



Non-standard amino acids

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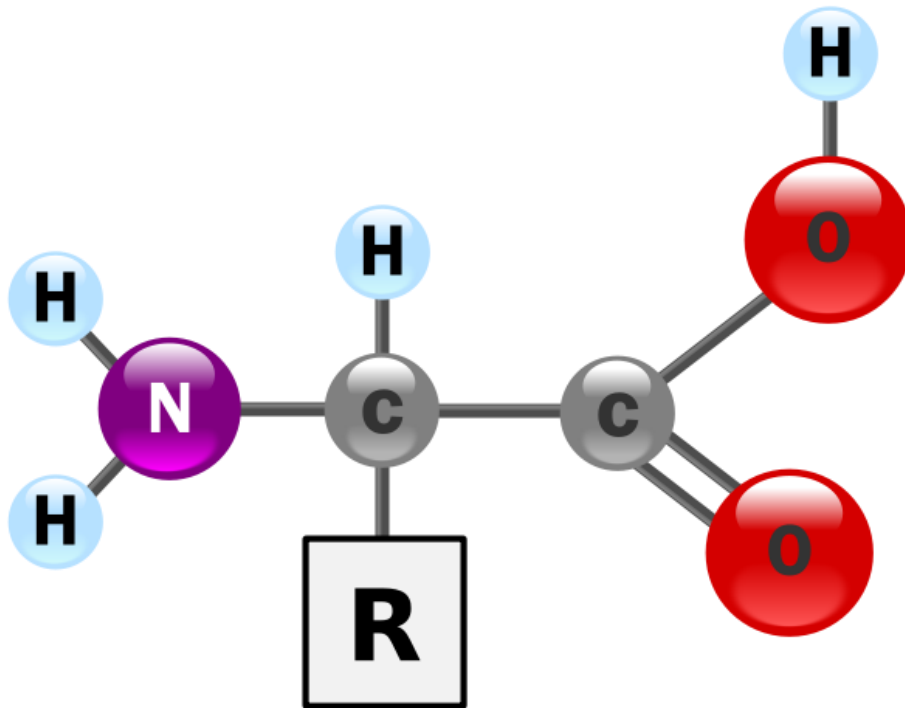
Outline

- ❑ Introduction
- ❑ Non-standard amino acids
 - ❑ Variations to the standard genetic code
 - ❑ Modifications to standard genetic code
 - ❑ PTM
- ❑ Summary

Introduction

AMINO ACID

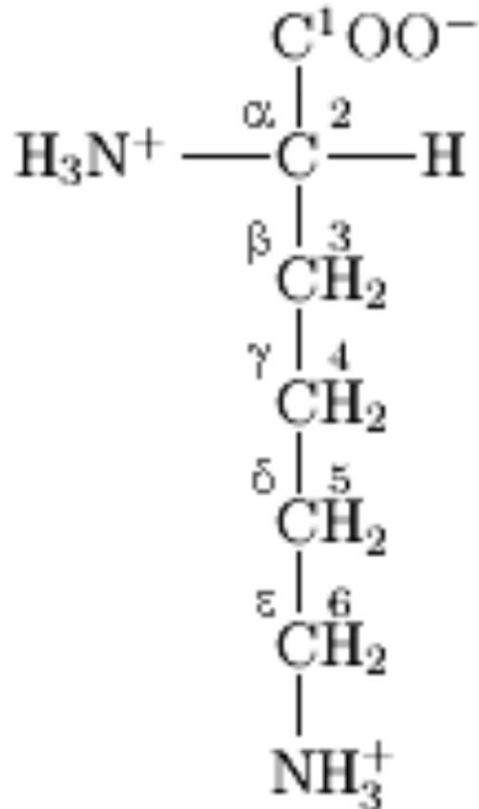
organic molecule with three main components: an amino group (-NH₂), a carboxylic acid group (-COOH), and an R group, or side chain, unique to each amino acid



Amino acids are critical to life, and have a variety of roles in metabolism

Introduction

GENERAL STRUCTURE



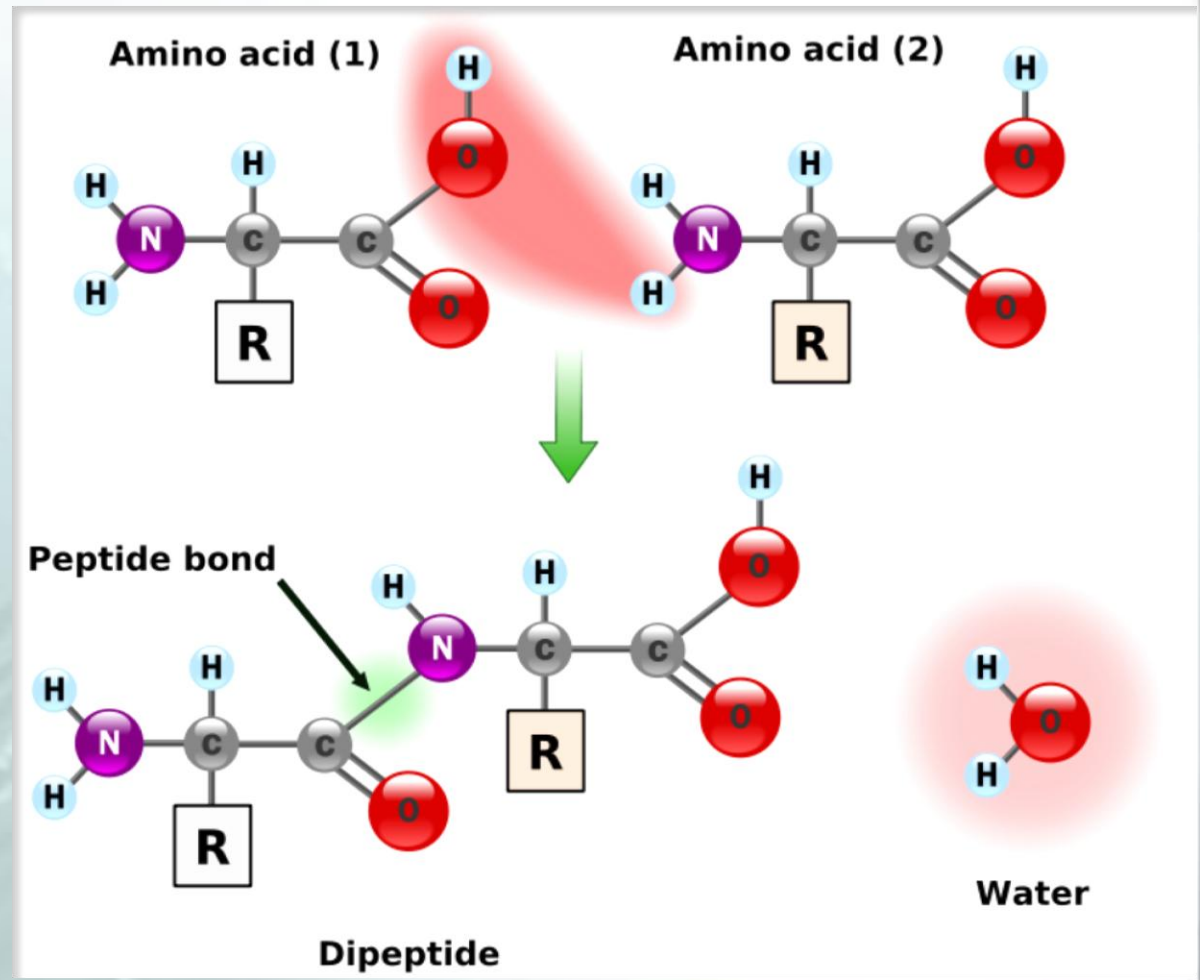
In amino acids that have a carbon chain attached to the α -carbon, as in lysine on the right, the carbons are labeled in order as α , β , γ , δ , and so on.

alpha amino acids are the most common form found in nature

Introduction

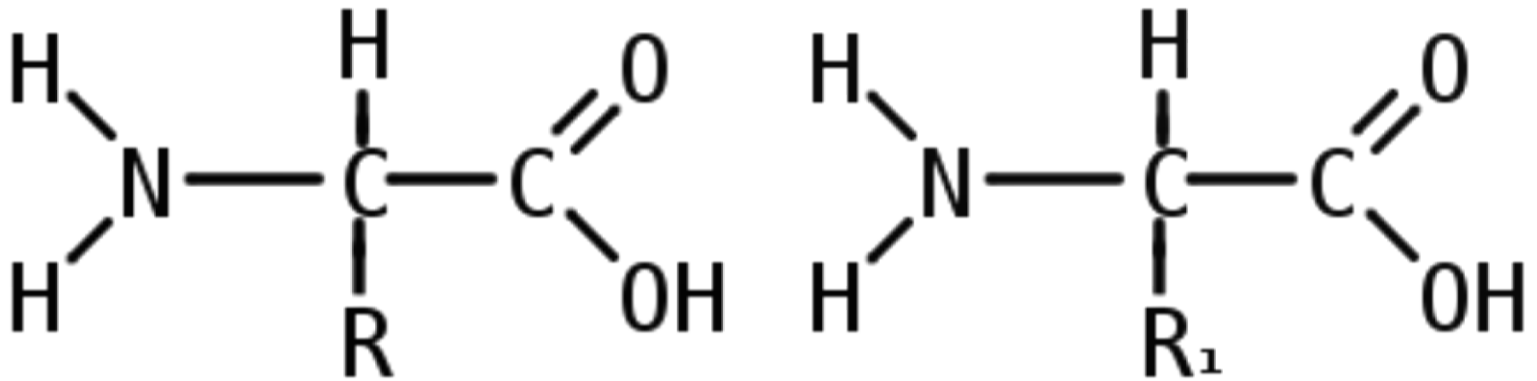
PEPTIDE BOND FORMATION

one amino acid molecule can react with another and become joined through an amide linkage



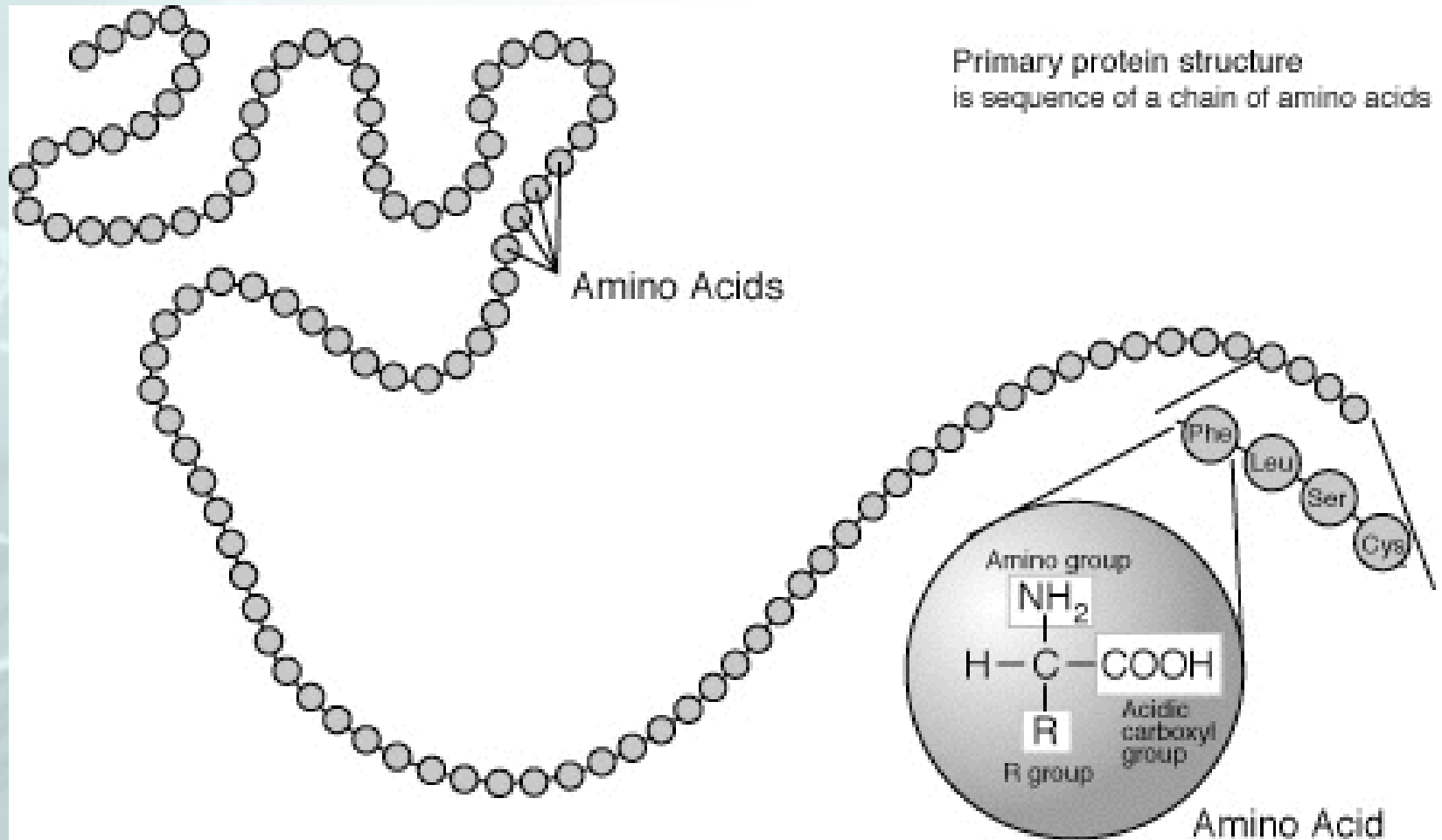
Introduction

Summary of the formation of a peptide bond.



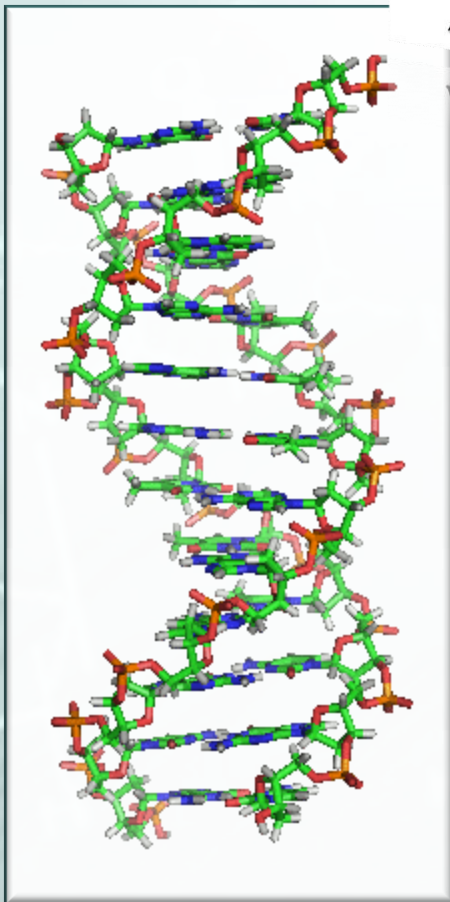
Introduction

This polymerization of amino acids is what creates proteins

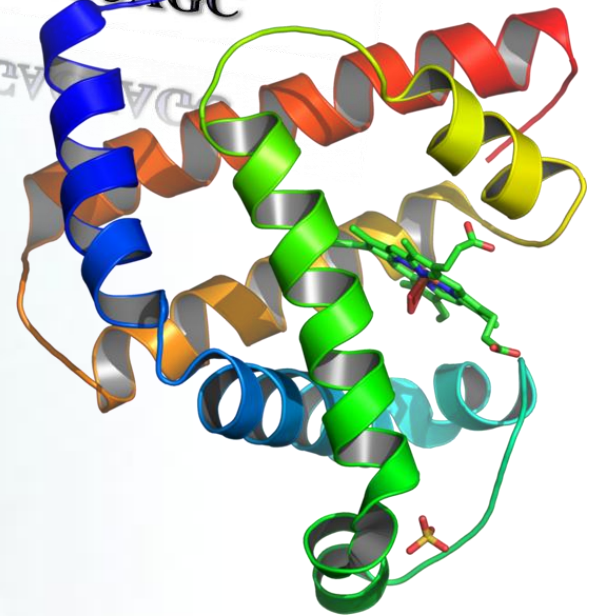


Introduction

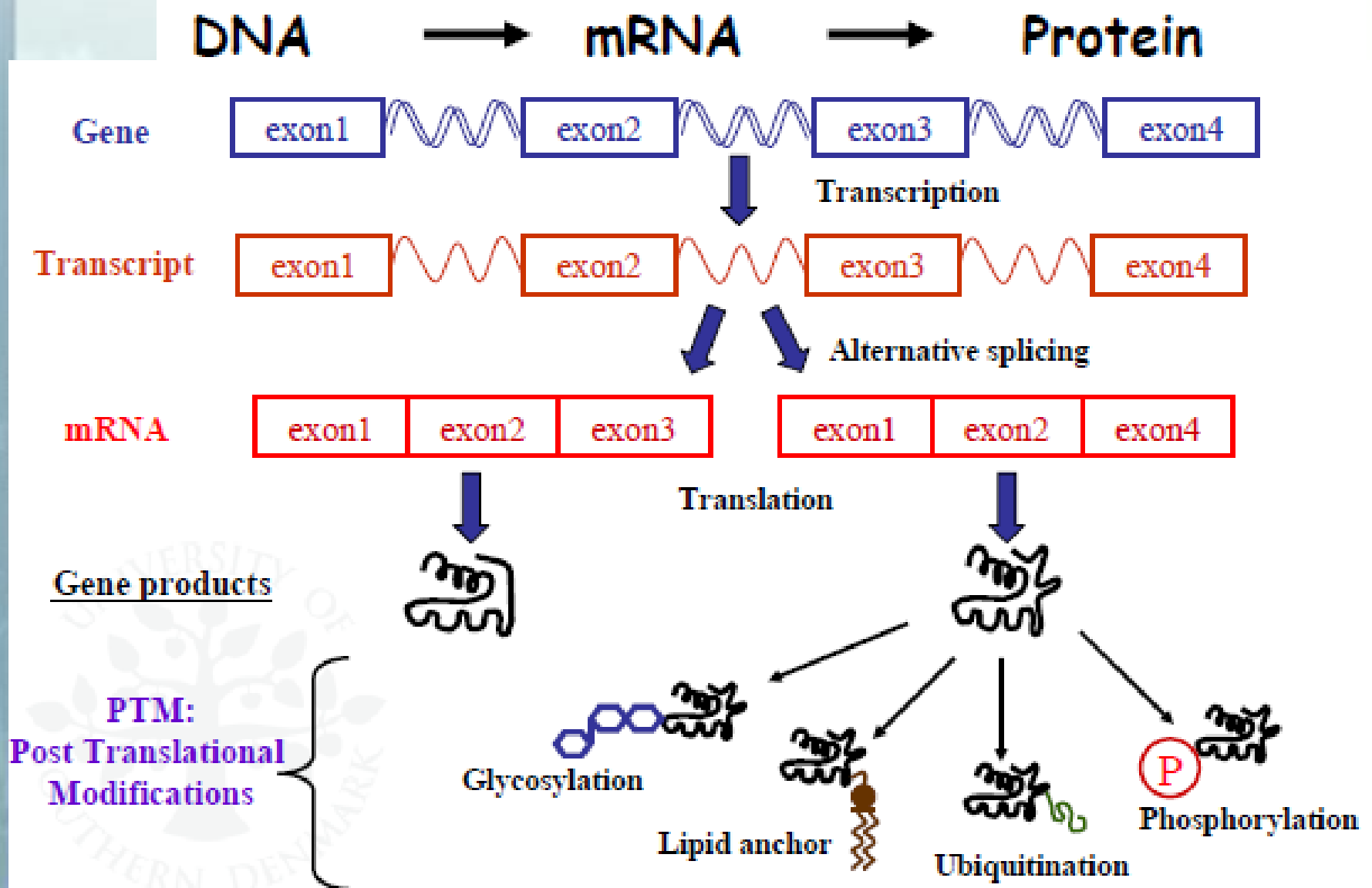
GENETIC CODE



AUGGAAGAG UGG UCUUCCUCAUCGAGUAGC

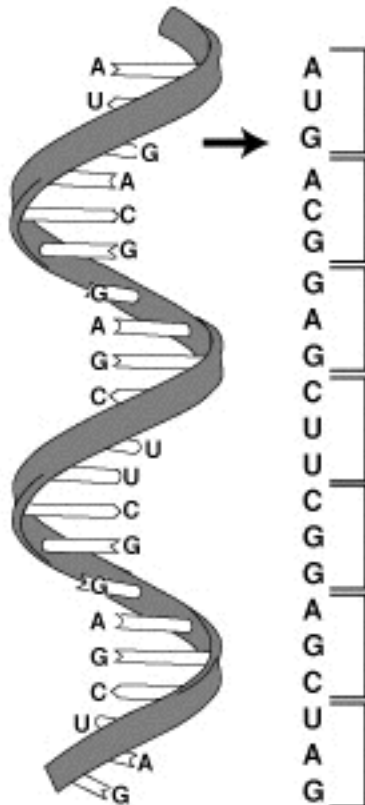


Introduction



Introduction

STANDARD AMINO ACIDS



RNA

Ribonucleic acid

nonpolar polar basic acidic

Codon 1

Codon 2

Codon 3

Codon 4

Codon 5

Codon 6

Codon 7

The table shows the 64 codons and the amino acid for each. The **direction** of the mRNA is 5' to 3'.

		2nd base			
		U	C	A	G
1st base	U	UUU (Phe/F) Phenylalanine	UCU (Ser/S) Serine	UAU (Tyr/Y) Tyrosine	UGU (Cys/C) Cysteine
		UUC (Phe/F) Phenylalanine	UCC (Ser/S) Serine	UAC (Tyr/Y) Tyrosine	UGC (Cys/C) Cysteine
		UUA (Leu/L) Leucine	UCA (Ser/S) Serine	UAA Ochre (<i>Stop</i>)	UGA Opal (<i>Stop</i>)
		UUG (Leu/L) Leucine	UCG (Ser/S) Serine	UAG Amber (<i>Stop</i>)	UGG (Trp/W) Tryptophan
	C	CUU (Leu/L) Leucine	CCU (Pro/P) Proline	CAU (His/H) Histidine	CGU (Arg/R) Arginine
		CUC (Leu/L) Leucine	CCC (Pro/P) Proline	CAC (His/H) Histidine	CGC (Arg/R) Arginine
		CUA (Leu/L) Leucine	CCA (Pro/P) Proline	CAA (Gln/Q) Glutamine	CGA (Arg/R) Arginine
		CUG (Leu/L) Leucine	CCG (Pro/P) Proline	CAG (Gln/Q) Glutamine	CGG (Arg/R) Arginine
	A	AUU (Ile/I) Isoleucine	ACU (Thr/T) Threonine	AAU (Asn/N) Asparagine	AGU (Ser/S) Serine
		AUC (Ile/I) Isoleucine	ACC (Thr/T) Threonine	AAC (Asn/N) Asparagine	AGC (Ser/S) Serine
		AUA (Ile/I) Isoleucine	ACA (Thr/T) Threonine	AAA (Lys/K) Lysine	AGA (Arg/R) Arginine
		AUG (Met/M) Methionine, Start ^[A]	ACG (Thr/T) Threonine	AAG (Lys/K) Lysine	AGG (Arg/R) Arginine
	G	GUU (Val/V) Valine	GCU (Ala/A) Alanine	GAU (Asp/D) Aspartic acid	GGU (Gly/G) Glycine
		GUC (Val/V) Valine	GCC (Ala/A) Alanine	GAC (Asp/D) Aspartic acid	GGC (Gly/G) Glycine
		GUA (Val/V) Valine	GCA (Ala/A) Alanine	GAA (Glu/E) Glutamic acid	GGA (Gly/G) Glycine
		GUG (Val/V) Valine	GCG (Ala/A) Alanine	GAG (Glu/E) Glutamic acid	GGG (Gly/G) Glycine

Introduction

Inverse table

Ala/A	GCU, GCC, GCA, GCG	Leu/L	UUA, UUG, CUU, CUC, CUA, CUG
Arg/R	CGU, CGC, CGA, CGG, AGA, AGG	Lys/K	AAA, AAG
Asn/N	AAU, AAC	Met/M	AUG
Asp/D	GAU, GAC	Phe/F	UUU, UUC
Cys/C	UGU, UGC	Pro/P	CCU, CCC, CCA, CCG
Gln/Q	CAA, CAG	Ser/S	UCU, UCC, UCA, UCG, AGU, AGC
Glu/E	GAA, GAG	Thr/T	ACU, ACC, ACA, ACG
Gly/G	GGU, GGC, GGA, GGG	Trp/W	UGG
His/H	CAU, CAC	Tyr/Y	UAU, UAC
Ile/I	AUU, AUC, AUA	Val/V	GUU, GUC, GUA, GUG
START	AUG	STOP	UAG, UGA, UAA

Introduction

ESSENTIAL AMINO ACIDS

Of the 20 standard amino acids, 8 are called essential amino acids because the human body cannot synthesize them

Essential: Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine

Nonessential: Alanine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine, Tyrosine, Arginine, Histidine,

Introduction

The question is why did life stop with 20 and why these particular 20?

Non-standard amino acids

- Variations to the standard genetic code (selenocysteine and pyrrolysine)
- Modifications to standard genetic code (O-methyl-L-tyrosine and L-homoglutamine)
- PTM (posttranslational modification)

Non-standard amino acids

VARIATIONS TO THE STANDARD GENETIC CODE

Numerous variations of the standard genetic code are found in mitochondria, which are energy-producing organelles

Many Small variants such as Mycoplasma translating the codon UGA as tryptophan.

In some bacteria and archaea, GUG and UUG are common start codons.

However, in rare cases, certain specific proteins may use alternative initiation (start) codons not normally used by that species.

A detailed description of variations in the genetic code can be found at the [NCBI web site](#).

Non-standard amino acids

SELENOCYSTEINE

encoded in a special way by a UGA codon

Selenocysteine is now viewed as the 21st amino acid

is present in several enzymes (for example glutathione peroxidases, tetraiodothyronine 5' deiodinases, thioredoxin reductases, formate dehydrogenases, glycine reductases and some hydrogenases).

Selenocysteine has a structure similar to cysteine, but with an atom of selenium taking the place of the usual sulfur. Proteins that contain one or more selenocysteine residues are called selenoproteins.

SECIS element (SElenoCysteine Insertion Sequence)

Non-standard amino acids

PYRROLYSINE

encoded in a special way by a UGA codon
(normally a stop codon named "amber")

Pyrrolysine is now viewed as the 21st amino acid

Pyrrolysine is an amino acid used by some archaea in enzymes that are part of their methane-producing metabolism.

Pyrrolysine is a lysine derivative encoded by the UAG codon in methylamine methyltransferase genes of *Methanosarcina barkeri*.

Non-standard amino acids

MODIFICATIONS TO STANDARD GENETIC CODE

An expanded genetic code with a functional quadruplet codon

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*

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Contributed by Peter G. Schultz, March 18, 2004

<http://www.pnas.org/content/101/20/7566.full>

Schultz and his colleagues a way to site-specifically incorporate novel amino acids into proteins expressed by the *Escherichia coli*

Non-standard amino acids

ABOUT THE SCRIPPS RESEARCH INSTITUTE

The Scripps Research Institute in La Jolla, California, is one of the world's largest, private, non-profit biomedical research organizations. It stands at the forefront of basic biomedical science that seeks to comprehend the most fundamental processes of life.

Scripps Research is internationally recognized for its research into immunology, molecular and cellular biology, chemistry, neurosciences, autoimmune diseases, cardiovascular diseases and synthetic vaccine development.

Non-standard amino acids

O-METHYL-L-TYROSINE

Starting with a tRNA/synthetase pair from the organism *Methanococcus jannaschii*, they created a library of *E. coli* cells, each encoding a mutant *M. jannaschii* synthetase, and they changed its specificity so that it could be used to recognize the unnatural amino acid **O-methyl-L-tyrosine**.

To do this, they devised a positive selection whereby only the cells that load the orthogonal tRNA with any amino acid would survive. Then they designed a negative selection whereby any cell that recognizes UAG using a tRNA loaded with anything other than O-methyl-L-tyrosine dies.

Non-standard amino acids

O-METHYL-L-TYROSINE

In so doing, they found their orthogonal synthetase mutants that load the orthogonal tRNA with only the desired unnatural amino acid. When a ribosome reading an mRNA within the *E. coli* cells encounters UAG, it inserts the unnatural amino acid O-methyl-L-tyrosine.

Furthermore, any codon in an mRNA that is switched to UAG will encode for the new amino acid in that place, giving Schultz and his colleagues a way to site-specifically incorporate novel amino acids into proteins expressed by the *E. coli*.

Non-standard amino acids

L-HOMOGLUTAMINE

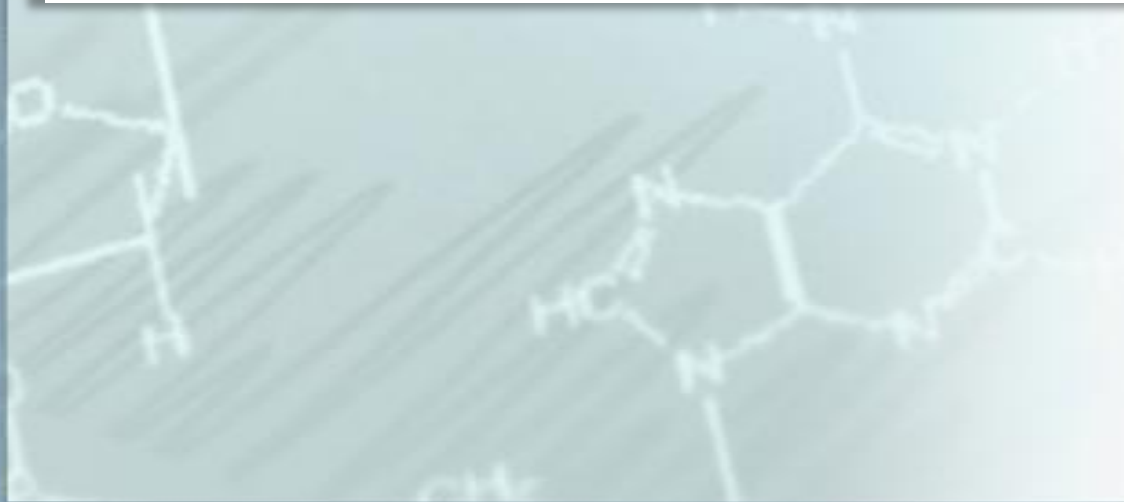
Similarly, Schultz and his colleagues made an engineered tRNA/synthetase orthogonal pair from the polar archaean organism *Pyrococcus horikoshii* that recognizes the four-base codon AGGA.

The tRNA has a four-base anticodon loop, and when a ribosome reading an mRNA within the *E. coli* cells encounter AGGA, it inserts the unnatural amino acid L-homoglutamine at that site.

Non-standard amino acids

L-HOMOGLUTAMINE

By placing both of these systems within the same *E. coli* cell, Schultz and his colleagues have demonstrated, as a proof of principle, that it is technically possible to have mutually orthogonal systems operating at once in the same cell. This opens up the possibility of doing multiple site substitution with additional unnatural amino acids in the future.



Non-standard amino acids

PTM (POSTTRANSLATIONAL MODIFICATION)

PTM is the chemical modification of a protein after its translation. It is one of the later steps in protein biosynthesis for many proteins.

PTMs involving addition include:

phosphorylation, acylation, O- and N-glycosidation, glycosylation, nucleosidylation, oxidation, methylation, vitamin K-dependent carboxylation, sulfation, ADP-ribosylation, hydroxylation, prenylation, selenoylation etc.

Post-translational modification of proteins are detected by mass spectrometry or Eastern blotting.

Non-standard amino acids

PHOSPHORYLATION

Phosphorylation is the addition of a phosphate (PO_4) group to a protein or other organic molecule. Protein phosphorylation in particular plays a significant role in a wide range of cellular processes.

Many enzymes and receptors are switched "on" or "off" by phosphorylation and dephosphorylation.

Phosphorylation usually occurs on serine, threonine, and tyrosine residues in eukaryotic proteins.

Reversible phosphorylation of proteins is an important regulatory mechanism that occurs in both prokaryotic and eukaryotic organisms

Non-standard amino acids

PHOSPHORYLATION

Regulatory roles of phosphorylation include:

- * Biological thermodynamics of energy-requiring reactions
 - o Phosphorylation of Na⁺/K⁺-ATPase during the transport of sodium (Na⁺) and potassium(K⁺) ions across the cell membrane in osmoregulation to maintain homeostasis of the body's water content.
- * Mediates enzyme inhibition
 - o Phosphorylation of the enzyme GSK-3 by AKT (Protein kinase B) as part of the insulin signaling pathway.

Non-standard amino acids

PHOSPHORYLATION

- * Important for protein-protein interaction via "recognition domains."

- o Phosphorylation of the cytosolic components of NADPH oxidase, a large membrane-bound, multi-protein enzyme present in phagocytic cells, plays an important role in the regulation of protein-protein interactions in the enzyme

- * Important in protein degradation.

Non-standard amino acids

GLYCOSYLATION

One of the most common post-translational modifications of proteins is glycosylation, the covalent attachment of oligosaccharides.

Carbohydrates in the form of asparagine-linked (N-linked) or serine/threonine-linked (O-linked) oligosaccharides are major structural components of many cell surface and secreted proteins.

Glycoproteins play crucial roles in cellular processes such as protein sorting, immune recognition, receptor binding, inflammation, and pathogenicity.

Non-standard amino acids

CARBOXYLATION

Carboxylation in biochemistry is a posttranslational modification of glutamate residues in proteins. It occurs primarily in proteins involved in the blood clotting cascade

The carboxylase requires vitamin K as a cofactor and performs the reaction in a processive manner.

γ -carboxyglutamate binds calcium, which is essential for its activity.

For example, in prothrombin, calcium binding allows the protein to associate with the plasma membrane in platelets, bringing it into close proximity with the proteins that cleave prothrombin to active thrombin after injury.

Summary

These rare exceptions aside, scientists have often looked for ways to incorporate unnatural amino acids into proteins in the test tube and in the context of living cells because such novel proteins are of great utility for basic biomedical research.

They provide a powerful tool for studying and controlling the biological processes that form the basis for some of the most intriguing problems in modern biophysics and cell biology, like signal transduction, protein trafficking in the cell, protein folding, and protein–protein interactions.

Summary

For example, there are novel amino acids that contain fluorescent groups that can be used to site-specifically label proteins with small fluorescent tags and observe them in vivo.

This is particularly useful now that the human genome has been solved and scientists are now turning their attention to what these genes are doing inside cells.

Summary

Other unnatural amino acids contain photoaffinity labels and other "crosslinkers" that could be used for trapping protein–protein interactions by forcing interacting proteins to be covalently attached to one another.

Purifying these linked proteins would allow scientists to see what proteins interact with in living cells -- even those with weak interactions that are difficult to detect by current methods.

Summary

Unnatural amino acids are also important in medicine, and many proteins used therapeutically need to be modified with chemical groups such as polymers, crosslinking agents, and cytotoxic molecules.

Last year, Schultz and his Scripps Research colleagues also showed that glycosylated amino acids could be incorporated site-specifically to make glycosylated proteins -- an important step in the preparation of some medicines.

Summary

Novel hydrophobic amino acids, heavy metal-binding amino acids, and amino acids that contain spin labels could be useful for probing the structures of proteins into which they are inserted.

And unusual amino acids that contain chemical moieties like "keto" groups, which are like LEGO blocks, could be used to attach other chemicals such as sugar molecules, which would be relevant to the production of therapeutic proteins.

THE END

Thank You for Your attention