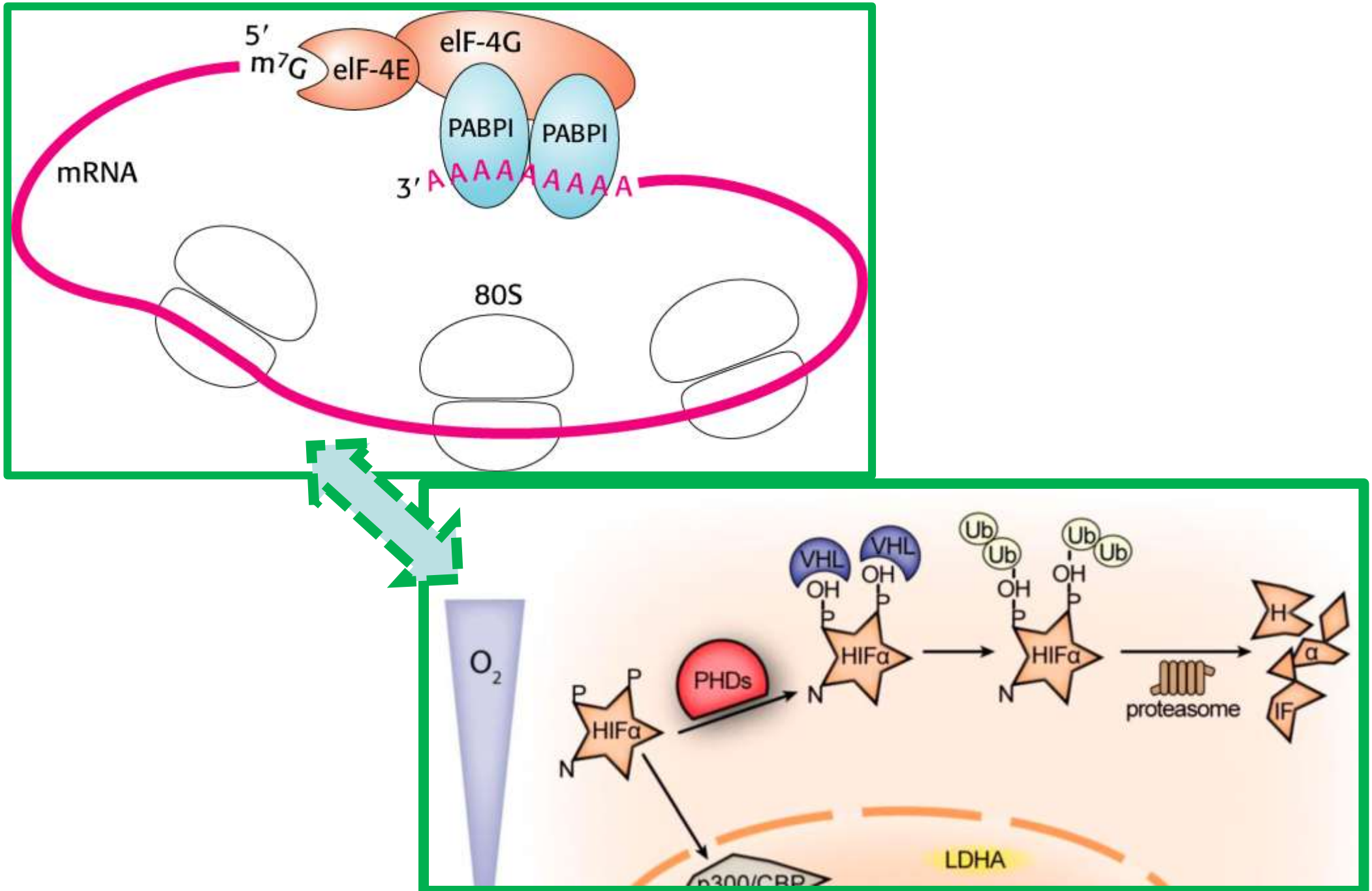
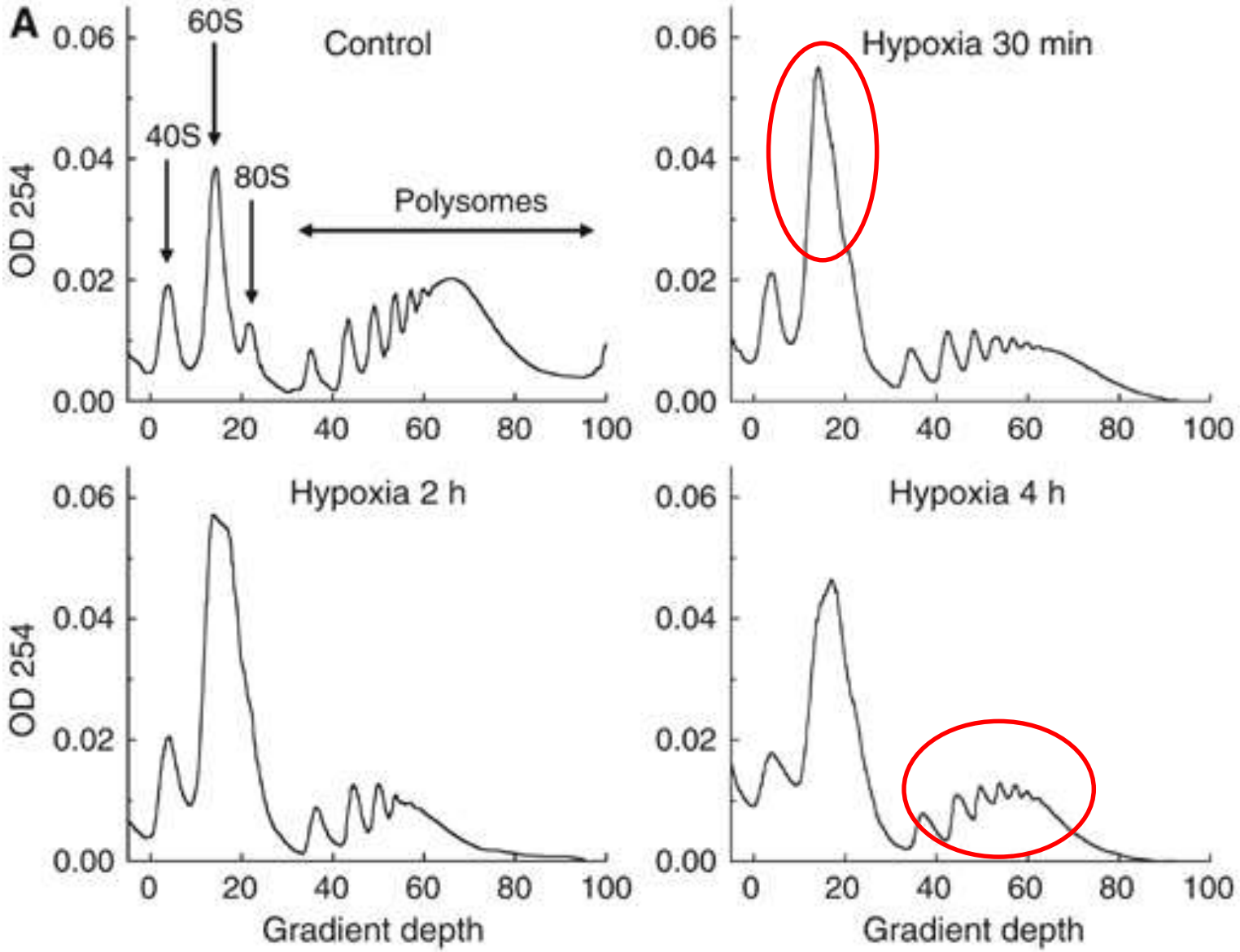
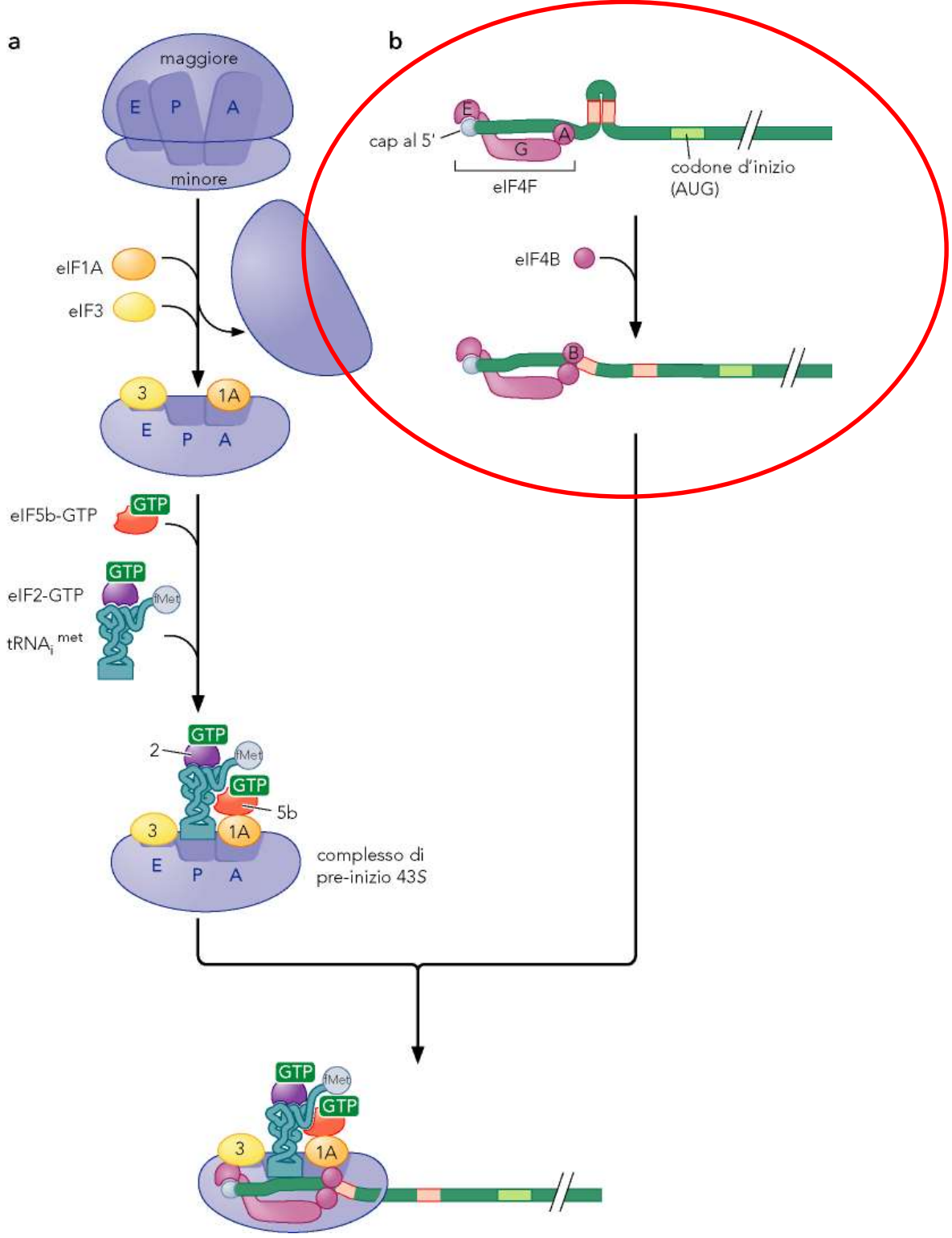
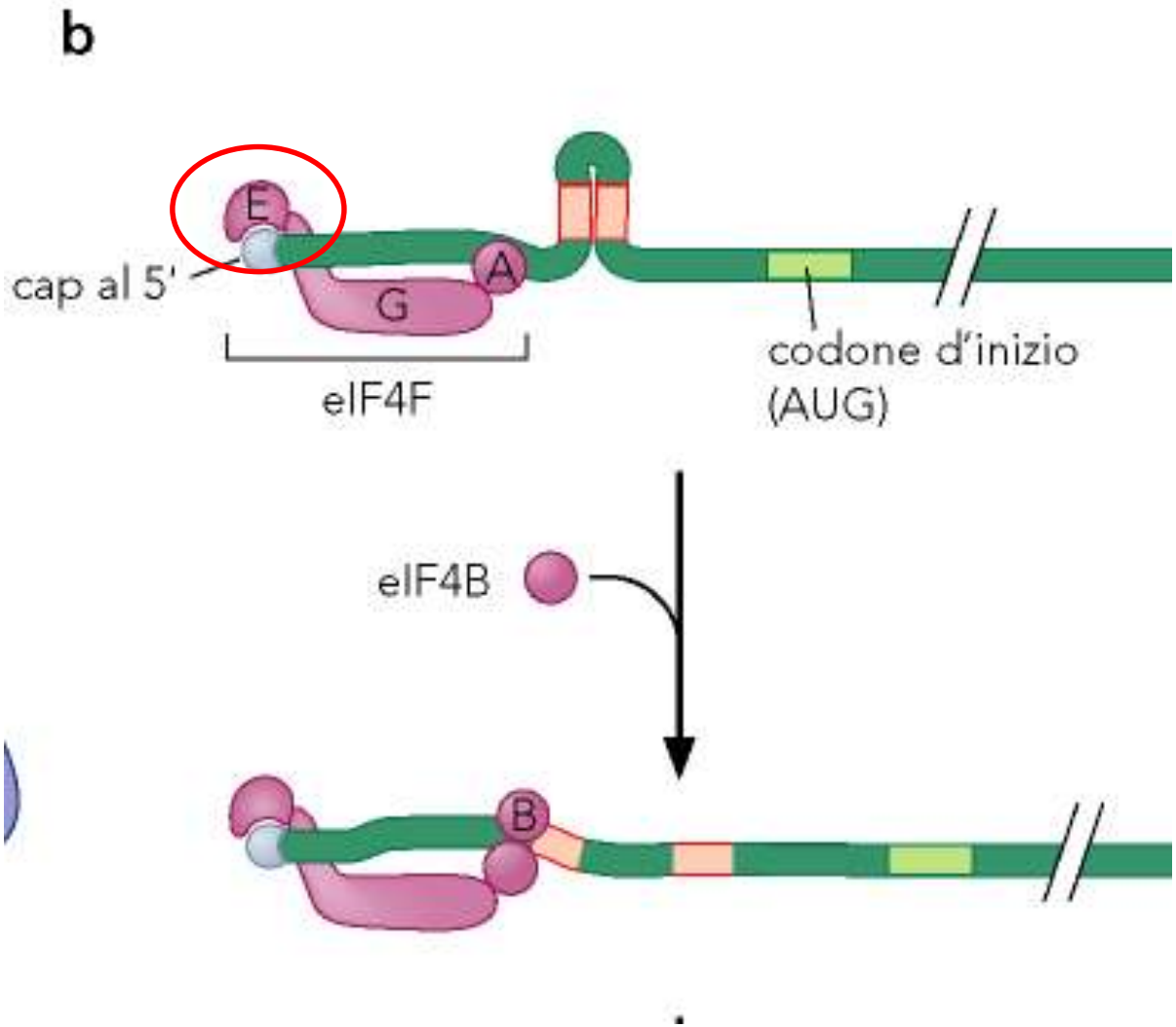
# Oxygen regulation of the protein synthesis machinery



# Hypoxia inhibits mRNA translation







- The initial step of protein synthesis is the binding of the eukaryotic translation initiation factor 4E (eIF4E) to the 7-methylguanosine (m7-GpppG) 5’ cap of messenger RNAs

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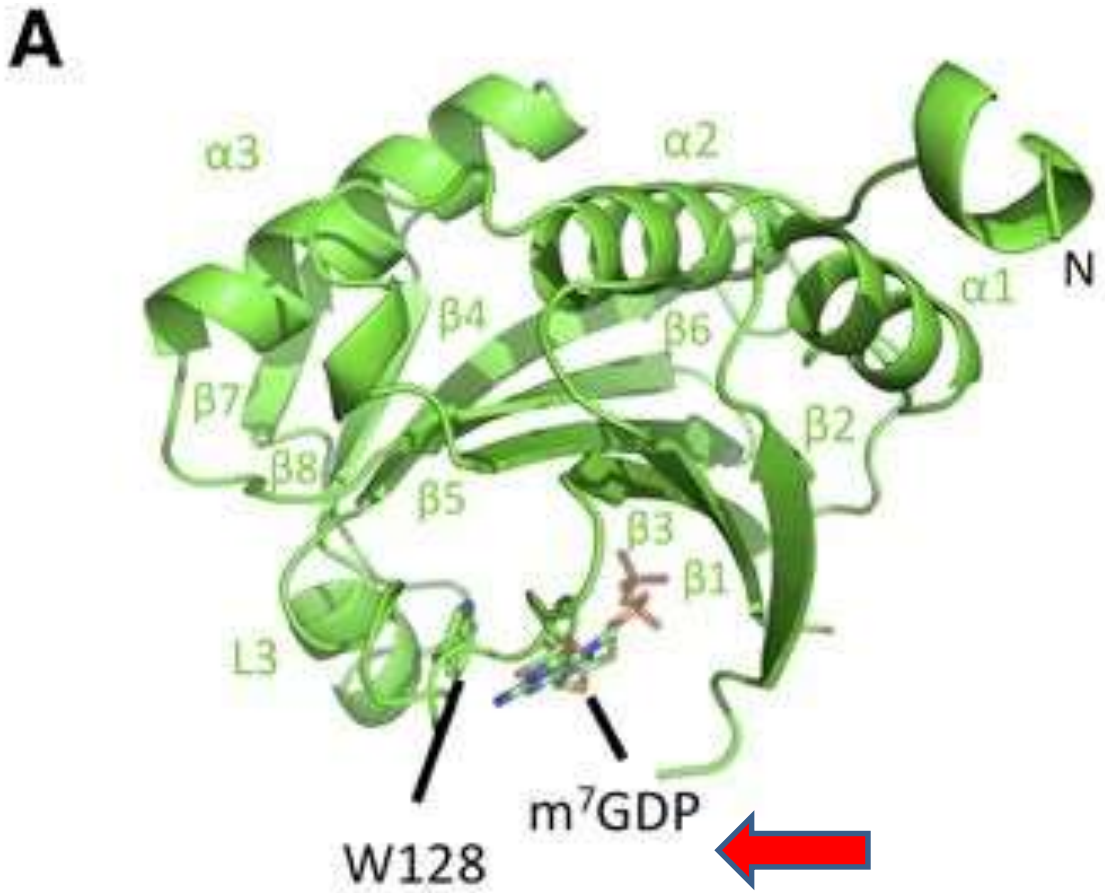
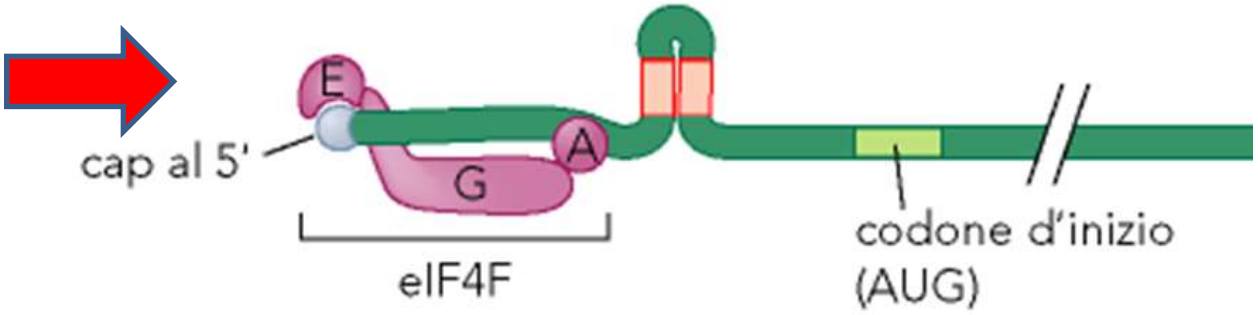
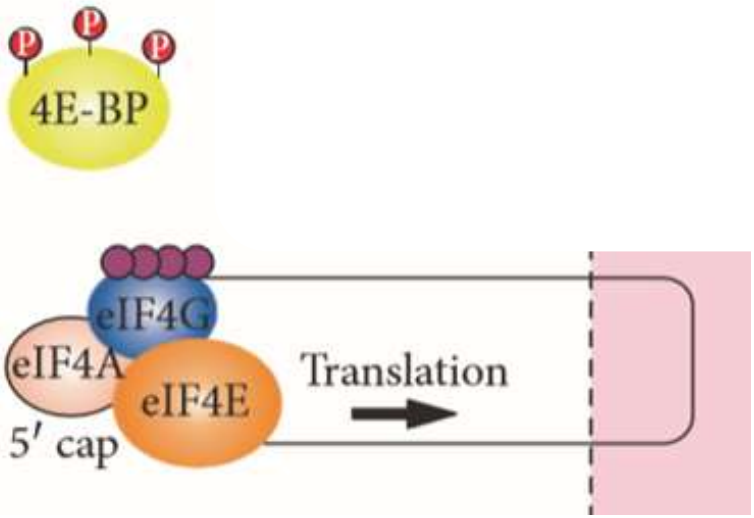


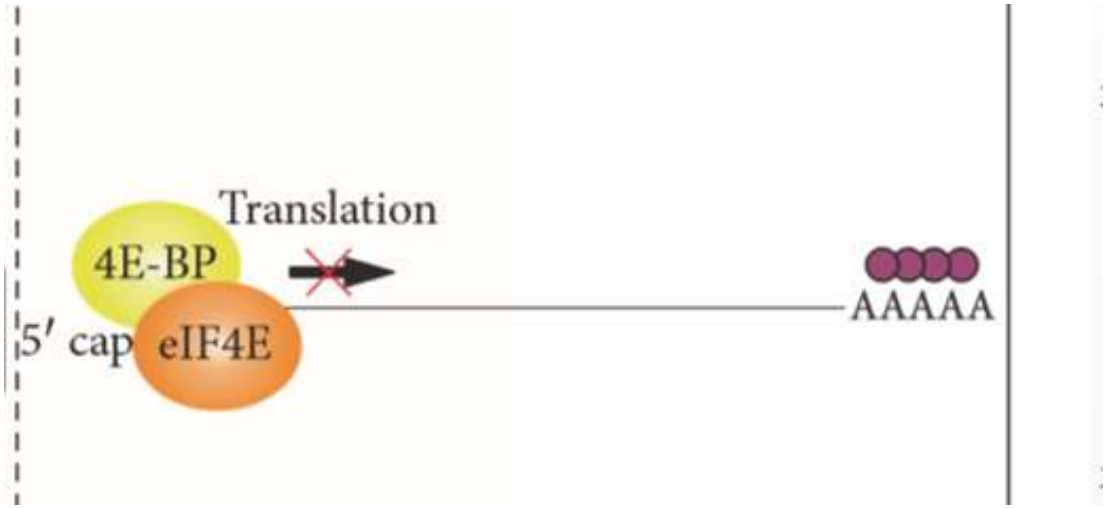

eIF4E 51-235 in complex with the m7GDP cap analog. The m7GDP is located in the cap-binding pocket. Residue W128, in direct interaction with the cap, is marked.

# Low oxygen tension (hypoxia) represses cap-mediated translation **by sequestering eIF4E**



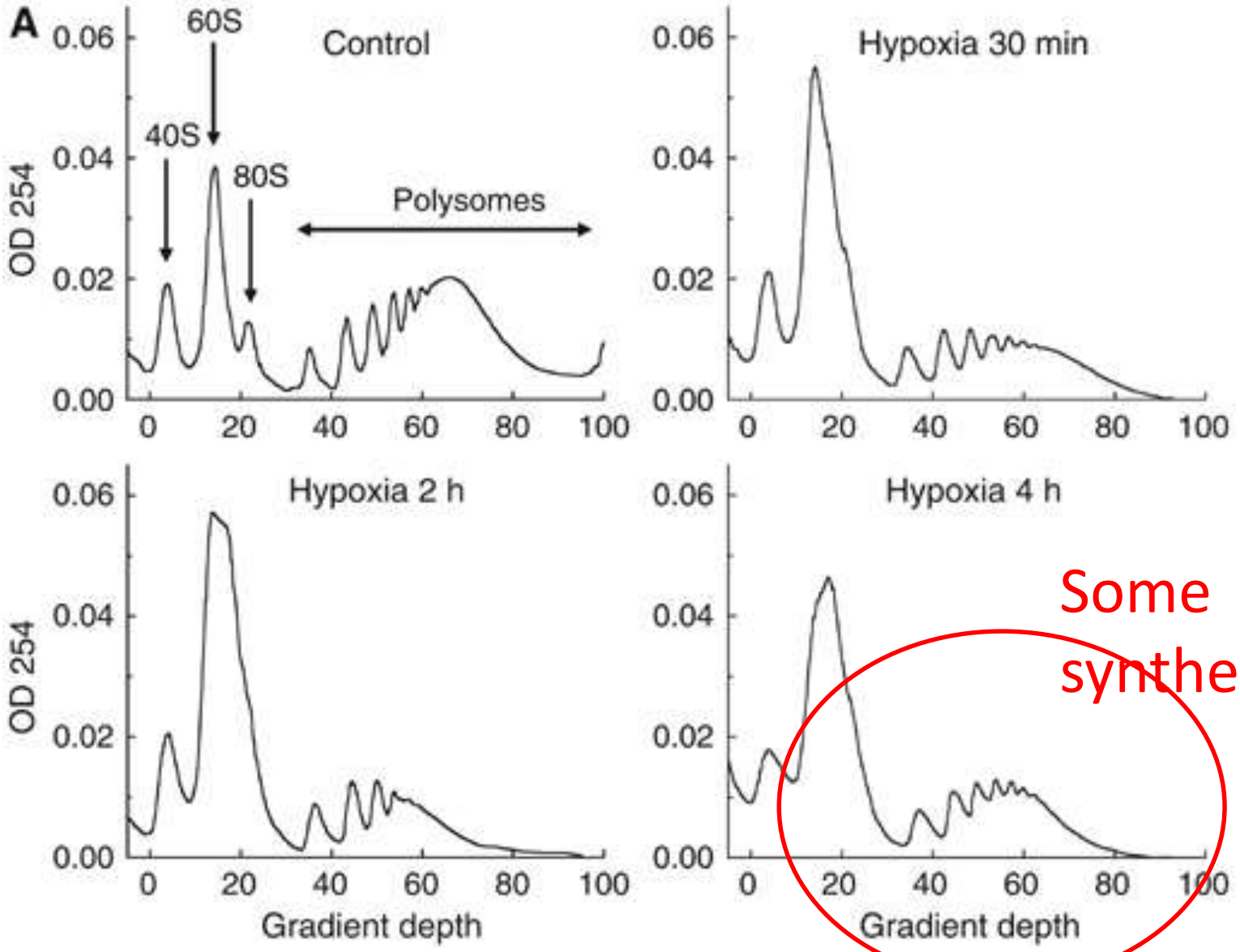

Dephosphorilated 4E-BP1 binds eIF4E and forms an inactive complex



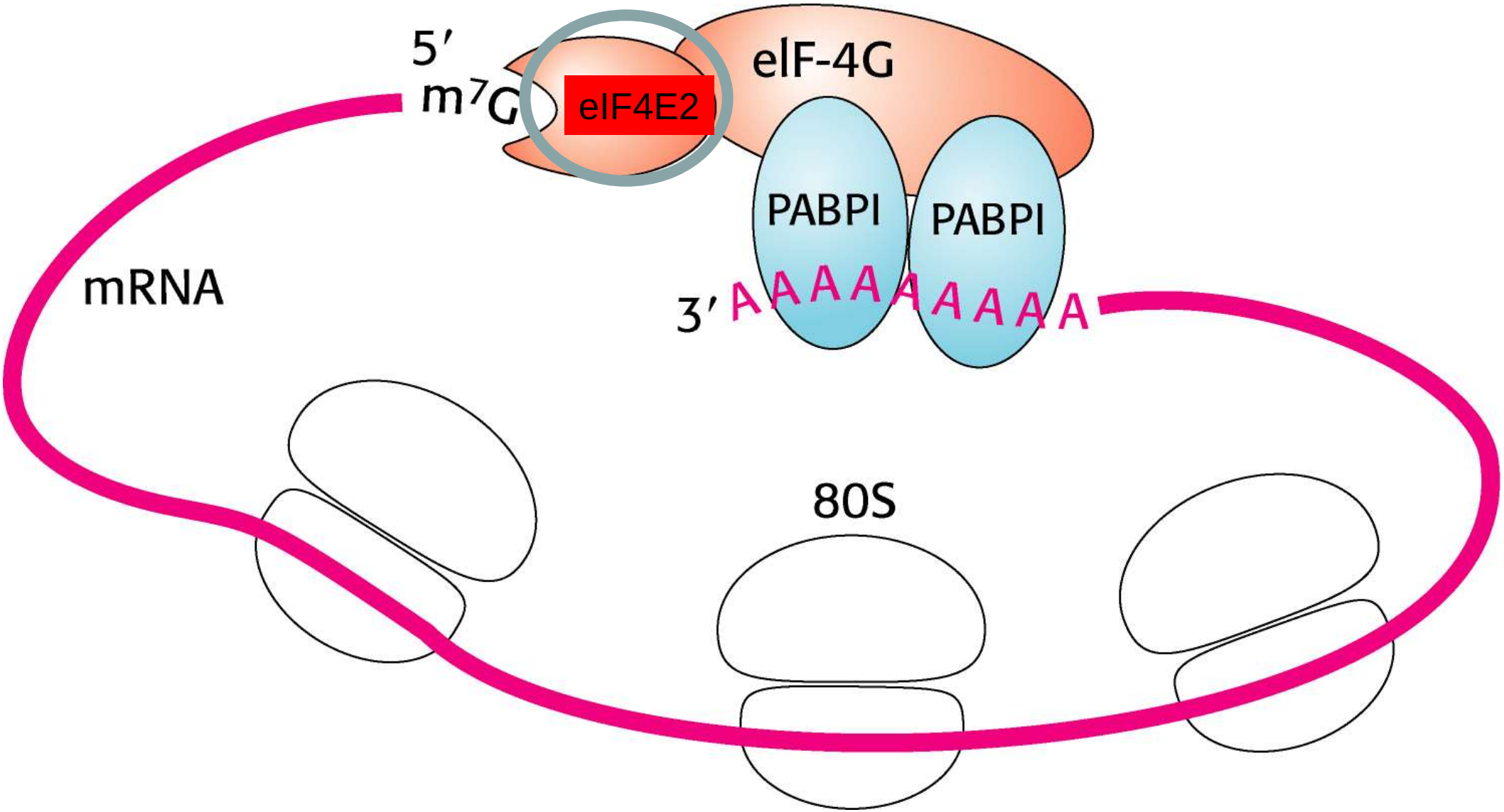



- A fundamental question in biology is as to how proteins are synthesized in periods of oxygen scarcity and eIF4E inhibition.

# Hypoxia inhibits mRNA translation

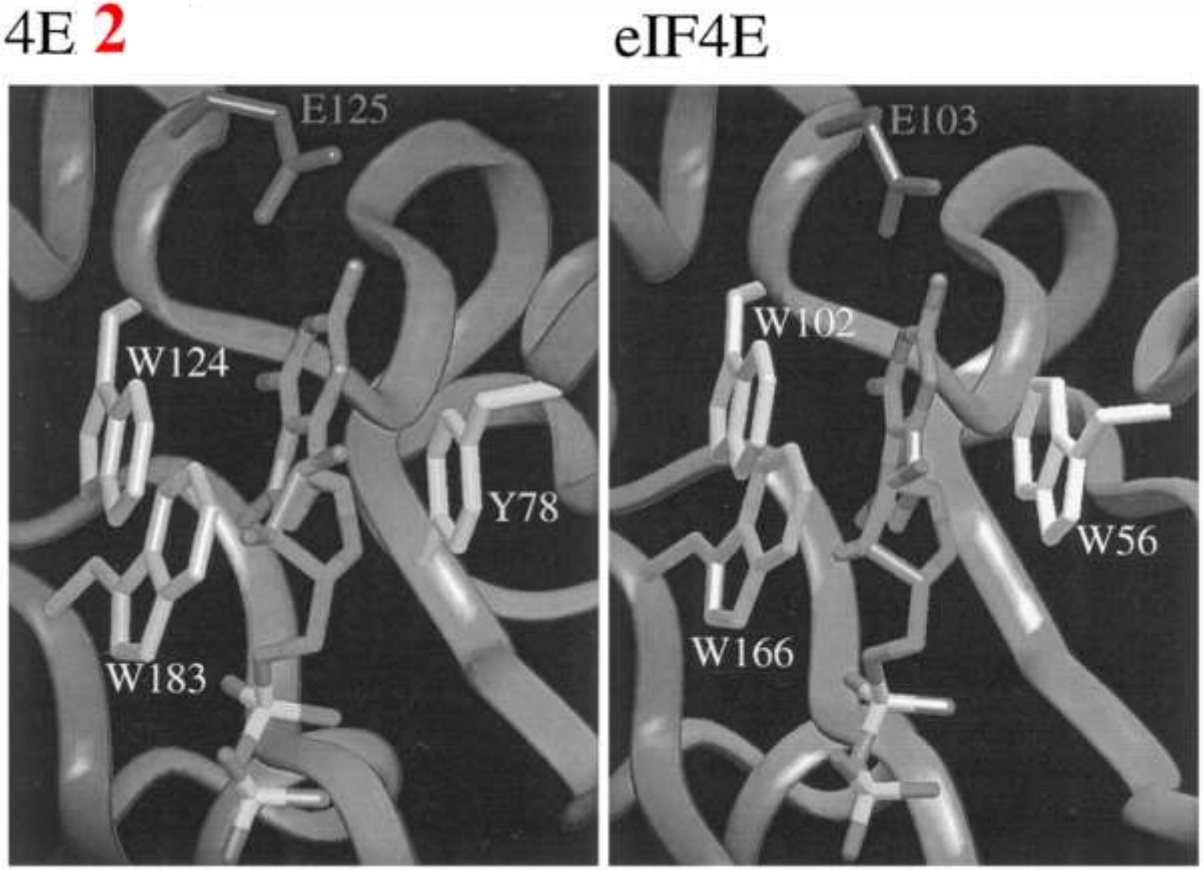



Some proteins synthesized

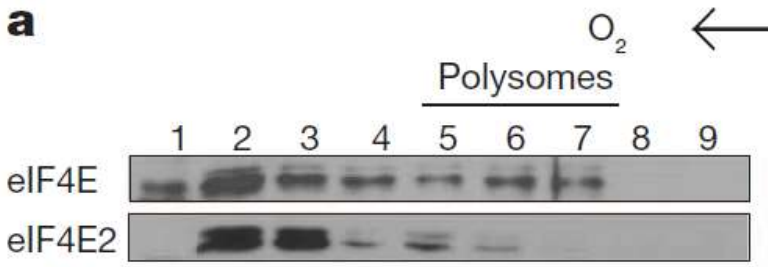


# eIF4E2

- similar to eIF4E.
- tissue distribution ubiquitous - at 10-fold lower levels
- eIF4E2 becomes available in the cytoplasm and increases in response to various forms of stress

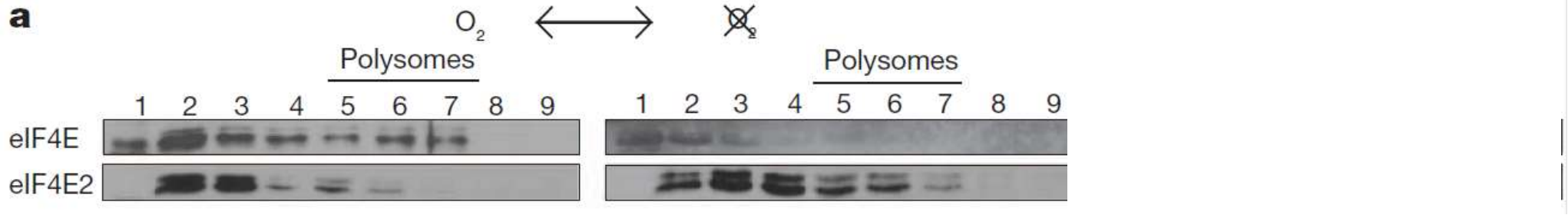


# An oxygen-regulated switch from eIF4E- to eIF4E2-dependent protein synthesis.

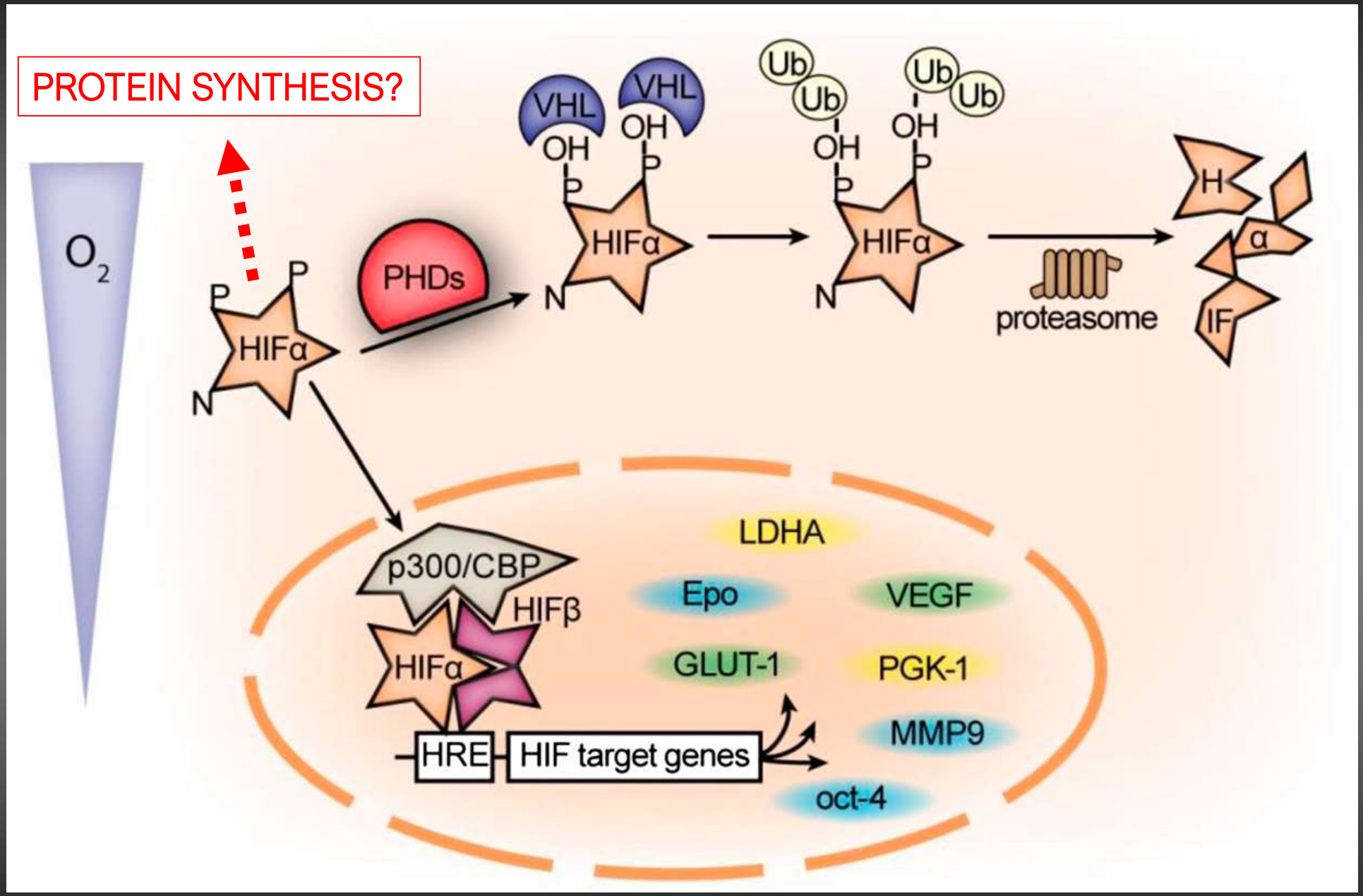


# An oxygen-regulated switch from eIF4E- to eIF4E2-dependent protein synthesis.

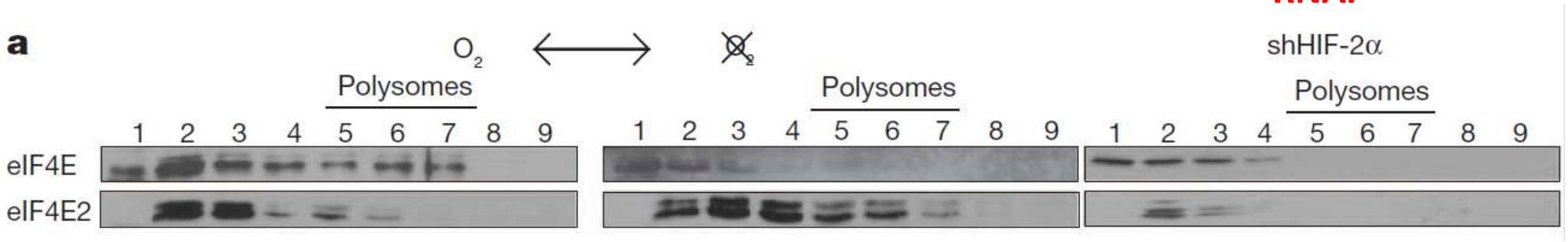
## “changing partners to keep dancing”




| eIF4E polysome association in normoxia | eIF4E2 polysome association in hypoxia |
| --- | --- |


PROTEIN SYNTHESIS?
$O_2$
HIFα
P
P
N
PHDs
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
proteasome
H
α
IF
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

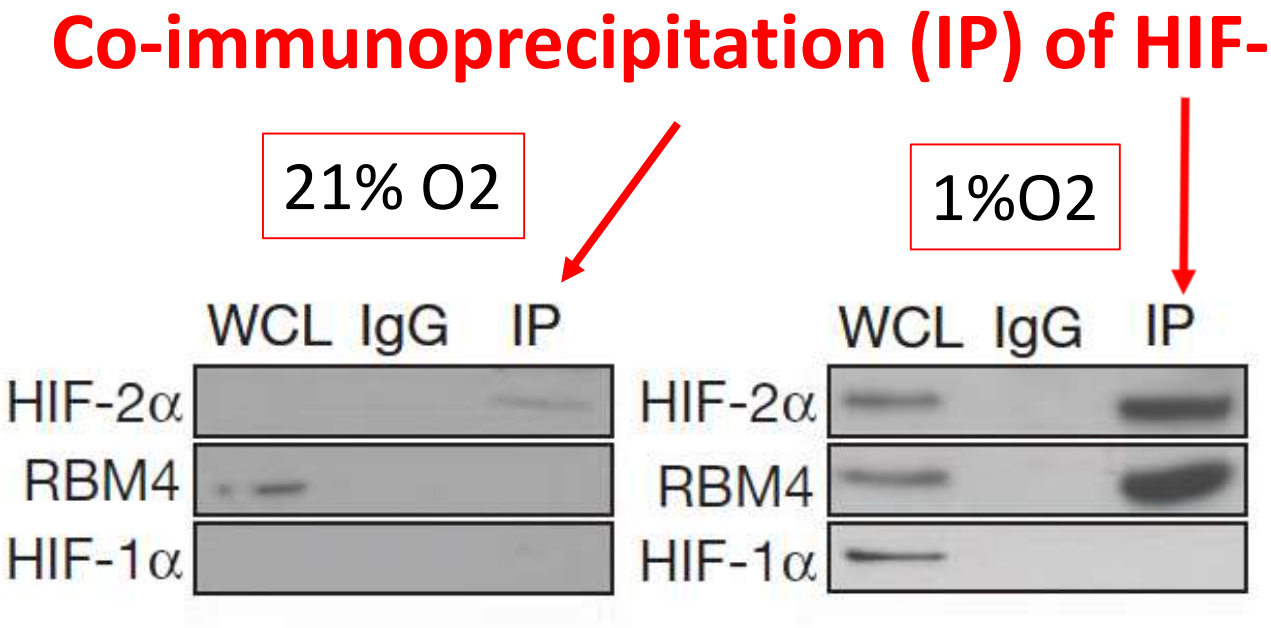
hypoxia stimulates the switch from the cap-binding eIF4E to to eIF4E2 omologue




**RNAi**

**dependent from the oxygen-regulated hypoxia-inducible factor 2a (HIF-2a)!**

# RNA-binding protein RBM4 recruits HIF-2a in hypoxia



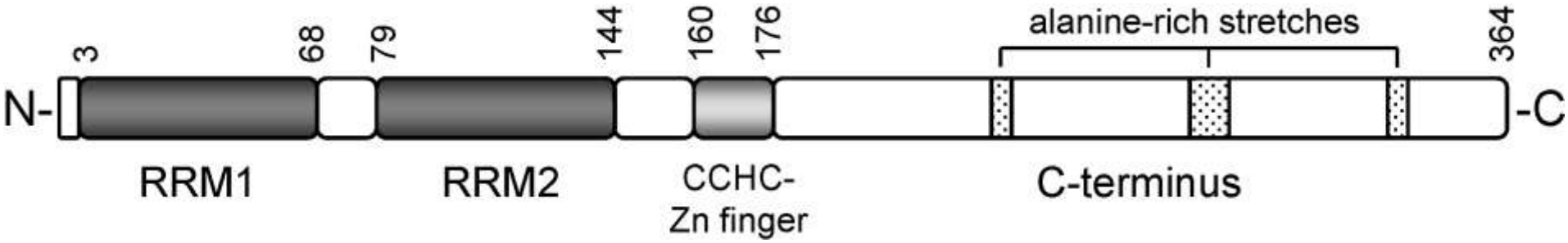

Co-immunoprecipitation of HIF-2a with RBM4 in hypoxia (right)

WCL, whole cell lysate

RNA-binding protein RBM4

oxygen-regulated hypoxia-inducible factor 2a (HIF-2a)

# RBM4




RNA recognition motifs (RRMs)

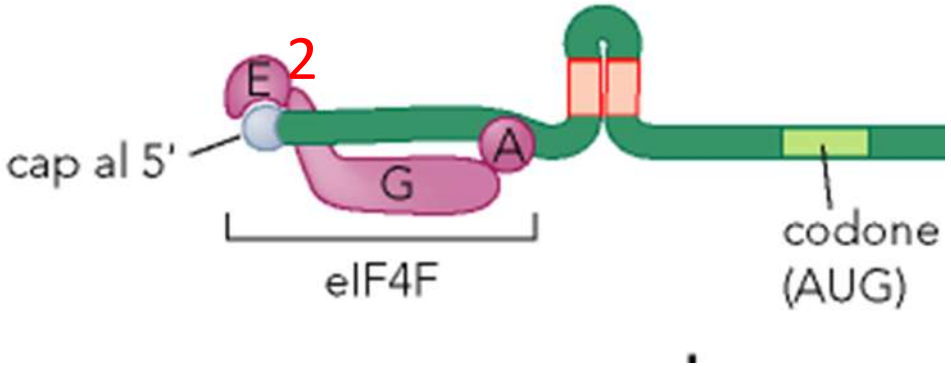
interaction of RBM4 with other proteins

- Qual' è la relazione tra RBM4 - HIF2a/ EIF4E2 - Cap m7-G?

RBM4

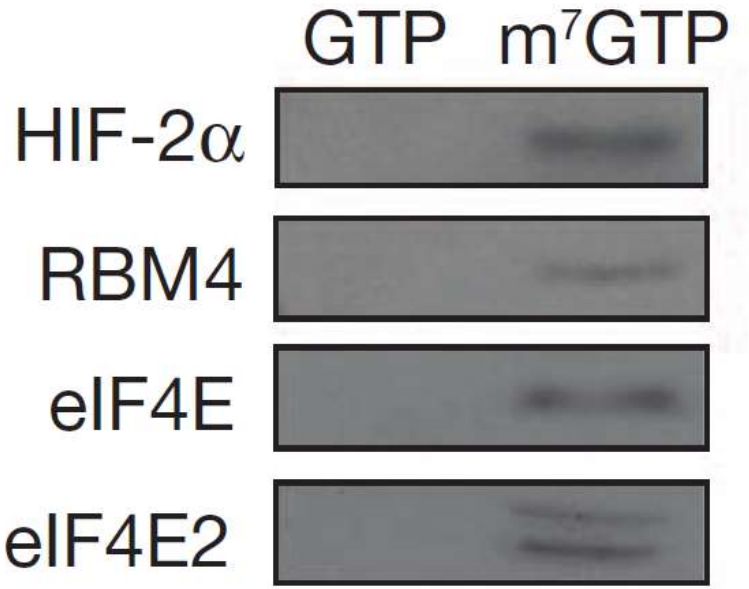
HIF2a

?



# Capture assays using m7-GTP beads in hypoxic cell lysates

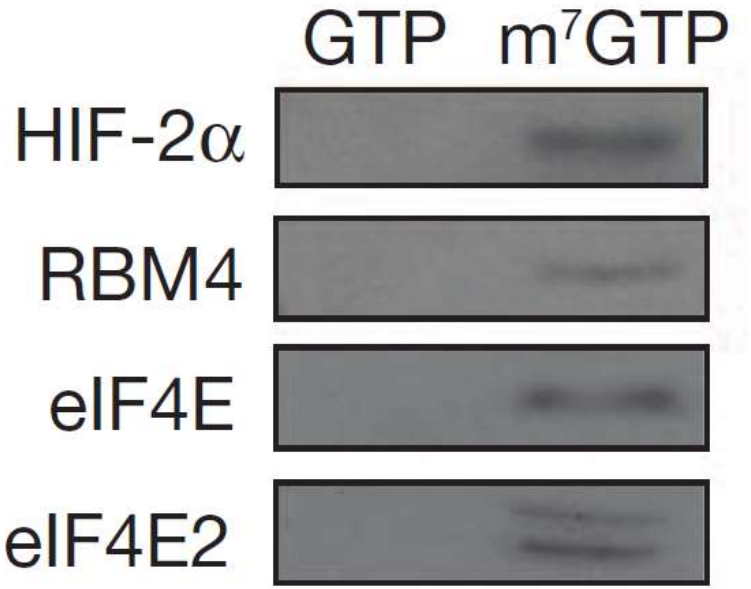
cromatografia di affinità



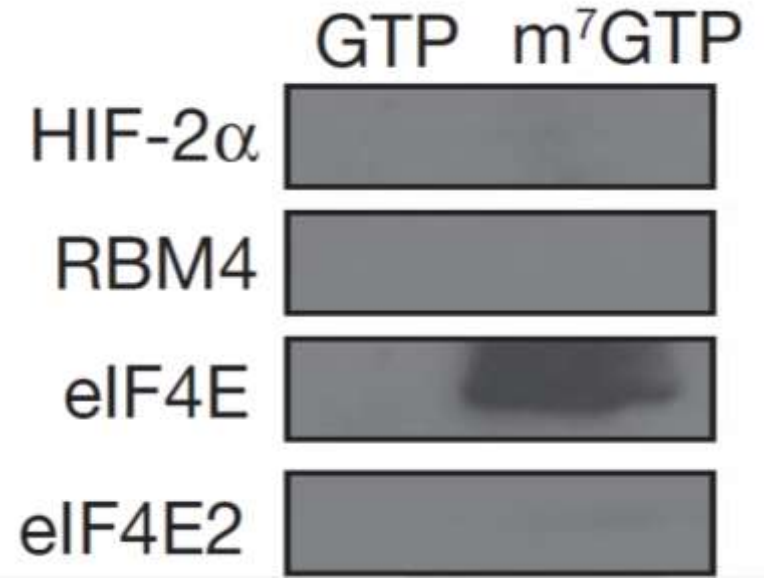

m7GTP, proteins bound to m7-GTP beads

# Capture assays using m7-GTP beads in hypoxic cell lysates

cromatografia di affinità




**eIF4E2 knockdown (sieIF4E2)**




**hypoxia stimulates the formation of a complex that includes**

**1 the oxygen-regulated hypoxia-inducible factor 2a (HIF-2a),**

**2 the RNA-binding protein RBM4 and**

**3 the cap-binding eIF4E2**

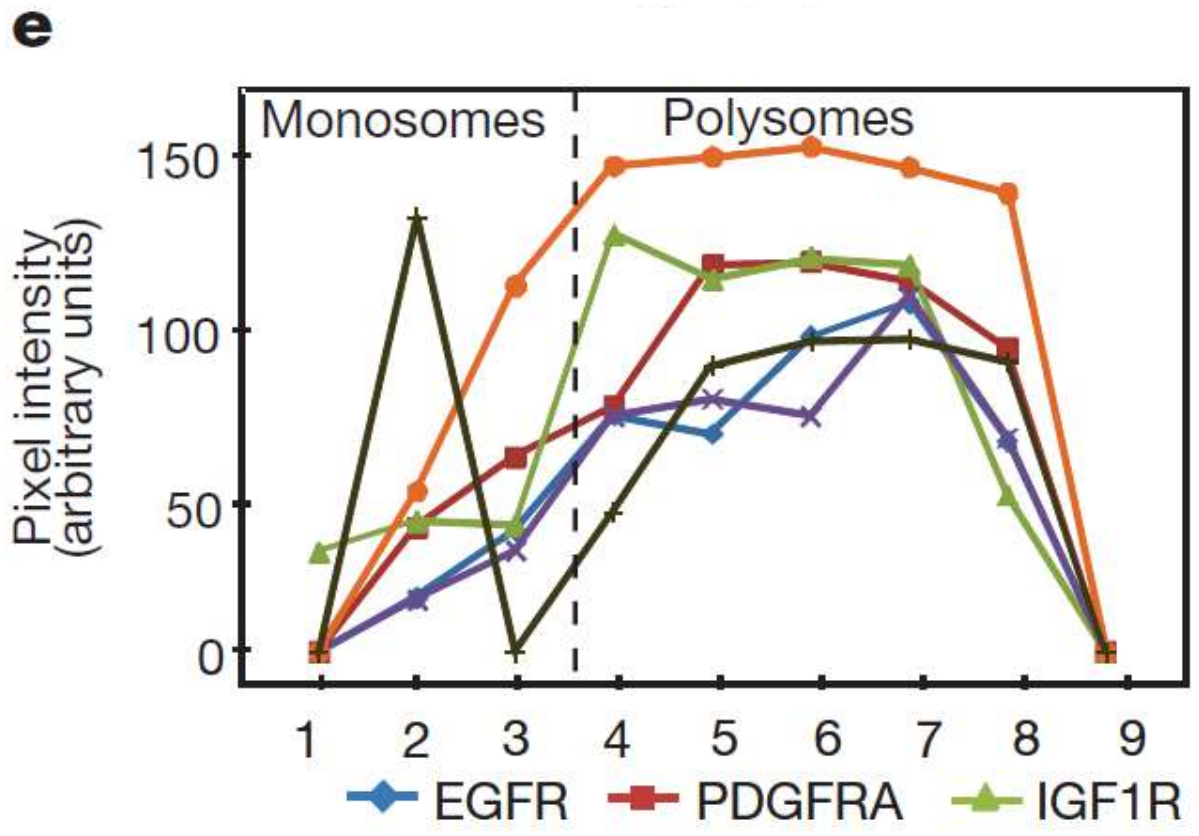
## Il Complesso

1 the oxygen-regulated hypoxia-inducible factor 2a (HIF-2a),
2 the RNA-binding protein RBM4 and
3 the cap-binding eIF4E2

## A quali mRNA si lega specificamente?

## A che sequenze?

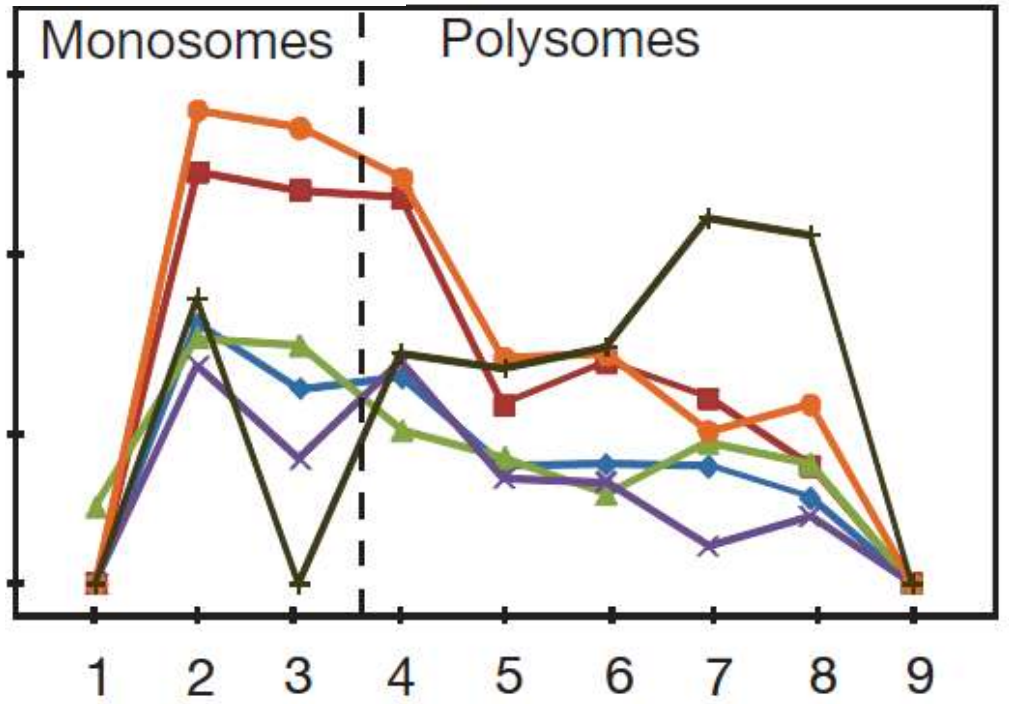
# mRNA and Polysomal distribution in hypoxic cells




EGFR (epidermal growth factor receptor)

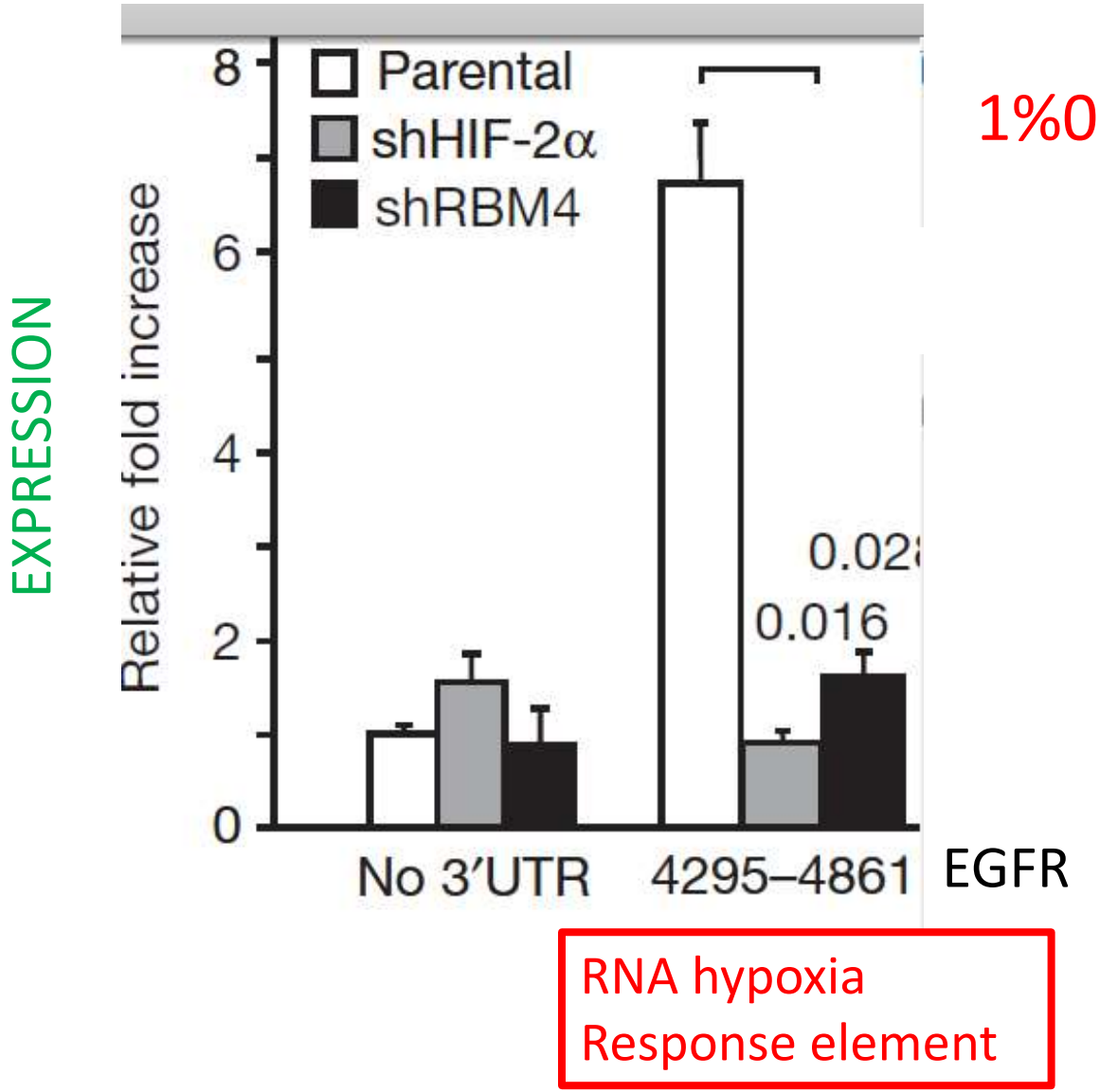
# mRNA and Polysomal distribution in hypoxic cells

**eIF4E2 knockdown (sieIF4E2)**



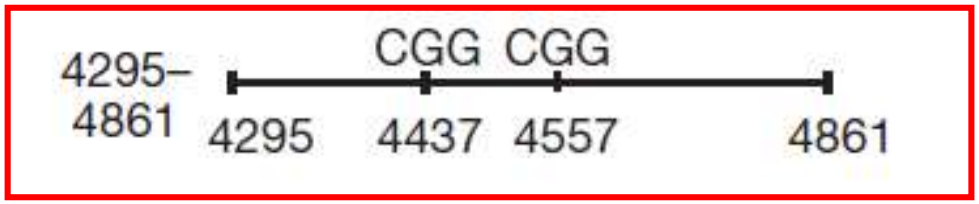

EGFR (epidermal growth factor receptor)

the HIF-2a–RBM4–eIF4E2 complex captures the 5’ cap and targets specific mRNAs to polysomes for active translation



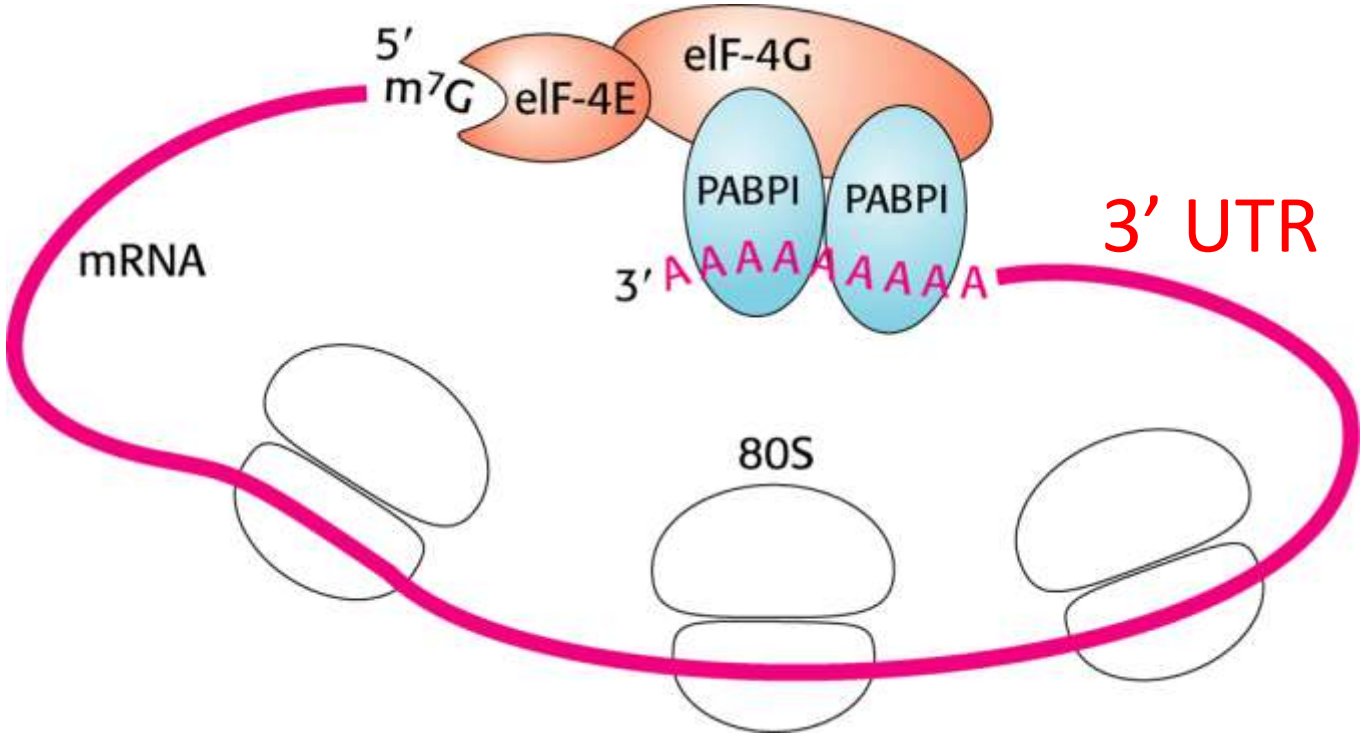

**RBM4 recruits HIF-2a to the 3'UTR for hypoxic translation**

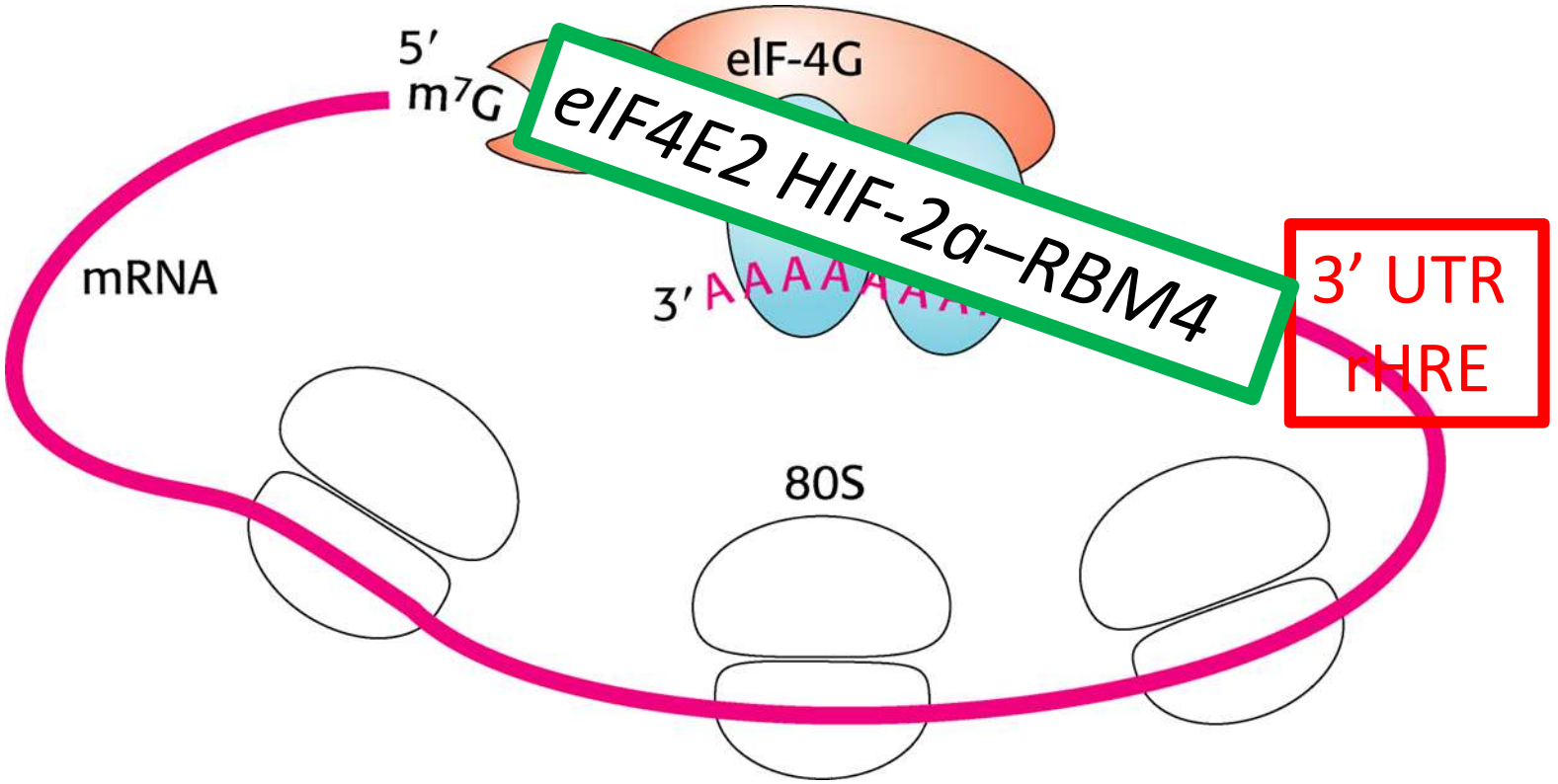
RNA hypoxia response element (rHRE) !!




EGFR 3’ UTR

rHRE Present in many mRNA regulated by 02





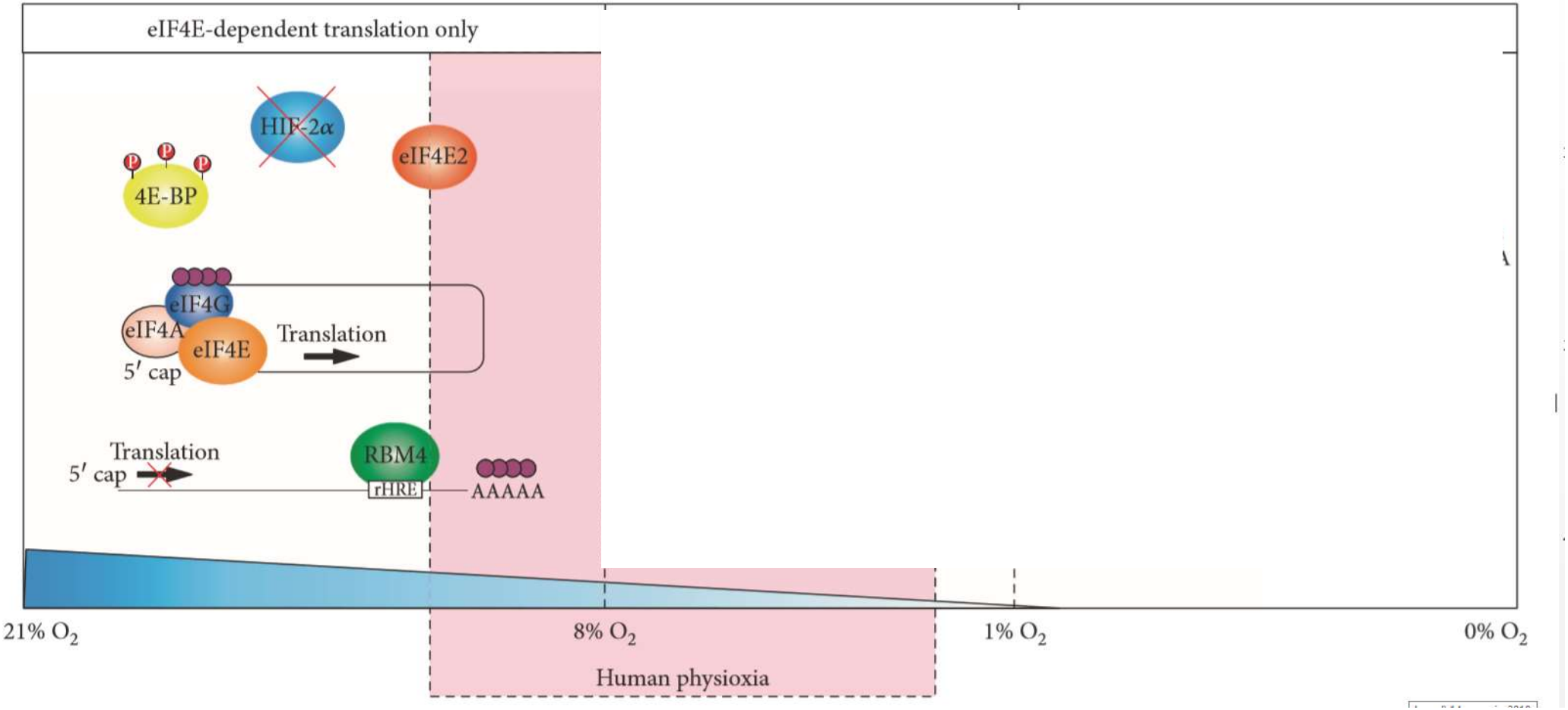

*1 Once assembled at the rHRE*

*2 the HIF-2a–RBM4–eIF4E2 complex captures the 5’ cap*

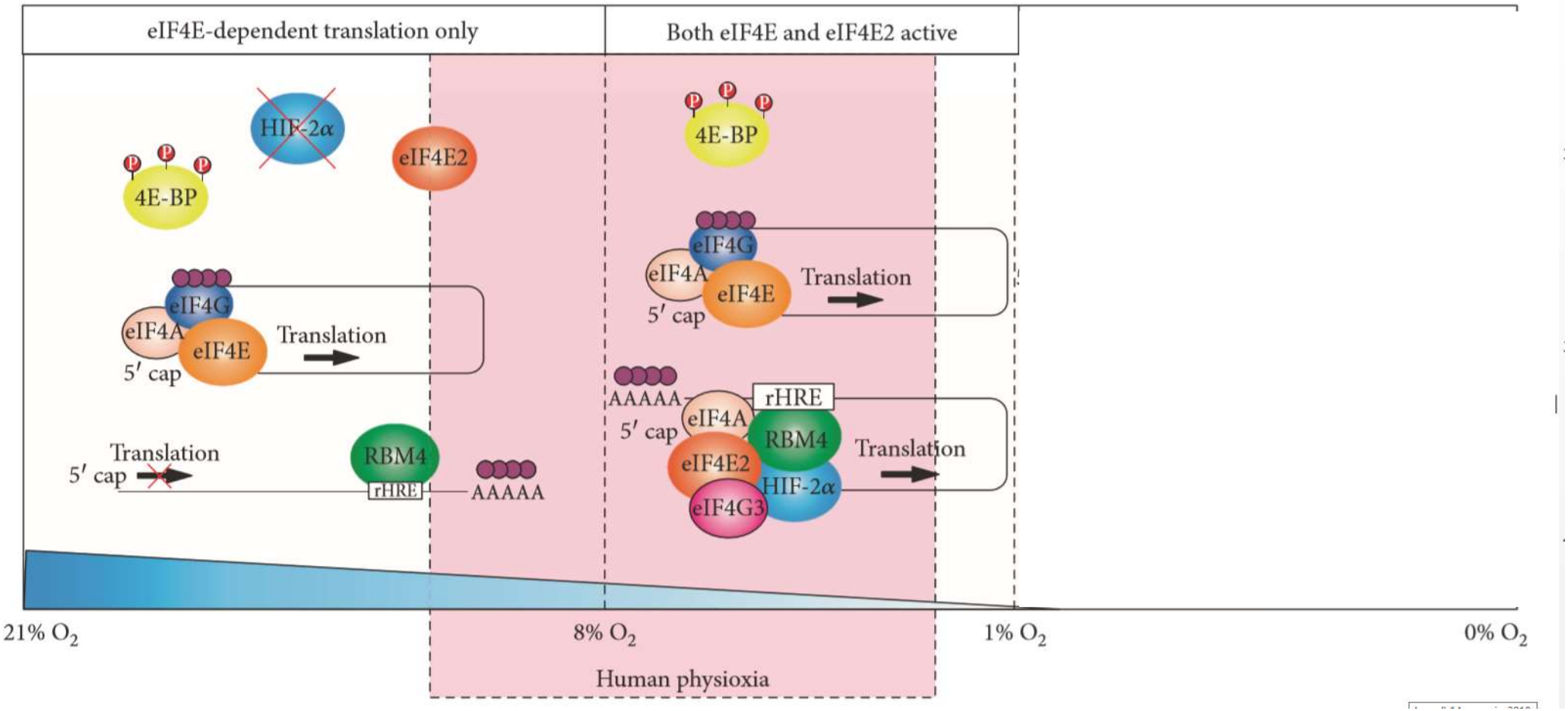
*3 and targets mRNAs to polysomes for active translation*

***evading hypoxia-induced repression of protein synthesis***

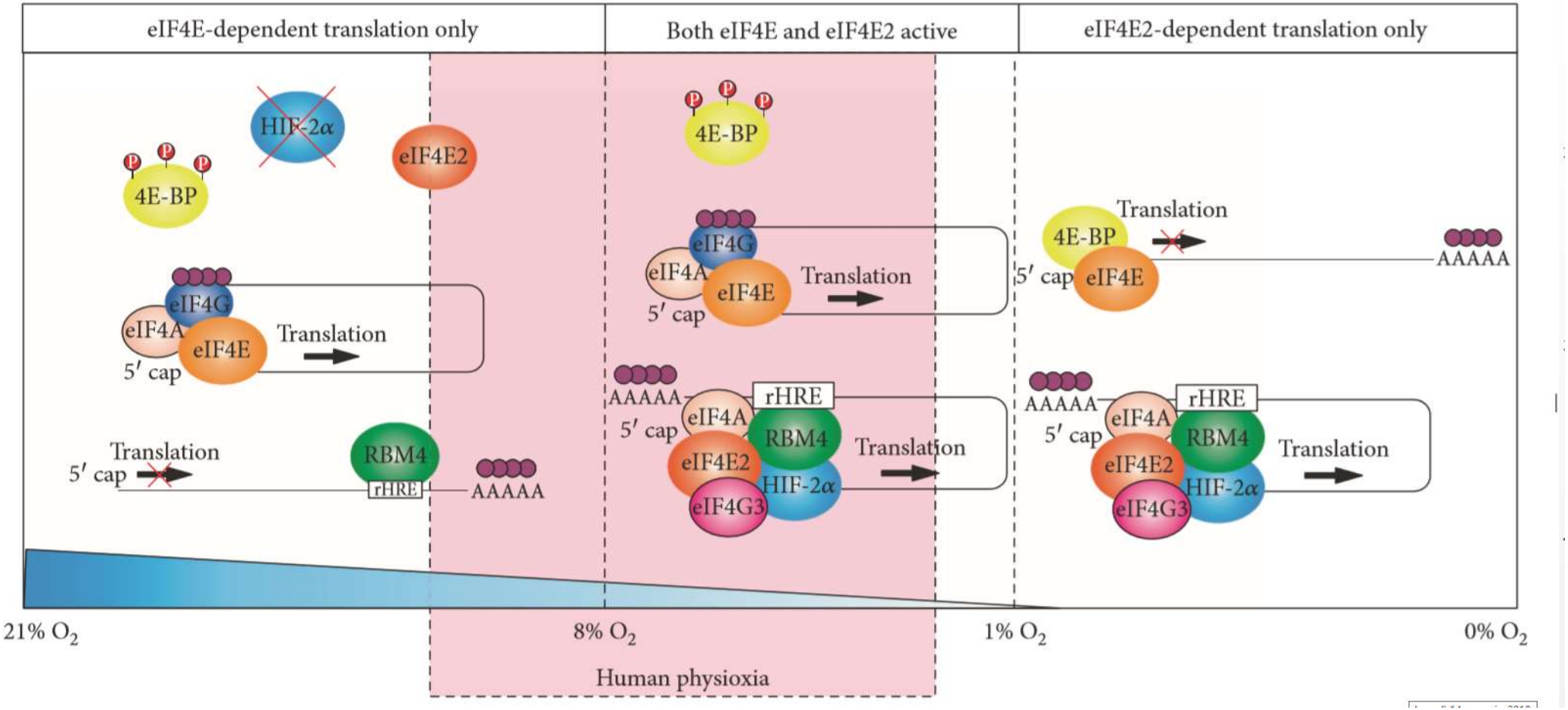
# The eIF4E2-dependent translation initiation does not act at high tissue oxygenation

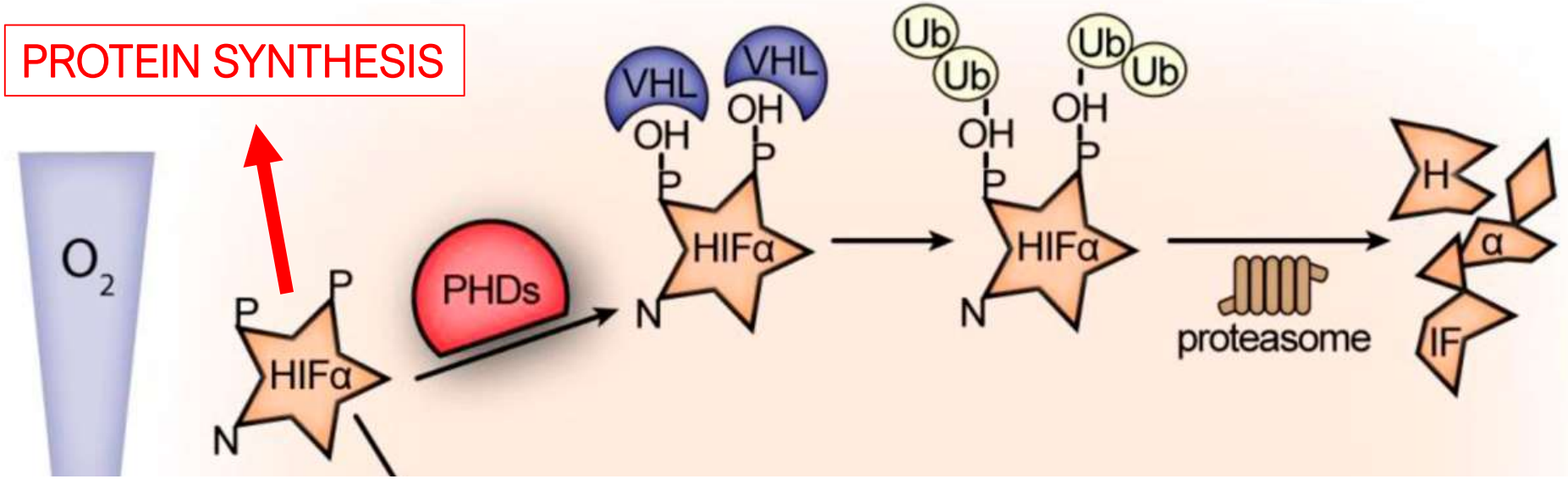


# Both eIF4E- and eIF4E2-dependent translation initiations are active in the range of physiological tissue oxygenation



# The eIF4E2-dependent translation initiation prevails at low tissue oxygenation






- Cells have evolved a program by which oxygen tension switches the basic translation initiation machinery