

Chapter 27 Protein Metabolism (That's synthesis and degradation)

Problems: 1, 2, 4, 7, 9, 12

27.0 Intro

Protein synthesis is complicated
70 proteins on ribosome
20 enzymes to activate AA's for synthesis
at least a dozen additional factors
100 more proteins for processing of different proteins
40 different tRNA

overall more than 300 different molecules involved

protein synthesis accounts for 90% of chemical E used in cell

in bacterial cell will have 15,000 ribosomes, 100,000 of the related factors,
200,000 tRNA

can take up to 35% of its dry weight

Yet 100 residue peptide synthesized in 5s

tightly regulated and controlled

so lots is involved

27.1 The Genetic Code

ribosomes identified as site of synthesis in 1950's by Zamecnik
fed radioactive AA's and looked where ended up in cell
Zamecnik & Hoagland then found that the amino acids were activated for
synthesis by getting attached to a heat stable RNA, later called t-RNA
process of making aminoacyl-tRNA's catalyzed by aminoacyl tRNA synthases
It was Crick who theorized the small nucleic acids like RNA might be the bridge
to reading a sequence of DNA into a protein

We call this process **Translation**

A. *Genetic Code was cracked using artificial mRNA templates*
during 1960's knew that needed at least a triplet code
2 letter code 4×4 only 16 AA's could be coded
3 letter code $4 \times 4 \times 4 = 64$ - more than enough

Call the three letter code a *codon*

Genetic code is continuous, no space between AA codes
so for many pieces of DNA can read 3 different ways depending on start

Proper starting spot established the **reading frame**

Frame of reference for reading the message

1961 Nirenberg & Matthaei

Made poly U with e coli extract that included all you needed

Got polyU using polynucleotide phosphorylase (last chapter)

Got poly phe

So know that UUU = Phe

Poly C gave poly pro

Poly A gave poly lys

Etc. until figure them all out

1964 Nirenburg & Leder

PolyG - formed tetraplexes back in chapter 10 so it didn't work

By varying synthetic mix could identify composition of almost every triplet but not exact sequence

Many other experiments eventually evolved until we came to the final code shown in [figure 27-7](#)

Code nearly universal

Some minor variations See Box 27-1 for details

Some Mitochondria, bacteria, single celled eukaryotes

Some codons special functions

AUG initiation in all cells

UAA, UAG, UGA stop codons

Based on random chance, 3 in 64 codons or about 1 in 20 in a reading frame is a stop codon

A reading frame that is not terminated in 50 codons is called an **open reading frame ORF**

Long open reading frames usually proteins

60,000 MW protein = about 500 AA

=500 codons = 1500 bases

Code is *degenerate* - some AA have more than 1 code

But not uniformly degenerate

Met, Trp 1 codon each

Leu, Ser, Arg, - 6 each

When several codons for 1 AA
 Difference usually at 3rd base 5' end
 First two letters are the primary
 interesting consequences

Note for Dr. Z. To keep straight

DNA promoter sequences were the sequence on the non-coding or non-template DNA strand

RNA is then made from DNA template, so RNA is non-template

tRNA binds to non-template RNA so we are back to template sequence

B. Wobble allows some tRNA's to recognize more than one codon

sequence on mRNA is called the codon

sequence on the tRNA is called the anticodon

see figure 27-8

Normal anti-parallel base pairing so

Uncertainty in 3 position (reading 5'-3' on mRNA)

Corresponds to 1 position (reading 5'-3' on tRNA)

Structures of A, Inosine and G

Remember Inosine is deaminated A so no surprise that binds U

Once deaminated looks sort of like G so that is why binds C

But I bond to A is funky two purines to large space!

If pairing was strictly Watson/Crick would need 64-3 stop or 61 different tRNA's

But somewhat different

remember those post transcriptional modifications of tRNA?

one is inosine

Inosine can make H-bonds to A, U or C but weak bonds

So single tRNA can bind 3 different codons

1st 2 bases for strong bonds, 3rd rather weak

Call the third the 'wobble' base

Crick's Wobble hypothesis

1. 1st 2 bases of mRNA for strong base pairs confirm most of specificity

2. 1st base of ANTICODON (tRNA determined # of codons recognized by tRNA

Figure 27-8b

C or A - specific - only 1 codon recognized

U or G - less specific - 2 codons recognized

I - least specific - 3 codons recognized

Table 27-4 if you want it

3. AA's specified by several codons
If first 2 bases of codons different, need different tRNAs
4. a minimum of 32 tRNA are required for all 61 codons
(Actually use about 40 codons)

C. Translational Frameshifting

Was originally thought that once the ORF was set, the AA were read of until the termination was hit, with no 'funny business' in between to change the reading frame

A few genes have been found where the ribosome 'hiccups' during translation to change the reading frame
May allow two different but related proteins to be made from 1 RNA
May be used to regulate synthesis

Best example gag and pol genes in Rous sarcoma virus **Figure 27-9**
Last chapter gag/pol usually synthesized as one mRNA and translated into one polypeptide
With gag part of peptide getting cleaved out into virus structural elements
Pol getting cleaved out for reverse transcriptase
Here gag/pol is again a single mRNA
But 19 out of 20 times the ribosome hits the stop sequence
And make only gag structural proteins
1 out of 20 times hiccups, misses the stop
Goes on and synthesizes the larger peptide that can be trimmed to make both the structural gag proteins and the revers transcriptase pol product

Some frame-shifting occurs when mRNA is edited
Mitochondrial and chloroplast genomes often include additions or deletions that change frame of message
Use guide RNA's (gRNA) to guide this process
As another class of specialized RNA

Might think of introns and exons as the ultimate in frame-shifting edits

Some frame shifting done by base modification
In discussing DNA damage talked about deamination
There is actually an enzyme in your body that deliberately does deamination
Called ADAR for adenosine deaminase that acts on RNA
Observed in low-density lipoprotein gene **Figure 27-12**
A 513 000 MW is synthesized in liver

A 250,000 MW is synthesized in intestine
 Intestine has ADAR
 Binds to the same mRNA at termination point
 Changes a CAA-Gln to a UAA termination to make shorter protein!

27.2 Protein Synthesis

as usual there will be an initiation, elongation and termination
 in addition will be activation of precursors and post synthetic modification

A. Overall process then:

The players in the process are shown in Table 27-5

Activation of AA's - activate COOH group, form link between AA and information bearing tRNA

Will take place in cytosol (not ribosome)

Will cost ATP energy

Initiation mRNA binds smaller of 2 pieces of ribosome, then initiating tRNA, then large ribosomal unit binds to make complete requires GTP and initiation factors

Elongation keep adding tRNA's and extending the chain requires elongation factors require GTP for ribosomal movement

Termination and Release ribosome moves over a termination codon release factors help in releasing the peptide chain

Folding and postranslational modification protein must fold, various modifications may occur; removal of AA's, addition methyl, acetyl, phosphoryl, carboxyl groups attachment of oligosaccharides or prosthetic groups

B. The Ribosome

15,000 or more in an e .coli

1/4 of dry weight

Bacterial 65% RNA, 35% protein

2 major units, one large one small

Classification

One unit called 30S

One unit called 50S

Combined called 70S

S refers to Svedberg unit - a measure of how fast it moves in a centrifugal field

Larger S #, Larger mass

Both units contain protein and at least 1 RNA (Table 27-6)

X-ray crystallography/electron microscopy and other methods used to make current picture

Figure 27-13

The RNA makes structural core that proteins are stuck to. (3 RNA's prokaryote, 4 RNA's eukaryote)

Predicted secondary structure (Fig 27-14) is largely seen in 3D structure, but 3D structure is lots more complex

No protein within 18A of active site! So enzymatic work done by RNA
(This thought is new this decade!)

Eukaryotic larger and more complex than prokaryotic Figure 27-15

Mitochondrial and chloroplast ribosomes slightly smaller and simpler than bacterial

But all ribosomes have similar structure

C. tRNA's

relatively small single stranded RNA folded in a precise 3D structure

73-93 nucleotides - 24,000-31,000 MW

(About same size as 250-300AA protein so stable core)

Mitochondrial and chloroplast tRNA distinctive, and smaller

as in Crick's wobble hypothesis all cells contain at least 32 tRNAs

Some have more

Yeast tRNA^{Ala} first to be sequenced

Figure 27-16

76 bases 10 modified

many common structural features Figure 27-17

8 or more nucleotides modified

Can be in base or sugar

Usually methylation

Most have pG cap at 5' end

(Not a special cap, just a G)

All have CCA at 3' end

H bonding pattern is 4 armed 'cloverleaf

Some, longer tRNA's, have a 5th arm

3D structure twisted L figure 27-18

L has two arms

Amino acid arm

AA esterified by COO to 2' or 3' OH of 3' A

Anticodon arm

Contains anticodon to bind to mRNA

Other arms of H bond structure

T ψ C and D occupy hinge between arms

Funky bases needed for funky base pairing to hold together

T ψ C arm interacts with large subunit of ribosome

Now that you know the players, let's look at protein synthesis

D. Aminoacyl-tRNA synthetases attach correct AA to tRNA

Each synthase specific for 1 AA and one or more tRNA

Most organisms a single synthase for each AA

(Even if use different anticodons)

All in E coli synthetases have been isolated and crystallized

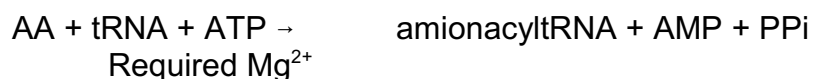
2 classes based on structure and mechanism

Figure 27-19

2 classes are seen in all organisms, no evidence of a common ancestor!

Why two classes for the same reaction? Who knows!?

Reaction catalyzed is:



2 step process

Step 1 COO⁻ of AA attacks α P of ATP

Release PPi and get AA -AMP through a high E mixed anhydride

Step 2 2' or 3' OH of tRNA displaces the AMP

Slightly lower E ester linkage

This is where we get the two different classes of mech, if used 2' or 3'

Net reaction -29 kJ/mole

but even higher since PPi hydrolyzed to 2Pi

Many synthetases have proofreading abilities

Note ILE and VAL are very similar C(CH₃)₂ and C(CH₃)CH₂CH₃

Only 1 methylene group difference
 While binds Ile 200 x better than Val
 Incorrect incorporation of val is ILE only 1 in 3,000

Why is this better than binding? How get such great selectivity?

1 nice tight binding for proper AA into proper site
 But that doesn't account for it all
 Enzyme has distinct second site that can bind INCORRECT VAL-AMP and hydrolyzes. Correct one doesn't fit and is not hydrolyzed

Thus uses two independent binding events so get increased specificity
 Say 1 binding event only goof to 1/100 have $1/100 \times 1/100 = 1/10000$

This is proofreading at the AA-AMP level, before the AA is even attached to the t-RNA. Many synthases also proofread the AA-tRNA complex and can hydrolyze that linkage if it is wrong.

Interesting synthases for AAs with no close structural analogs, like Cys, have little or no proofreading activities!

Overall error rate on protein synthesis 1/10,000
 Not as good as DNA
 But most proteins will not contain an error
 And bad protein is not passed on to next generation
 So don't spend as much time or energy correcting protein errors

Interaction between Aminoacyl tRNA synthase and tRNA a 'second genetic code'

How does the synthase know it has the right tRNA?

The anticodon does some of the specificity (27-12)
 But if you look at the structure of a tRNA bound to a synthase you will see that protein can use entire side of a tRNA so interactions are spread out on several places on the tRNA

In fact, with Ala-tRNA can cut out just about everything INCLUDING the anticodon loop and get it to work, all you need is a single G-U pair in the AA arm! (Figure 27-23)

E. Stage 2: Initiation

First some additional details on AA activation

Always occurs at AUG

This is Met

Met only has only 1 anticodon AUG

yet are 2 tRNA's

One for initiation AUG, one for internal AUG

In bacteria

tRNA^{Met} and tRNA^{fMet}

Initiation uses fMet

F met is n-formyl methionine

Structure page 1088

Cannot be used internally

Met synthase attached Met to both tRNA's

Separate enzyme called transformylase

Binds tRNA^{fMet} only

Formylates N using N¹⁰ formyltetrahydrofolate

tRNA^{fMet} is the only tRNA that binds to ribosome at a special initiation site

In Eukaryote using cytosolic ribosomes

2 separate tRNA met's

But initiation Met not formylated

In mitochondrial and chloroplastial ribosomes IS formylated

I. Initiation Step 1 (figure 27-25) Prokariots

30S ribosome binds two initiation factors IF-1 & IF-3

IF-3 keeps 50S large unit from coming in too soon

mRNA binds to 30S ribosome complex

Find right spot on mRNA using Shine-Dalgarno sequence

8-13 bases upstream from AUG

See figure 27-26

Region at end of 16S rRNA has consensus match with region just upstream from start codon

This Shine-Dalgarno sequence has is what

distinguishes an interior MET from an initiation Met

Prokariotic ribosomes have 3 sites to bind t-RNA's

(figure 27-25 step 3)

Aminoacyl or A site

Peptidyl or P site

Exit or E site

A&P sites part on both 30S and 50S

E site mostly on 50S

During initiation EF-1 binds to 30S ribosome A site so nothing can get in

The AUG start codon is moved so it is at the P site on the 30S

This is only site the tRNA^{fMet} can bind
And it is the only tRNA that binds at this site first!

Initiation step 2

Now bind IF-2·GTP and fMet-tRNA^{fMet}
And get everything aligned

Initiation step 3

50s ribosome binds
IF-2·GTP is hydrolyzed to GDP + Pi
All three IF's leave

Now have function 70S ribosome, called *initiation complex*

II. *Initiation in Eukaryotic cells*

A bit more complicated

mRNA bound to complex by several specific binding proteins
3' end had poly A tail, bound by poly(A) binding protein
(PAB)

Others as well

At least 9 initiation factors

1 complex called eIF4F

Contains eIF4G, eIF4E and others

eIF4E binds to 5' cap region of mRNA

protein eIF4G (part of complex)

Binds both eIF4E(5' end of mRNA)

And

PAB (binds at 3' end)

See figure 27-27

Having 2 ends of RNA is part of control mech (next chapter)

Initiation site NOT by shine -Dalgarno

but by first AUG from the 5' end

(Just scans until it is found)

Table of what is known right now is 27-8

Not complete - don't need to know

F. *Stage 3: Elongation (peptide bond formation)*

Again start with bacteria

Need

Initiation complex

Aminoacyl-tRNA's

3 soluble factors

EF-Tu, EF-Ts, EF-G

And GTP
following 3 steps repeated until protein is made

I. Elongation step 1: Binding of incoming aminoacyl-tRNA

Figure 27-28

Correct AA-tRNA is bound to EF-Tu/GTP complex
(All 20 complex are out there, but only the correct one enters the site)

AA-tRNA/EF-Tu/GTP complex enters A site of 70S ribosome
GTP hydrolyzed, EF-Tu/GDP released
Separate reaction with EF-Ts to regenerate Tu-GTP complex

II. Elongation step 2: Peptide bond formation Figure 27-29

Amine of AA in A site acts as nucleophile
Form bonds with C=O of AA in P site
Bond between P site AA and P site t-RNA opens up
But P site tRNA still bound
Enzymatic activity associates with this reaction called *peptidyl transferase and is 23S RNA activity!*

III. Elongation step 3: Translocation Figure 27-30

Ribosome shifts 1 codon toward the 3' end
This shifts empty tRNA to E site
Where it is released to cytosol
Peptide/tRNA to P site
Open up A site for next AA-tRNA
Requires EF-G cofactor (also called translocase)
Accompanied by GTP hydrolysis
Mech not known, may involve EF-G binding like a AA-tRNA to A- site to pull things along

Cycle now repeats

Elongation in Eukaryote similar

3 factors eEF1 α , eEF1 $\beta\gamma$, and eEF2 (Tu, Ts, G respectively)
No E site, tRNA expelled directly

IV. Proofreading

No actual proofreading function
Just the time it takes to bring the AA-tRNA/EF-Tu/GTP complex and hydrolyzes

If incorrect AA, will dissociate before process is complete

Can slow down by using GTP γ S (S in place of O see [right column page 1093](#))

React slower, has more time to proofread,
fidelity increased
Synthesis rate decreased

Assume that mother nature has found optimum compromise between fidelity and synthesis rate (or intelligent design?)

G. Stage 4: Termination [Figure 27-31](#)

Continue as above until hit a stop codon
(UAA, UAG, or UGA)

Now need 3 factors

RF₁ recognizes UAG and UAA

Rf₂ recognizes UGA and UAA

Either RF1 or RF2 binds to stop codon

Induces peptidyl transferase to use water (instead of NH of next AA), hence separating peptide from tRNA. Peptide may then float off

RF₃ thought to release ribosomal units but not certain

Eukaryote has a single eRF that recognizes all three codons

Recycling to start over

After RF1 or RF2 and peptide released

EF-G and ribosomal recycling factor (RRF) bind

EF-G hydrolyzes GTP

30S and 50S units separate

EF-G and RRF replaced by IF-3 and ready to begin again

I. Energy Cost

2 ATP to bind AA to t-RNA

(1 ATP used if incorrect AA hydrolyzed in proofreading step)

2 GTP / AA in synthesis

1 in elongation and 1 in translocation)

Net 4 ATP/peptide bond (or higher if edited out a bad AA)

4 x 30.5 = 122 kJ E used

Actual E of peptide bond -21 kJ

So have wasted about 100 kJ/bond

Books says the 100 kJ is used to make sure is low error rate
I personally think is done for increase in speed.

II. Rapid translation of a single message in polysomes

Large clusters of 10-100 ribosomes
Found in both Eukaryotes and Prokaryotes
Single RNA several ribosomes on it at once

Figure 27-32

In bacteria even more tightly coupled
Protein synthesized on mRNA, even as RNA is still being
synthesized from the DNA! Figure 27-33
Can't happen in Eukars - mRNA must leave nucleus

Bacteria have to be fast, mRNA has short lifetime so quickly
degraded

H. Stage 5: Folding and processing

assume protein starts folding even while being synthesized

Sometimes can't fold until additional processing is completed

Post Translational Modification

NH₂ and COOH termini modifications

In prokaryotes all protein have N terminal fMet
Often this and additional AA removed by peptide
cleavage
COOH terminal residue also sometimes removed

Also happens in Eukaryotes

In addition about 50% of eukaryotic proteins have N-acetyl
added to N terminus

Some COOH terminal modifications as well

Loss of Signal Sequences

15-30 residues at N terminus used to target protein to proper
cellular compartment. Get this process started and remove
this signal (more in a bit)

Modification of AA's (Fig 27-34)

Some Ser, Thr, Tyr phosphorylated
 Extra COOH to glu
 Mono and dimethyl lys
 Etc

Attachment of carbohydrate

Saw in proteins that add carbohydrate
 This done during or shortly after synthesis

Addition of Isoprenyl Groups

Used to anchor proteins in membrane
 Added to cys of protein

Figure 27-35

Proteins modified in this way include Ras proteins and G proteins and lamins

Ras proteins are product of ras oncogenes
 Carcinogenic activity of ras gene lost when this reaction is blocked!

Addition of prosthetic groups

Proteolytic cleavage
 Formation of disulfides

I. Protein synthesis inhibited by many antibiotics and toxins

Central process in cell physiology
 natural target for antibiotic and toxins
 enough difference between prokaryotes and eukaryotes that can target one and not the other

Some listed here target prokaryotes, harmless to eukaryote

Puromycin Fig 27-36

Made by mold
 Structure similar to 3' end of aminoacyl-tRNA
 Bind to A site
 Gets linked to growing peptide
 Peptide synthesis stops and ends

Tetracyclines right column page 1098

Block A site, can't bind t-RNA's

Chloramphenicols block peptidyl transferase

Cyloheximide - blocks peptidyl transferase in 80S of eukaryotes but not prokaryotes or mitochondria or chloroplasts

Streptomycin - misreading of genetic code at low conc
Inhibits synthesis at higher concentrations

Diphtheria toxin - modifies eEF2 stopping synthesis

Ricin(from castor beans) -inactivates 60S ribosome of eukaryote by depurinating an A in the 23 S rRNA

27.3 Protein Targeting and Degradation

almost all proteins synthesized on cytosolic ribosomes
yet many end up in specific organelles - How is this accomplished?

Proteins for secretion or integration into PM or into lysosomes
Share first few steps as targeted for ER
Proteins mito, chloro and nucleus have 3 separate pathways
Cytosolic just stay where synthesized

One important element is *signal sequence*
small piece of protein on N terminus, removed as processed

degradation a second process - molecular signals imbedded in protein itself?

A. For many proteins *posttranslational modification begins in ER*
most lysosomal, membrane or secreted proteins have N terminal signal
see [figure 27-37](#)
has been seen on 100's of proteins
varies from 13-36 AA's
1 or more + charge near N terminus
10-15 hydrophobic
Short sequence near cleavage site
Polar and short side chains often Ala

Starts with synthesis in free cytosolic ribosomes ([figure 27-38](#))
As sequence emerges from ribosome
Ribosome, mRNA, and peptide bound up in *signal recognition particle (SRP)*
SRP binds GTP, halts elongation after about 70 AA emerged
Binds to cytosolic face of ER at peptide translocation complex at an SRP receptor site
GTP hydrolyzed
Elongation resumes

Protein fed through membrane into ER lumen
 Signal sequence clipped off by peptidase in ER lumen
 At end ribosome released to do it again

B. Glycosylation role in protein targeting

further modifications

Protein fold in lumen

Disulfides form

Glycosylated

Often N linked (asn)

Glycosylation pattern and mech diverse but share same initial step

A core 14 sugar oligosaccharide built on lipid then transferred en toto from dolichol-phosphate to chosen ASN

Figure 27-39

Transferrals on lumen side of ER go can't do anything to cytosolic proteins

Once on protein, can be trimmed and added to

Always retains a pentasaccharide core

Several antibiotics mess with this process and have helped piece it together

Best characterized *tunicamycin* (**structure 1102**)

Mimic UDP N-acetylglucosamine

First sugar added in entire process

Back to proteins

Protein in ER lumen moved to Golgi in transport vesicles

Figure 27-40

In golgi further processing

O-linked sugars added

N- linked further modified

By unknown mech proteins sorted and sent to destinations

Don't know what the key is, can't be the signal peptide because that is long gone!

One piece we know is sorting of hydrolyases for lysosomes

As hydrolyases arrive in Golgi from ER some unknown signal

Triggers phosphorylation of certain mannose in oligo part

This phosphorylated mannose is bound by a receptor to hold in place

Eventually this buds off of Golgi and goes to sorting vesicle

Sorting vesicle has lower pH
 This dissociates protein from receptor
 Phosphate is also removed from mannose
 Receptor recycled to Golgi
 Vesicle buds and moves to lysosome

If treat with tunicamycin, hydrolases that should be targeted to lysosome are secreted
 (Don't get first N-linked sugar so signal messed up)

C. Proteins targeted to Mito and Chloroplasts

Protein for these organelles have amino terminal sequence that is bound by a cytosolic chaperonin
 Complex delivered to receptor on outside surface of organelle
 then to protein channel that spans both inner and outer membrane of organelle
 translocation through channel required ATP or GTP and H⁺ gradient.
 Once inside signal gets cleaved and protein refolds

Additional Information from 3rd ED text

Mitochondrial signal sequence

20-35 AA's
 Rich in Ser, Thr and basic

Chaperones that bind are either HSP70 or MSF (mitochondrial import stimulation factor) Figure 27-39

Stabilize unfolded protein so doesn't ppt
 Hsp70 general found in bacteria and eukaryotes
 MSF specific for mito

Receptor on Mito called TOM

Transport across Outer Membrane
 Second TOM binds for transport across outer
 Then TIM binds and transports across inner
 (Transport across Inner Membrane)

Chaperones inside mito if protein needs help folding

Integral mito membrane protein also contain a stop transfer sequence

When get to this spot, TIM and TOM are released and protein gets stuck in membrane where it belongs

For protein in inner membrane space get stuck on outer membrane, the cleaved off of membrane!

D. Signal sequence for Nuclear protein are not cleaved

Some really tricky things going on here

Ribosomes

Protein mRNA transported to cytosol and protein made

Protein goes back to nucleus through nuclear pore

Protein and rRNA assembled to 60S and 40S ribosomes

Intact 40 & 60S ribosomes go to cytosol through pores

general proteins

Histones, polymerases, topoisomerases etc

signal sequence not removed because during cell division nuclear

membrane disappears so nuclear proteins dispersed in cytosol

after division complete, nuclear membrane reassembles and need to bring the proteins back in

Signal to go to nucleus is called the *nuclear localization sequence NLS*

Can occur anywhere in sequence

4-8 residues with several consecutive Arg or Lys

A number of proteins involved in process (figure 27-42)

Importin α , Importin β , and a GTPase called Ran

Importin α/β binds to NLS region of protein in cytosol

(α is what binds to the NLS)

Complex goes to nuclear pore (Take energy)

In nucleus separate Ran GTPases binds to both α & β importins

When the protein CAS (cellular apoptosis susceptibility)

protein binds to α importin, it releases the NLS region of the imported protein.

RAN-GTP-importin complex then transported out of nucleus

Once in cytosol the GTP get hydrolyzed to GDP

Ran fall off the importins

And cycle can begin again

Ran returned to nucleus by Nuclear Transport Factor 2 (NTF-2)

In nucleus Ran Guanosine exchange factor exchange GDP to GTP to Ran is ready to go.

E. Bacteria also use signal sequences

Don't need as many signals, just inner membrane, outer membrane, and periplasmic space

Main pathway shown in figure 27-44
sample sequence shown in figure 27-43

Proteins for export thought to fold slowly (perhaps signal sequence interferes)

Protein bound by a chaperone called Sec B
 complex delivers to SecA associated with inner surface of cytoplasmic membrane

Acts as both receptor, translocase and ATPase

SecB released, Sec Y, Sec E and Sec G join in the complex for translocation (the SecYEG complex)

Moves protein in step of about 20 AA

Each step takes an ATP

Some bacterial transport follows a pathway more like a SRP

Signal peptide

Synthesis arrested on the ribosome

Ribosome/mRNA complex delivered to membrane where synthesis resumes

F. Cell - protein IMPORT Figure 27-45

some protein imported into eukaryotic cells

Low-density lipoproteins (LDL)

Iron carrying protein transferrin

Peptide hormones

Circulating proteins that are to be degraded

Proteins bind to receptors in membrane in invaginations called *coated pits*

Get concentrated there then endocytosed into cell

cytosolic side of pit is composed of *clathrin* that forms a polyhedral structure (*figure 27-46*)

as more receptor bind protein, clathrin builds until vesicle buds to inside

Once inside cytosol, clathrin removed by uncoating enzyme

membrane fuses with an endosome

ATPases pump inside full of H⁺

pH makes receptors and protein dissociate

things fall apart and go their separate ways

Also a similar pathway using caveolins

Some things recycled, other degraded after have their effect

Some toxins and viruses exploit this mech to get into cell

Diphtheria toxin, cholera toxin, and influenza viruses

G. Protein Degradation

need to remove unwanted old or abnormal protein so don't build up in cell
 ½ life of eukaryotic proteins 30 sec to many days

defective proteins usually turned over quickly in both prokar's and eukaryots's

ATP-dependent cytosolic system

In Mammals a second system using lysosomes to recycles membrane proteins, extracellular proteins, and long-lived proteins

E coli ATP system

ATP dependent proteins called Lon

Called lon for long-form of proteins seen when this guy isn't around

Protease activated by presence of protein to be destroyed
 used 2 ATP's for each peptide bond cleaved
 mech not known

after lon cuts into small pieces, then other ATP dependent proteases take it to AA's

Eukaryotic ATP system

uses *ubiquitin* a highly conserved 76 residue protein
 essentially identical between yeast and humans
 gets attached to proteins for degradation by 3 proteins E1, E2, and E3

see figure 27-48

How protein is recognized is not known

protein is then bound to 26S *proteasome* complex (1×10^6 in size)
 32 different subunits

Won't worry about details of structure of complexes
 and degraded **Figure 27-48**

With use of ATP

One trigger mech for ubiquitination has been observed

Depend on n-terminal AA (after post-Translational processing)

If AA s Met, Gly, Ala, Ser, Thr, Val will last a long time

If other will degrade quickly (see table 27-9)

Other more complex signals still being investigated

Ubiquitin dependent destruction important for cell cycle

Proteins required for a given part of cycle must be quickly destroyed at end of that cell phase

E3 and E3 components are actually families of proteins
Different targets and different specificities are localized in
different cell compartment and different tissues and different
cell cycles

Defects in this pathway implicated in a large number of disease
states