tRNA-Ile (^{k2}**CAU**) occurs first; **AUA** - **unassigned**



AUG**GAU**ACCUACAGGUCAAUAA

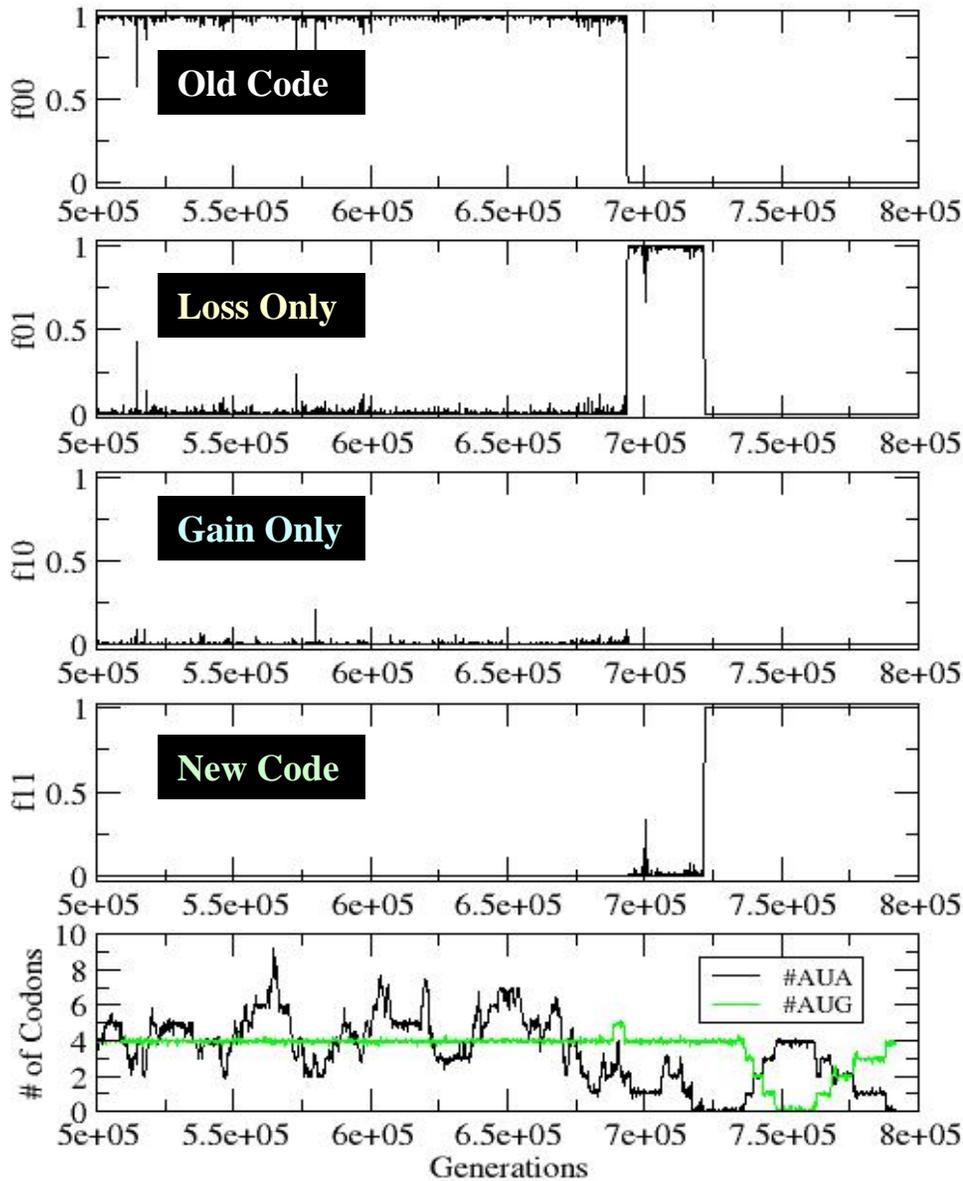
Translated as **Ile** by tRNA-Ile (**GAU**) but with *low* efficiency

A new tRNA acquires the ability to efficiently decode the codon.

Eg.: **Gain** of function of tRNA-Met(**UAU** or ^{f5}**CAU**)

Codon gets new meaning ; new genetic code results.

Eg.: AUA translated as **Met**



Evidence of Codon Reassignment initiated by the **Loss** of tRNA.

Parameters

$N=1000$; $u=0.0001$;

$s_{\text{unas}}=0.007$;

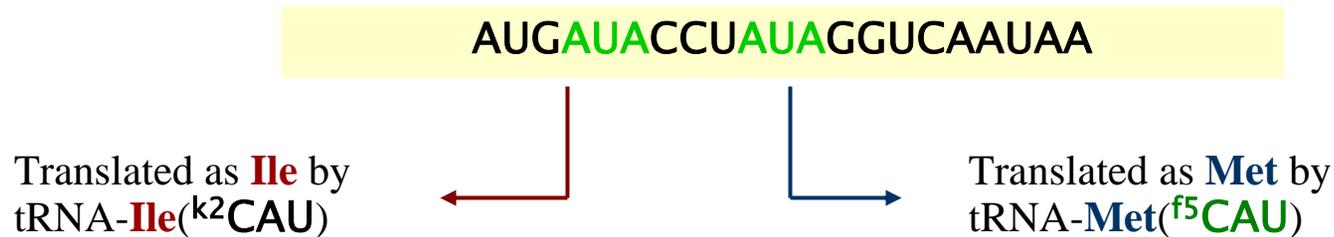
$s=s_{\text{stop}}=0.02$; #AUA=4.

Ambiguous Intermediate (AI) [Schultz & Yarus]

New tRNA acquires the ability to decode the codon.

Eg.: **Gain** of function of tRNA-Met(**UAU** or **^{f5}CAU**)

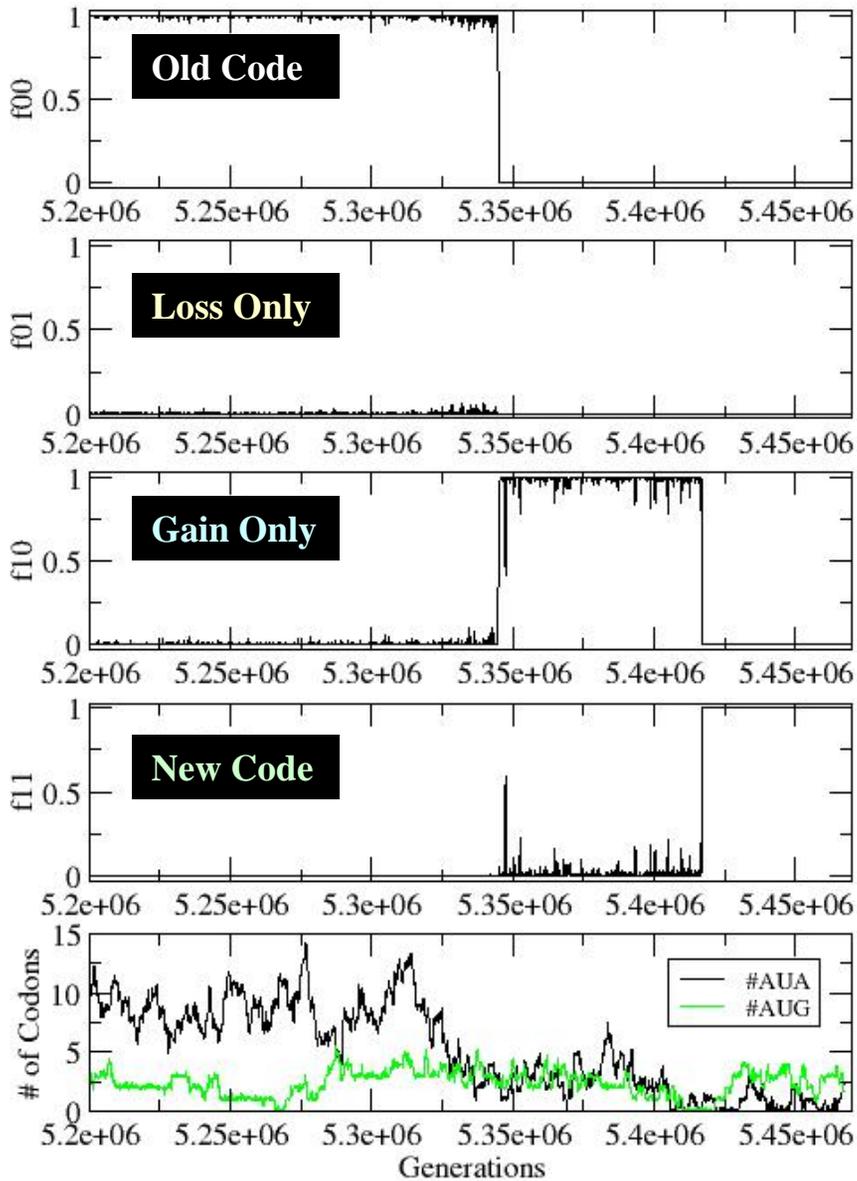
Codon undergoing reassignment is translated as **two distinct** amino acids during a **transient intermediate** stage.



New code is established when one meaning is **selected** over the other.

Eg.: **Loss** (deletion) of tRNA-Ile (**^{k2}CAU**) ; **ambiguity removed**.

AUA uniquely translated as **Met**.



Evidence of codon reassignment occurring via the **Ambiguous Intermediate** mechanism.

36 **Ile** codons (including 12 AUA's) and 4 **Met's** were present in the original sequence.

Parameters

$N=1000$; $u=0.0001$;
 $s_{\text{sense}}=s_{\text{stop}}=s_{\text{unas}}=0.01$; #AUA=12.

Compensatory Change

(Kimura)

Changes in the Gain-Loss gene used to represent the state of the tRNA

Gain-Loss

00

Initial State



0 → **1** mutation in the Loss gene

01

Lower Fitness

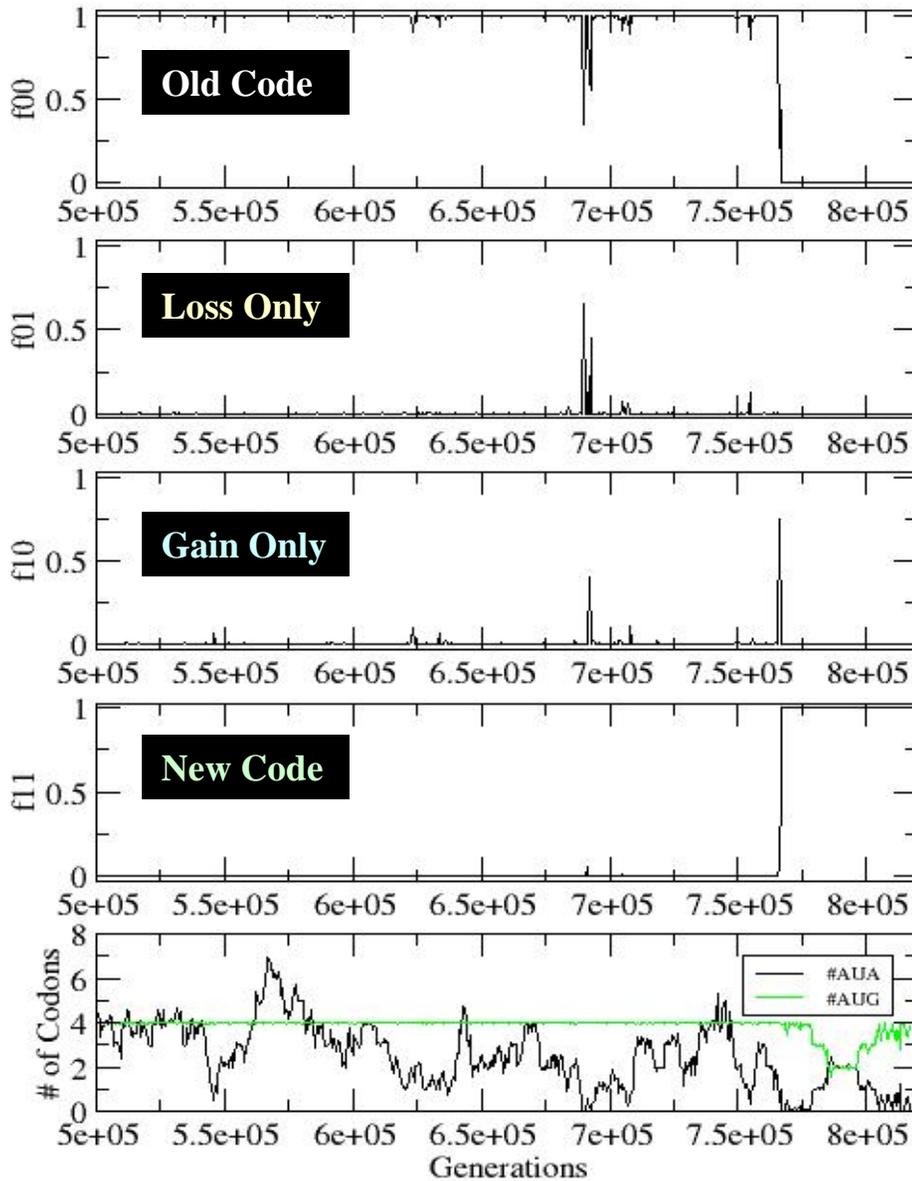


0 → **1** mutation in the Gain gene

11

Increase in Fitness

The selective disadvantage of the intermediate stage is compensated for by a subsequent mutation.



Evidence of codon reassignment occurring via the **Compensatory Change** mechanism.

Parameters

$N=1000$; $u=0.0001$;
 $s_{\text{sense}}=s_{\text{stop}}=s_{\text{unas}}=0.05$;
 $\#AUA=4$.

Model Predictions

Ambiguous Intermediate mechanism seems more likely for reassignment involving sense codons.

Codon Disappearance mechanism could be more relevant for reassignment involving stop codons.

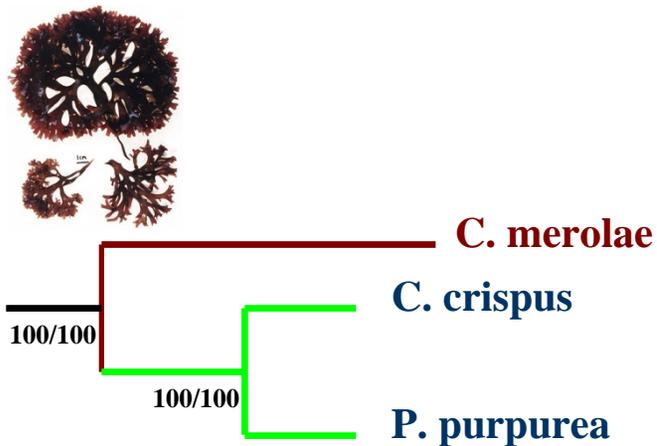
Reassignment initiated by **tRNA Loss** is likely in scenarios in which a codon can be translated by a near-cognate tRNA without substantial fitness cost.

Compensatory Change mechanism is more likely when penalty against ambiguous & unassigned states are large.

Genomic Analysis of Changes in the Mitochondrial Genetic Code

SS, X. Yang & P. Higgs (J.Mol.Evol. 64:662-688 (2007))

- Determine location of each codon reassignment on a phylogenetic tree.
- Correlate location of codon reassignment with codon usage and tRNA types available for the specific codon(s) undergoing reassignment.



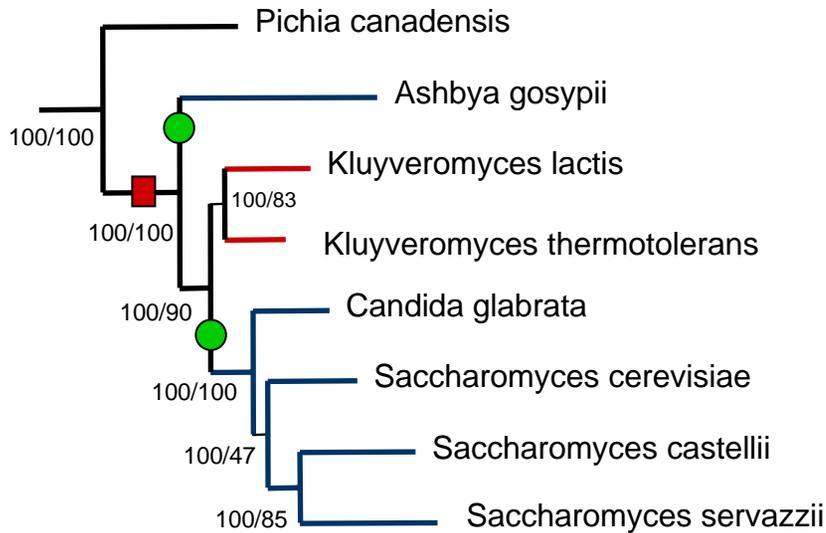
UGA: Stop to Trp

<u>Species</u>	# UGA	tRNA-Trp anticodon	$P_{dis=} (1-f_{UGA})^N$
<i>C. merolae</i>	2 (stop)	CCA	
<i>C. crispus</i>	101 (Trp)	UCA	0.036
<i>P. purpurea</i>	117 (Trp)	UCA	0.0014

- UGA disappeared in the common ancestor of *C. crispus* and *P. purpurea*.
- Anticodon of tRNA-Trp mutated from CCA to UCA

➡ UGA on reappearance is translated as Trp.

AUA: **Ile** to **Met** in Fungi



■ Location of disappearance of tRNA-**Ile**(^{k2}CAU)

● AUA: **Ile** to **Met**

<u>Species</u>	#AUA	tRNA Ile (^{k2} CAU)	Gain of tRNA Met (CAU)
P.canadensis	562	Present	No
A.gosypii	95	Absent	Yes
K.lactis	7	Absent	No
K.thermotolerans	2	Absent	No
C.glabrata	16	Absent	Yes
S.cerevisiae	60	Absent	Yes
S.castellii	101	Absent	Yes
S.servazzii	95	Absent	Yes

Reassignment initiated by **deletion** of tRNA-**Ile**(^{k2}CAU)

In **K.Lactis** & **K. Thermotolerans** AUA translated as **Ile** by tRNA-**Ile**(GAU)

Completed after **gain** of function of tRNA-**Met**(CAU)  AUA translated as **Met**

Predictions of Codon Reassignment Mechanisms in Mitochondria

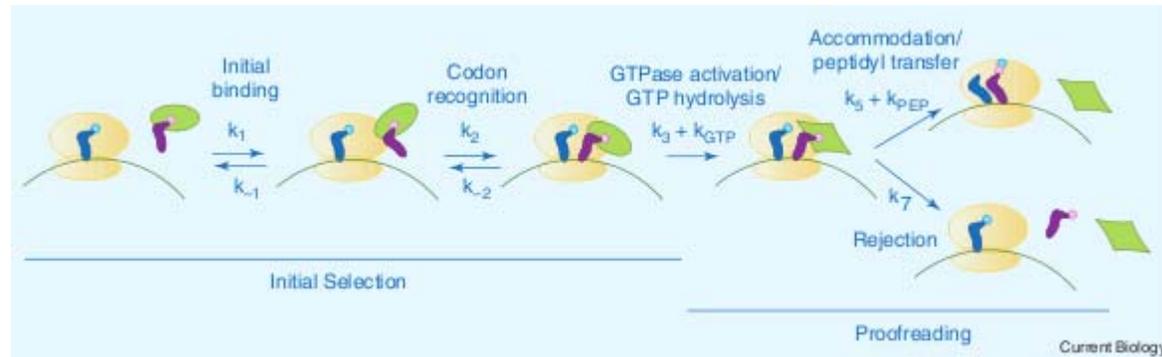
Codon Reassignment	# of times	Mechanism
UAG: Stop → Leu	2	CD
UAG: Stop → Ala	1	CD
UGA: Stop → Trp	12	CD
CUU/CUC/CUA/CUG: Leu → Thr	1	CD
CGU/CGC/CGA/CGC: Arg → Unassigned	5	CD
AUA: Ile → Met	5	UI
AAA: Lys → Asn or Unassigned	3	AI
AGA/AGG: Arg → Ser	1	UI
AGA/AGG: Ser → Stop	1	AI
AGA/AGG: Ser → Gly	1	AI
UUA: Leu → Stop	1	UI/AI
UCA: Ser → Stop	1	UI/AI

- AI and UI mechanisms can only be *indirectly* inferred after ruling out the CD mechanism
- For some reassignments, its *not* possible to determine whether the AI/UI mechanism was responsible.

Testing the viability of the AI & UI mechanisms *in vitro*

Translational Error correction *before* Peptide bond formation

(W. Wintermeyer, M. Rodnina, R. Green)



Source : Cochella & Green; Current Biology : 15 (2005); R536

Aminoacyl-tRNA's arrive at the ribosome in a ternary complex with GTPase EF-Tu and GTP.

✚ A **cognate** ternary complex hydrolyzes with greater rate ($k_3 > k_{-2}$) than a **non-cognate** ternary complex

A **non-cognate** ternary complex is more likely to dissociate ($k_{-2} > k_3$) than hydrolyze.

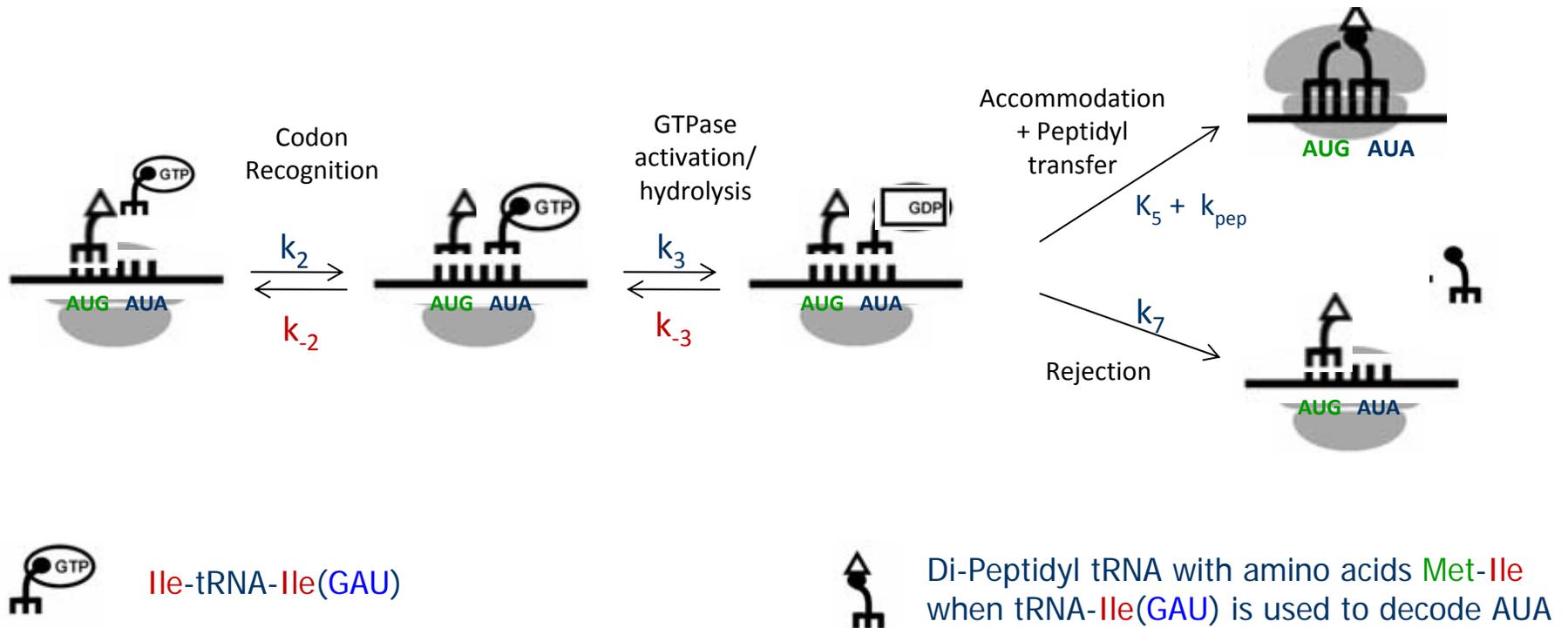
✚ A **cognate** ternary complex undergoes the peptidyl transfer reaction with greater rate ($k_5 > k_7$) than a **non-cognate** ternary complex.

✚ **Conformational changes** at the ribosome *and* in the **cognate** / **non-cognate** tRNA is induced by the pairing between the codon and anticodon and **affects** the peptidyl transfer reaction rate.

Such conformational changes increases the **rejection rate** ($k_7 > k_5$) of a **non-cognate** ternary complex from the ribosome.

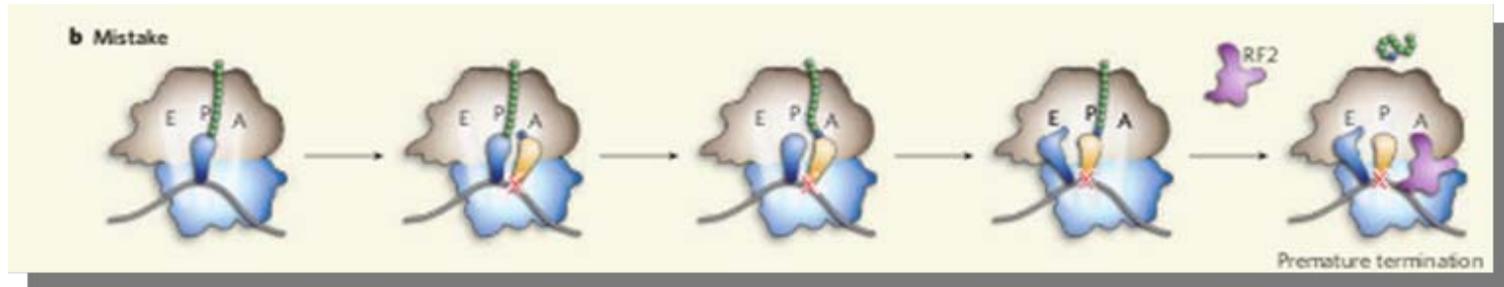
Testing the UI mechanism : Effect on Translation Rates

- Produce a ribosomal nascent chain (RNC) complex   tRNA-fMet
- Produce a ternary complex of EF-Tu-GTP-Ile-tRNA-Ile(GAU) for translating the AUA codon
- Determine the rates (k_{-2} , k_3 , k_7 , k_{pep}) of AUA codon recognition by the tRNA.



Translational Error Correction *after* Peptide bond formation

Zaher & Green; Nature 457 (2009) 161



Source : Fredrick & Ibba; Nature : 457 (2009); 157-158

The ribosomal complex becomes more susceptible to premature termination induced by RF2

➔ The partial formed erroneous sequence is prematurely released and eventually degraded.

During *ambiguous* translation of AAA by tRNA-Asn(GUU) a “wrong” amino acid Asn can be inserted into the peptide chain ?

How viable is *ambiguous* translation of AAA by tRNA-Asn(GUU) and tRNA-Lys(UUU) ?

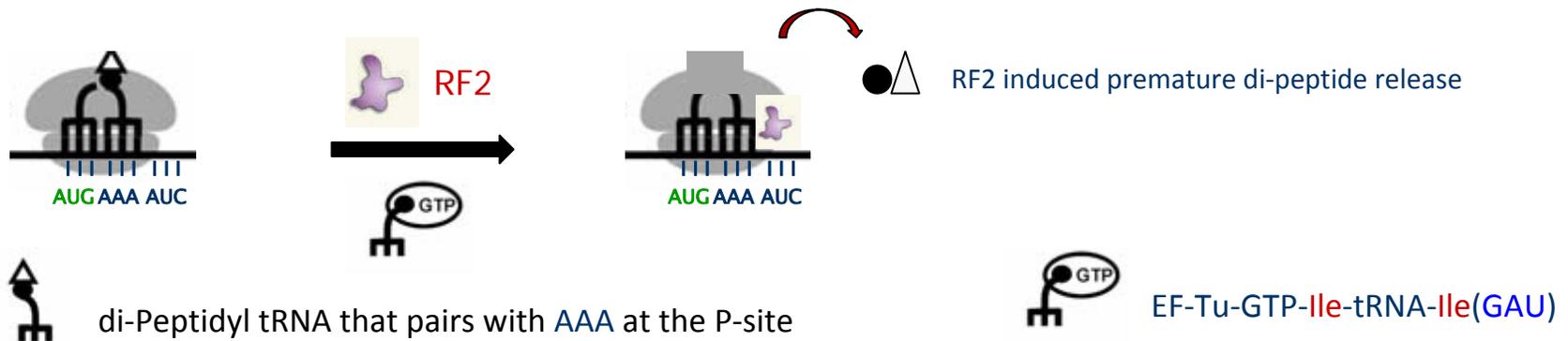
Retrospective Error Correction during Ambiguous Translation of AAA

✚ Create two di-peptidyl-tRNA-ribosome complexes both of which carry a sense codon (AUC) at the A-site.

✚ “Wrong” di-Peptidyl tRNA with amino acids Met-Asn when tRNA-Asn(GUU) is used to decode AAA

Correct di-Peptidyl tRNA has amino acids Met-Lys when tRNA-Lys(UUU) is used to decode AAA

✚ Determine if recognition of AAA by tRNA-Asn(GUU) leads to RF2 induced peptide release.



✚ A comparison of the **differential** rates of translation by the tRNA-Asn(GUU) and tRNA-Lys(UUU) will determine the extent of ambiguous decoding AAA.

Conclusions

■ The Genetic Code is *still* evolving

■ The evolutionary pathways leading to codon reassignment can be deciphered

Codon Disappearance mechanism is responsible for **all** reassignments involving **loss** of **stop** codons.

Unassigned Intermediate and **Ambiguous Intermediate** mechanisms are mostly responsible for reassignments involving sense codons.

■ *in vitro* experiments can be used to test the viability of **AI** & **UI** mechanisms.

■ Open Question : tRNA import into mitochondria and its connection to codon reassignment

✚ Understanding the tRNA import machinery and how it varies across organisms ?

✚ When did the tRNA import machinery evolve and did it evolve independently multiple times ?

✚ Why are some tRNA's imported and not others ?

Acknowledgements

Paul Higgs – Genetic Code evolution model, genomic analysis of codon reassignments.

Xiaoguang Yang – Genomic analysis of codon reassignments.

Ashutosh Vishwabandhu – codon reassignment in bacterial and nuclear genomes

Howsun Jow

Cendrine Hudelot

Vivek Gowrishankar

Magnus Rataray

Daniel Jameson

Bin Tang

Wei Xu

PHASE – PHylogenetics And Sequence Evolution package



- Database for mitochondrial genomes
drake.physics.mcmaster.ca/ogre