

TRANSLATION is the conversion of the nucleotide sequence information in RNA into amino acid sequence information in protein

It follows the "genetic code"

The code has evolved to be as resistant to mutations as possible

Since 1980, we know that mitochondria (which have their own small genomes) use a slightly different genetic code

It is (nearly) universal

So do some other unicellular organisms

The third position contributes least to specificity (WOBBLE)

If the second AA is a PURINE, then generally the AA is POLAR

The most common type of mutation is a TRANSITION (purine → purine or pyrimidine → pyrimidine). TRANSVERSIONS are less common

In the THIRD position, TRANSITIONS mostly have no effect at all, and TRANSVERSIONS only have an effect half the time, in which case they specify a similar type of amino acid

In the SECOND position, TRANSITIONS will usually cause a change to a different type of amino acid, but TRANSVERSIONS will change the type of AA

DEGENERATE - more than one codon codes for each amino acid

Not all codons lead to amino acids - some are "STOP" codons

Introduction

How was the genetic code elucidated?

Logic

Pairs of bases wouldn't be enough - only 16 possibilities

Triplets are better - 64 possibilities, only 21 if w/guacs are needed - not 'neat', but there we are

Experiment

Observations

Loss-of-function mutations introduced by ACRIDINE are common even in the tolerant region - they named these "FCO" +/-

These can be made phenotypically wild-type again if they pick up a second acridine-induced mutation at a DIFFERENT SITE (a SUPPRESSOR mutation) - they called these +/- mutants

These two mutations could be segregated by HOMOLOGOUS RECOMBINATION - it was found that the suppressor themselves were also loss-of-function mutants

Crossing 2 +/- mutants gave a mutant phenotype

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Crossing a +/- and a +/- mutant yielded a wild-type phenotype

Interpretation

They knew that ACRIDINE could induce insertions and deletions in DNA

They reasoned that +/- mutations might correspond to insertions, while +/- mutations might correspond to deletions

Either of these would have resulted in a shift in the reading frame, and therefore in a mutation. BOTH of these (in the TOLERANT region) would have restored the reading frame, leading to a wild-type phenotype

They knew that combinations of +/- or +/- yielded mutant phenotypes, because of a shift of reading frame by +2

However, if they generated TRIPLE mutants, in which the reading frame was shifted by +3, then they found that the wild-type was restored

Therefore, the reading frame consists of 3 bp - codons are TRIPLETS

HOW MANY nt PER AA?

Structure

irRNAs are the ADAPTOR molecules that deliver AAs to the ribosomes and decode the information in mRNA

"Clover-leaf" structure (though more complex in reality)

Contains a large number of modified bases (modified post-transcriptionally)

The 3' end is the AMINO ACID ATTACHMENT SITE, at which '-CCA' is post-transcriptionally added

At the bottom of a molecule is an ANTICODON LOOP, which pairs with whatever codon it is meant to read

The 1st position of the anticodon (3rd position of codon) is often a MODIFIED BASE (DIHYDROURIDINE)

Post-transcriptionally modified adenosine in 3rd position

Done by ANTICODON DEAMINASE - converts 6-amino group to keto-group

This can base-pair to C, U or A - WHICH of these codons exactly is in the third position never matters

This is known as WOBBLE, and it is a way to deal with degeneracy without too many tRNA molecules

Even if not with U, in general, there is more freedom with the rules of base-pairing in that position, because this base is able to undergo more movement than the others

tRNA

irRNAs need to be "charged" with the correct amino acid to become AMINOACYL-tRNAs

First attaches AMP to the CARBOXYL GROUP of the AA, forming a high energy AMINOACYL ADENYLATE (driven by hydrolysis of the resulting pyrophosphate)

In the second step, this reacts with the CORRECT, UNCHARGED tRNA and attaches the AA acid to an -OH of the "A" in the attachment site

The resulting AMINOACYL-tRNA is of higher energy than a peptide bond, and so helps drive protein synthesis

This is done by very specific enzymes called aminoacyl-tRNA synthetase

"Loading"

The synthetases distinguish between the ~40 similarly shaped but different tRNAs using particular parts of the tRNA, IDENTITY ELEMENTS that are different in each tRNA

This is not necessarily the anticodon sequence

It often includes base pairs in the acceptor stem

Some synthetases that have to distinguish between two CHEMICALLY SIMILAR AAs can carry out a PROOFREADING STEP

if they accidentally carry out step 1 on the wrong AA, then they will not carry out step 2 but hydrolyse the aminoacyl adenylate

Proofreading

This is only necessary when a single-recognition step is not sufficiently discriminating

Principles of translation

The ANTICODON at one end of tRNA interacts with the complementary triplet base on mRNA, the CODON, when they are brought together in the CLEFT of the ribosome - the interaction is ANTIPARALLEL in nature

Sometimes, more than one ribosome can attach to mRNA while one ribosome is already translating it

This is fairly common, and these are called POLYSOMES

There can be as many as 50 on some mRNA

They need to be no closer than 80nt from each other

In PROKARYOTES, translation can sometimes begin before TRANSCRIPTION is finished, and the two processes are therefore COUPLED

This is because in prokaryotes, there is no nuclear membrane and translation and transcription are not spatially resolved

In eukaryotes, however, they are, and this allows RNA to be modified before translation

Hence the whole intron thing (see later)

NIRENBERG - roughly 1961

HOMOPOLYMERS of RNA (strings of identical bases), made using polynucleotide phosphorylase

LYSED BACTERIAL CELLS, treated with DNase to prevent further translation

Different radiolabelled amino acids (in separate tubes)

Mix together

Precipitate in TRICHLOROACETIC ACID (non polymerised AAs are soluble therein - only proteins precipitate)

Cell-free translation of HOMOPOLYMERIC tracts of DNA

See which precipitate is radioactive

Elucidated UUU, CCC and AAA

Interestingly, poly(G) could not be translated

Use polynucleotide phosphorylase to polymerise a MIXTURE of two nucleotides in an UNEVEN RATIO (say U:G in 0.76:0.24)

The most common triplet will then be UUU, the least common GGG

The next most common will be something with two Gs and one U

Cell-free translation of CO-POLYMERS

By looking at the frequency of incorporation of particular AAs using these RANDOM CO-POLYMERS as mRNA, it was possible to determine the COMPOSITION of codons for many AAs

Later, it was found that SYNTHETIC TRINUCLEOTIDES could bind to ribosome and the corresponding tRNA from a mixture

When subsequently passed through a membrane, only the complexes of ribosome-mRNA-tRNA stayed behind

TRIPLET binding to RIBOSOMES

This could identify triplets to specific amino acids

WHICH CODON CORRESPONDS TO WHICH AA?