REGULATION OF EUKARYOTIC TRANSLATION

EuReg 1

Mainly at the level of TRANSLATION INITIATION

by **PHOSPORYLATION** of INITIATION FACTORS:

1) eIF2, in the form eIF2·GTP binds initiator Met-tRNA$_i^{Met}$ to the 40S subunit

eIF2 + Ⓟ → **INHIBITION OF INITIATION**

↑ kinases: HRI, PKR, PERK, etc, **AMPK**

2) eIF4E = cap binding protein (CBP)

eIF4E + Ⓟ → **STIMULATION OF INITIATION**

↑ kinases

3) different mechanisms

eIF2 phosphorylation:

a) absence of heme
b) viral infection - ds RNA → interferons
c) thermal shock, UV irrad.
d) absence of growing factors
e) entry into M-phase of cell-cycle
f) absence of amino acids*
g) low ATP, high AMP (⇒AMPK)

} ACTIVATION OF SPECIFIC KINASES (eIF2 kinases)

} PHOSPHORYLATION OF eIF2 at Ser 51 of its α-subunit

} BLOCK OF PROTEIN SYNTHESIS (not *)

→ APOPTOSIS!

KINASES ACTING ON eIF2 (1)

(Composition : one conserved kinase domain, various regulatory domains)

eIF2 functional cycle : two forms of eIF2 :

GDP

eIF2 · GTP = active in initiation (binds $Met\text{-}tRNA_i^{Met}$)

REGENERATION OF ↑ INITIATION ↓

BY eIF2B

eIF2 · GDP = inactive in initiation

GEF — GTP

eIF2B ≪ eIF2 !!

Ada) HRI = heme regulated inhibitor (of translation) = kinase

= heme sensor, it binds heme (in reticulocytes)

REGULATION OF GLOBIN BIOSYNTHESIS BY HEME AVAILABILITY

low heme → HRI activation (through autophosphorylation induced by release of heme from one of HRI four binding sites ... on the kinase heterodimer)

HRI

eIF2 · GDP → eIF2 · GDP + ADP

ATP

addition of heme

heme pro-HRI inactive ← low heme — HRI active (P) + heme

ATP ADP

eIF2 · GDP — eIF2 (P) · GDP

(eIF2 phosphatase) (regeneration)

eIF-2B ⇌ eIF2 (P) · GDP / eIF2B

NO FREE eIF2B

PROTEIN SYNTHESIS STOP

polysome disassembly

formation of stress granules

tight complex !

STRATEGY:

hemoglobin

heme — globin

low heme → globin synthesis should be stopped

high heme → globin synthesis should proceed

REGENERATION OF BOTH INTACT AND PHOSPHO-eIF2 · GDP BY eIF2B IS PREVENTED BECAUSE OF SEQUESTERING OF eIF2B BY THE eIF2 (P) · GDP

KINASES ACTING ON eIF2 (2)

Add b) **PKR = (DAI)** = protein kinase activated by double stranded RNA (double stranded RNA activated inhibitor)

DEFENSE AGAINST VIRAL INFECTION by RNA viruses:
e.g. picornaviruses

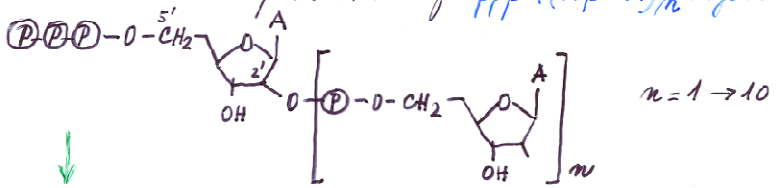
e.g. poliomyelitis, morbilli (spalničky), rabies (vzteklina), influenza, rubeola (zarděnky) | nasopharyngitis, tick encephalitis, mumps (parotitis ep.), slintavka – kulhavka = foot + mouth disease

During infection of cells by DNA and RNA viruses ds RNA is generated (or when a synthetic ds RNA longer than ~30 bp is added): induction of synthesis of interferons (cytokines). This converts the cells to an antiviral state – virus infested individuals become resistant to infection by a second type of virus

Mechanism of the antiviral effect mediated by INTERFERONS:

1) induction + activation (autophosphorylation + dimerization) of PKR → eIF2–Ⓟ → sequestering of eIF2B → PROTEIN SYNTHESIS: STOP (no production of new viral and cellular proteins)

2) induction of synthesis of (2',5')-OLIGOADENYLATE SYNTHETASE → production of ppp A(2'p5'A)$_n$ oligonucleotide



→ activation of RNase L → (viral) mRNA degradation → PROTEIN SYNTHESIS: STOP

KINASES ACTING ON eIF2 (3)

Ad c) **PERK** = PKR-like endoplasmic reticulum kinase
it is normally kept in an inactive state (inhibited) by chaperone **BiP**

Thermal shock ⇒ excess of unfolded (denatured) proteins
↓
BiP dissociates from PERK
↓
eIF2 – (P) → sequestering of eIF2B
↓
PROTEIN SYNTHESIS STOP

"ENDOPLASMIC RETICULUM STRESS"

PROTECTION OF CELLS FROM THE IRREVERSIBLE DAMAGE CAUSED BY ACCUMULATION OF UNFOLDED PROTEINS IN ER

polysome disassembly
accumulation of untranslated mRNA
aggregation
assembly of STRESS GRANULES (P-bodies)
reprogramming of mRNA

Ad 2) **eIF4E phosphorylation**:

cytokines, mitogens, growth f.
hormones (insulin)
phorbol esters
↓
ACTIVATION OF MAPK(ERK) KINASE → KINASE MNK1 → PHOSPHORYLATION OF eIF4E
↓
PI3K → PKB (Akt) → mTOR (FRAP)

PHOSPHORYLATION OF:
- 4E BP1 (PHAS I)
- 4E BP2 (PHAS II)
- 4E BP3

AMPK

eIF4E – (P): increased affinity to Cap
↓
STIMULATION OF INITIATION TRANSLATION: YES

eIF4E complex inactive in initiation
↓
release of free eIF4E:
it can bind eIF4G to form initiation complex with 40S + eIF2 etc

eIF4E + 4E-BP (INACTIVE) → phosphorylation process → eIF4E – (P) ACTIVE + 4E-BP – (P)

1. Phosphorylation of Shc (Section 19-3C) results in stimulation of a MAP-kinase cascade (Section 19-3D), ultimately affecting growth and differentiation.

2. Phosphorylation of **Gab-1 (Grb2-associated binder-1)** similarly activates this MAP-kinase cascade.

3. Phosphorylation of insulin receptor substrate (IRS) proteins (Section 19-3C) activates a phosphoinositide cascade via a PI3K (Section 19-4D), ultimately stimulating a variety of metabolic processes including glycogen synthesis (Section 18-3E) and glucose transport (Section 20-2E), as well as cell growth and differentiation.

PI3K pathway – essential signal transduction pathway for proliferation, apoptosis, metabolism, drug resistance. Alterations or mutations of PI3K pathway components often result in its deregulation & often a hallmark of cancer development and progression. The PI3K signalling also initiated by cytokines or growth factors via additional RTKs.

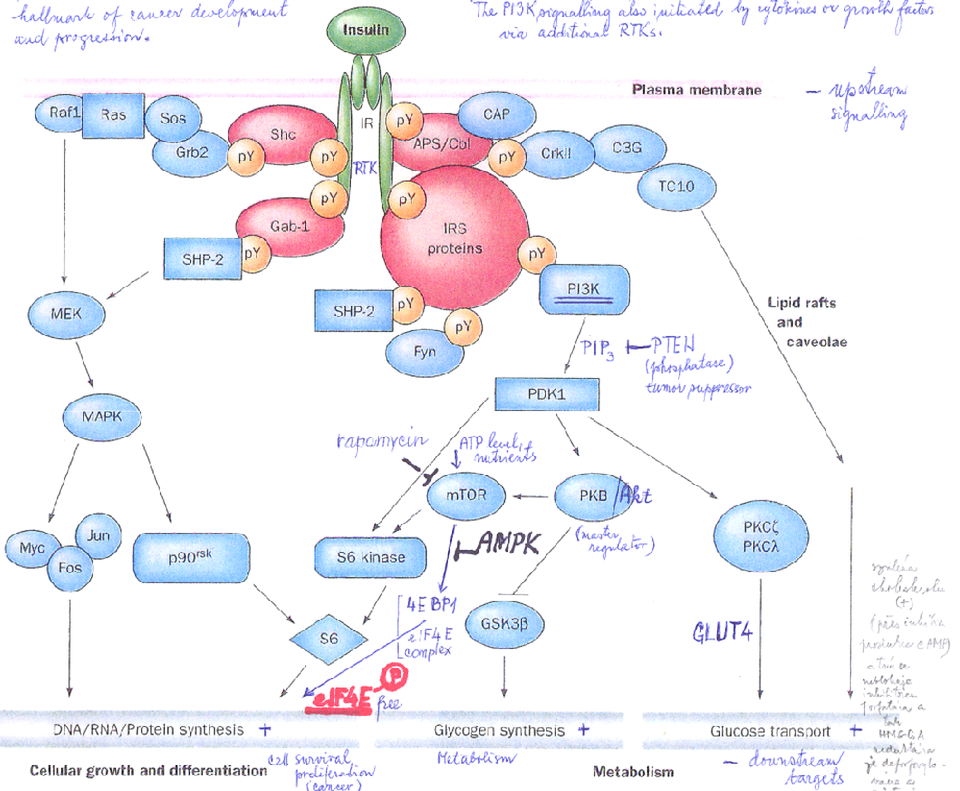


FIGURE 19-64 Insulin signal transduction. The binding of insulin to the insulin receptor (**IR**) induces its autophosphorylation at several Tyr residues on its β subunits. Several proteins, including Shc, Gab-1, the APS/Cbl complex, and IRS proteins, bind to these pY residues where they are Tyr-phosphorylated by the activated insulin receptor, thereby activating MAPK and PI3K phosphorylation cascades as well as a lipid raft and caveolae-associated regulation process. The MAPK cascade regulates the expression of genes involved in cellular growth and differentiation. The PI3K cascade leads to changes in the phosphorylation states of several enzymes, so as to stimulate glycogen synthesis, as well as other pathways. The PI3K cascade also participates in the control of vesicle trafficking, leading to the translocation of the **GLUT4** glucose transporter to the cell surface and thus increasing the rate of glucose transport into the cell (Section 20-2E) via a system in a PI3K-independent manner involving lipid rafts and caveolae. Other symbols: Myc, Fos, and Jun (transcription factors; Section 19-3D), SHP-2 (an SH2-containing PTP; Section 19-3F), **CAP** (Cbl-associated protein), **C3G** [a guanine nucleotide exchange factor (GEF)], **CrkII** [an SH2/SH3-containing adapter protein), **PDK1** (phosphoinositide-dependent protein kinase-1; Section 19-4C), **PKB** (protein kinase B, also named Akt; Section 19-4D, **mTOR** [for *m*ammalian *t*arget *o*f *r*apamycin, a PI3K-related protein kinase; **rapamycin** is an immunosupressant similar to FK506 (Section 9-2B); mTOR is also known as **FKBP12-rapamycin-associated protein (FRAP)**], **S6** (a protein subunit of the eukaryotic ribosome's small subunit (Section 32-3A; its phosphorylation stimulates translation], and PKCζ and PKCλ (atypical isoforms of protein kinase C; Section 19-4D) [After Zick, Y., *Trends Cell*

REGULATION OF EUKARYOTIC TRANSLATION

Micro-RNA (miRNA)

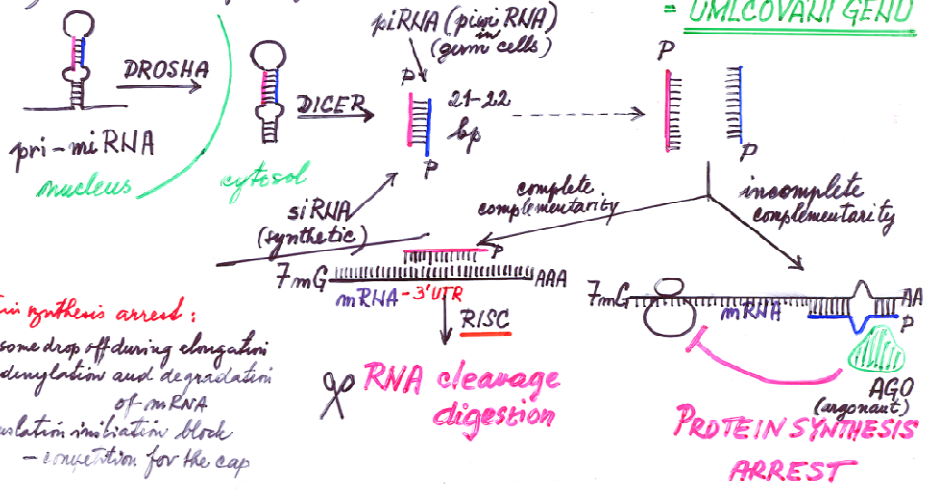
inhibition of translation of specific transcripts by (complementary) short RNAs

Cellular mRNAs have ds regions usually shorter < 10 bp.

If RNAs with longer ds regions are present they result from: replication of RNA viruses
transcription of repetitive sequences
some natural mRNAs
} they are subjected to degradation by the process called RNA interference (RNAi)

RNAi process

target RNA molecules hybridize with complementary single-stranded small (21, 22, 31 nt) inhibitory RNAs (siRNAs) originating from miRNAs. This results either into
1) degradation of the target RNAs or
2) inhibition of target RNAs translation
} GENE KNOCK-DOWN = UMLČOVÁNÍ GENŮ



Protein synthesis arrest:
- ribosome drop off during elongation
- deadenylation and degradation of mRNA
- translation inhibition block – competition for the cap

RNA i system works both under

a) standard conditions ~ 25% of mammalian genes are regulated by miRNA as well as (over 1/3 of human genes)

b) in response to stress situations

↓

active role in stress fighting

↓

reprogramming of gene expression

1) stress-activated TFs induce expression of specific miRNAs

(change in the spectrum of protected and inhibited RNAs)

2) stress activates dsRNA editing mechanisms (enzymes) to target miRNA precursors to alter stability, processing, nuclear export, or target specificity of selected miRNAs

↓

STRESS-INDUCED REPAIR miRNA→ENZYMES ARE PREFERENTIALLY TRANSLATED (chaperons)

REGULATION OF PROKARYOTIC TRANSLATION

Mechanisms are based on typical prokaryotic features of gene expression processes:

- Production of polycistronic mRNAs
- Coupling of transcription and translation

1) Modulation of translation of mRNAs coding for ribosomal proteins (by the level of ribosomal r-proteins)
= TRANSLATION REPRESSION

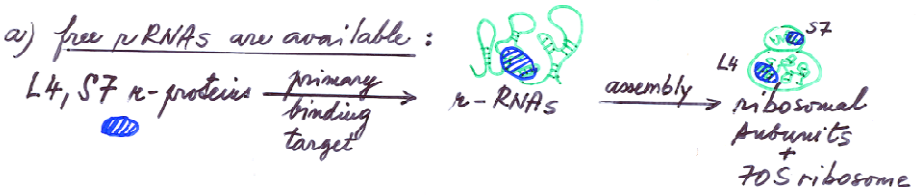
2) Modulation of production (transcription) of mRNAs coding for amino acid biosynthetic enzymes (regulating biosynthesis of some amino acids: Trp, Phe, His):
= ATTENUATION

ad 1) TRANSLATION REPRESSION

To maintain equilibrium between the level of free ribosomal RNA and the level of ribosomal proteins in order to ensure an efficient and economical assembly of ribosomal subunits.

MECHANISM: Some ribosomal proteins can bind not only to rRNA but, when in excess, to its mRNA and thus repress its translation.

a) free rRNAs are available:

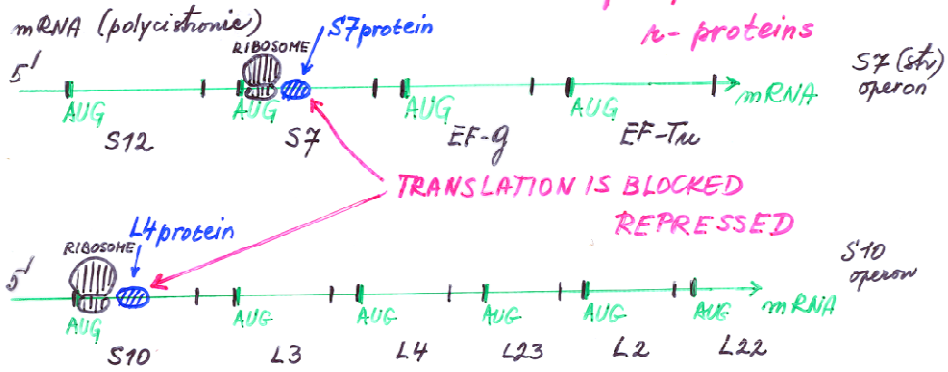


→ translation of mRNAs coding for L4 and S7 (+ other r-proteins) SHOULD CONTINUE to make new ribosomes

b) free rRNAs are not available:

L4, S7 —~~primary binding target~~→ (no rRNAs available)

secondary binding target → mRNAs coding for L4, S7 + other r-proteins → steric blocks in the movement of the ribosome on mRNAs → NO PRODUCTION of L4, S7 and other r-proteins



feed-back repression

Ad 2) ATTENUATION

To correlate de novo production of some amino acids with the needs of protein synthesis

MECHANISM: RNA polymerase transcription progression is modulated by nascent mRNA secondary structure. The secondary structure of this mRNA depends on the extent of readibility - translation of its 5' terminus called LEADER (sequence). The extent of this translation is determined by the availability of amino acid the production of which is catalyzed by enzymes coded by this mRNA.

a) amino acids are present in sufficient amounts – their de novo synthesis is not required ⇒ synthesis of mRNAs coding for their biosynthetic enzymes should be prevented

b) amino acids are not available – their de novo synthesis is required ⇒ synthesis of mRNAs coding for their biosynthetic enzymes should be allowed + promoted

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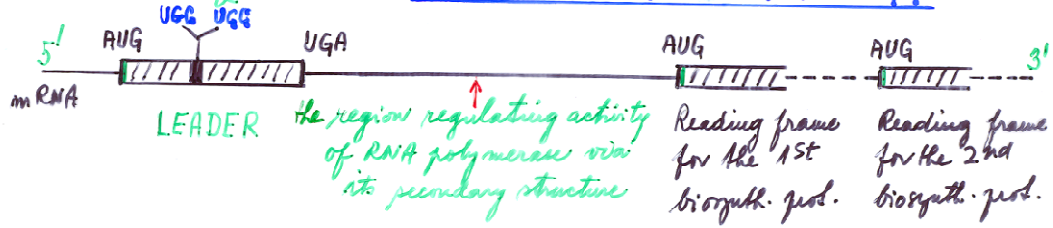
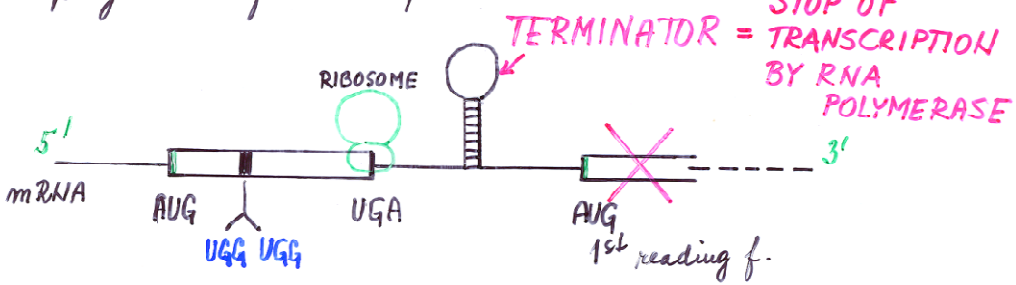
EXAMPLE: **REGULATION OF BIOSYNTHESIS OF TRYPTOPHAN**

POLYCISTRONIC mRNA coding for biosynthetic enzymes catalyzing biosynthesis of TRYPTOPHAN (~ 7000nt, 5 enzymes). Its translatable LEADER sequence has TWO TRYPTOPHAN CODONS UGG.



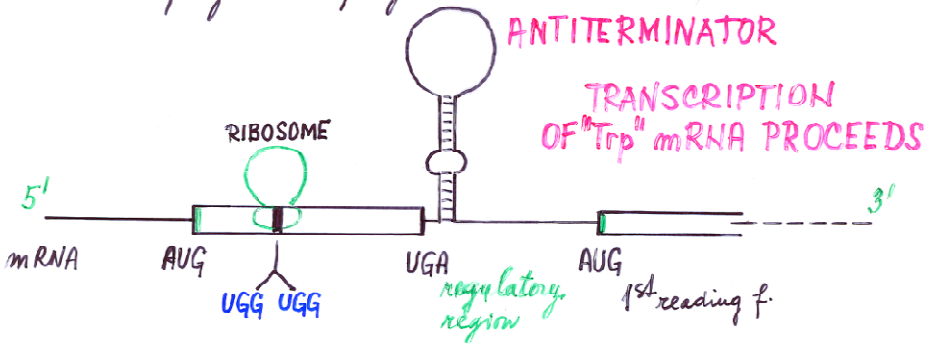
Ad a) free tryptophan (for $tRNA_{Trp}$ charging) is available:

- UGG codons in the LEADER can be translated by the ribosome which can proceed up to the UGA stop codon
- thus, the regulatory region adopts a secondary structure called terminator preventing RNA polymerase from progression of transcription



Ad b) no tryptophan (for $tRNA_{Trp}$ charging) is available
no $Trp\text{-}tRNA_{Trp}$ can be formed to read UGG codons in the LEADER

- UGG codons in the LEADER cannot be translated and the ribosome becomes stalled at these codons
- thus, the regulatory region adopts a different secondary structure called anti-terminator not affecting the RNA polymerase progression



enzymes catalyzing biosynthesis of Trp can be synthesized

mRNA regulates its own production by changing its conformation

Applies also for regulation of biosyntheses of:

Phe (3+3+1 Phe codons in the leader)
His (7 His codons together in the leader)