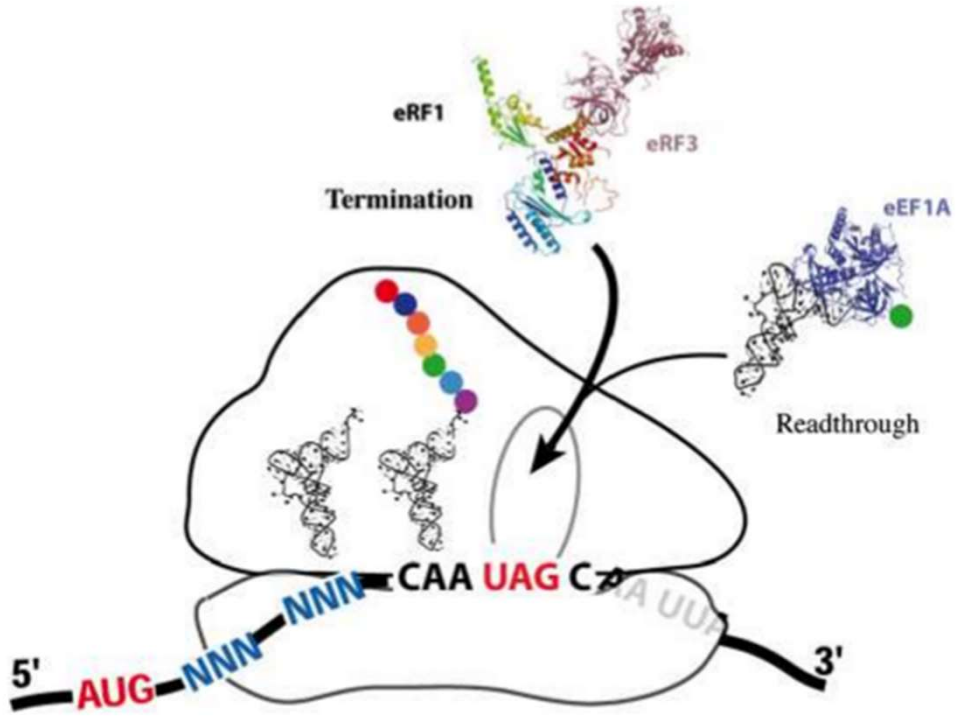
Ribosome readthrough



Starting from the base…PROTEIN SYNTHESIS

Eukaryotic translation can be divided into four stages: Initiation, Elongation, Termination and Recycling

During translation, the ribosome catalyzes the sequential addition of amino acids to a growing polypeptide chain, using an mRNA as template and aminoacyl-tRNAs as substrates

Correct base pairing between the three bases of the codon on mRNA and those of the anticodon of the aa-tRNA dictates the sequence of the polypeptide chain

Translation termination

When the ribosome arrives at a **stop codon**, there is **no corresponding tRNA**. Instead, a **release factor** enters the assembly site and **synthesis is terminated**, releasing the completed polypeptide from the ribosome.

Translation termination

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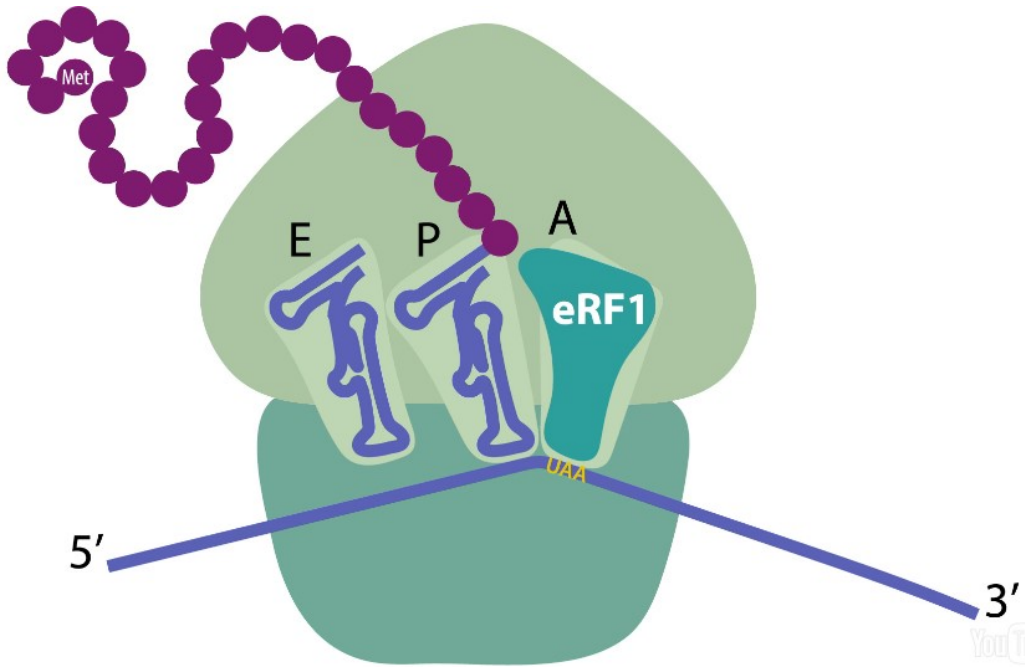
Translation termination in eukaryotes is mediated by two release factors:

- **eRF1** recognizes each of the three stop codons (UAG, UAA, and UGA) and facilitates release of the nascent polypeptide chain
- **eRF3** is a GTP binding protein that facilitate the termination process

Translation termination

1. A complex of eRF1 and eRF3-GTP enters the ribosome
2. The stable interaction between eRF1 and a stop codon in the ribosomal A site stimulates GTP hydrolysis by eRF3 (a not perfect interaction cause the dissociation of the release complex)
3. GTP hydrolysis **activates eRF1** so that it can efficiently stimulate nascent chain release



Translation termination is normally a highly efficient process

BUT occasionally stop codon recognition by eRF1 can be superseded by selected aminoacyl-tRNAs, resulting in **stop codon suppression**

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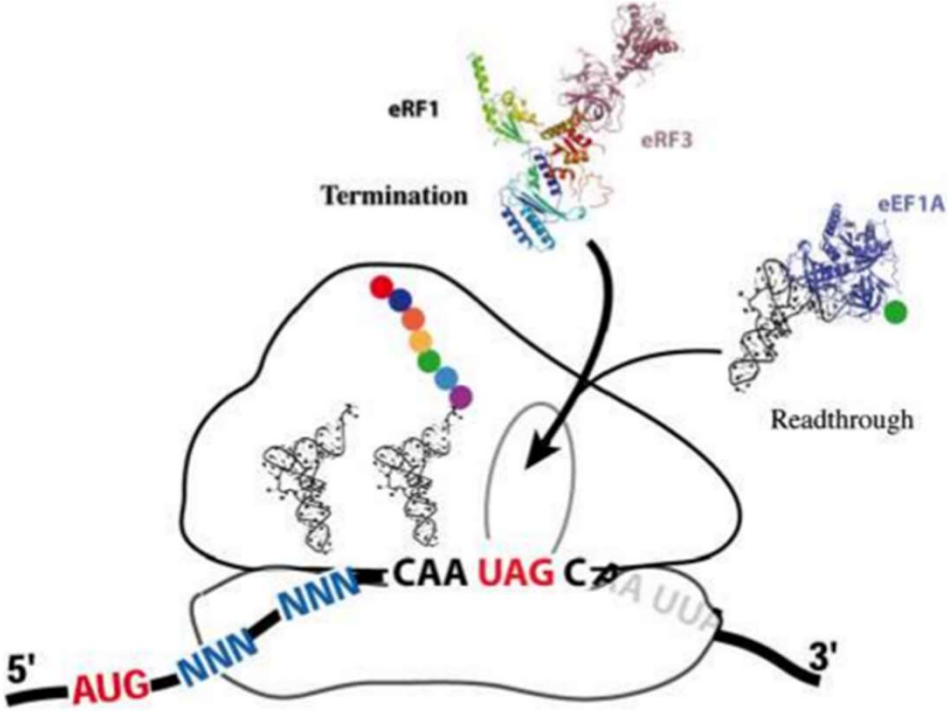


This event is called **READTHROUGH** and corresponds to the **incorporation of a tRNA**, or natural suppressor**, at the stop codon**, allowing translation to continue in the same frame until the ribosome reaches the next stop

The more efficient is translation termination, the less frequent is readthrough (and viceversa)

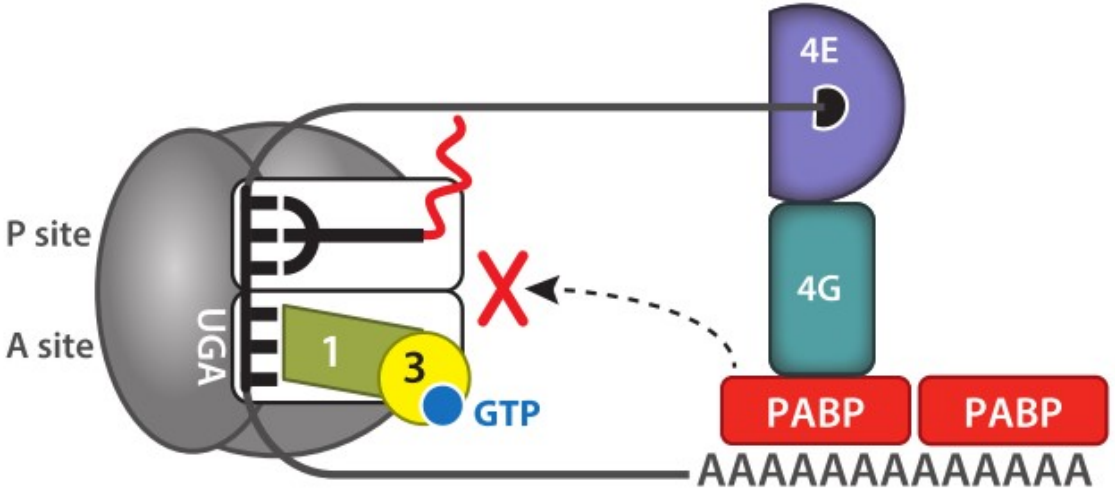
The efficiency of translation termination (and the occurrence of readthrough) can vary depending on many factors

The efficiency of translation termination (and the occurrence of readthrough) can vary depending on:

1) The efficiency of termination differs between normal stop codons and premature termination codons (PTC)

Premature STOP codon

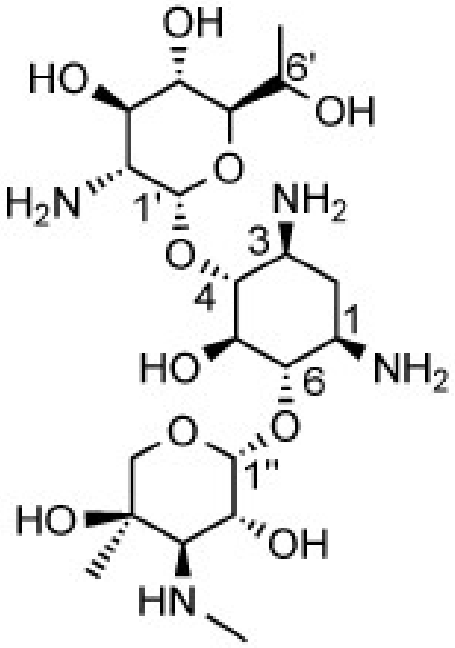
PABP can not interact with eRF3, leading to prolonged ribosomal pausing at PTC and increasing aa-tRNA sampling



Keeling et al. 2014

2) Aminoglycosides can decrease the fidelity of translation, causing higher frequencies of readthrough

Aminoglycosides are a class of antibiotics that interfere with bacterial-protein synthesis. They all have a common 2-deoxystreptamine ring structure, which binds to the ribosome decoding center.



Geneticin (G418)

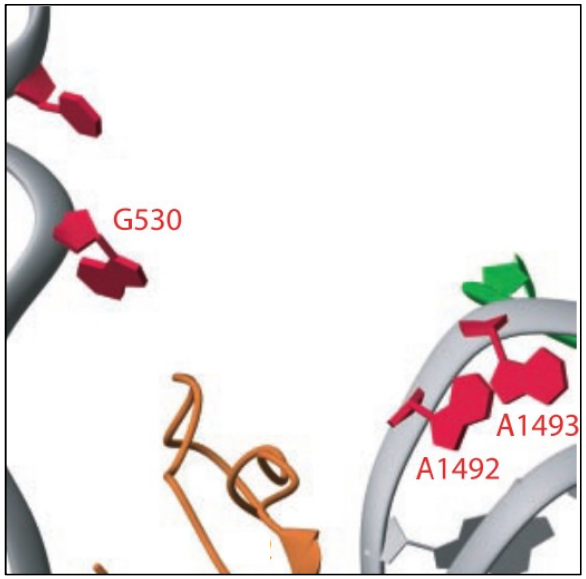
Ribosome is composed by **two subunits**:

- The large subunit contains the peptidyl transferase center, in which peptide bond are formed
- The **small subunit** contains the **decoding center**, a region in which the correct codon-anticodon pairing between mRNA and tRNAs is monitored

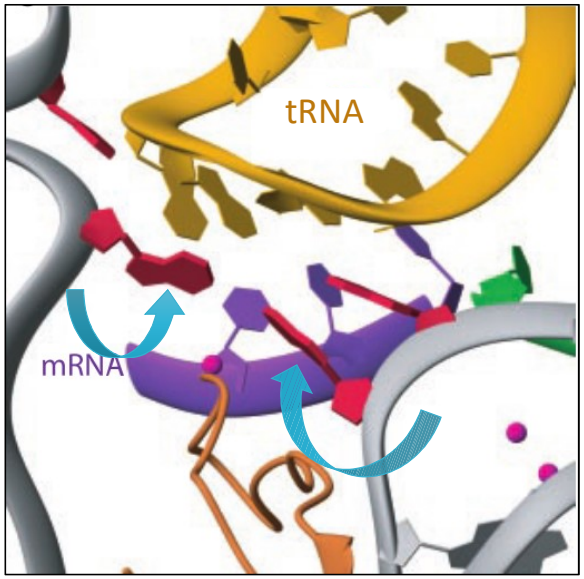
2) Aminoglycosides can decrease the fidelity of translation, causing higher frequencies of readthrough

Mechanism of action: aminoglycosides binding to ribosome decoding site induces a conformational change similar to the transition caused by a tRNA binding

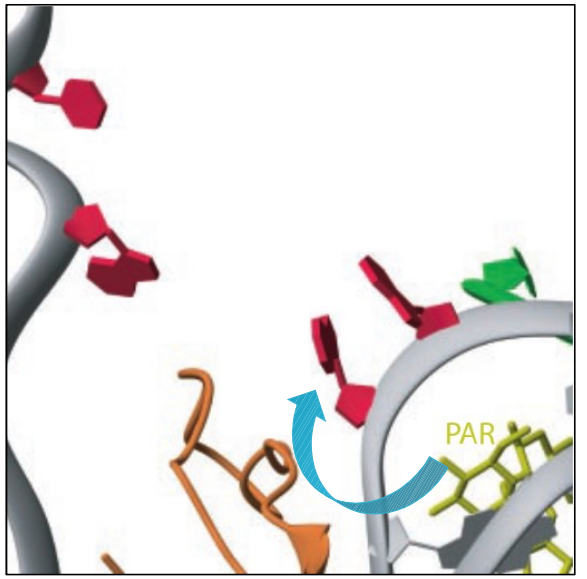
NATIVE STRUCTURE



+ mRNA + tRNA



+ PAROMOMYCIN



Ogle et al. 2001

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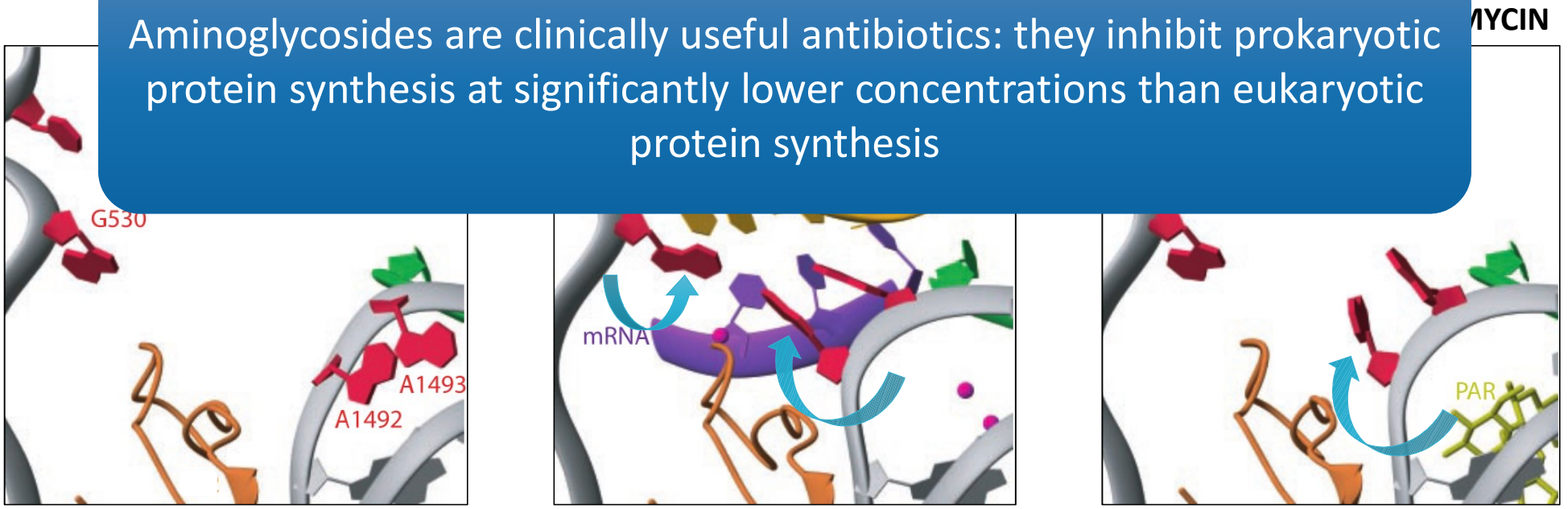
Aminoglycosides are clinically useful antibiotics: they inhibit prokaryotic protein synthesis at significantly lower concentrations than eukaryotic protein synthesis



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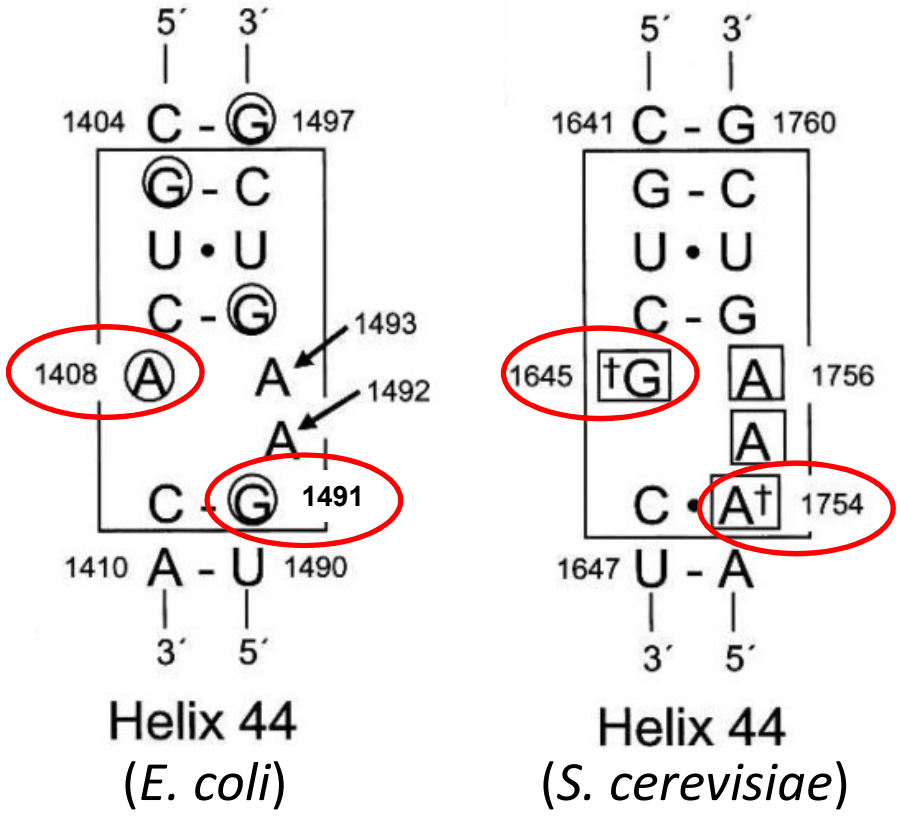
2) Aminoglycosides can decrease the fidelity of translation, causing higher frequencies of readthrough

The major determinants of the **differential** aminoglycoside **sensitivity** between prokaryotes and eukaryotes are **two non conserved residues** of the **decoding center**

A1408 and **G1491** **VS** **G1645** and **A1754**



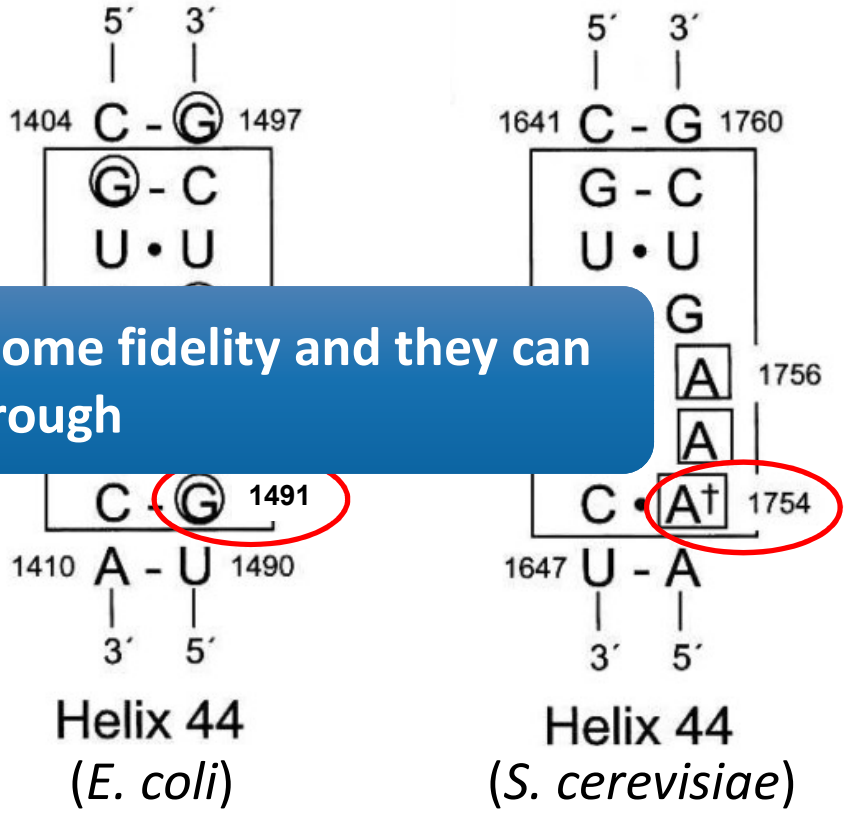
Fan-Minogue et al. 2008

2) Aminoglycosides can decrease the fidelity of translation, causing higher frequencies of readthrough

The major determinants of the **differential** aminoglycoside **sensitivity** between prokaryotes and eukaryotes are **two non con**

In eukaryotes aminoglycosides decrease ribosome fidelity and they can be used to induce readthrough

A1408 and **G1491** **VS** **G1645** and **A1754**



Fan-Minogue et al. 2008

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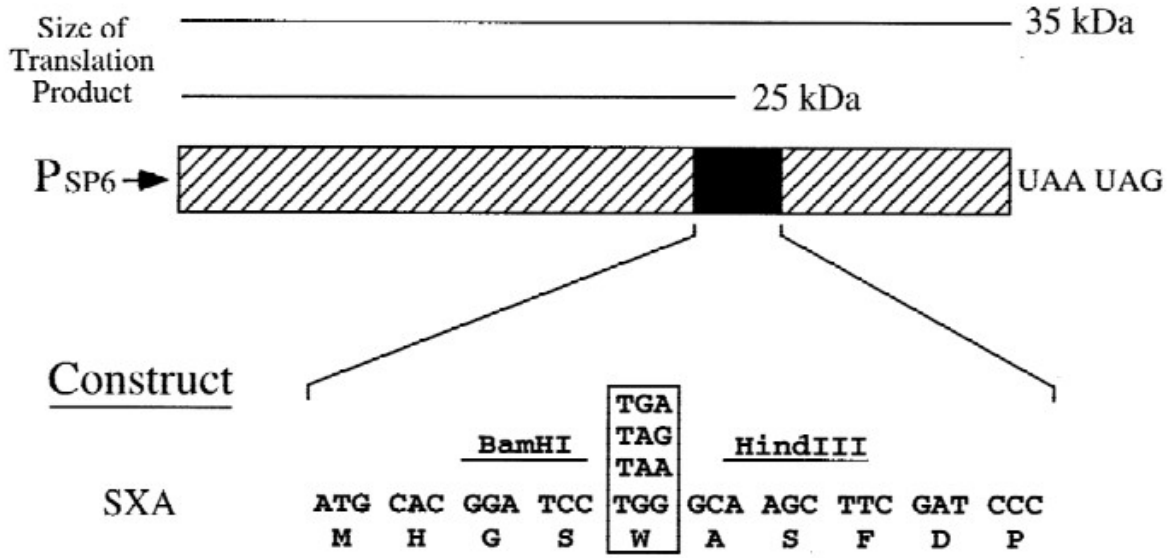
Different stop codons promote translation termination with different efficiencies

Readthrough reporter system:



Manuvakhova et al. 2000

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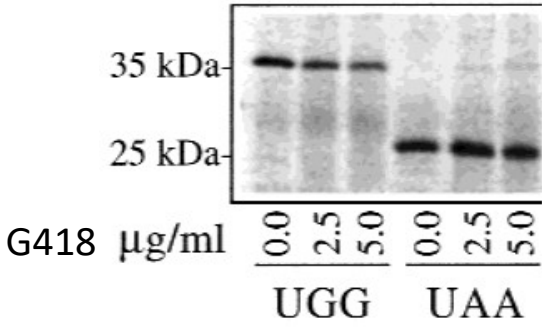
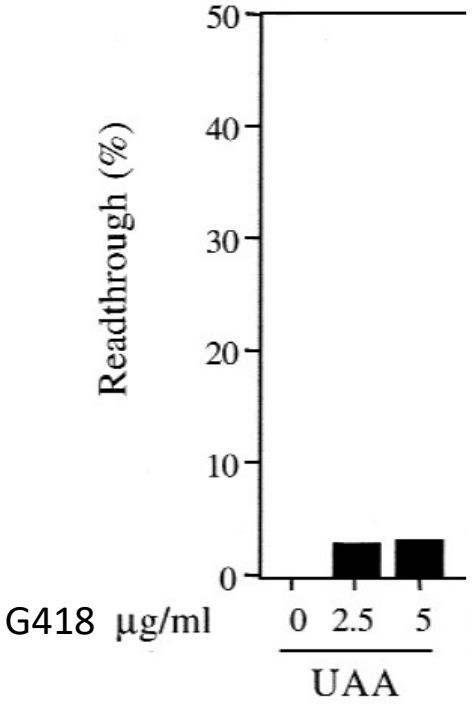
UAA





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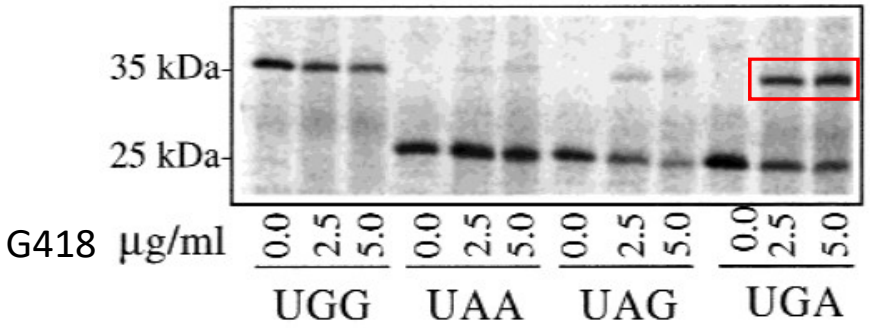
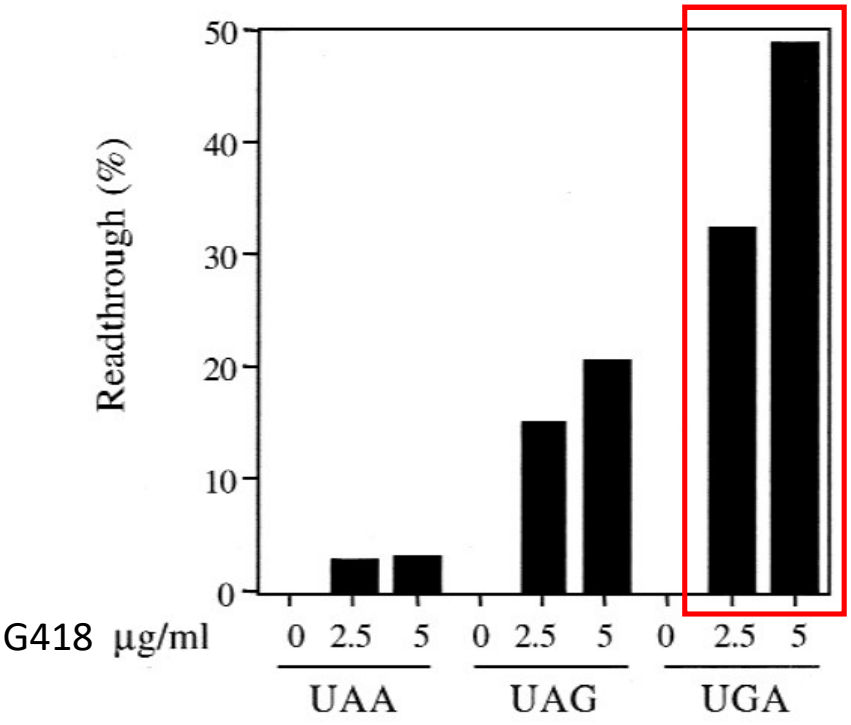
UAA > UAG ≥ UGA





Manuvakhova et al. 2000

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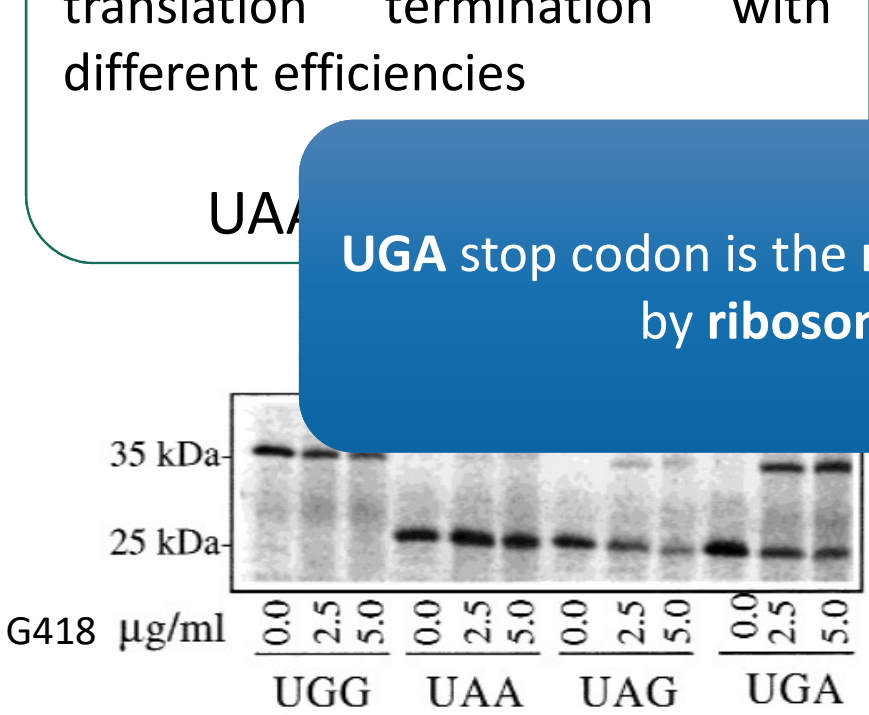
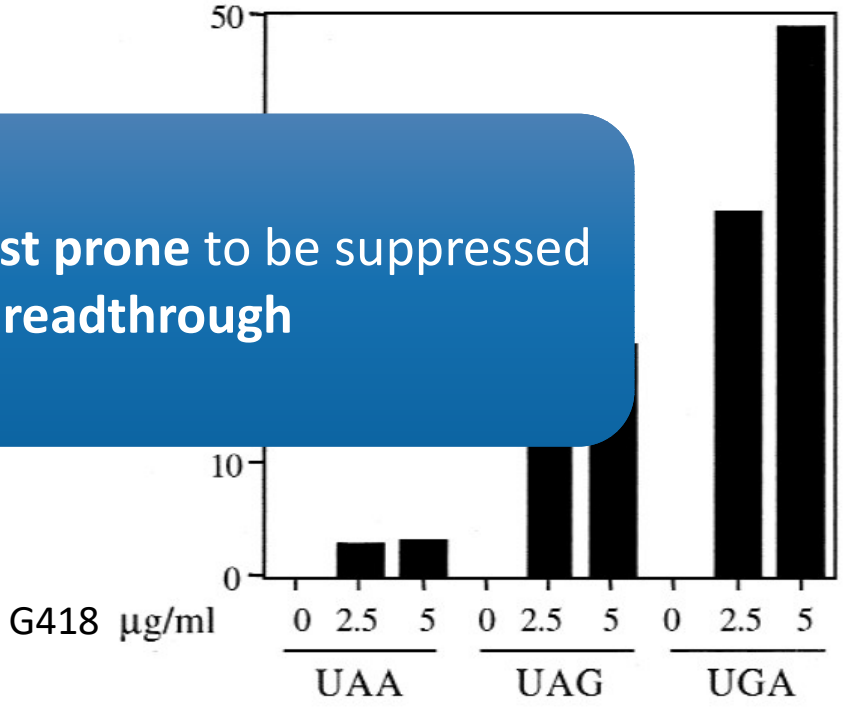
UAA

UGA stop codon is the **most prone** to be suppressed by **ribosome readthrough**





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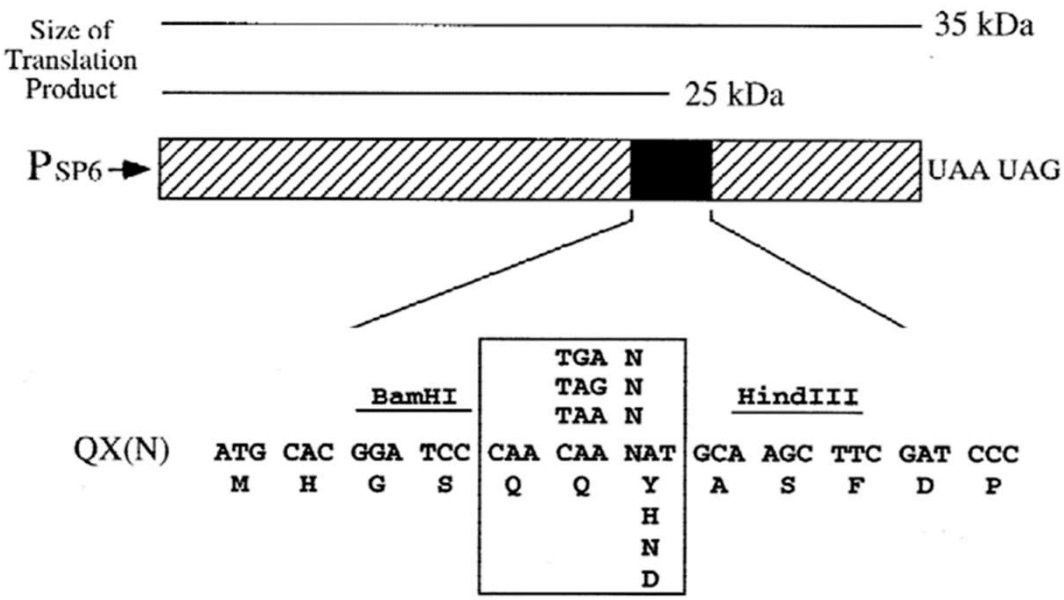
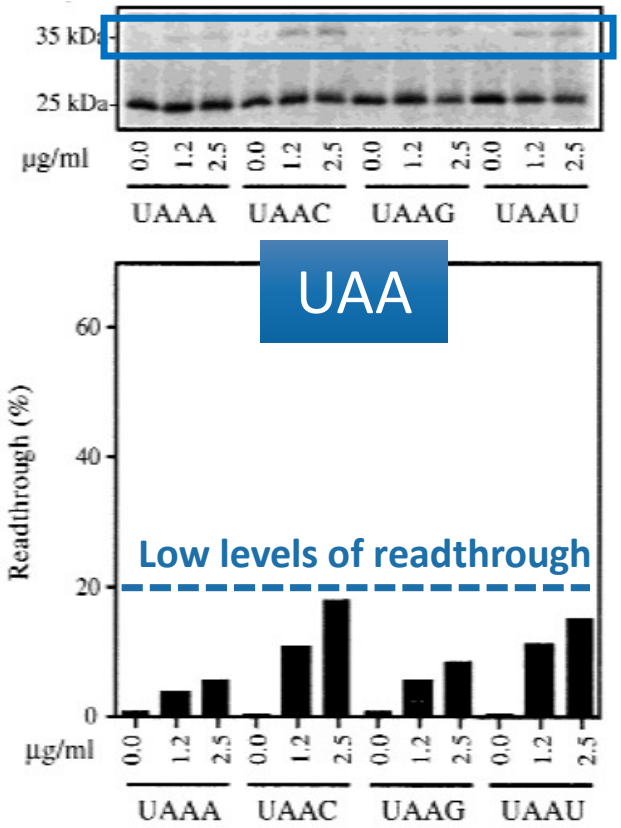


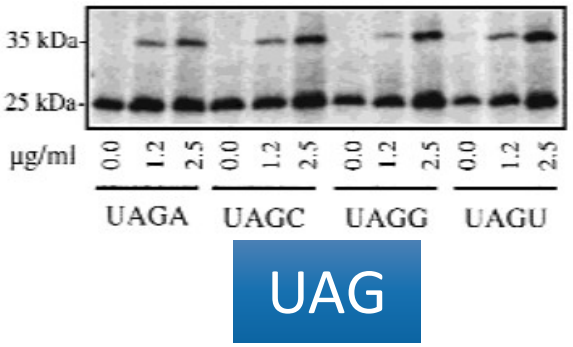
All possible combinations of stop codon and 4th nucleotide

Manuvakhova et al. 2000

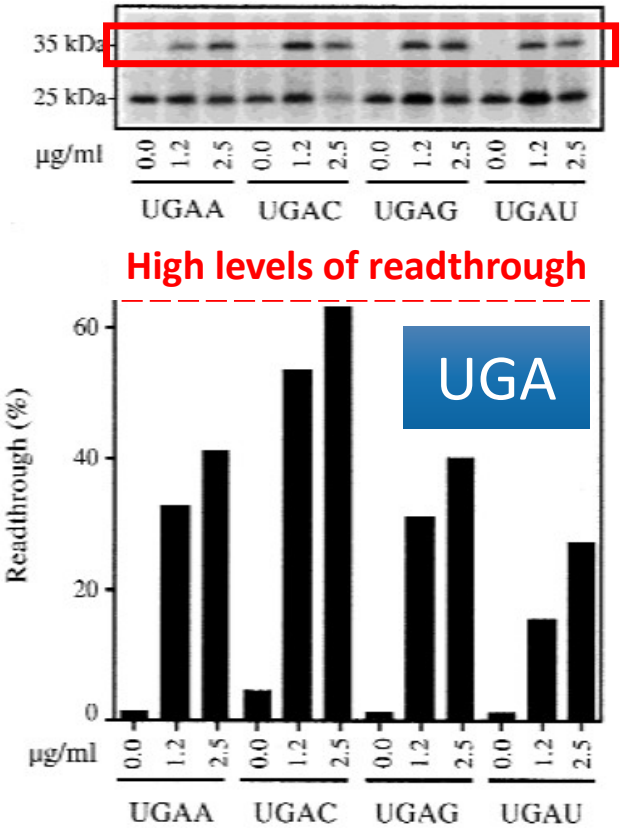
Also the 4th nucleotide strongly influences the occurrence of readthrough

Efficient translation termination





Less efficient translation termination



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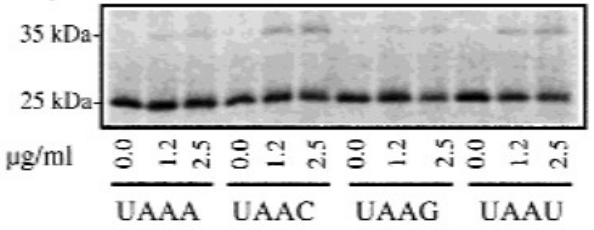
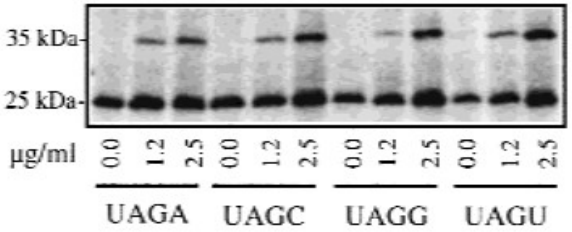
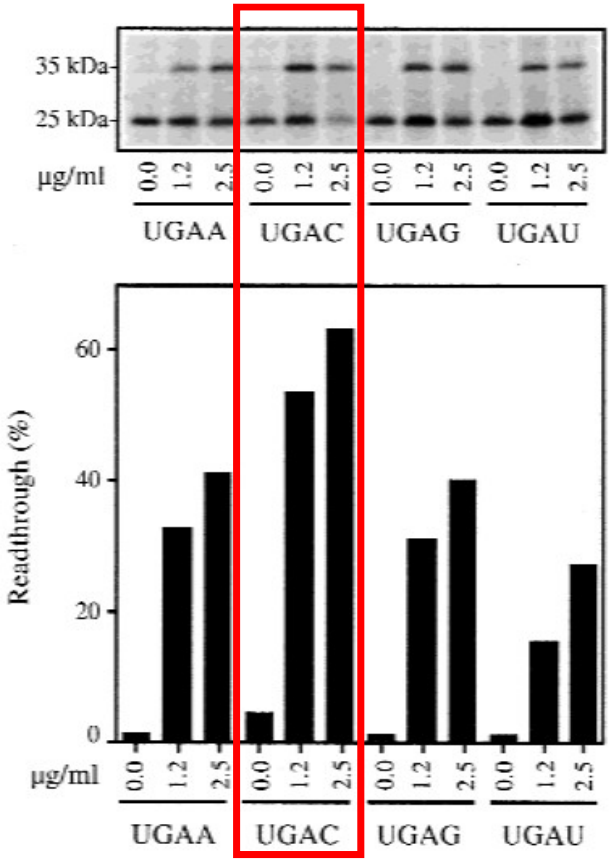




The tetranucleotide **UGAC** shows the most frequent stop codon suppression, with readthrough occurring at a frequency of 3–4% (spontaneous) and 63% (G418-induced)



Manuvakhova et al. 2000

The efficiency of translation termination (and the occurrence of readthrough) can vary depending on many factors

1) The efficiency of termination differs between normal stop codons and premature termination codons
2) Aminoglycosides can decrease the fidelity of translation, causing higher frequencies of readthrough
3) The stop codon type and the 4^{th} nucleotide strongly influence efficiency of translation termination and, as a consequence, occurrence of readthrough

The restoration of protein activity depends strongly on the identity of the amino acid inserted during PTC readthrough

Published online 23 July 2014 *Nucleic Acids Research, 2014, Vol. 42, No. 15* ***10061–10072***
doi: 10.1093/nar/gku663

New insights into the incorporation of natural suppressor tRNAs at stop codons in *Saccharomyces cerevisiae*

Sandra Blanchet[1], David Cornu[2], Manuela Argentini[2] and Olivier Namy[1,3,*]

Development of an *in vivo* reporter system to study amino acid insertion at all stop codons

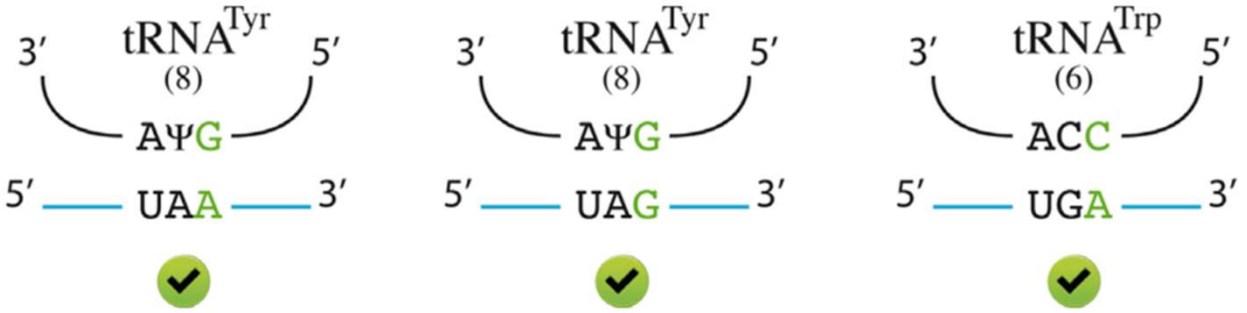
The restoration of protein activity depends strongly on the identity of the amino acid inserted during PTC readthrough

Results

- The main determinant of amino acid incorporation is the sequence of the stop codon
- Only a subset of predictable suppressor tRNAs are actually incorporated at the various stop codons

Tyrosine, **glutamine** and **lysine** can be inserted at **UAA** and **UAG** codons

Tryptophan, **cysteine** and **arginine** can be inserted at **UGA** codons



Near-cognate tRNAs: different in only one position of the codon-anticodon pairing

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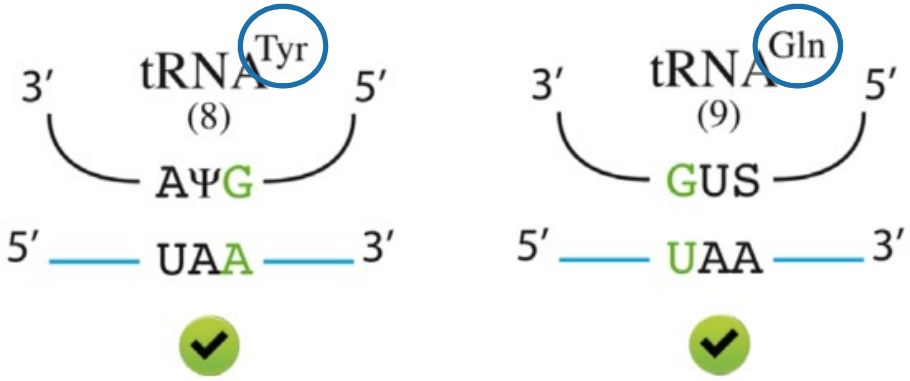
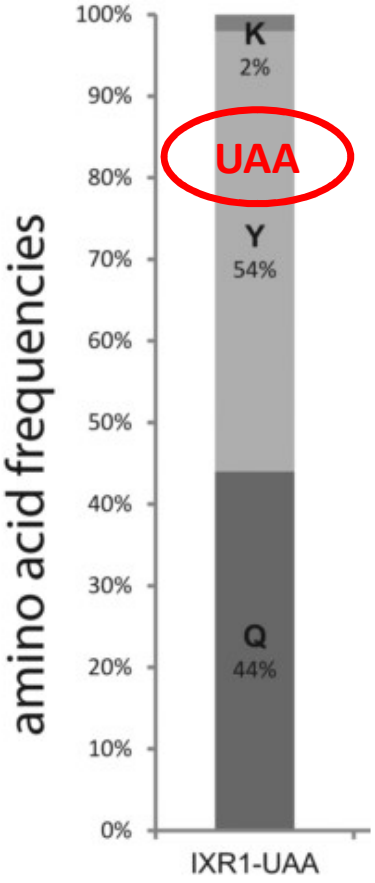
- Suppressor tRNAs are not incorporated at the same frequency at each stop codon





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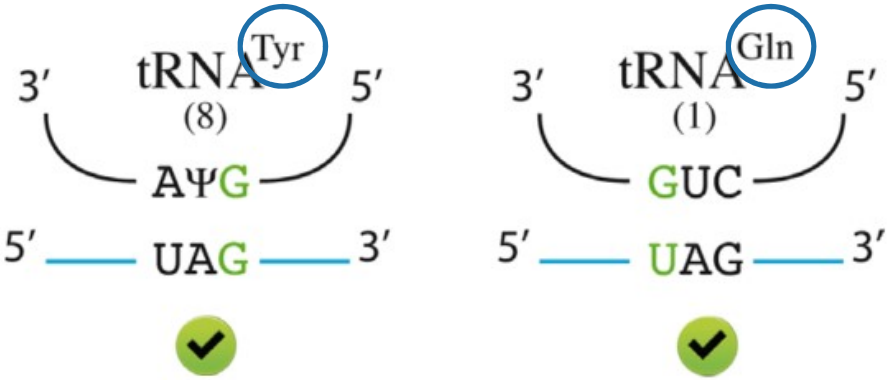

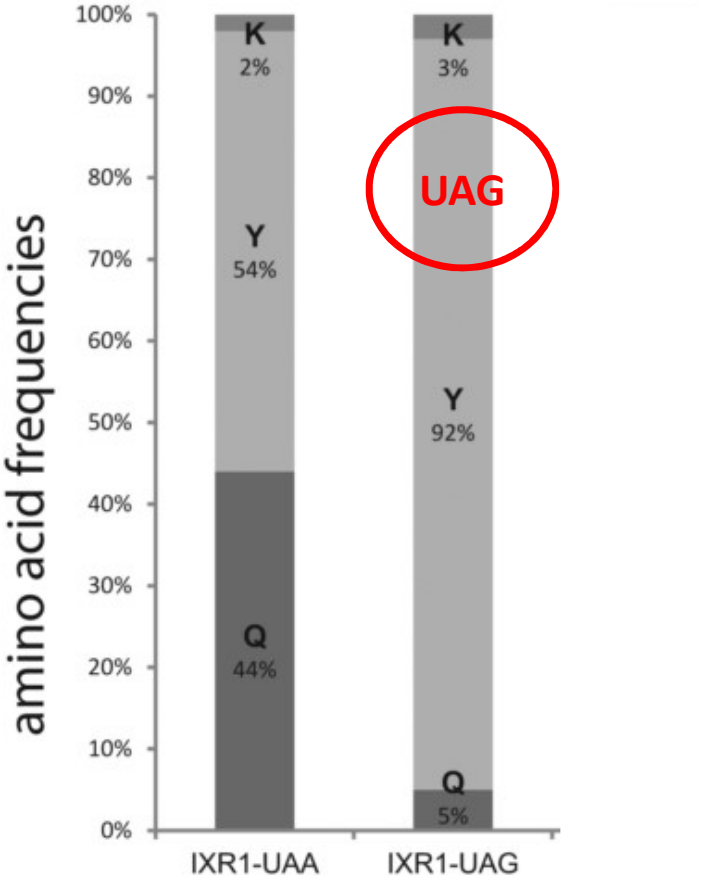

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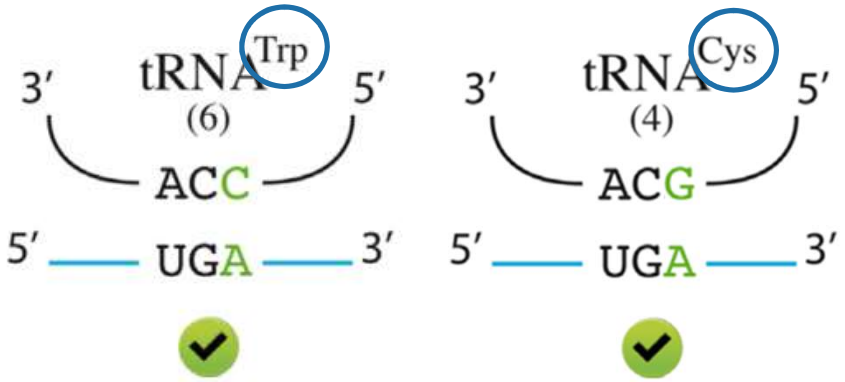
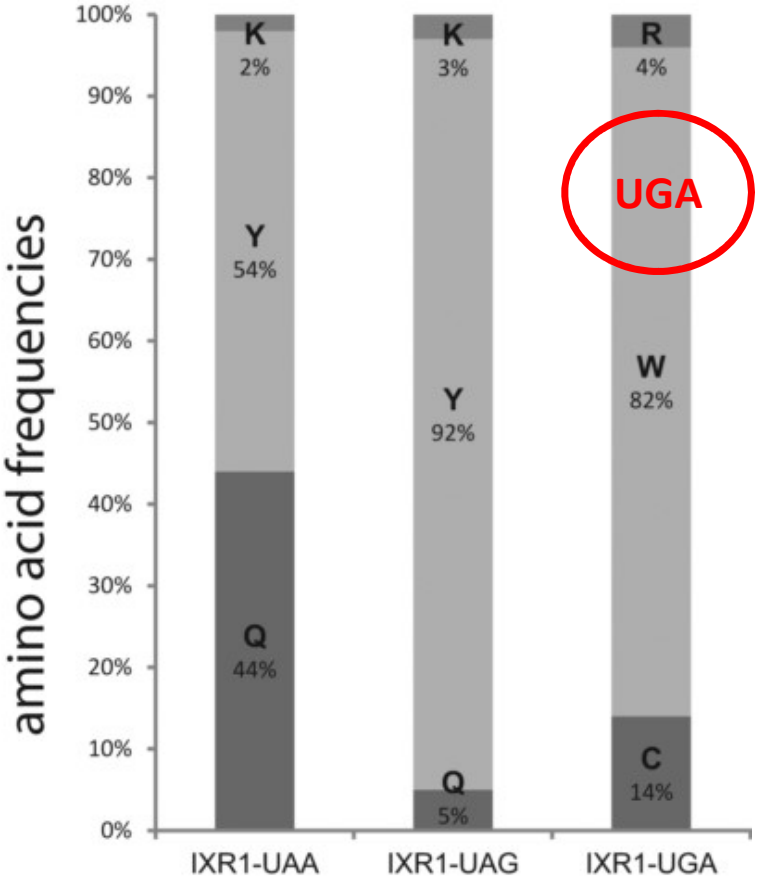
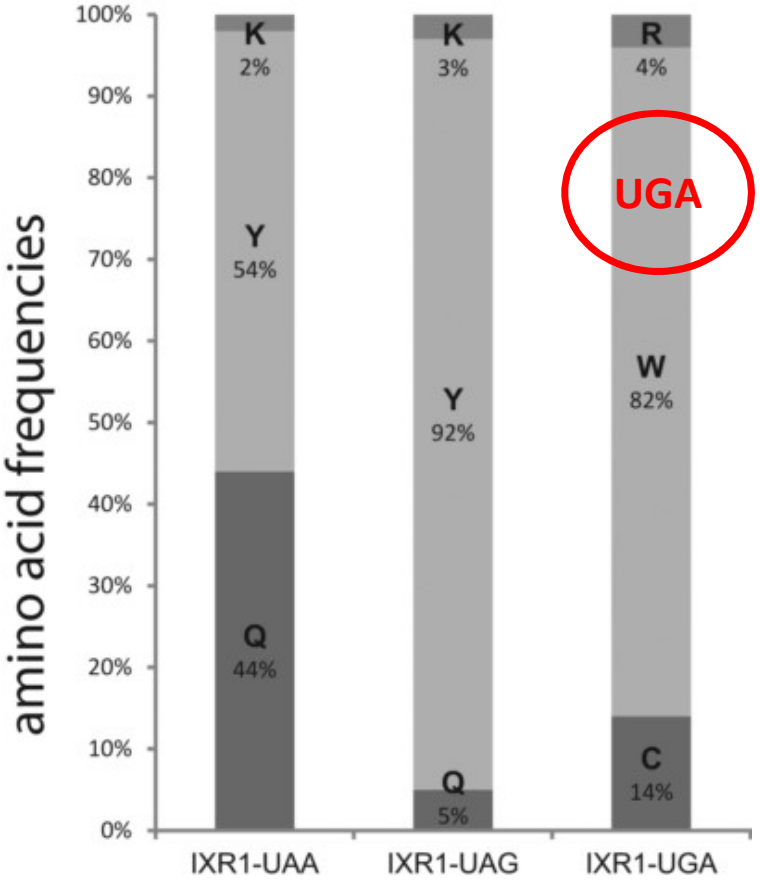




The restoration of protein activity depends strongly on the identity of the amino acid inserted during PTC readthrough

- Suppressor tRNAs are not incorporated at the same frequency at each stop codon

It is possible to predict the probable protein sequences arising from a readthrough event on the basis of the stop codon present



In 1996 PTC suppression was first described as a potential therapy for diseases caused by nonsense mutations

Since that, approximately 100 studies have investigated the effectiveness of nonsense suppression as a possible treatment for nearly 40 different diseases

Journal of Thrombosis and Haemostasis, **14**: 1994–2000 DOI: 10.1111/jth.13443

BRIEF REPORT

Differential functional readthrough over homozygous nonsense mutations contributes to the bleeding phenotype in coagulation factor VII deficiency

A. BRANCHINI,*† M. FERRARESE,* S. LOMBARDI,* R. MARI,‡ F. BERNARDI*† and M. PINOTTI*†

Objective

Evaluate the spontaneous and drug-induced readthrough levels of two nonsense mutations in coagulation factor VII (FVII) : **p.Ser112X** and **p.Cys132X**

Expected to be lethal

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Life-threatening bleeding symptoms

Moderate bleeding symptoms

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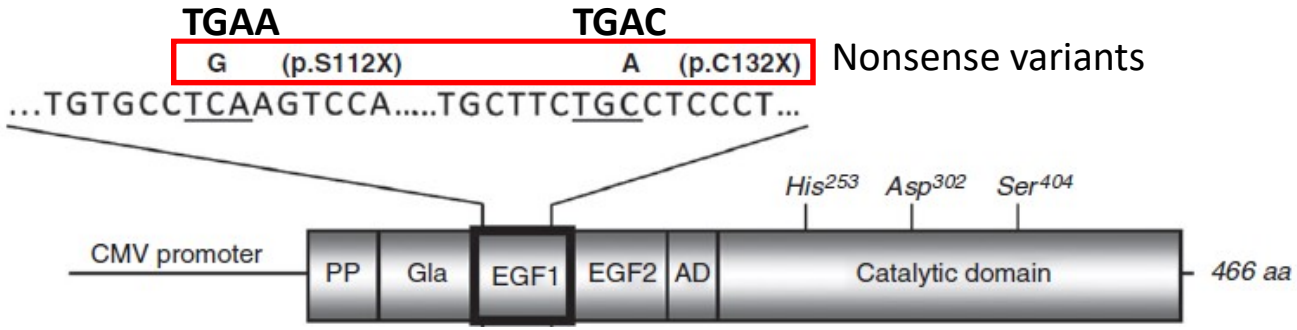
Methods

1. Creation of a cellular model through the transient expression of recombinant FVII **nonsense** variants

2. Evaluation of secreted levels of rFVII by ELISA



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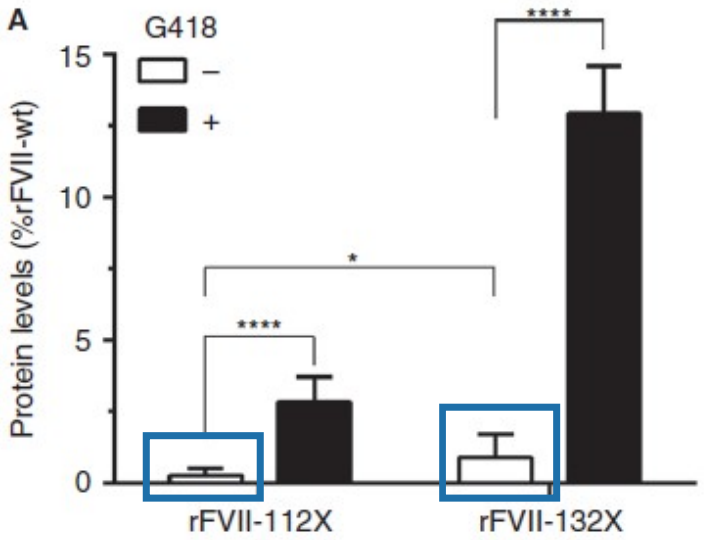
Secretion levels of rFVII nonsense variants



SPONTANEOUS

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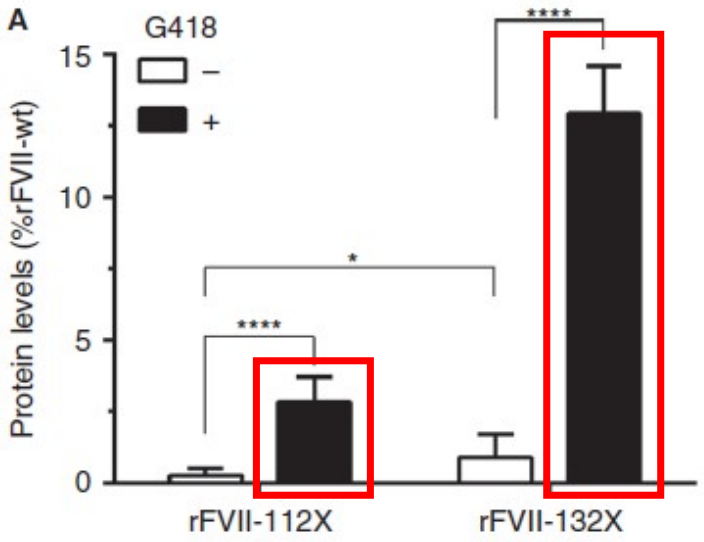

G418-induced

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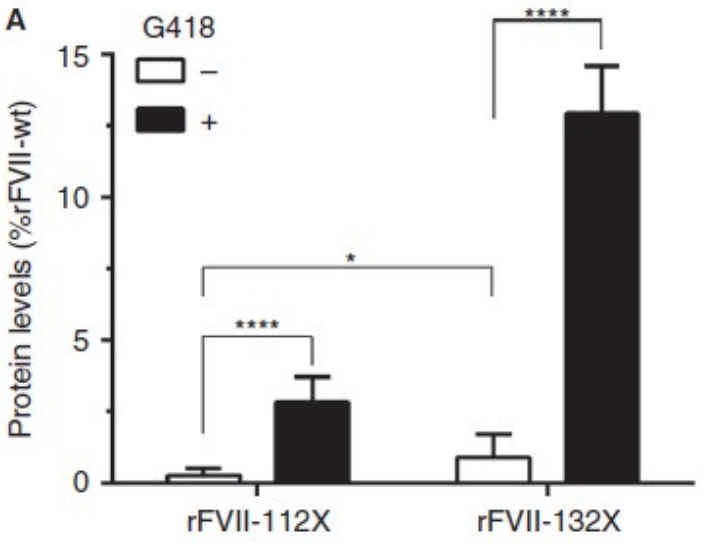
Secretion and activity levels of rFVII nonsense variants

rFVII C132X shows the higher degree of suppression, probably due to the more readthrough-favourable sequence context



TGA A **TGA C**

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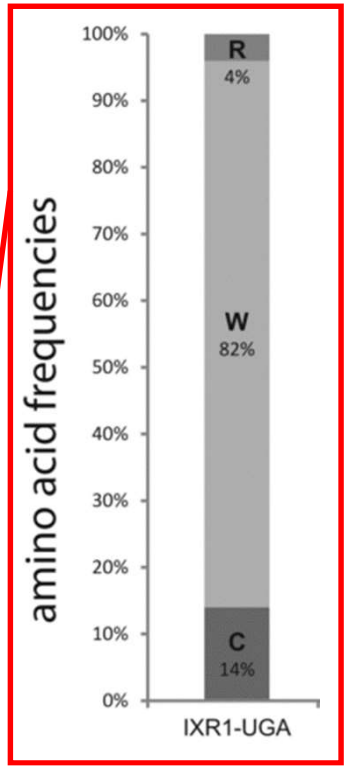
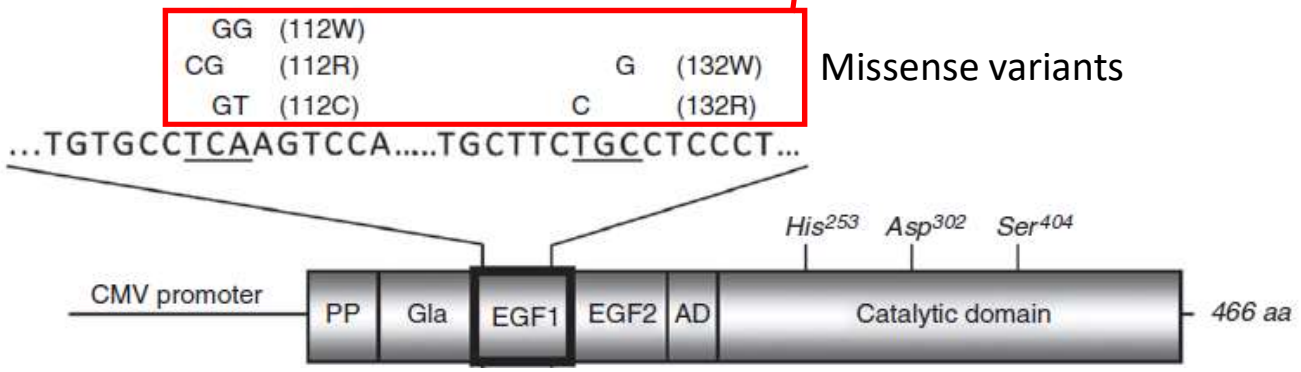
1. Creation of a cellular model through the transient expression of recombinant FVII **missense** variants

2. Evaluation of rFVII protein and **functional** levels by ELISA and activity assays





Missense variants

Journal of Thrombosis and Haemostasis, **14**: 1994–2000 DOI: 10.1111/jth.13443

BRIEF REPORT

Differential functional readthrough over homozygous nonsense mutations contributes to the bleeding phenotype in coagulation factor VII deficiency

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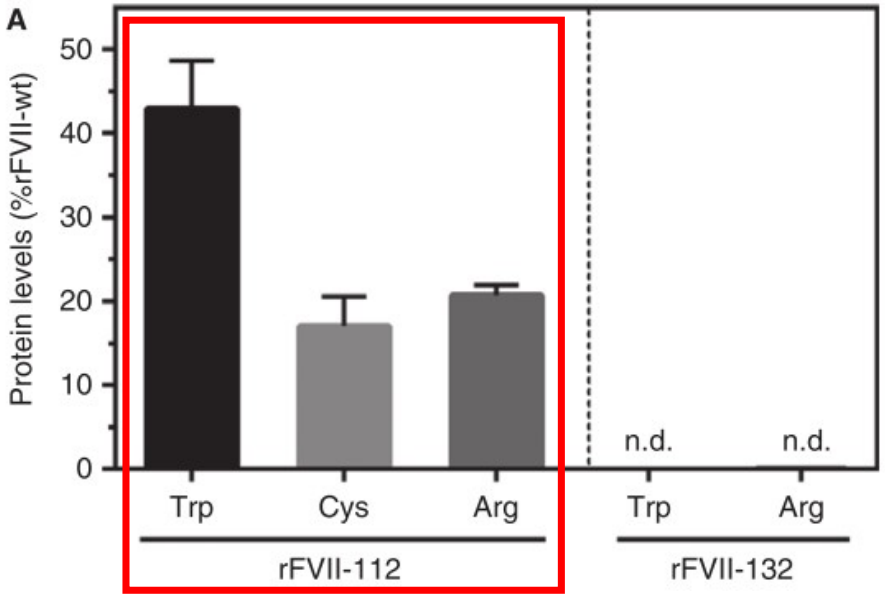
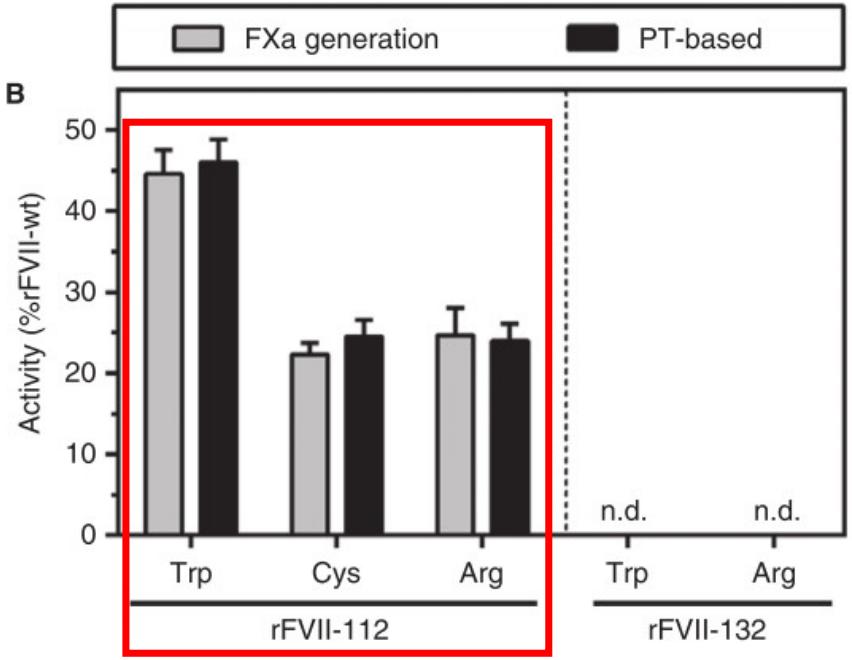
Results

Secretion and activity levels of rFVII missense variants





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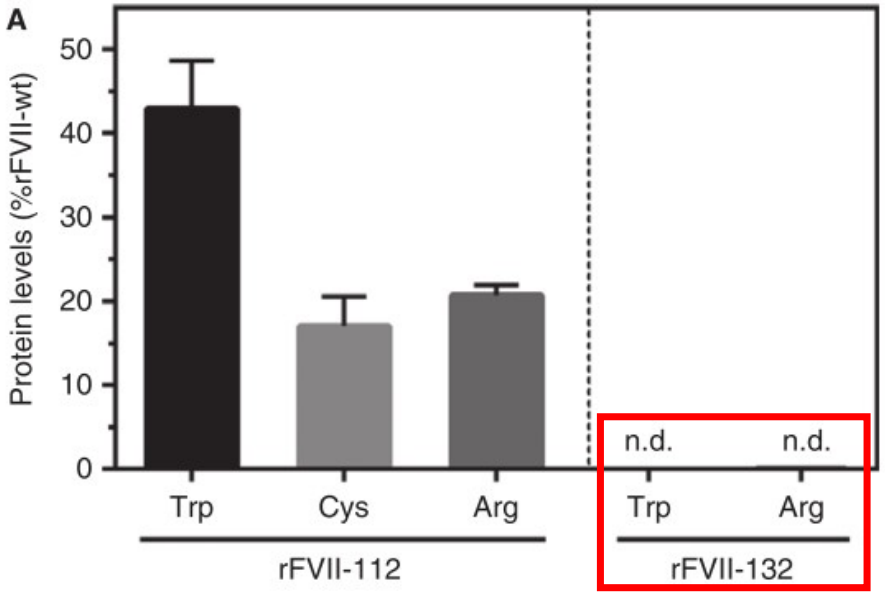

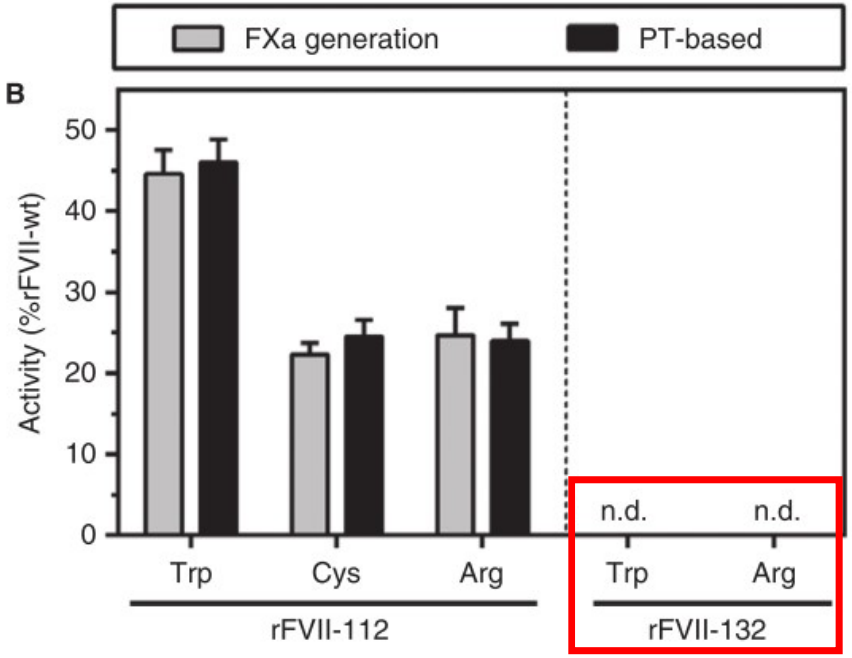

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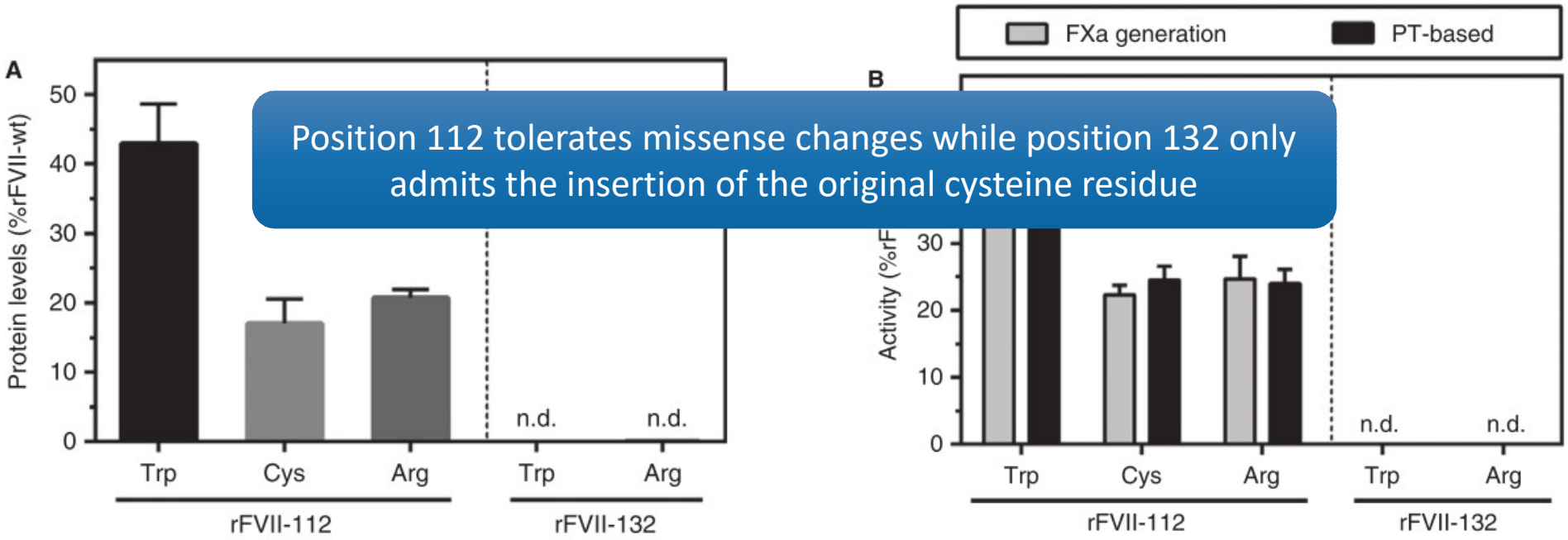

Position 112 tolerates missense changes while position 132 only admits the insertion of the original cysteine residue

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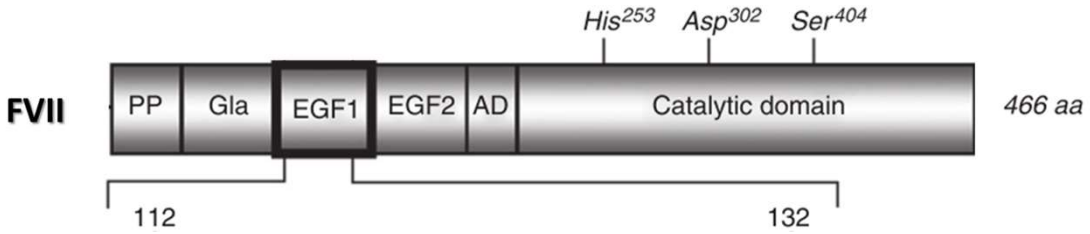
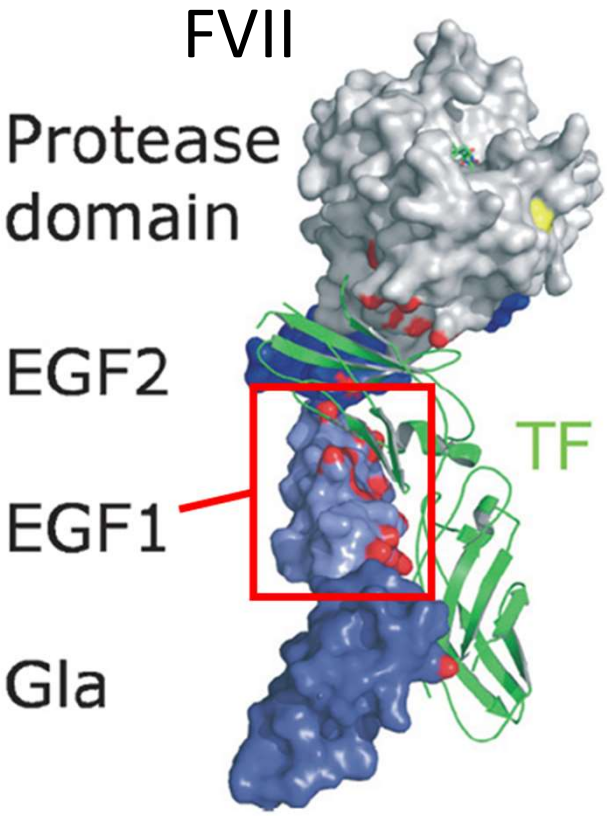


Both residues are part of the first EGF domain of FVII, which is involved in the interaction with tissue factor (TF, the FVII cofactor)



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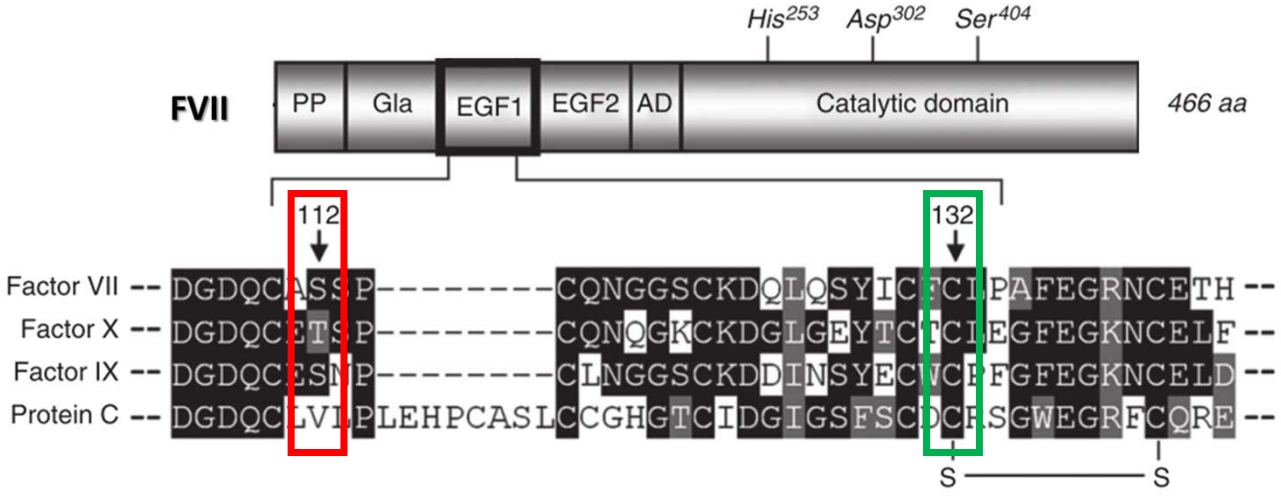
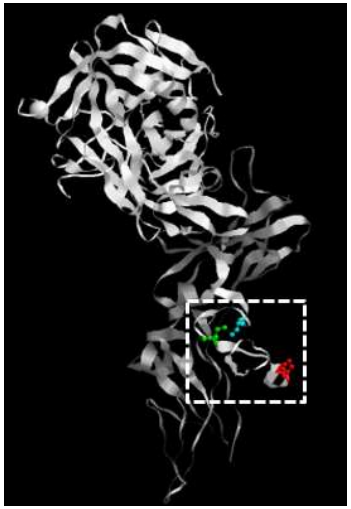
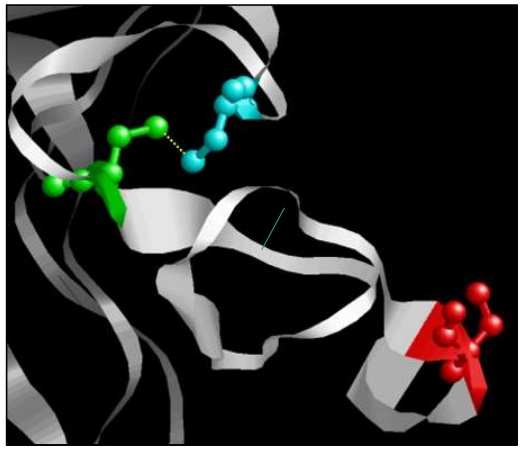


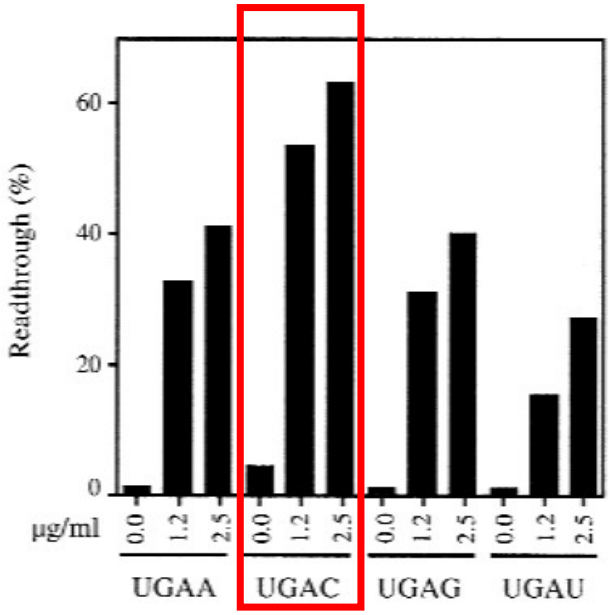


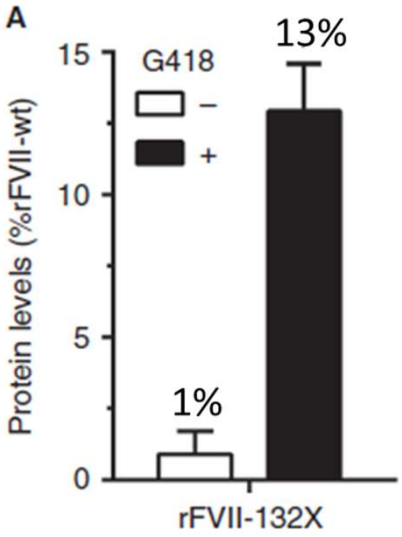


But whereas **Ser112** is surface-exposed and only partially conserved among others coagulation factors, **Cys132** forms a disulfide bridge with **Cys141** within EGF1 and is fully conserved.

In conclusion:

- **p.C132X** variant shows the **most readthrough favourable sequence context**

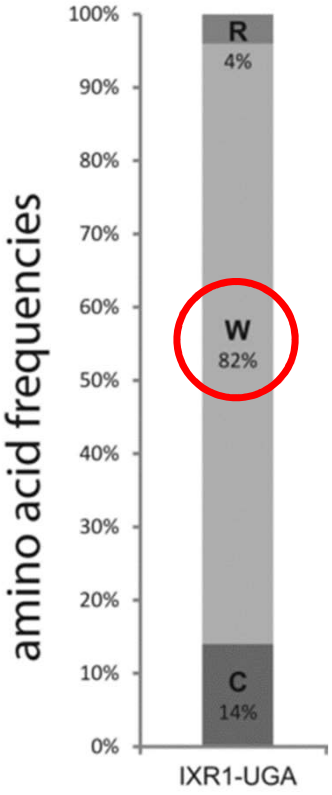


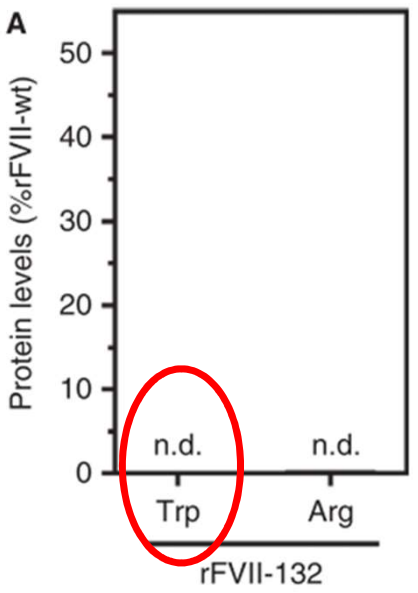


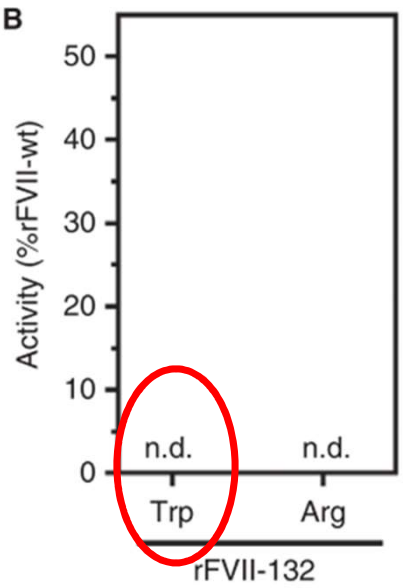
Moderate bleeding phenotype

In conclusion:

- **p.C132X** variant shows the most readthrough favourable sequence context
- PTC suppression can reinsert the original amino acid, thus leading to the production of wild-type FVII, whereas **other amino acids in this position are not tolerated** for protein secretion and function







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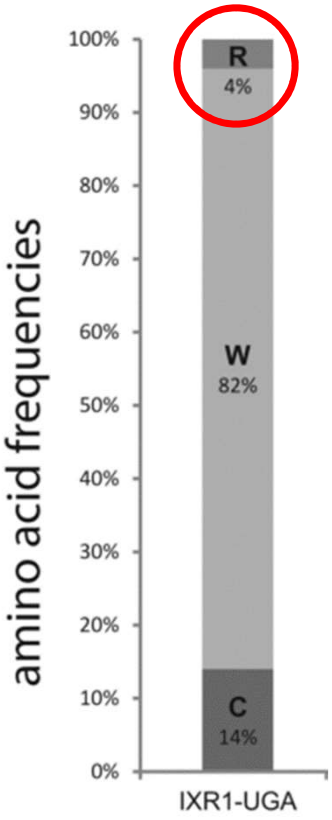

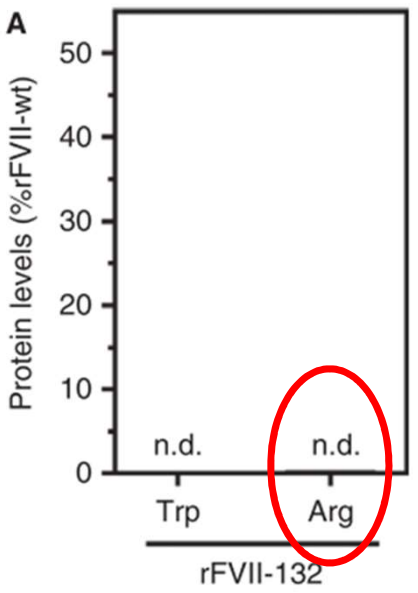

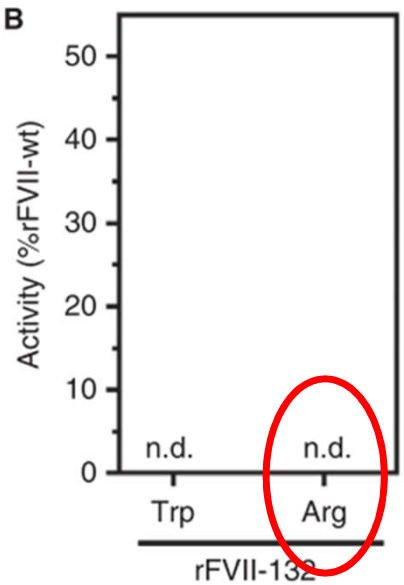

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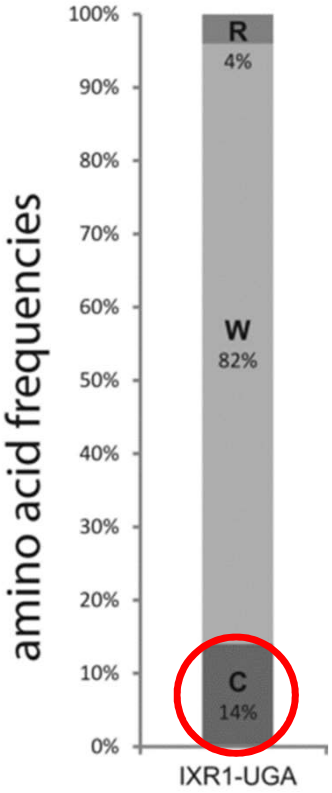
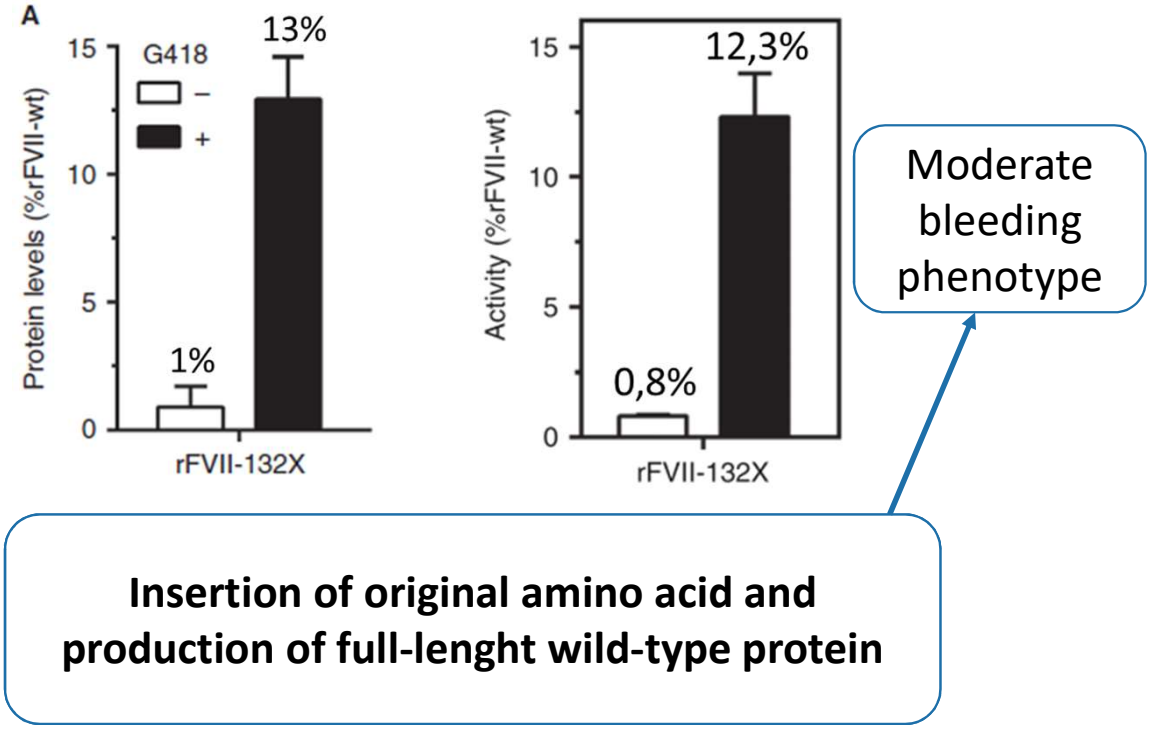
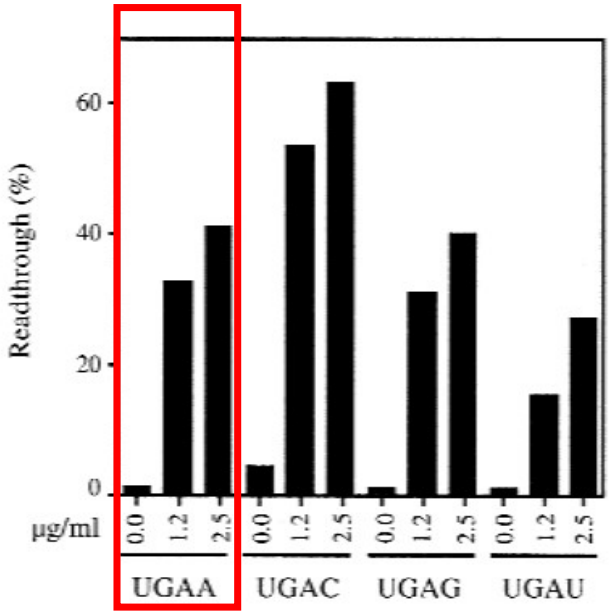
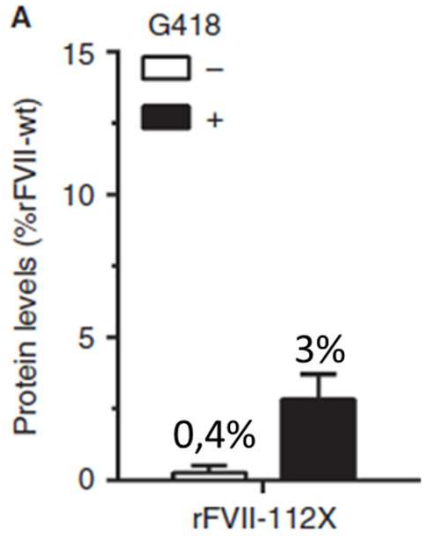




In conclusion:

- In the context of **p.S112X** the readthrough is predicted to occur with **lower efficiency**

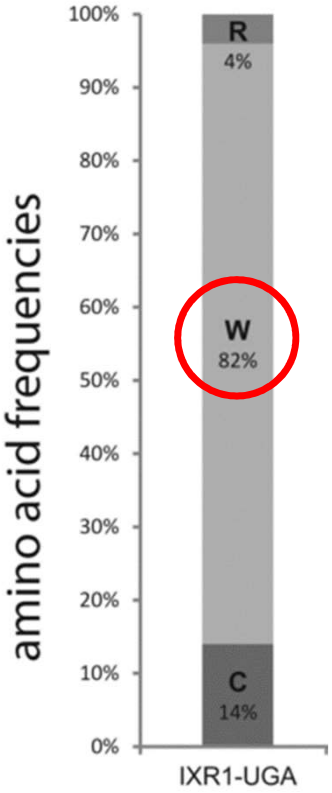


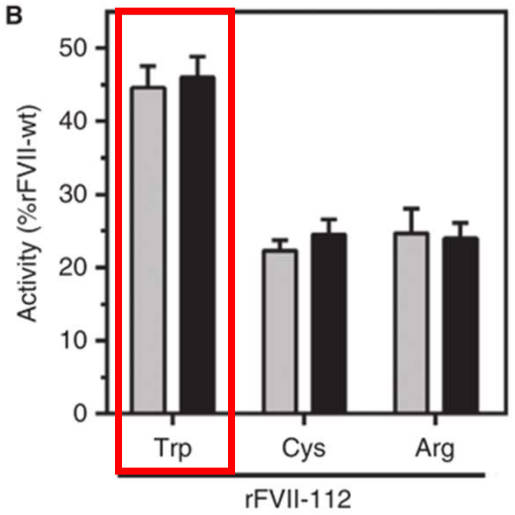


Life-threatening bleeding phenotype

In conclusion:

- In the context of **p.S112X** the readthrough is predicted to occur with **lower efficiency**
- The original amino acid (serine) can not be re-inserted by readthrough but this position significantly **tolerates** the most probable amino acid **substitutions**





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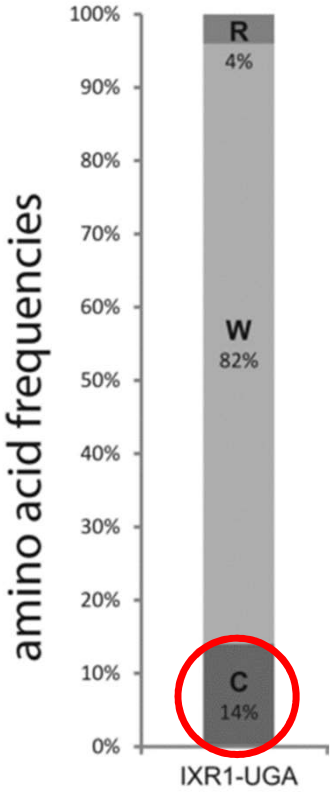

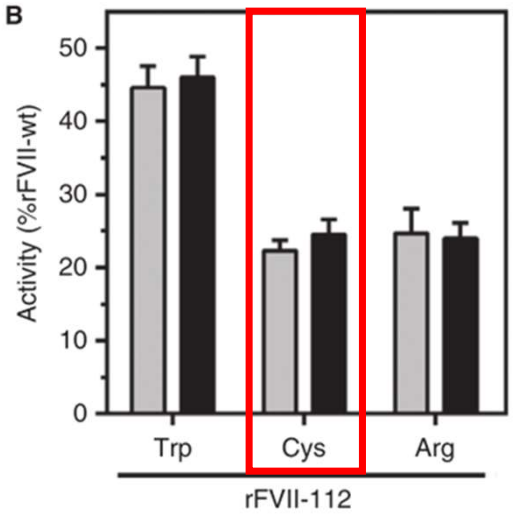

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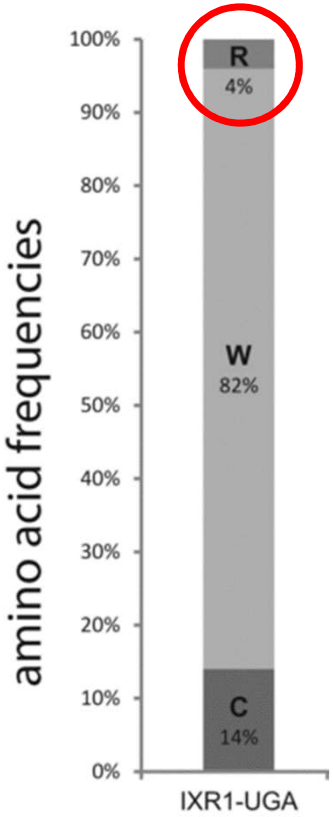

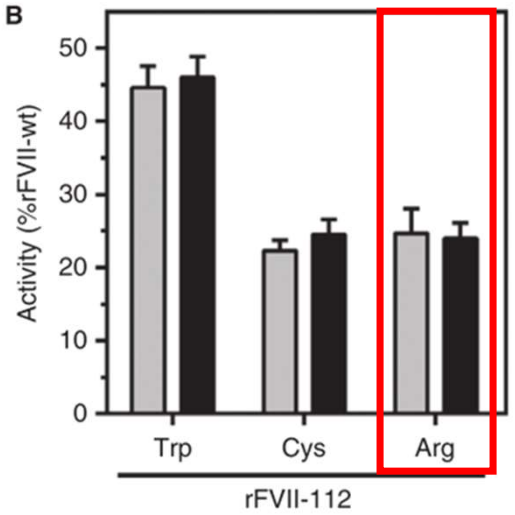

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Experimental findings are consistent with the complex and integrated scenario depicted above and support the notion that many elements account for readthrough efficiency and protein function restoration