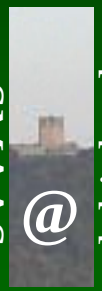
Molecular Studies on Complex Biological Systems:

Sources for Transcriptome and Proteome Complexity



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October 17, 2007

Dr. Stefan Maas, BioS Lehigh U.

What's the molecular difference ?

Nature 9/7/2006

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What accounts for the often massive and seemingly arbitrary differences in genome size observed among eukaryotic organisms?

The fruit fly
Drosophila melanogaster

180 Mb

The mountain grasshopper
Podisma pedestris

18,000 Mb

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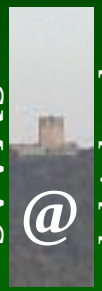
Is an Expansion in Gene Number driving Evolution of Higher Organisms?



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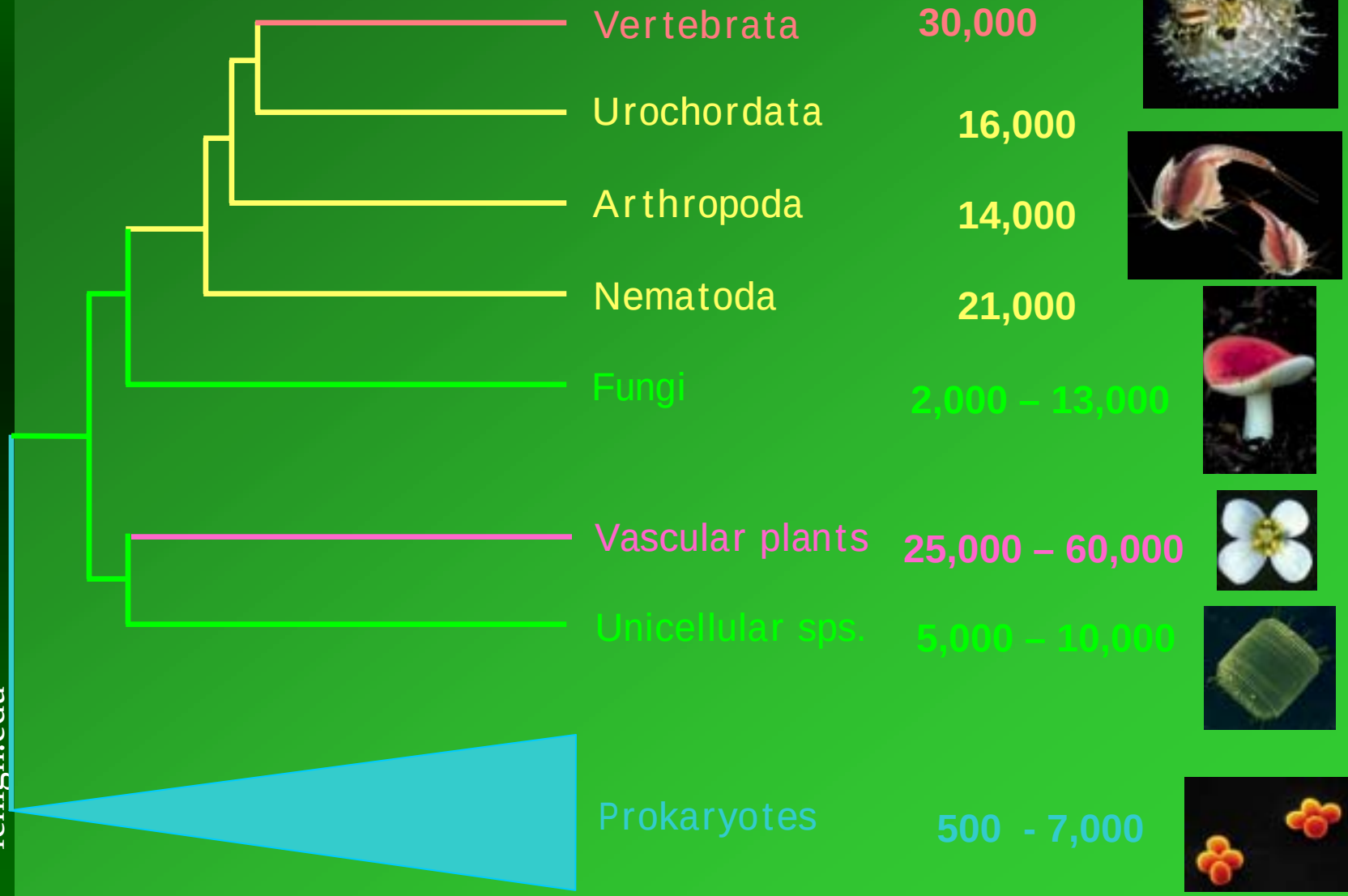
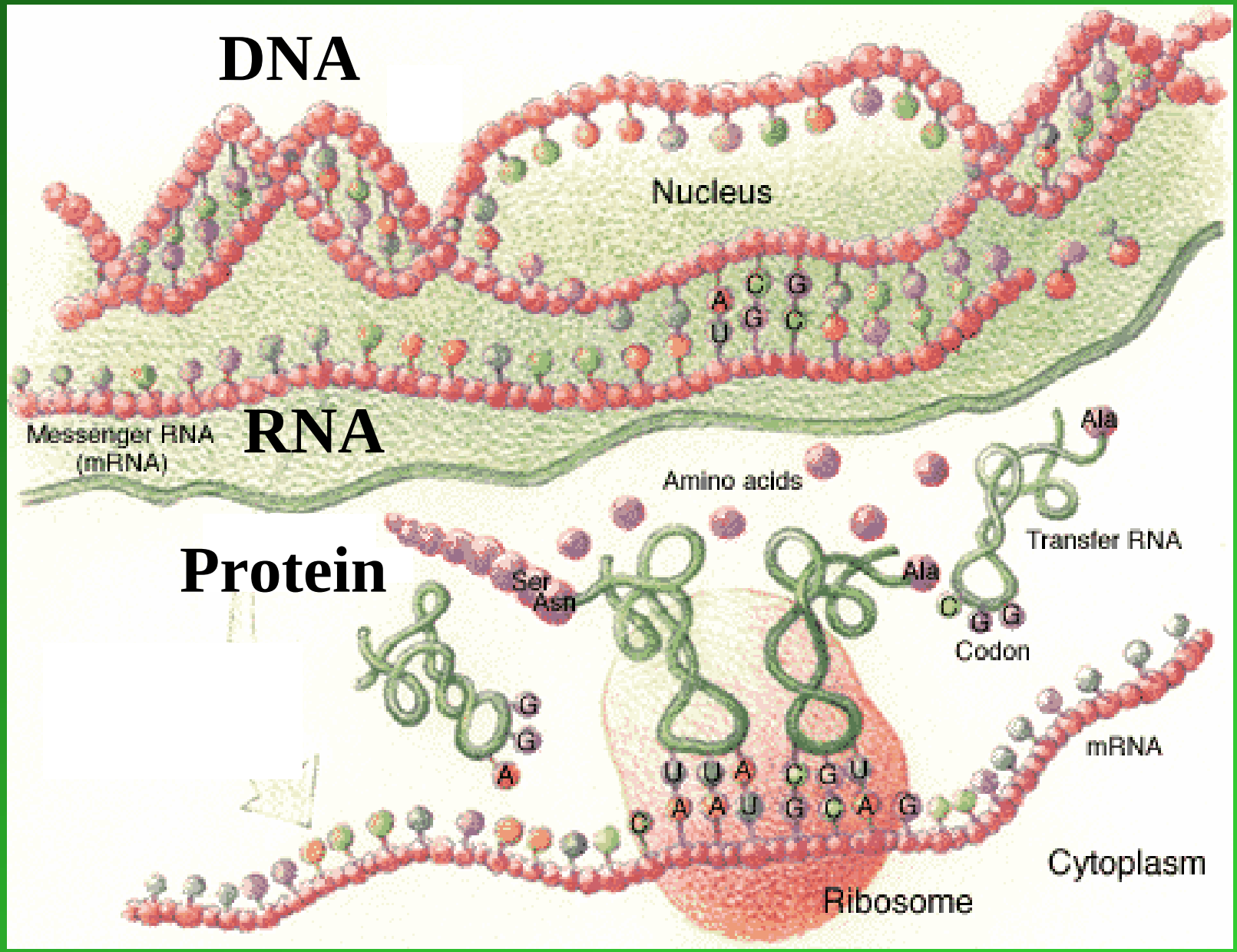


From genes to proteins



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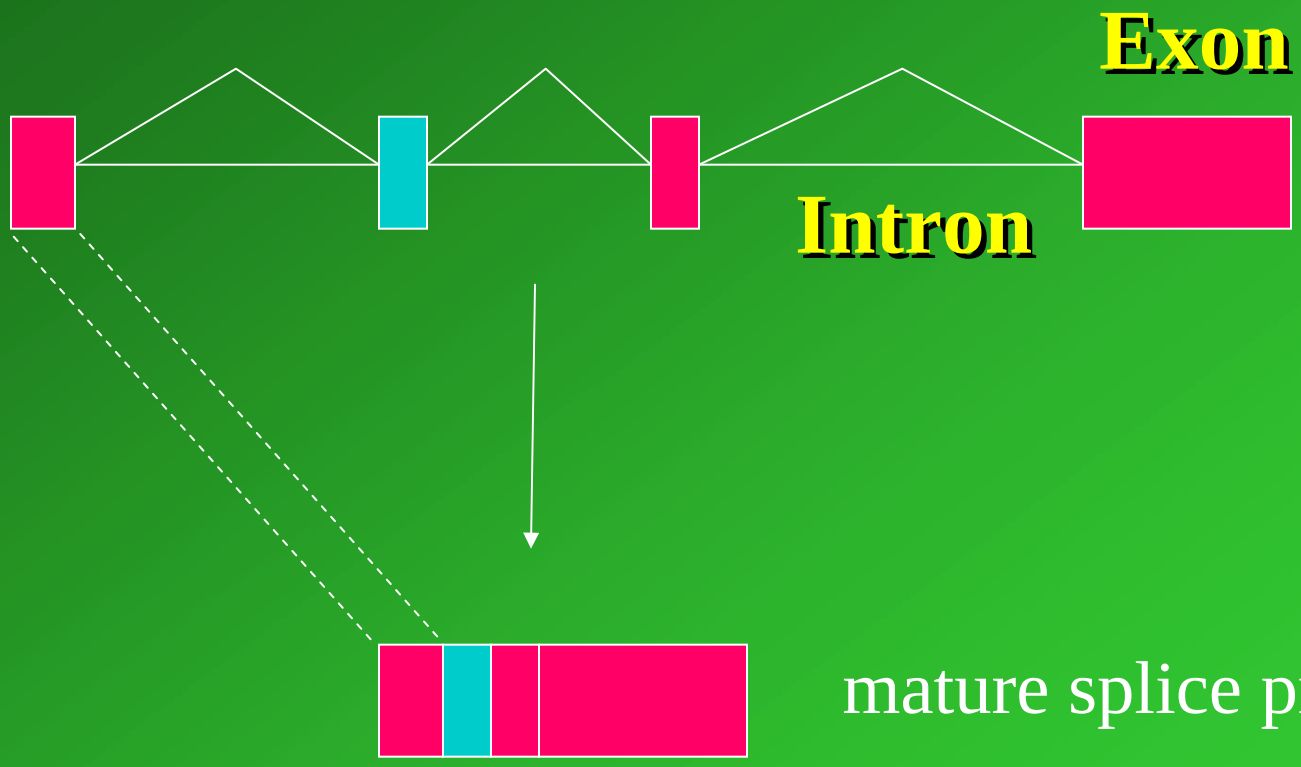
Gene splicing:
Removal of non-coding introns



mature splice product

PROTEIN

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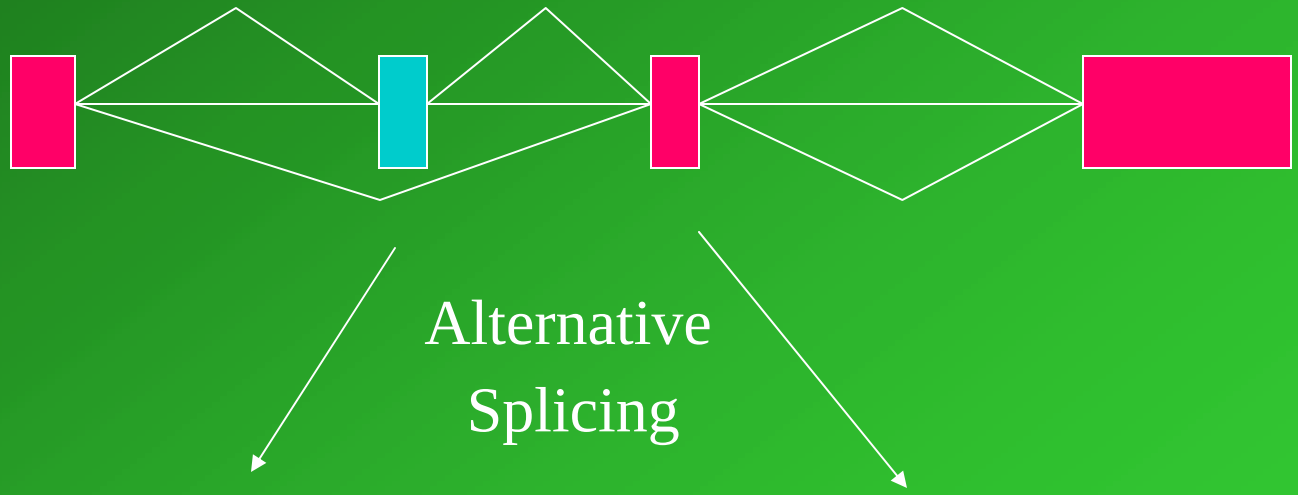
Alternative splicing: One gene, several proteins!



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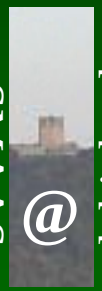

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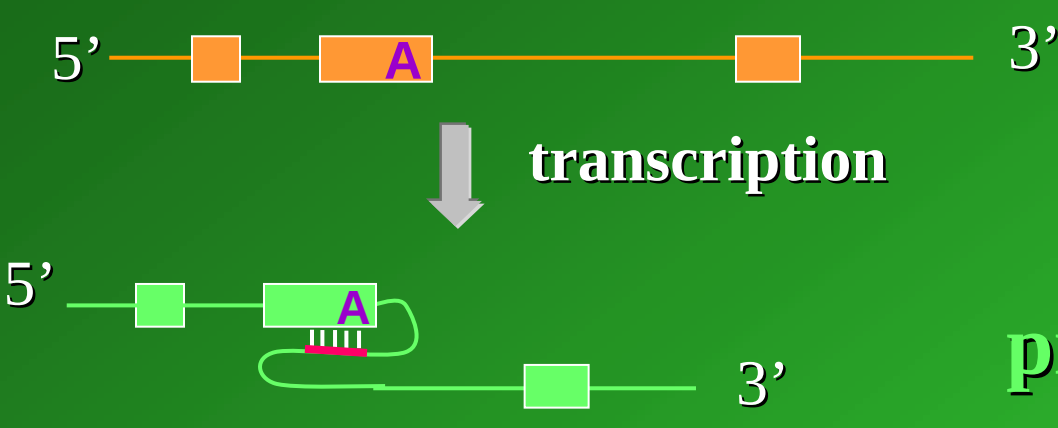
I A M T H E Q S R T V F I R S T Q P B A S R T V S E C O N D Q A B D E C T V A L T E R N A T I V E

Priciples of Alternative Splicing

n

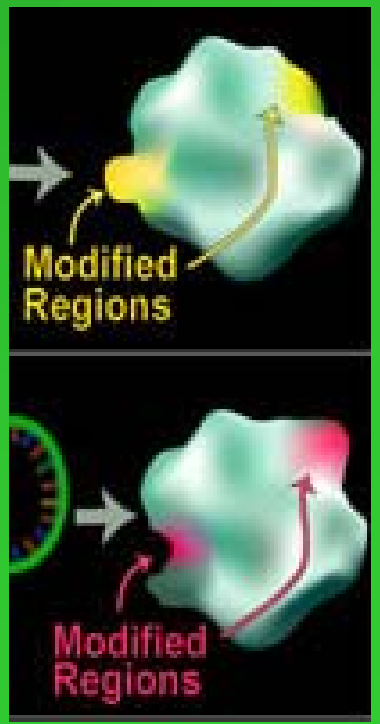
RNA editing



genomic DNA

pre-mRNA

splicing + translation



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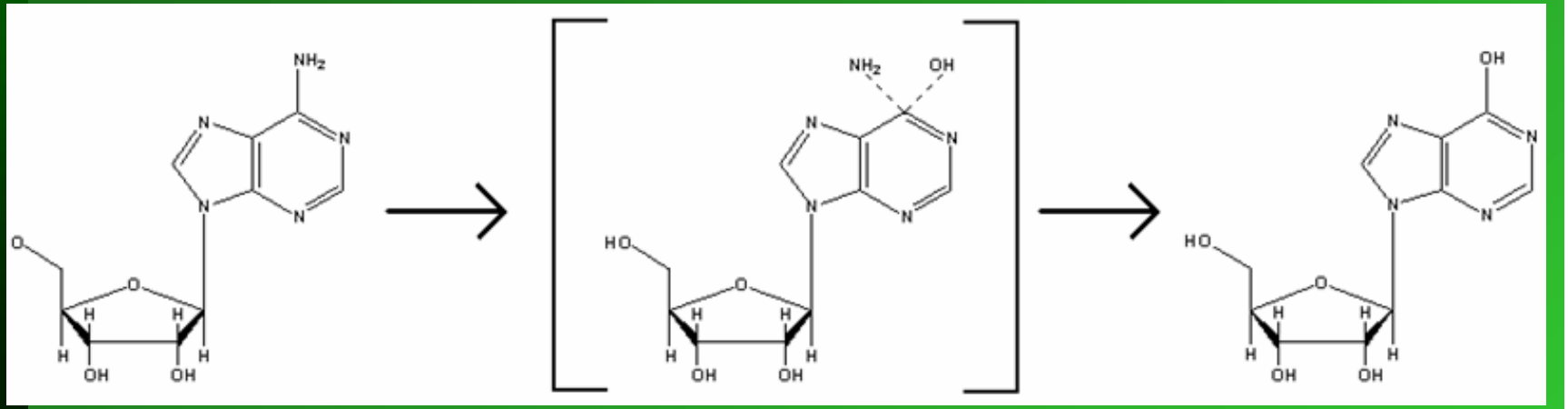
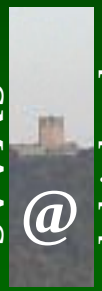
Effects of Editing



Adenosine

Inosine

- Adenosine converted to Inosine
- Interpreted as Guanosine
- Expand the proteome



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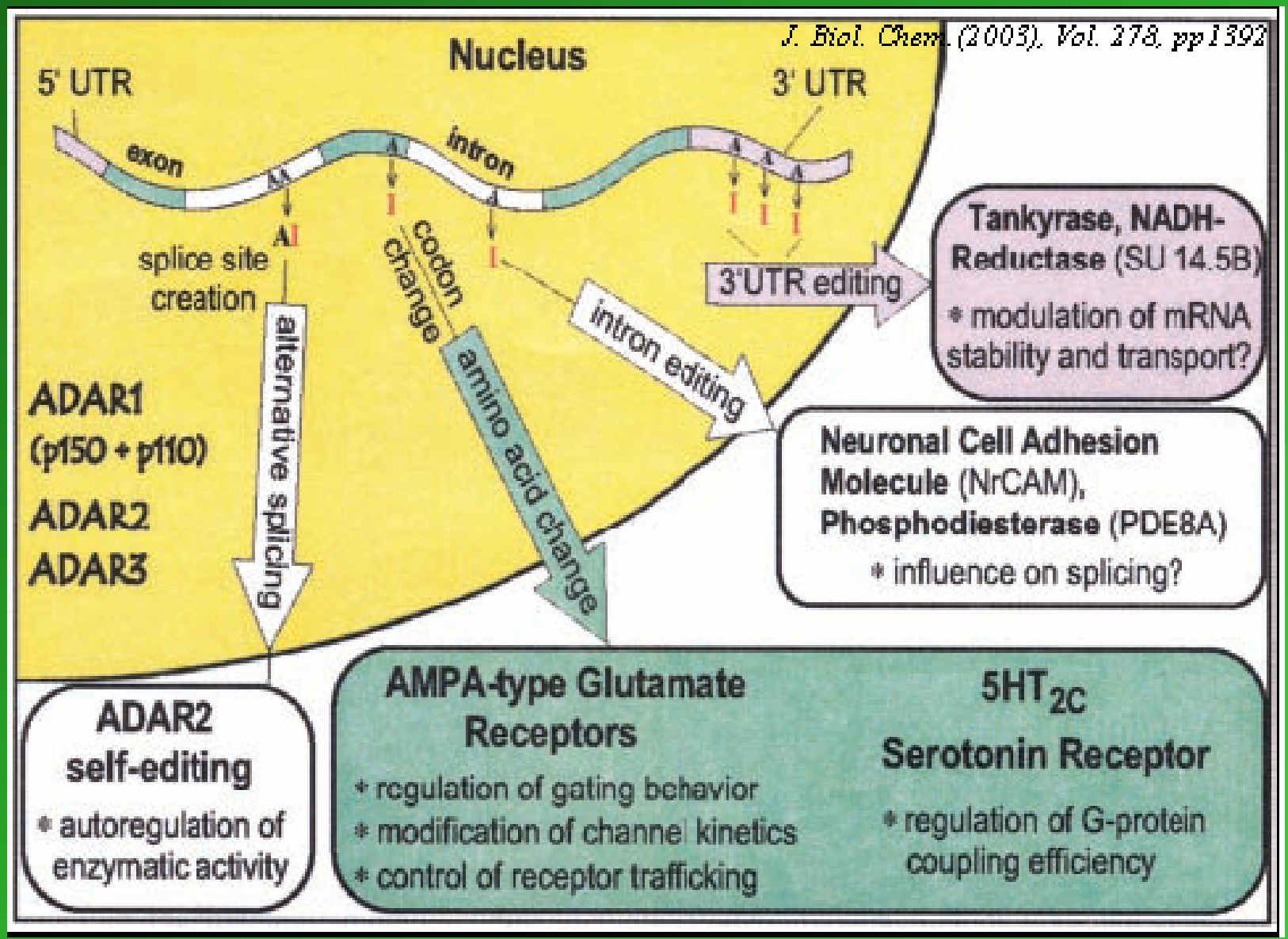
Consequences of Editing



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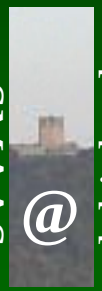
Mammalian substrates of A-to-I pre-mRNA editing

Gene	codon	amino acid	editing [%]
GluR-B	CAG/C**I**G	Q/**R**	**100**
GluR-B,-C,-D	AAG/A**I**G	R/**G**	**60-80**
GluR-5,-6	CAG/C**I**G	Q/**R**	**40-80**
GluR-6	AUU/**I**UU	I/**V**	**80**
	UAC/U**I**C	Y/**C**	**80**
5-HT_{2C} Serotonin-receptor	AUA/**I**UA	I/**V**	**40-90**
	AAU/A**I**U	N/**S**	**35-40**
	AUU/**I**UU	I/**V**	**45-75**



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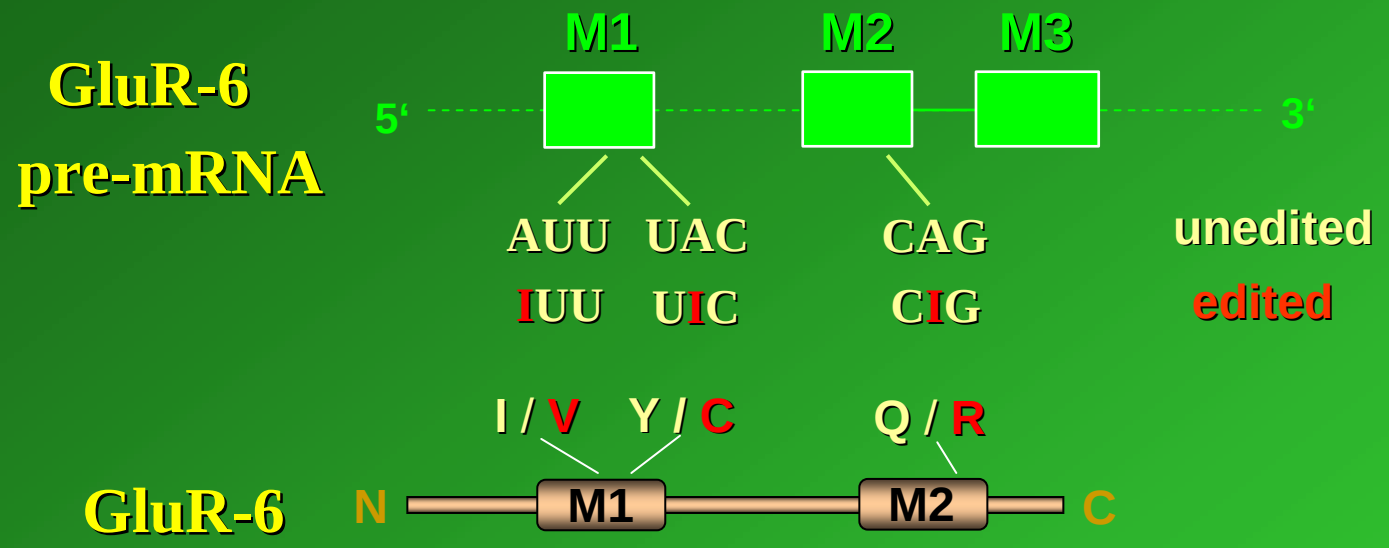
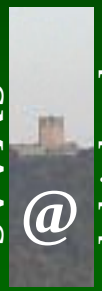
Diversity through RNA editing





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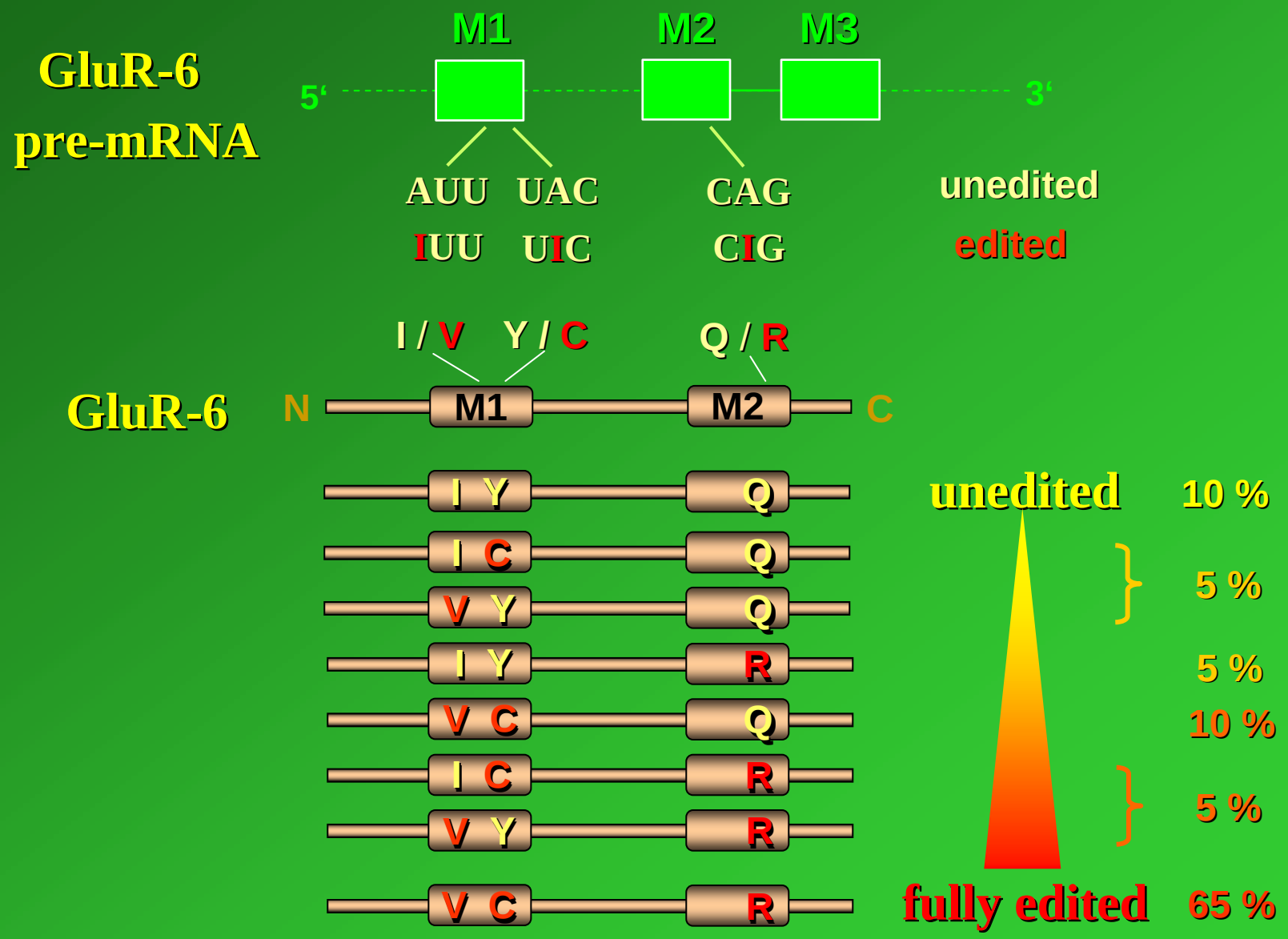
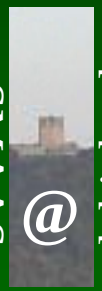
Diversity through RNA editing





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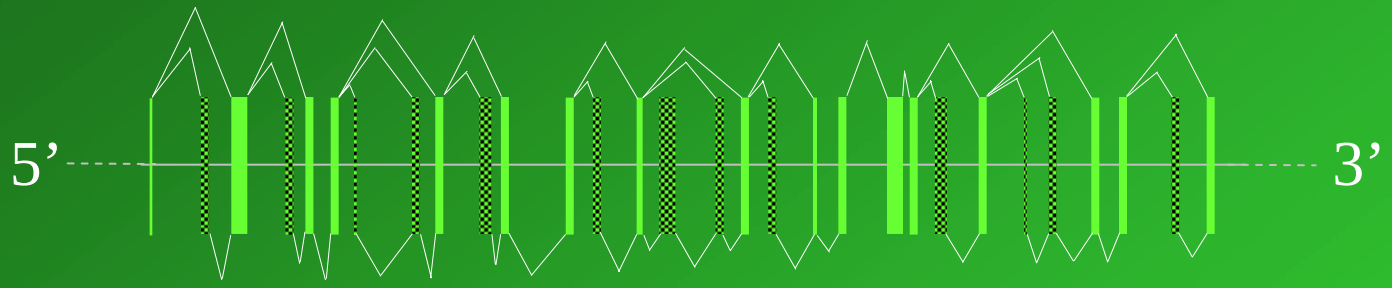
Even more diversity

***paralytic* pre-mRNA**



constitutive exon

alternative exon

alternative splicing

1 536 variants

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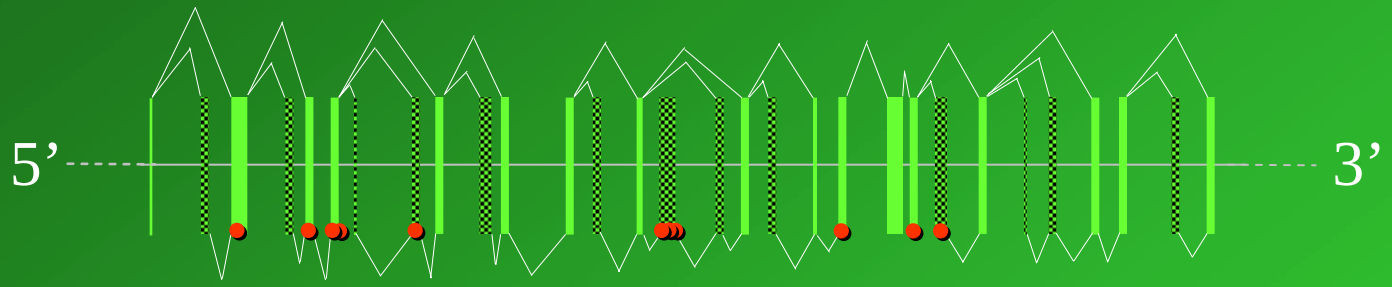
Even more diversity

***paralytic* pre-mRNA**



alternative splicing

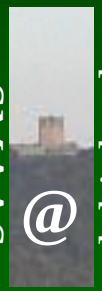
RNA editing

constitutive exon

alternative exon

editing site

1 032 192 variants



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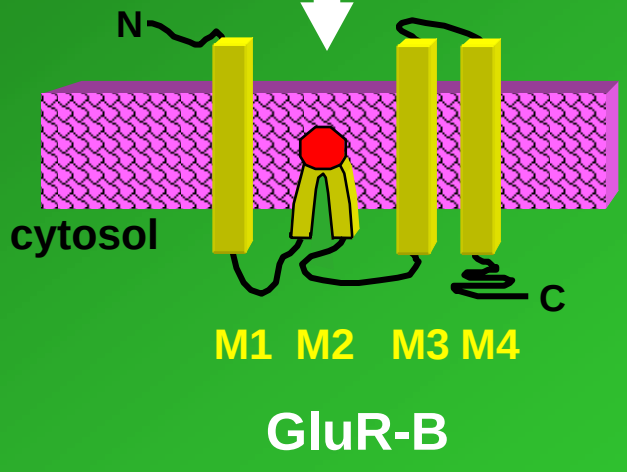
Q/R-site editing of glutamate receptor subunit GluR-B

mRNA

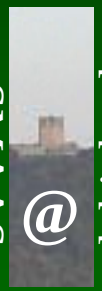
I

-UUU AUG CAG CAA GGA-

F M Q/R Q G



GluR-B



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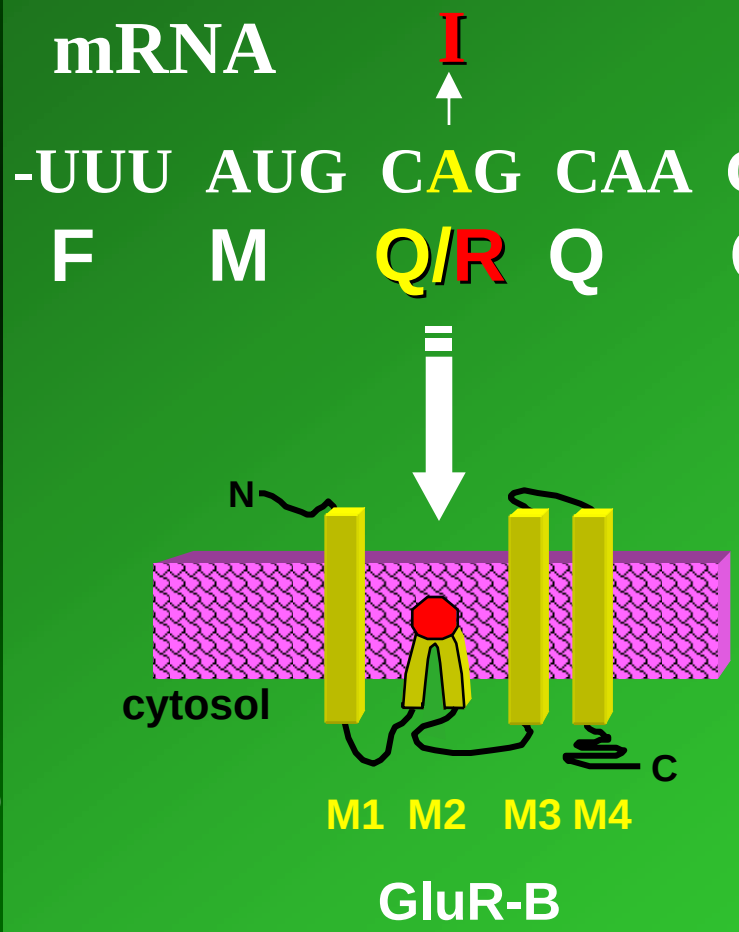
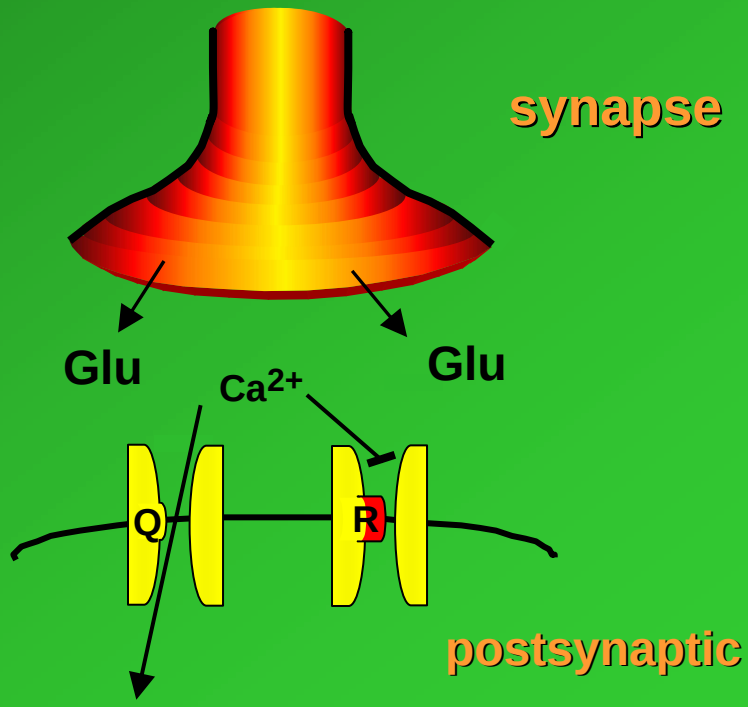
Q/R-site editing of glutamate receptor subunit GluR-B





>99.9% R

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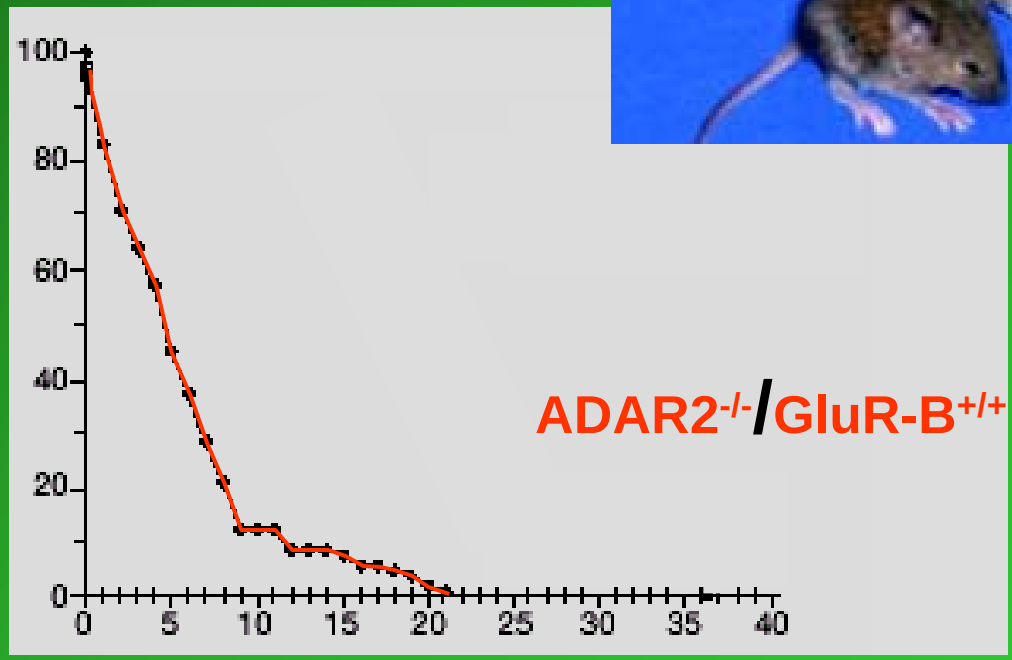
RNA editing enzyme deficient mice



(Higuchi, Maas, Single, Hartner et al., 2000, *Nature* 406, 78-81)

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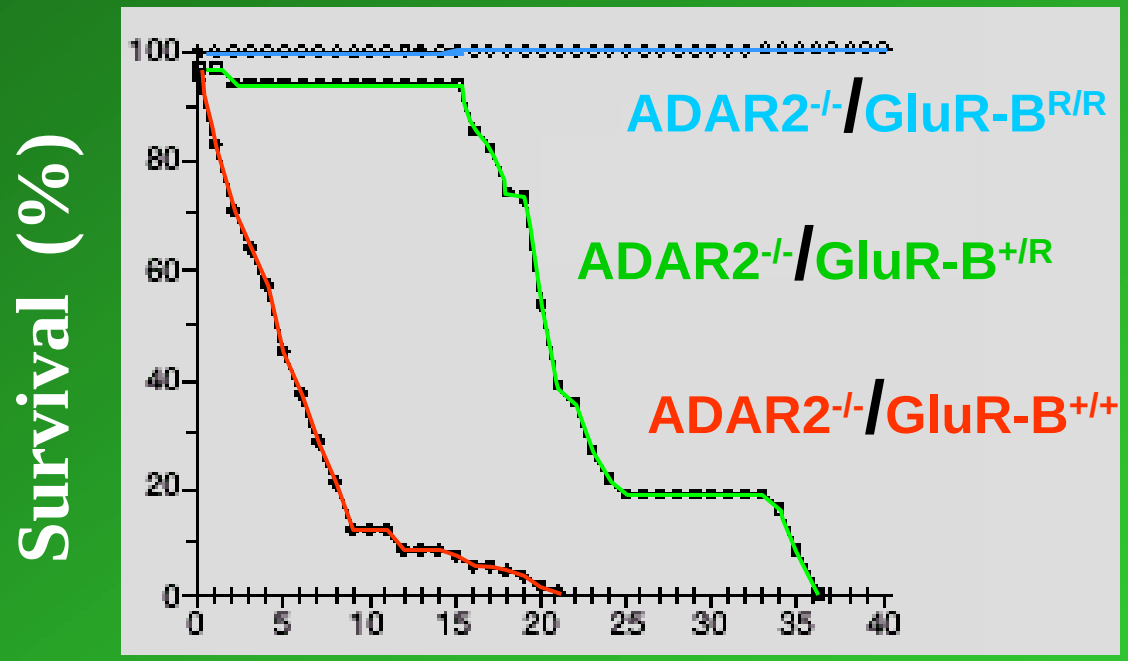
RNA editing enzyme deficient mice: Rescue by GluR-B point mutation



(Higuchi, Maas, Single, Hartner et al., 2000, *Nature* 406, 78-81)

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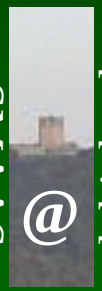
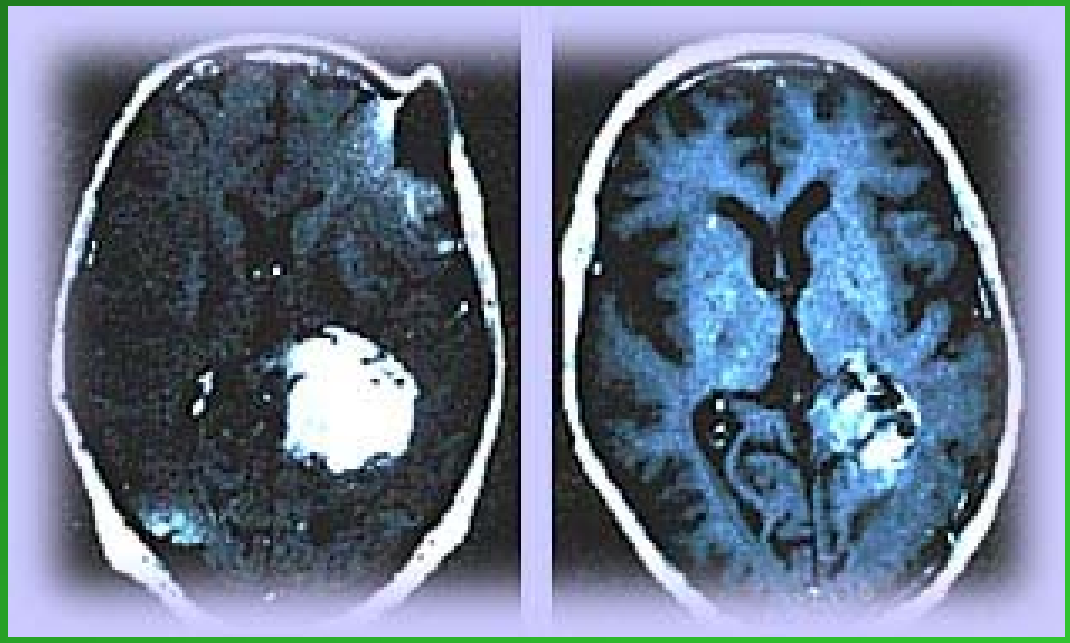
Question:

- Could too much or too little RNA editing cause disease or alter the progression of known diseases?



Glioblastoma multiforme (GBM)



http://www.thejohnphilpthompsonfoundation.org/GlioblastomaMultiforme_1_.jpg

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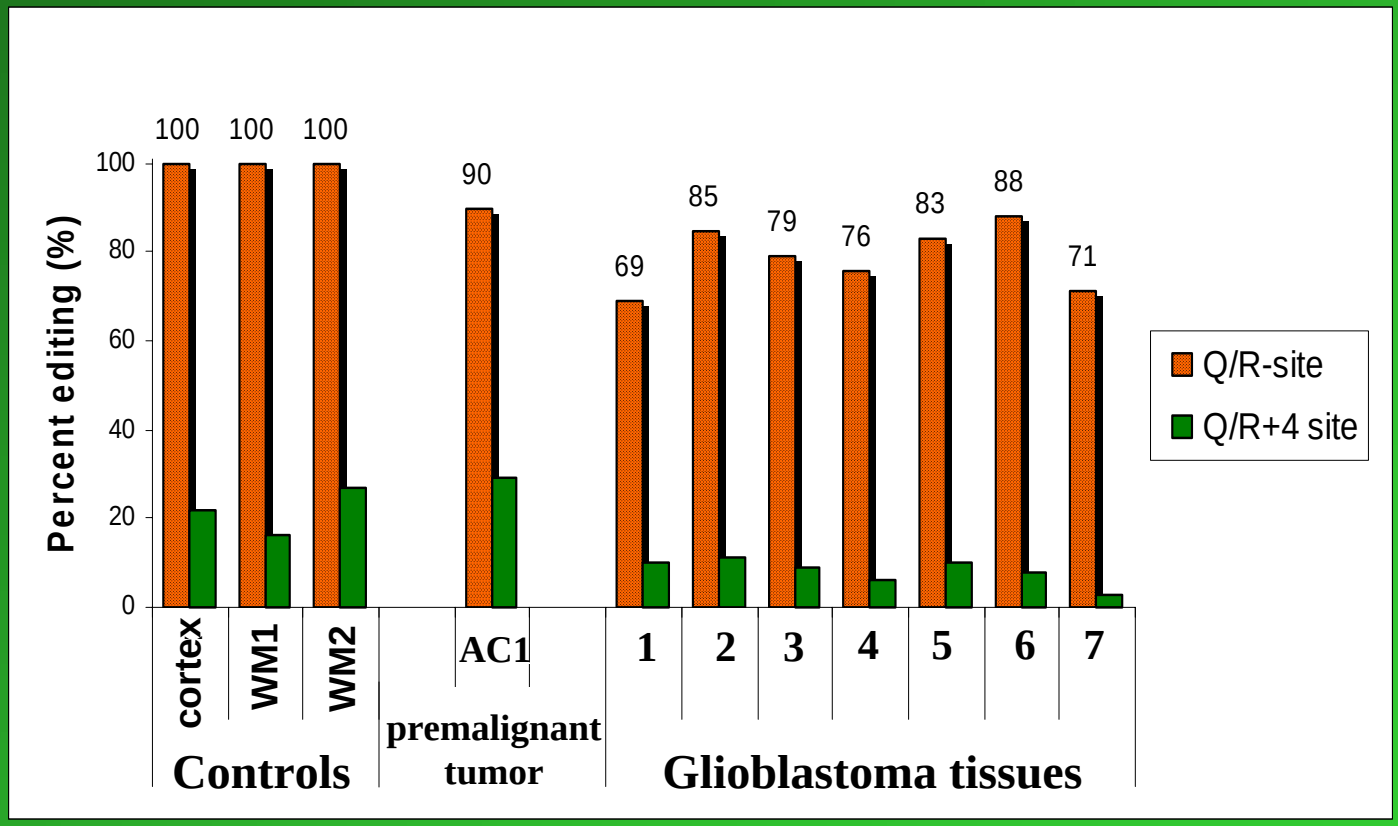
Q/R site editing in normal human brain and gliomas



(Maas et al., 2001, *PNAS* 98, 14687-92)

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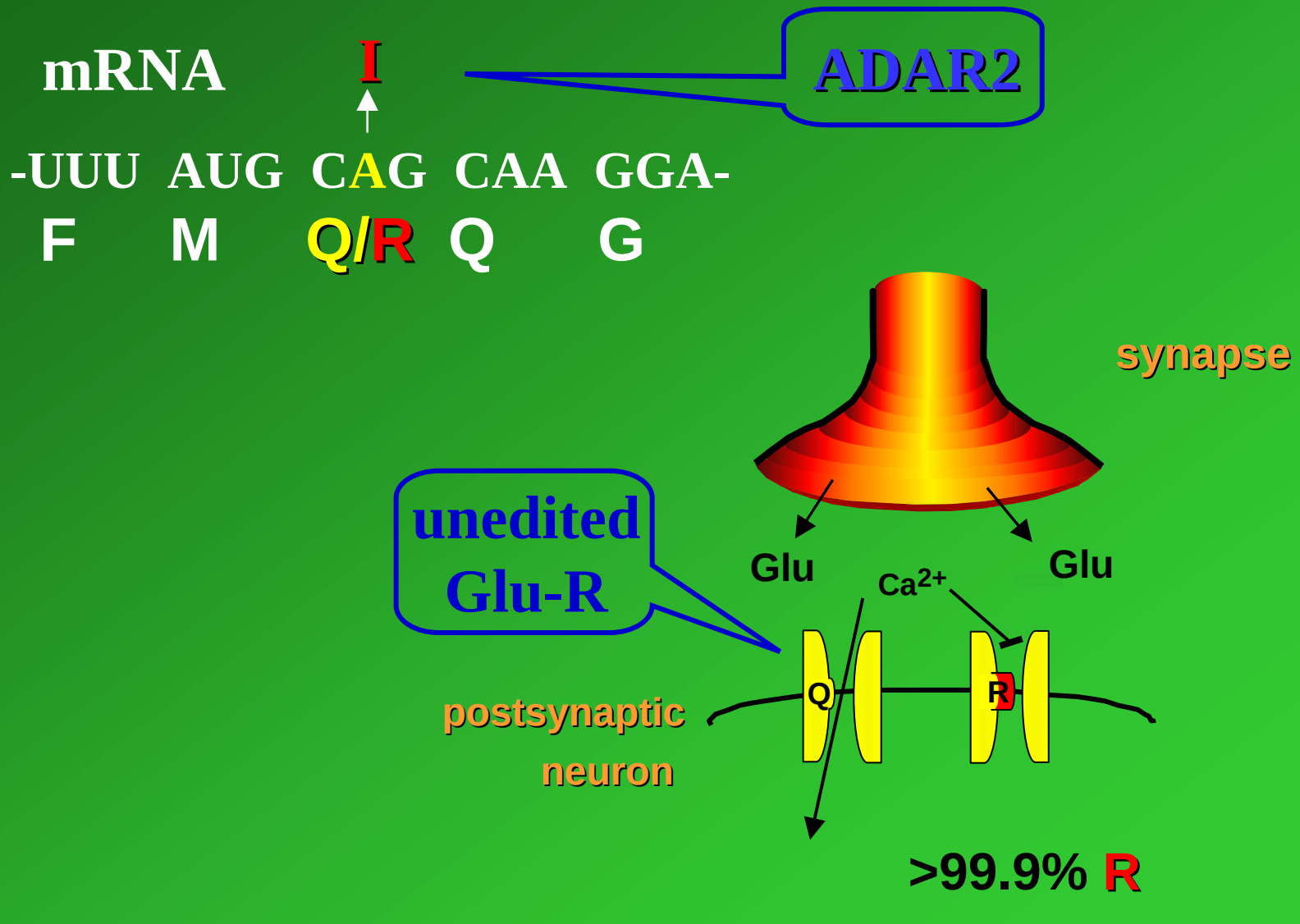
RNA editing of ion channel GluR-B



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RNA editing and cancer

Too little editing:

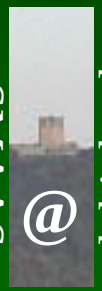
- Malignant growth,
- Activation of oncogenes/repression of tumor suppressor genes

[Glioblastoma (GluR-B)]

Too much editing:

- activation of oncogenes or
- inhibition of tumor suppressor genes

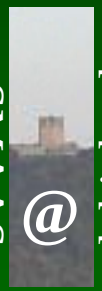
[BC10, leukemia (PTPN6), pancreatic cancer (prox1); hepatoma (APOBEC1), neurofibromatosis (NF1), Wilm's tumor (WT1)]



A-to-I RNA editing and human diseases

- Glioblastoma multiforme => GluR-B underediting due to ADAR2 deficiency
  (Maas et al PNAS 2001)
- Amyotrophic Lateral Sclerosis (ALS) => GluR-B Q/R-site under-editing in motor neurons
  (Kawahara et al Nature 2004)
- Suicidal Depression => Change in Serotonin receptor editing
  (Gurevich et al., Neuron 2002)

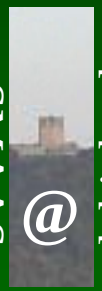
Hyperediting phenotypes: Lupus erythematosis, inflammatory lung disease



Question:

How many genes are subject to RNA editing in the human genome?

➔ Investigate using the complete human genome sequence as an important tool.



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The "Smoking Gun" of A-to-I RNA editing

-TTT ATG CAG CAA GGA- genomic DNA

-UUU AUG CAG CAA GGA- unedited

mRNA

-UUU AUG CIG CAA GGA- edited

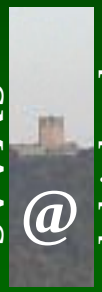
Reverse transcription

-TTT ATG CAG CAA GGA-

\+

-TTT ATG CGG CAA GGA-

cDNA



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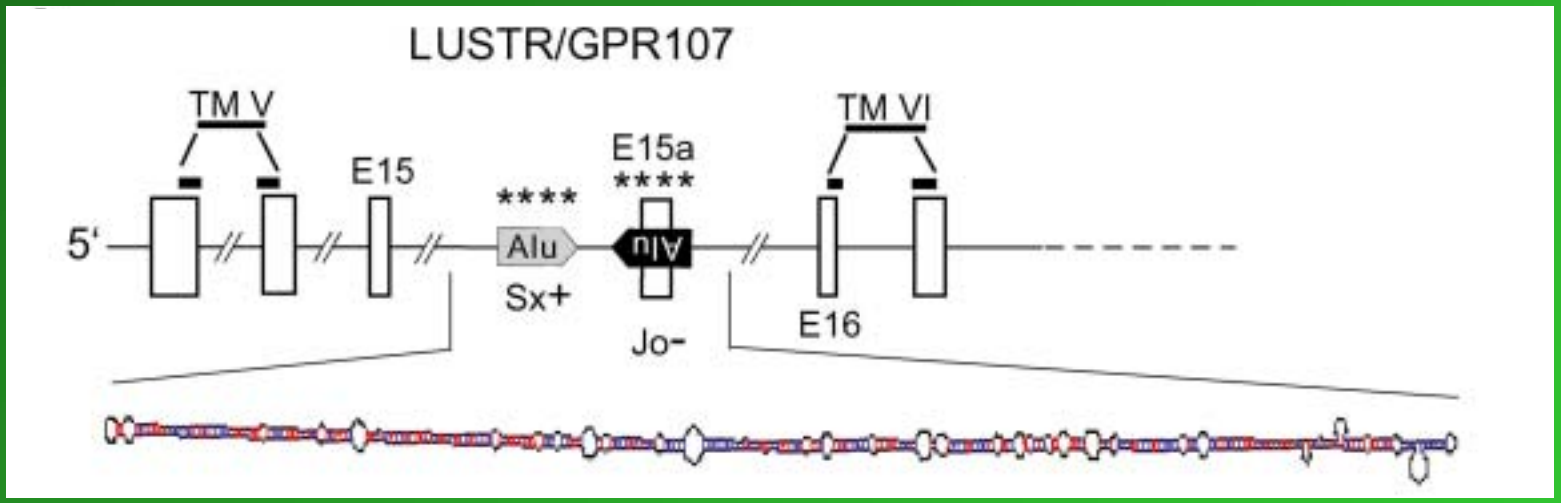
In silico evidence of RNA editing



Athanasiadis et al., *PLoS Biology* 2004

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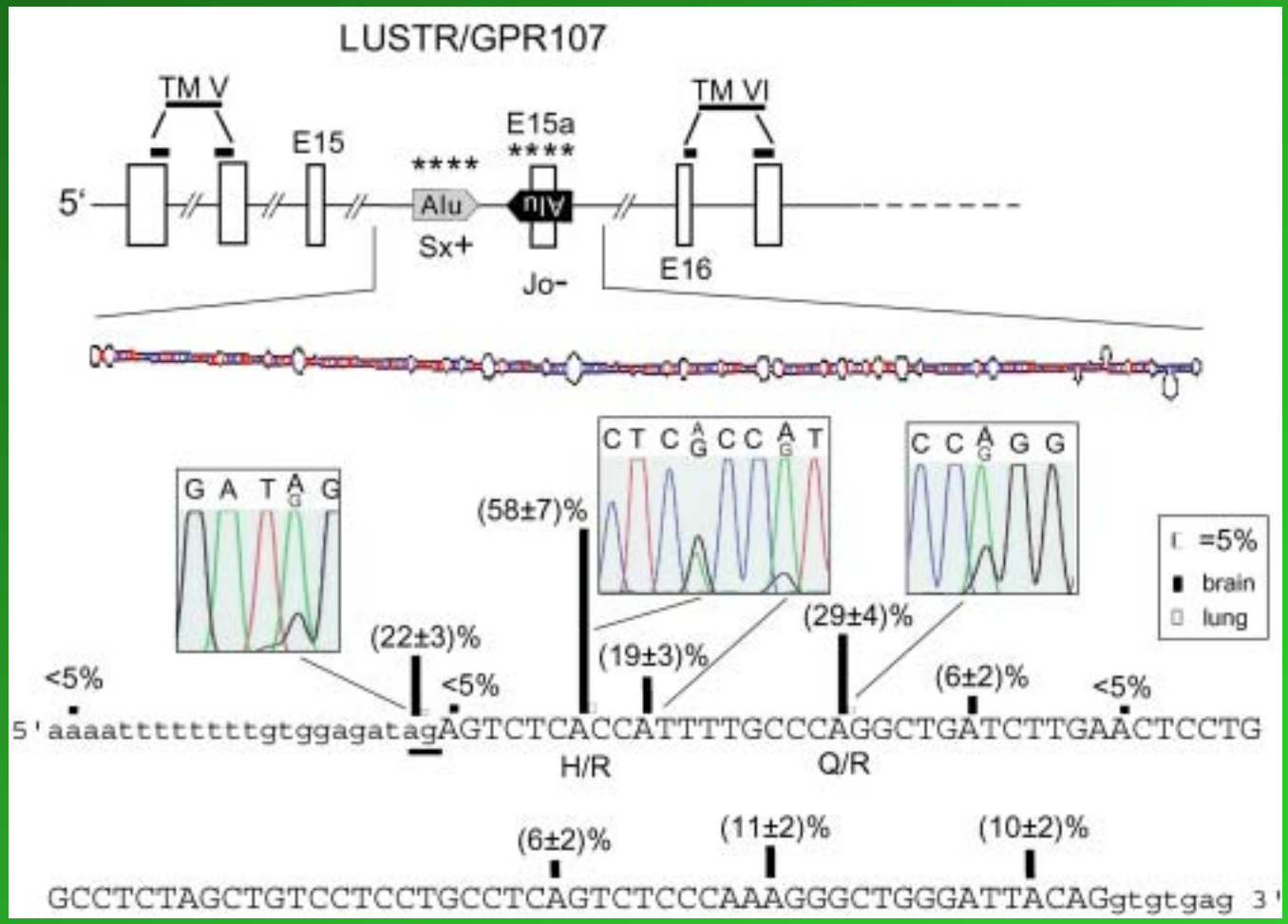
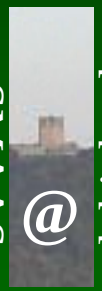
Experimental validation



Athanasiadis et al., *PLoS Biology* 2004

Number of edited sites exceeds predicted numbers



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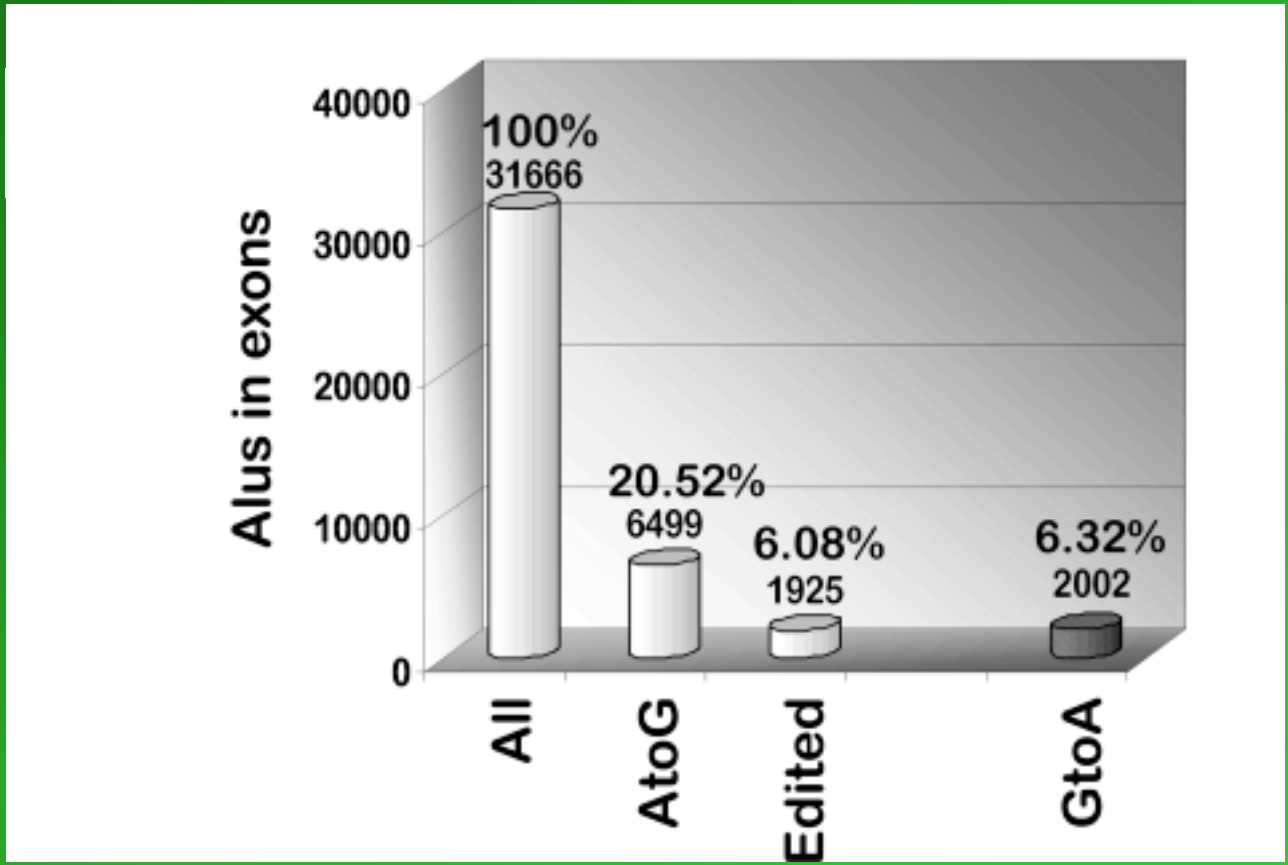
Analysis of 103,723 human cDNA sequences

17, 406 contain one or more Alu



Athanasiadis et al., *PLoS Biology* 2004

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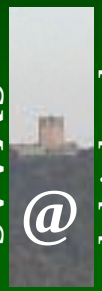
Transposable elements in the human genome

Type	Number of copies	Percentage of total genome
SINEs	1,558,000	13.1
AluI	1,090,000	10.6
LINEs	868,000	20.4
LINE1	516,000	16.9
LTR elements	443,000	8.3
DNA elements	294,000	2.8
mariner	14,000	0.1
Unclassified	3,000	0.1
Total of all types		**44.7**

Source: Data from E. S. Lander et al. 2001. *Nature* 409: 860.



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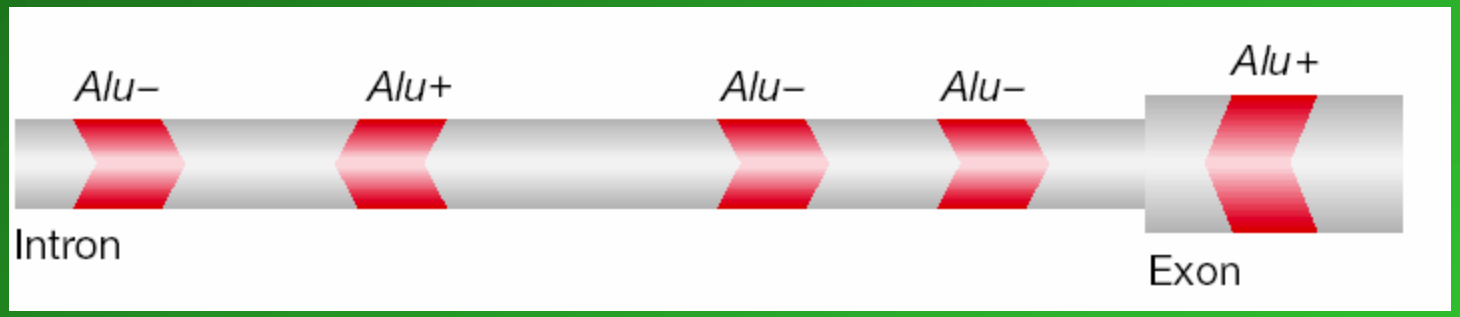
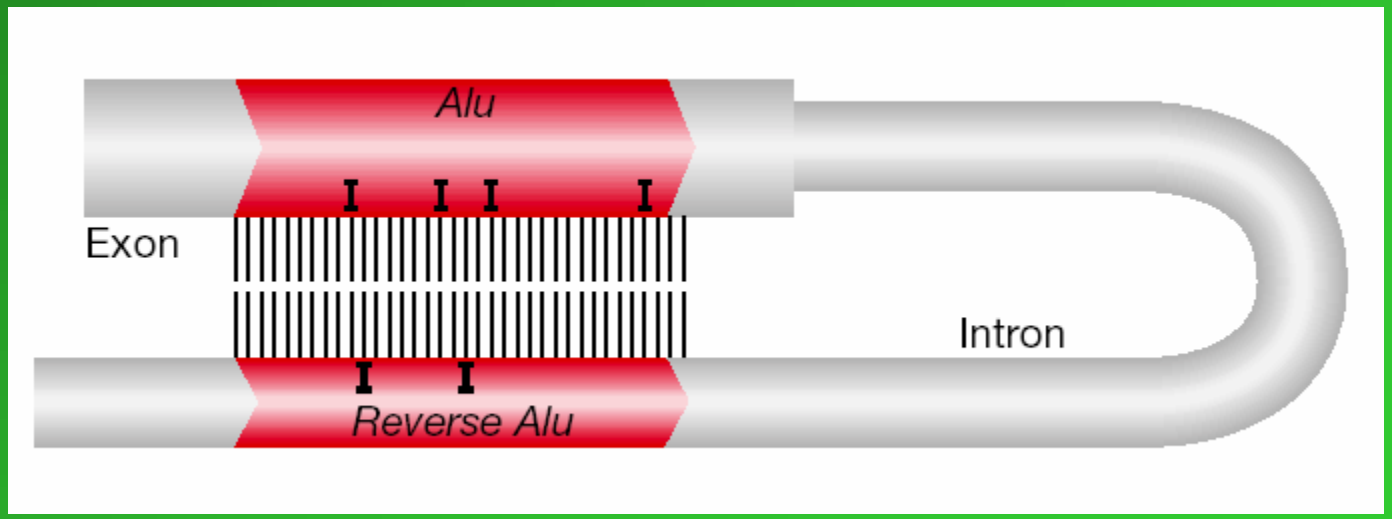
Alu-mediated RNA foldback structures





Levanon et al. EMBO Rep. 2005

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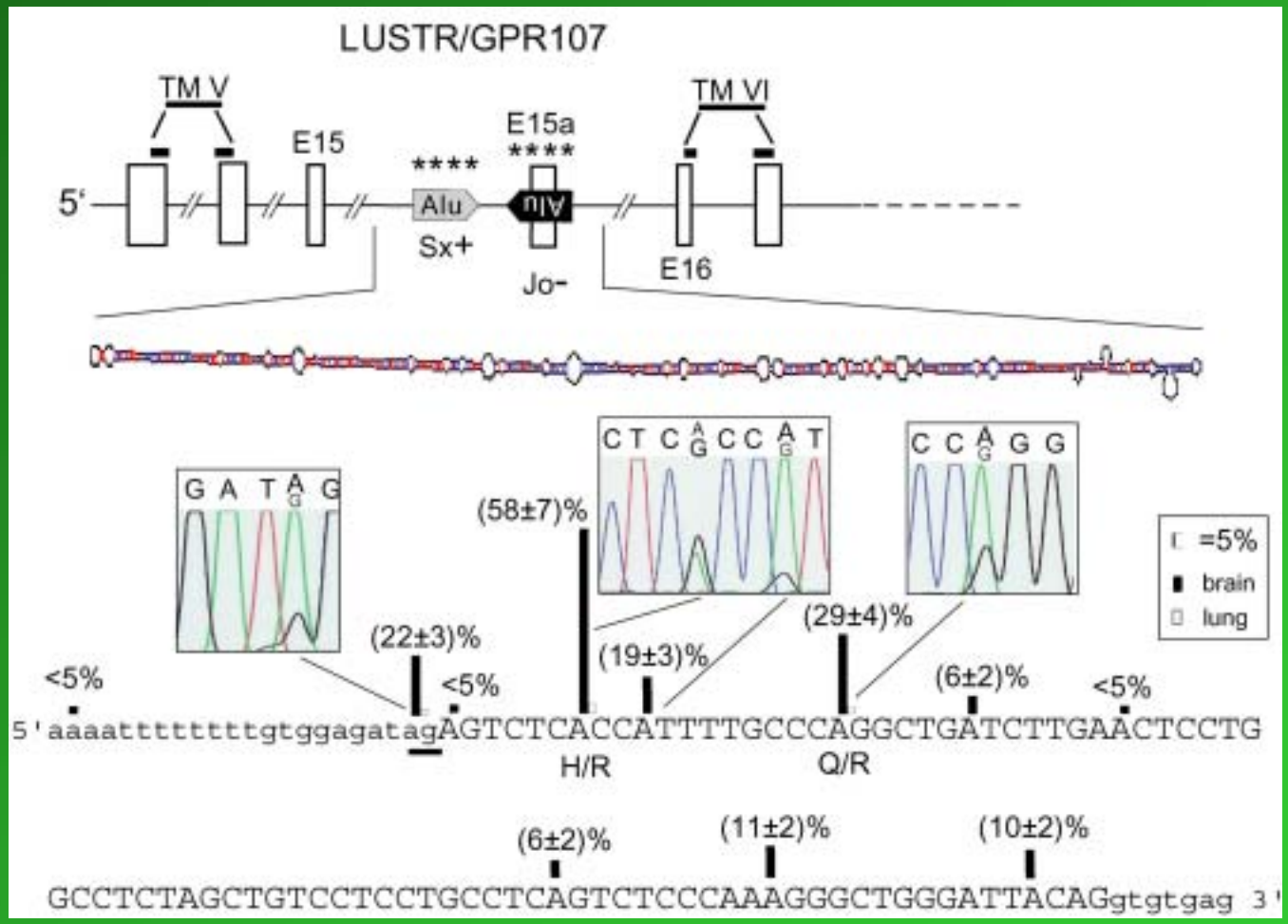
Experimental validation



Athanasiadis et al., *PLoS Biology* 2004

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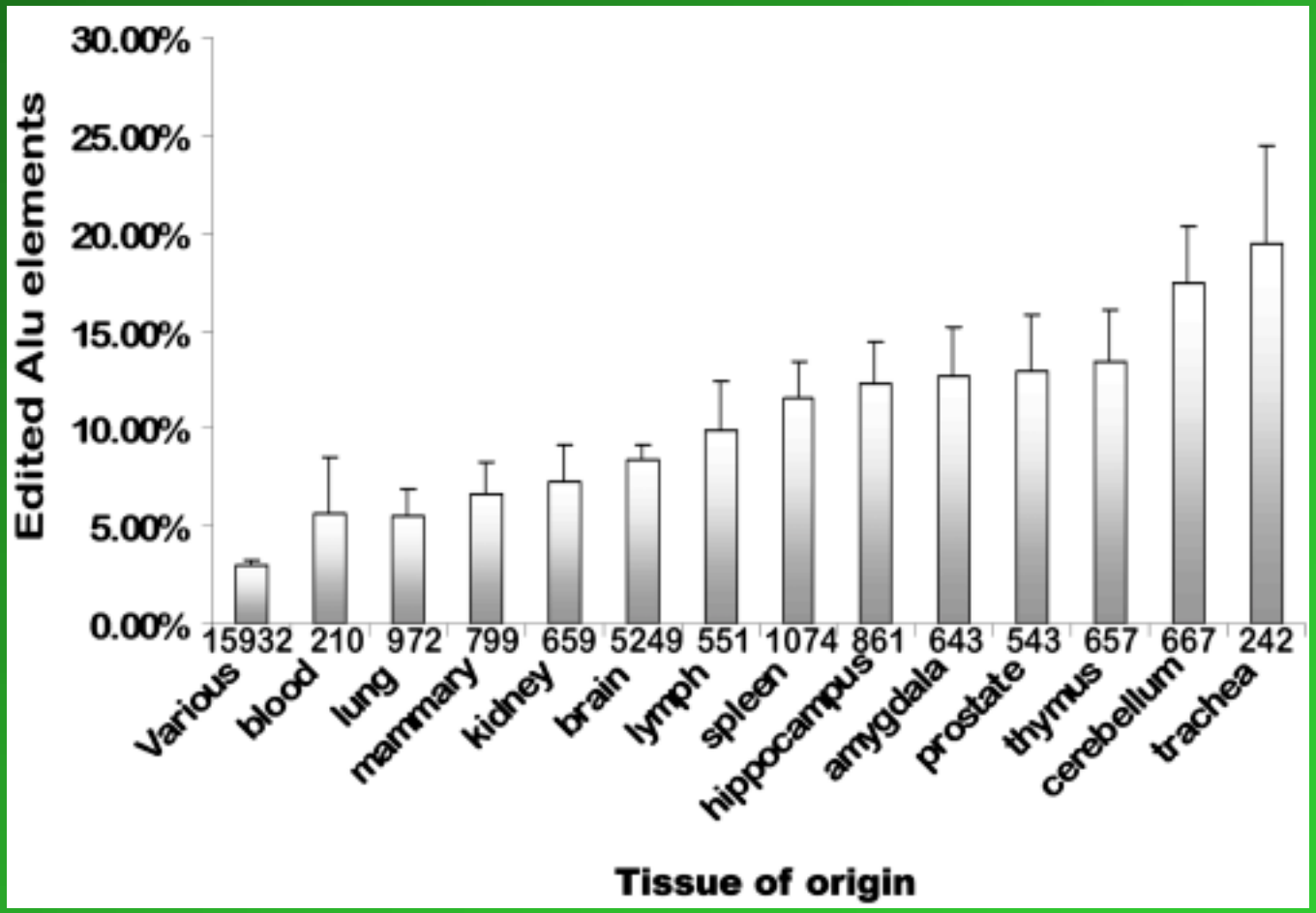
Tissue origin of Alus determines editing extent



Athanasiadis et al., *PLoS Biology* 2004

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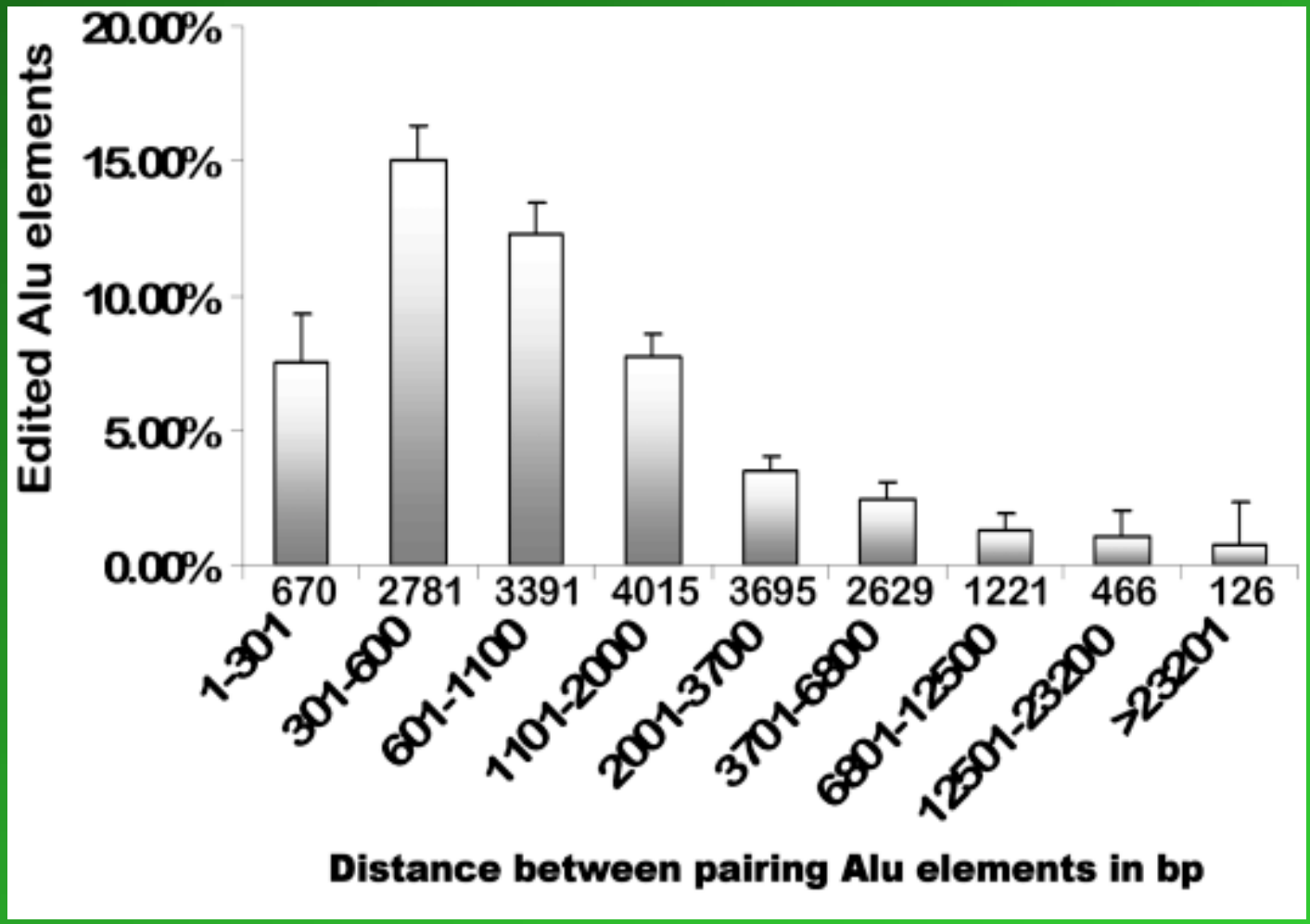
Distance between inverted Alu pairs



Athanasiadis et al., *PLoS Biology* 2004

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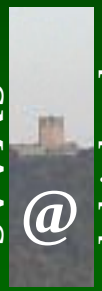
Functions (?)

- None
- Accelerated evolution
- Alu biology
- prevent RNAi
- RNA transport, stability, translation



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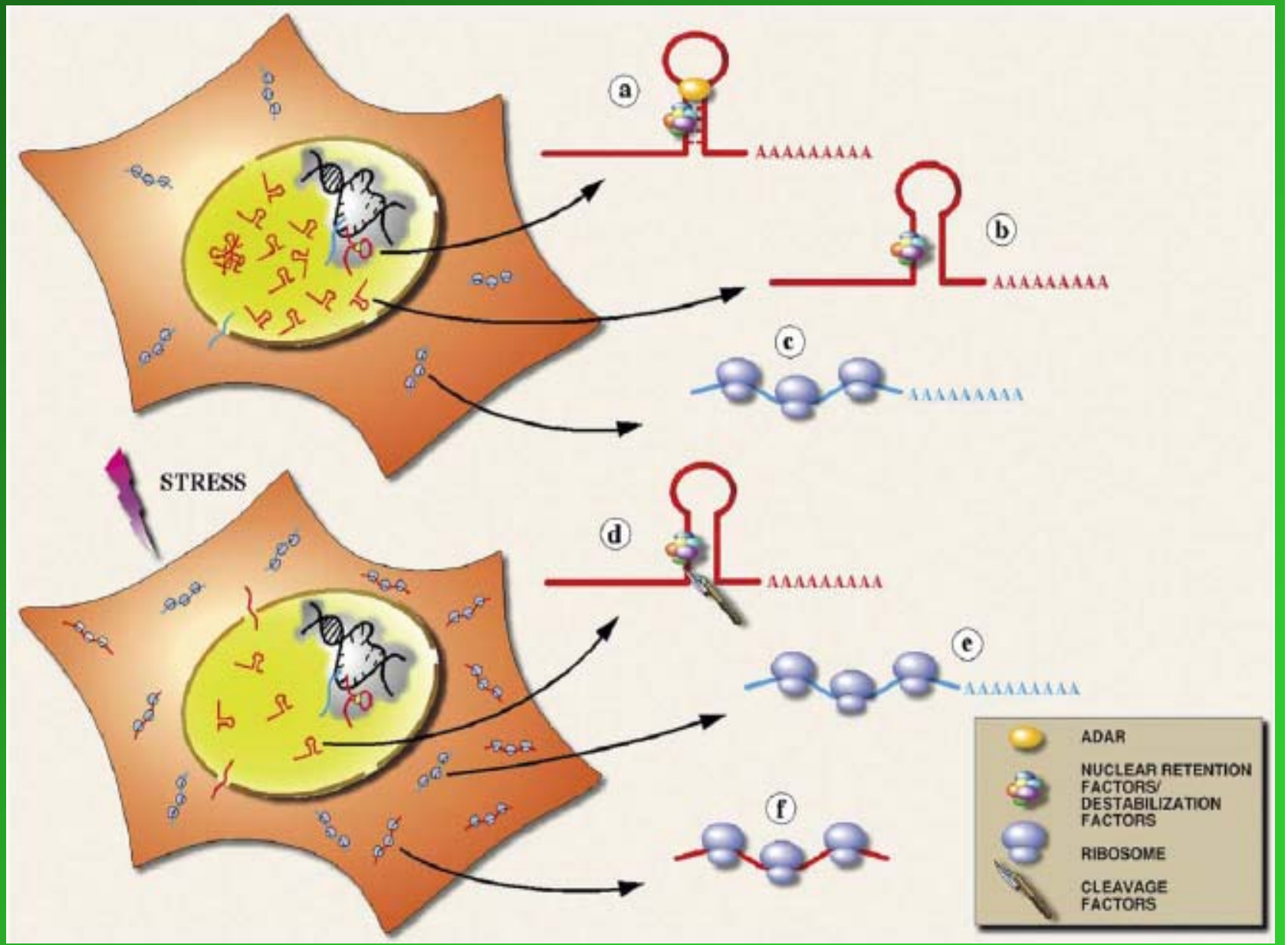

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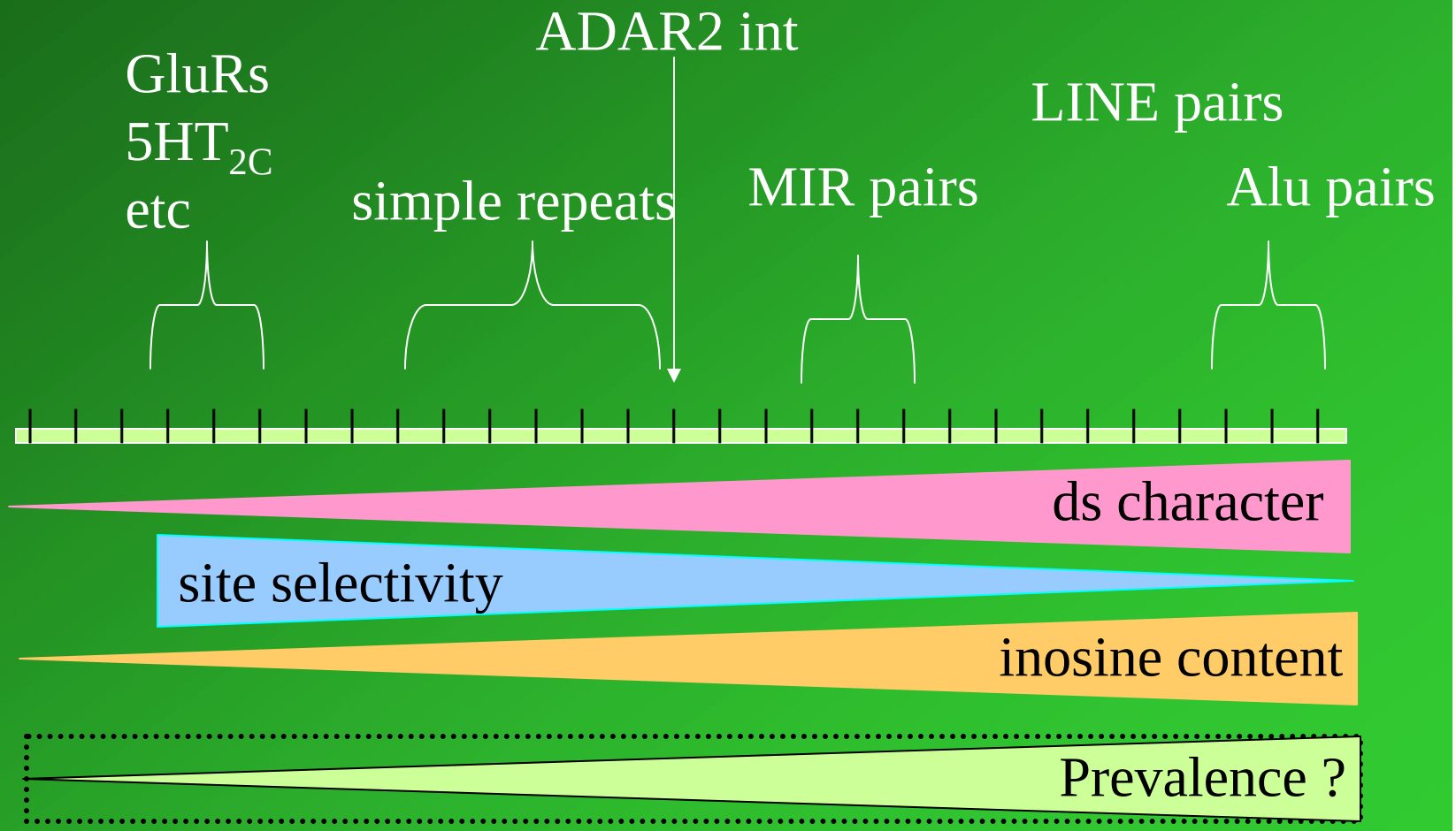
Spectrum of edited human RNAs



Average for each class

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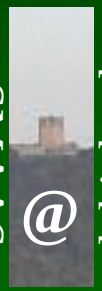
Summary - Conclusions

We do know:

- Complexity is generated by processes of alternative splicing and RNA editing
- RNA editing regulates the function of genes through recoding and probably through other mechanisms
- We can use genomic and transcriptomic sequence information to search for RNA variations

We don't know:

- What is the total impact of RNA editing on complexity ? How is it regulated in vivo ?
- Are there other molecular phenomena that contribute to complexity ?
- What is all the non-coding RNA (and DNA) doing ?



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