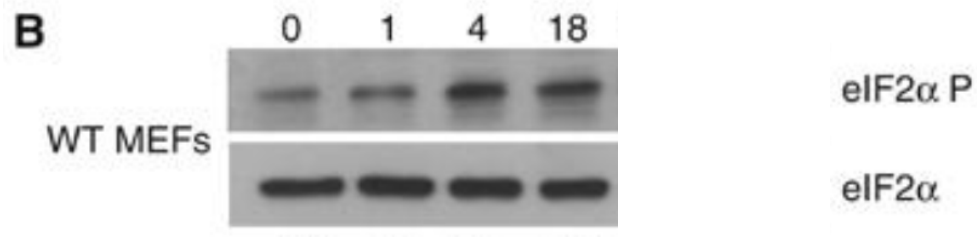


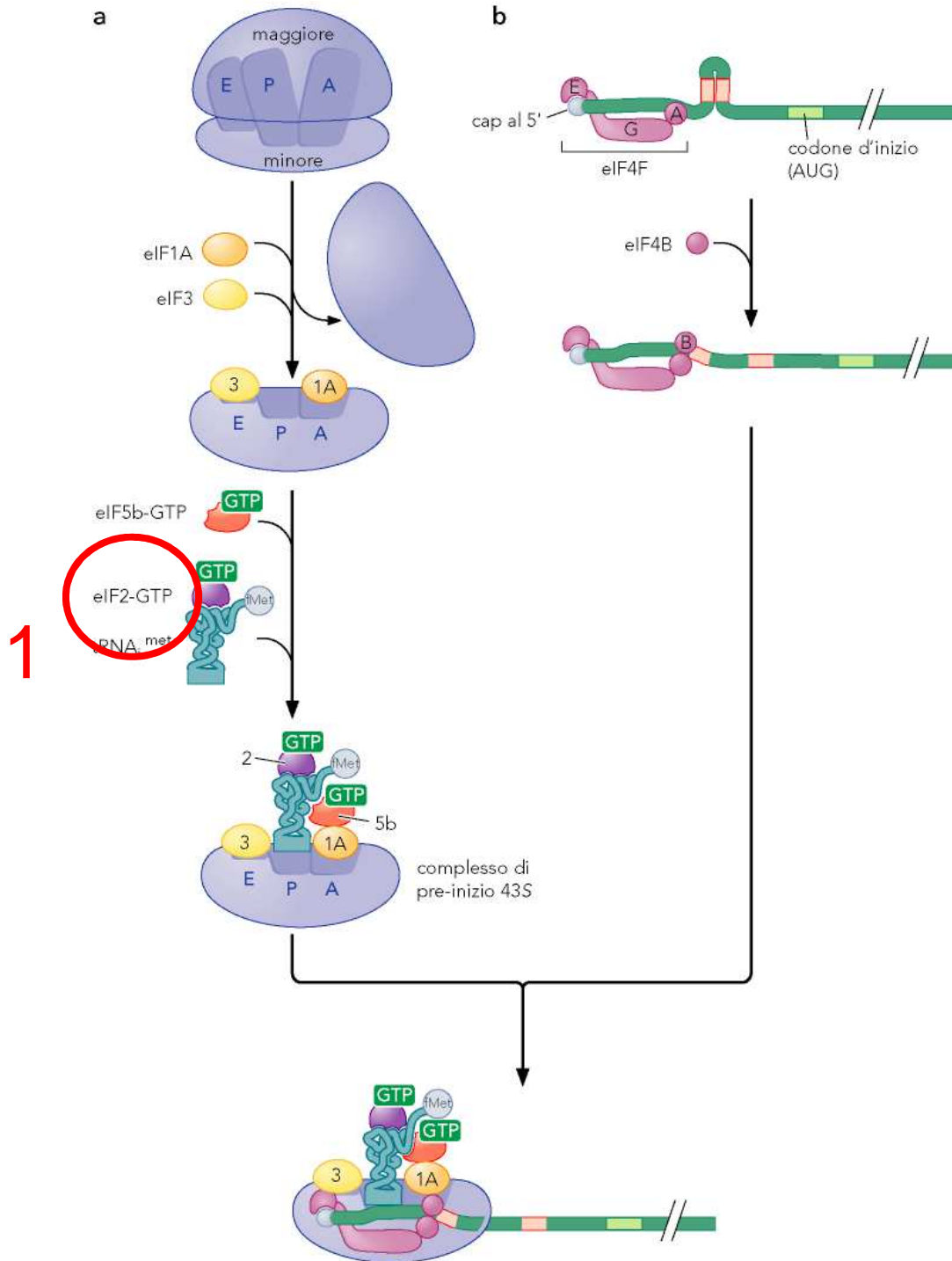
An oxygen-regulated switch in  
the protein synthesis machinery

1

Inhibition of translation during **acute** hypoxia is mediated by eIF2 $\alpha$  phosphorylation

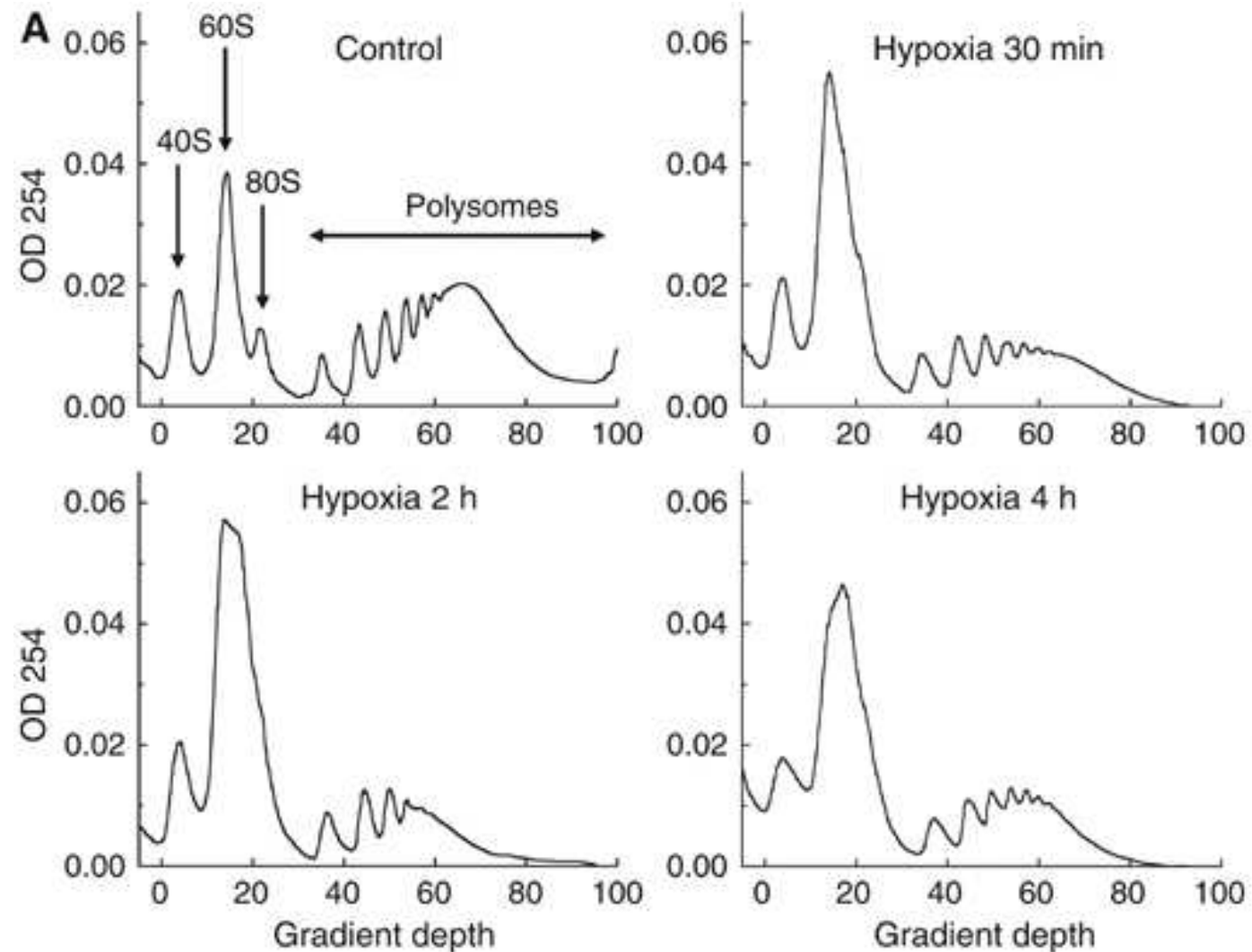


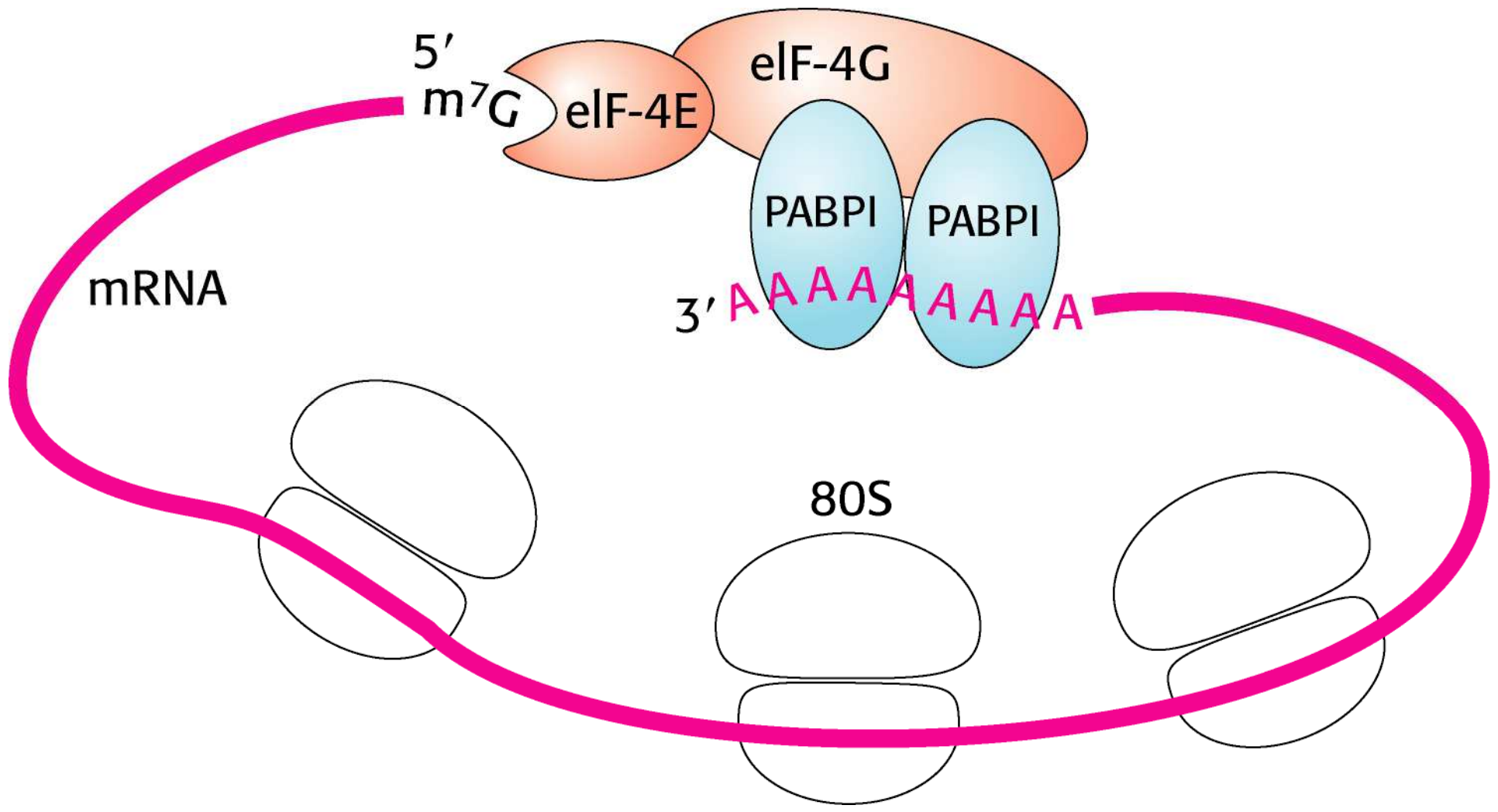
mouse embryo fibroblasts (MEFs)



1

# Prolonged Hypoxia inhibits mRNA translation

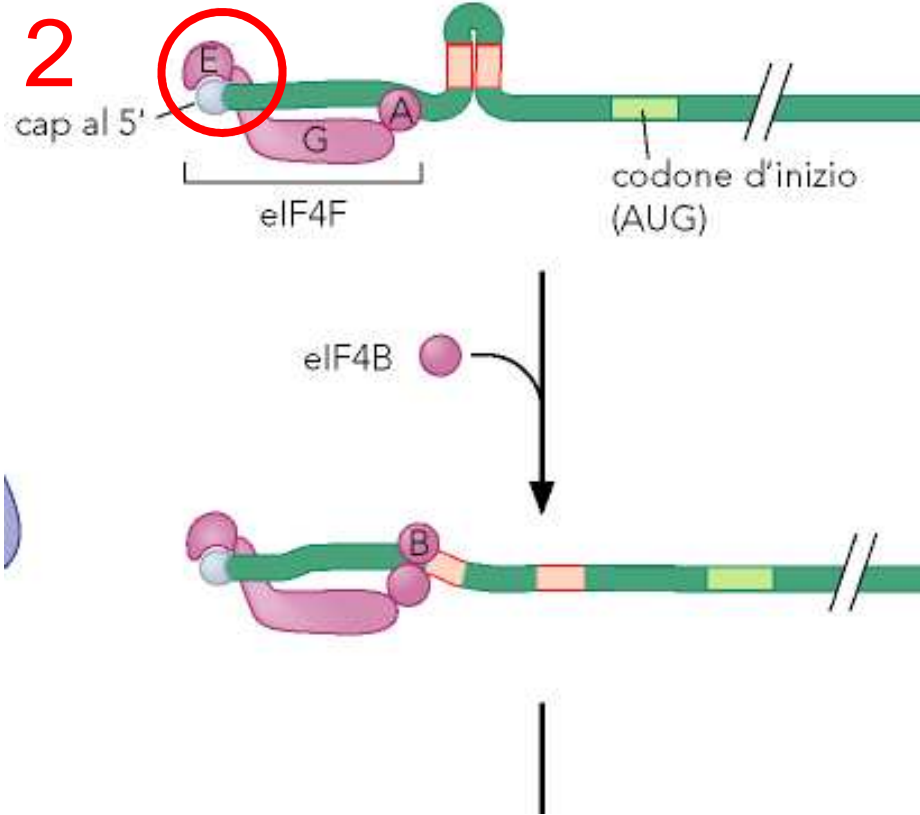




- The initial step of protein synthesis is the binding of the eukaryotic translation initiation factor 4E (eIF4E) to the 7-methylguanosine (m<sup>7</sup>-GpppG) 5' cap of messenger RNAs



b

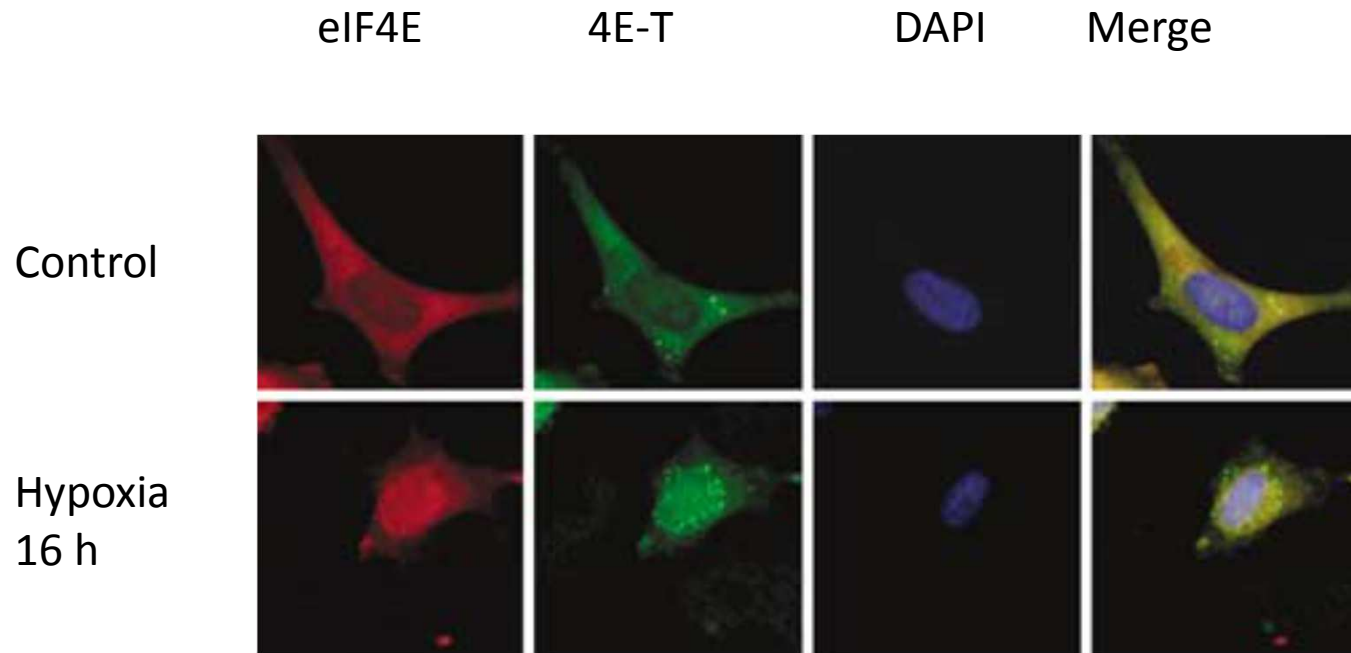


## 2

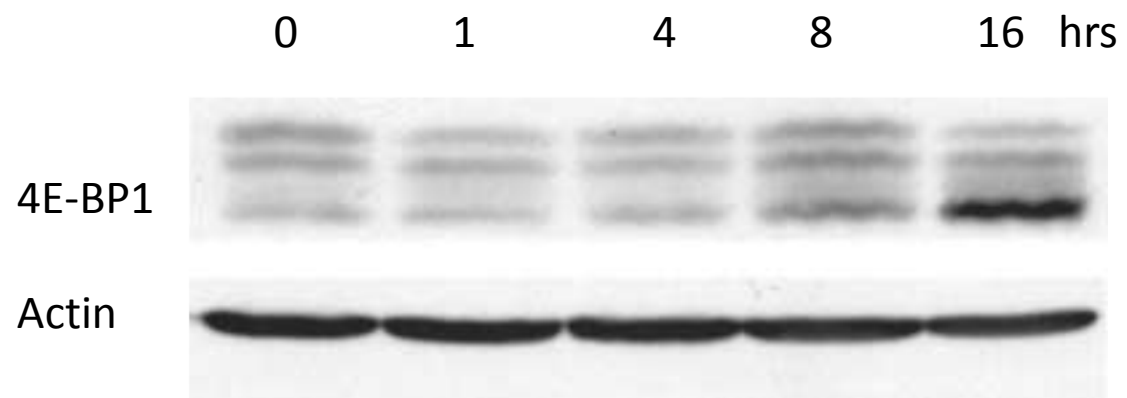
Low oxygen tension (hypoxia) represses cap-mediated translation by sequestering eIF4E.

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# eIF4E relocates during hypoxia

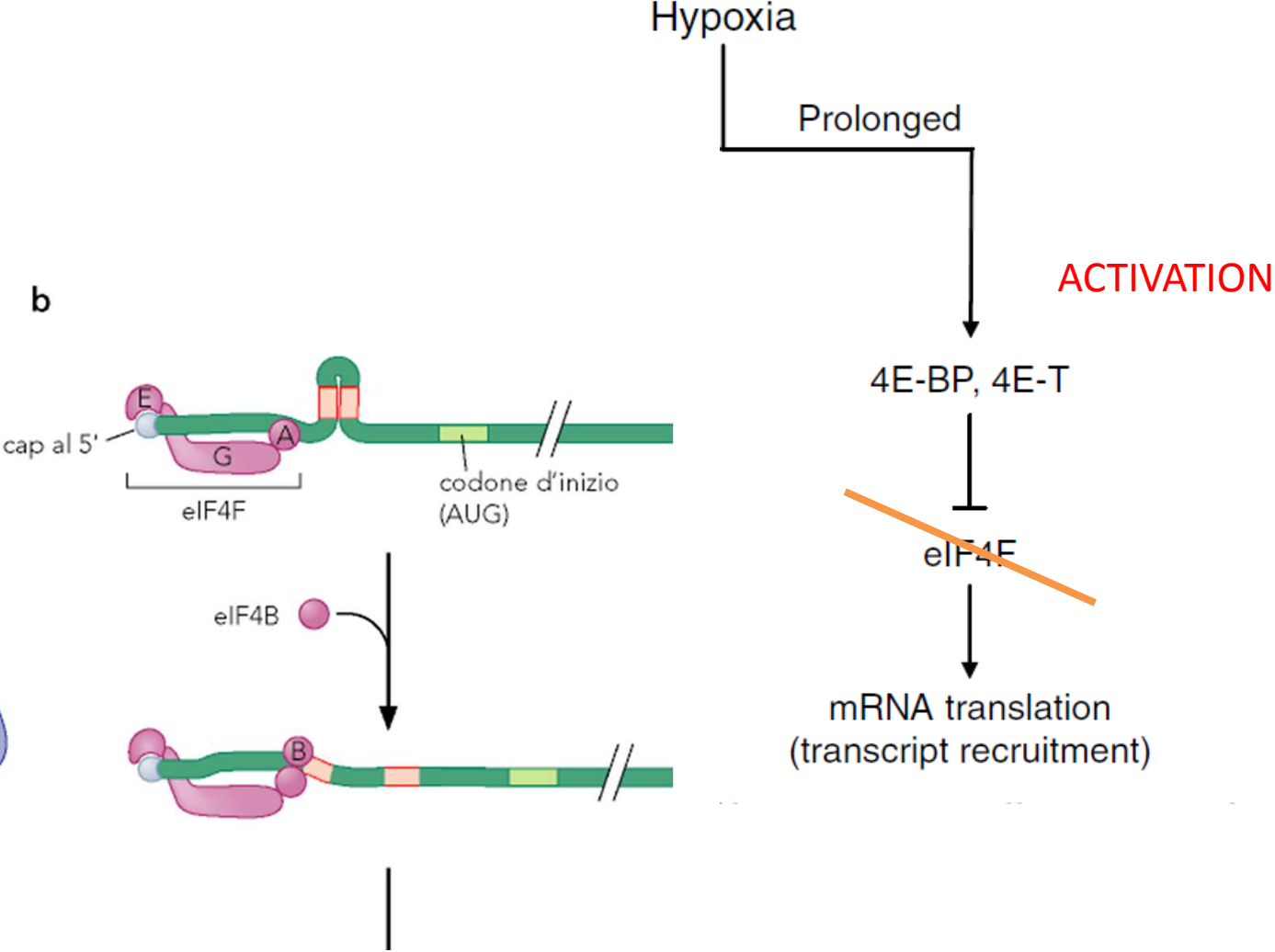


The shuttling protein 4E-T is a known regulator of eIF4E localization and is capable of binding and transporting it to the cell nucleus  
Correlation with the gradual dephosphorylation of 4E-T

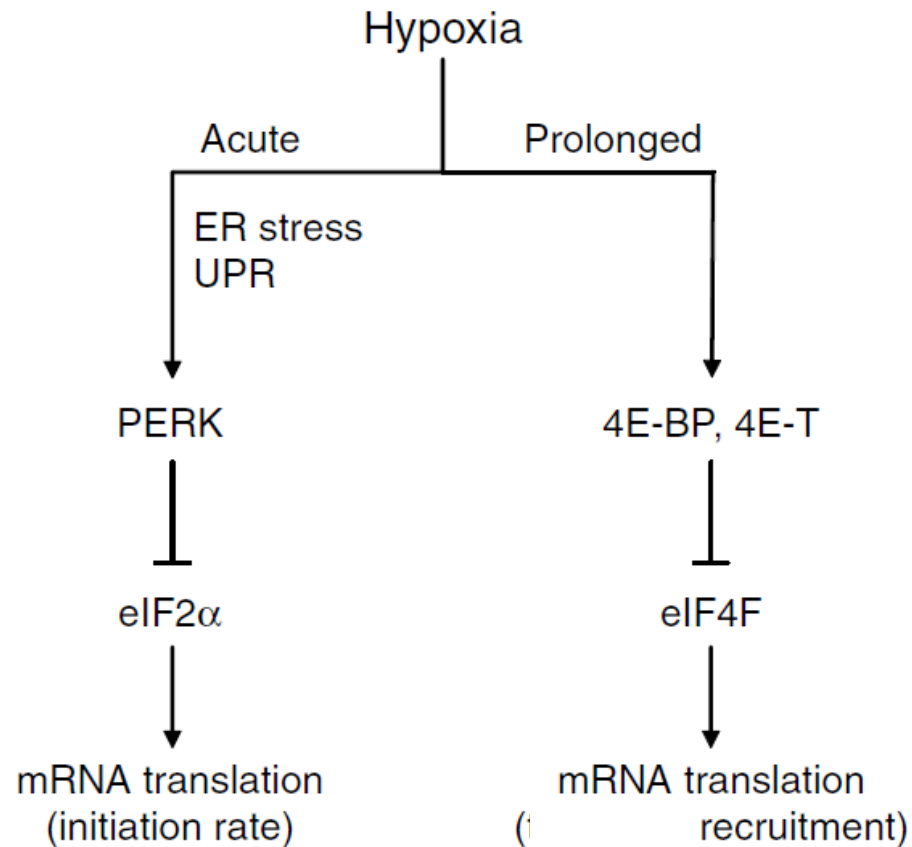


4E-BP1 an inactive complex shows induction after hypoxia

# effects of hypoxia on mRNA translation



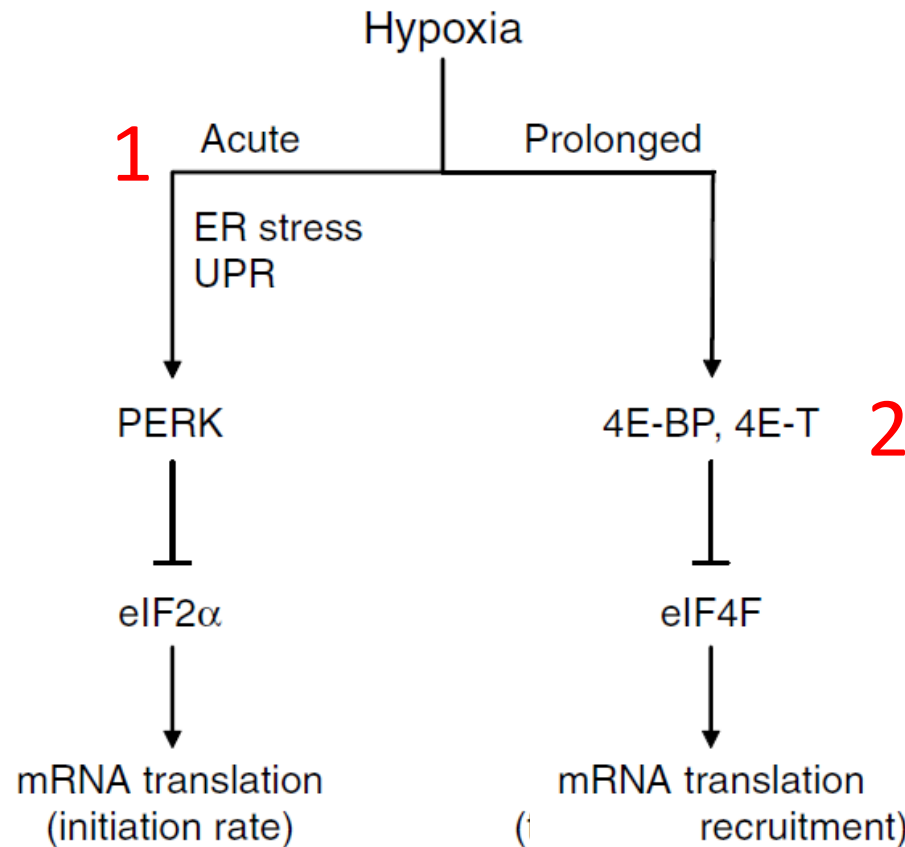
# effects of hypoxia on mRNA translation



Acute hypoxia causes transient eIF2 $\alpha$  phosphorylation due to PERK activation as a part of the UPR. This results in inhibition of the rate of translation initiation.

Following prolonged hypoxic conditions, activation of 4E-BP and 4E-T causes disruption of eIF4F, which inhibits the recruitment of mRNA to polysomes.

# effects of hypoxia on mRNA translation



Acute hypoxia causes transient eIF2a phosphorylation due to PERK activation as a part of the UPR. This results in inhibition of the rate of translation initiation.

Following prolonged hypoxic conditions, activation of 4E-BP and 4E-T causes disruption of eIF4F, which inhibits the recruitment of mRNA to polysomes.

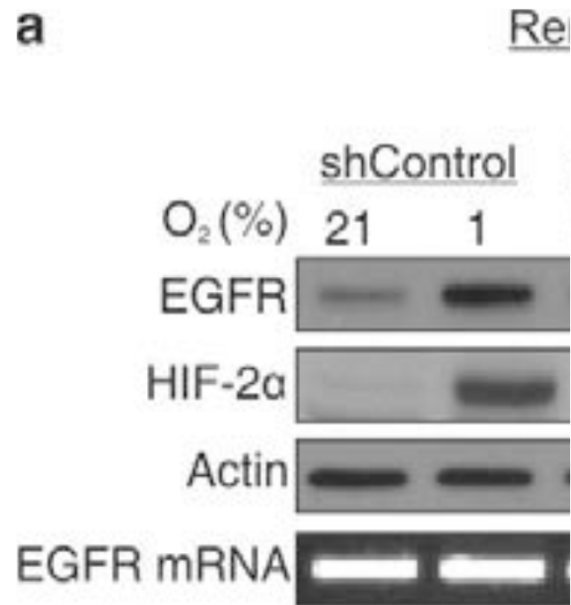
Both molecular mechanisms affect specific mRNAs to varying degrees, resulting in differential gene expression.





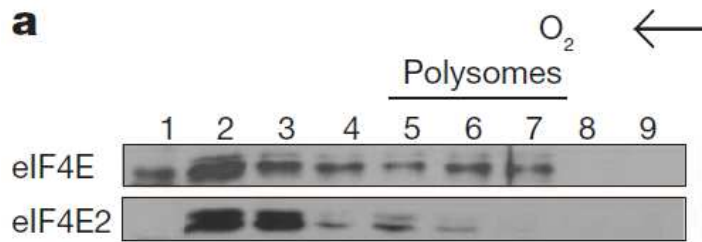
- A fundamental question in biology is as to how proteins are synthesized in periods of oxygen scarcity and eIF4E inhibition

# EGFR levels increase!! in hypoxic cells

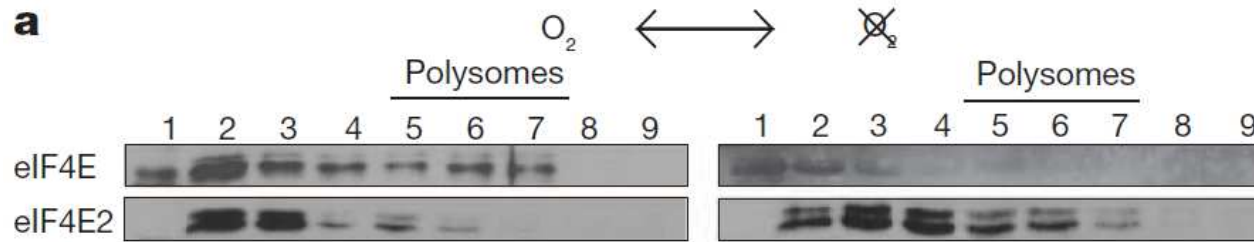


human renal proximal tubular epithelial cells

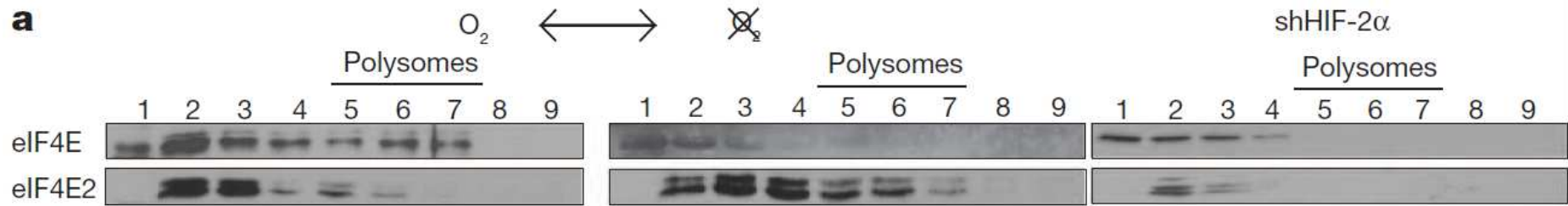
# An oxygen-regulated **switch** from eIF4E- to eIF4E2- dependent protein synthesis.



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

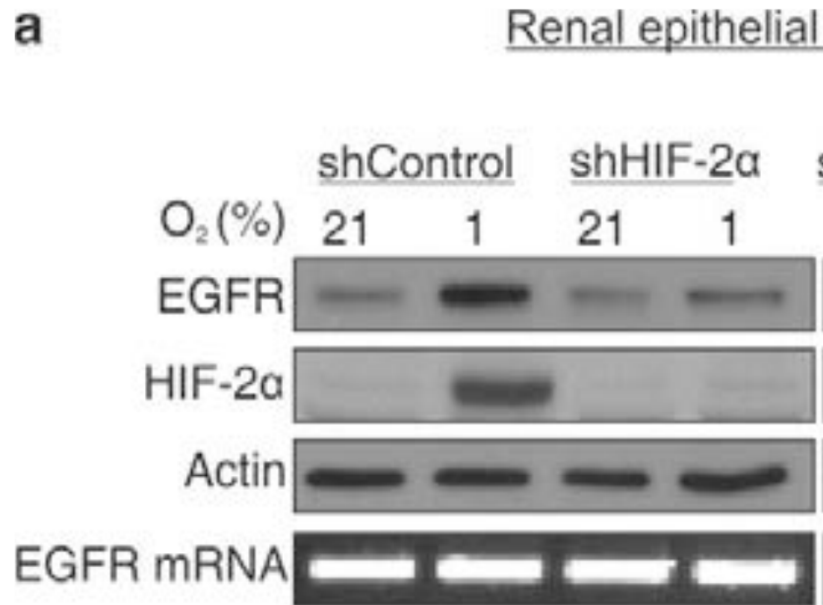


eIF4E and eIF4E2 polysome association in normoxia  
and hypoxia



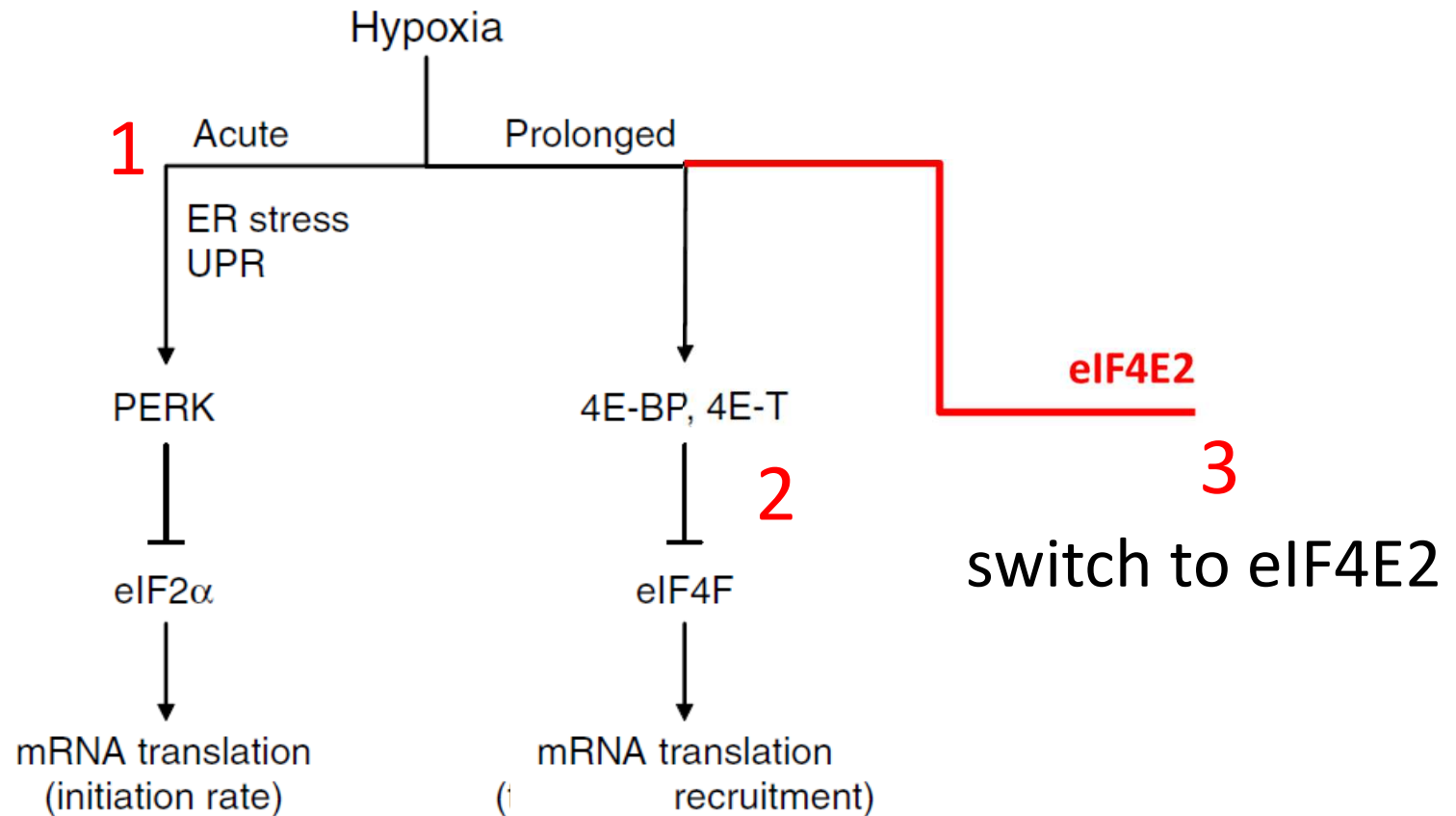
hypoxia stimulates the switch from the cap-binding eIF4E to its eIF4E2  
 homologue  
 dependent from the oxygen-regulated hypoxia-inducible factor 2a (HIF-2 $\alpha$ )

# EGFR levels in hypoxic cells do not increase when HIF-2 alpha is inhibited



human renal proximal tubular epithelial cells

# effects of hypoxia on mRNA translation



Acute hypoxia causes transient eIF2a phosphorylation due to PERK activation as a part of the UPR. This results in inhibition of the rate of translation initiation.

Following prolonged hypoxic conditions, activation of 4E-BP and 4E-T causes disruption of eIF4F, which inhibits the recruitment of mRNA to polysomes.

hypoxia stimulates the switch from the cap-binding eIF4E to to eIF4E2 homologue dependent from the oxygen-regulated hypoxia-inducible factor 2a (HIF-2alpha) **3**

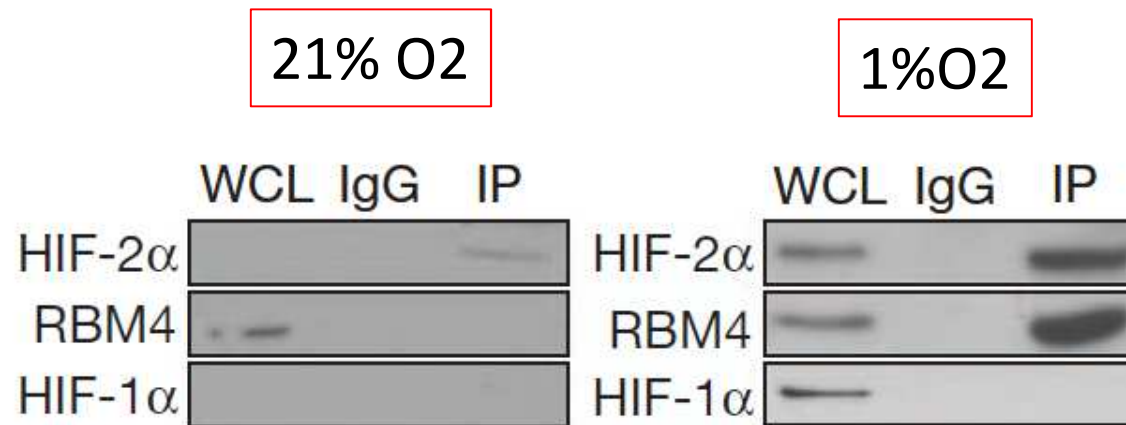
# Meccanismo molecolare di HIF 2 alpha nella sintesi proteica

- an oxygen-regulated translation initiation complex that mediates selective **cap-dependent** protein synthesis



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

Co-immunoprecipitation of HIF-2a



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

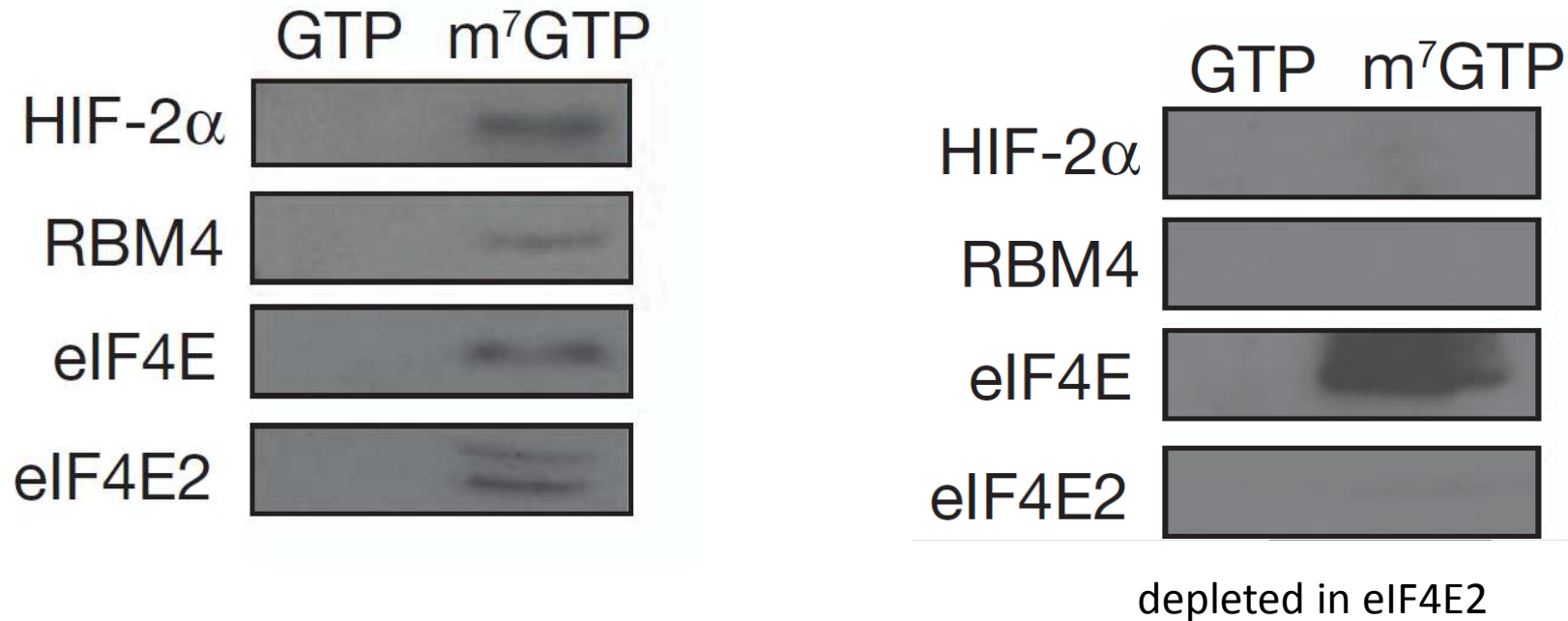
WCL, whole cell lysate

RNA-binding protein RBM4

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

HIF-2a–RBM4 recruits the m7-GTP cap by means of an interaction with eIF4E2

Capture assays using m7-GTP beads in hypoxic cell lysates

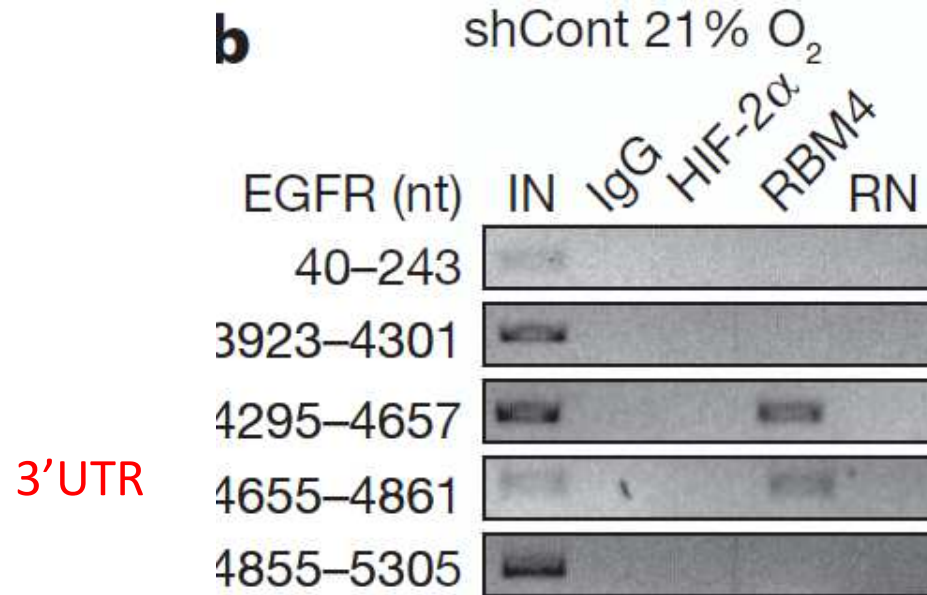


GTP, proteins dislodged from the beads by GTP; m7GTP, proteins bound to m7-GTP beads after GTP wash

hypoxia stimulates the formation of a complex that includes the oxygen-regulated hypoxia-inducible factor 2a (HIF-2a), the RNA-binding protein RBM4 and the cap-binding eIF4E2

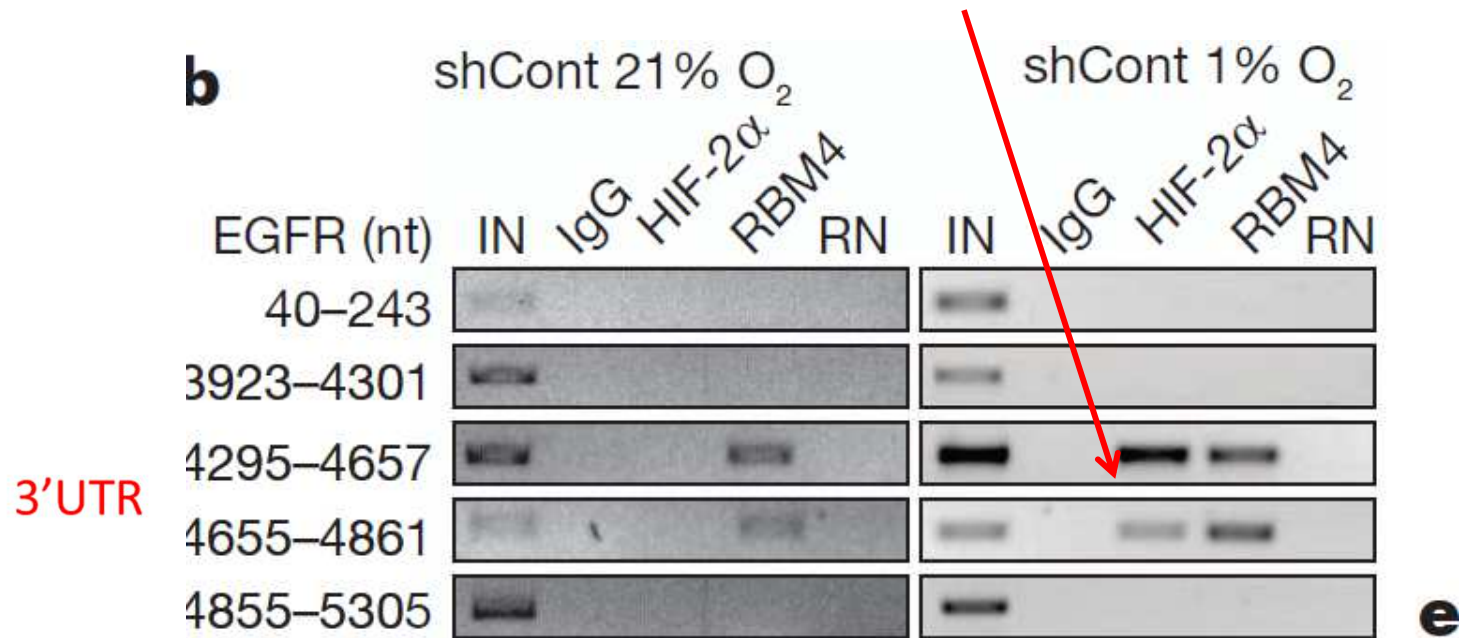
- Determinanti per la selezione di specifici messaggeri tradotti nella ipossia

## RBM4 recruits HIF-2alpha to specific regions of 3'UTR for hypoxic translation



RNA immunoprecipitation of HIF-2a and RBM4  
IN, input; nt, nucleotides; RN, RNase-treated

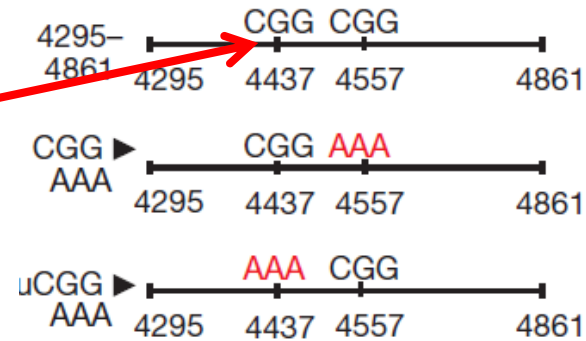
RBM4 recruits HIF-2alpha to specific regions of 3'UTR for hypoxic translation



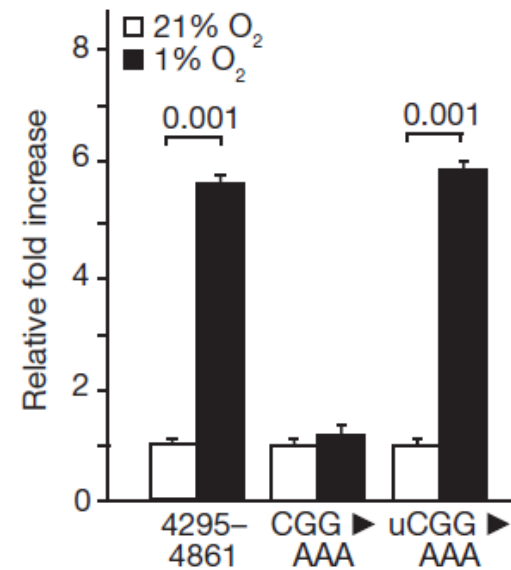
RNA immunoprecipitation of HIF-2a and RBM4  
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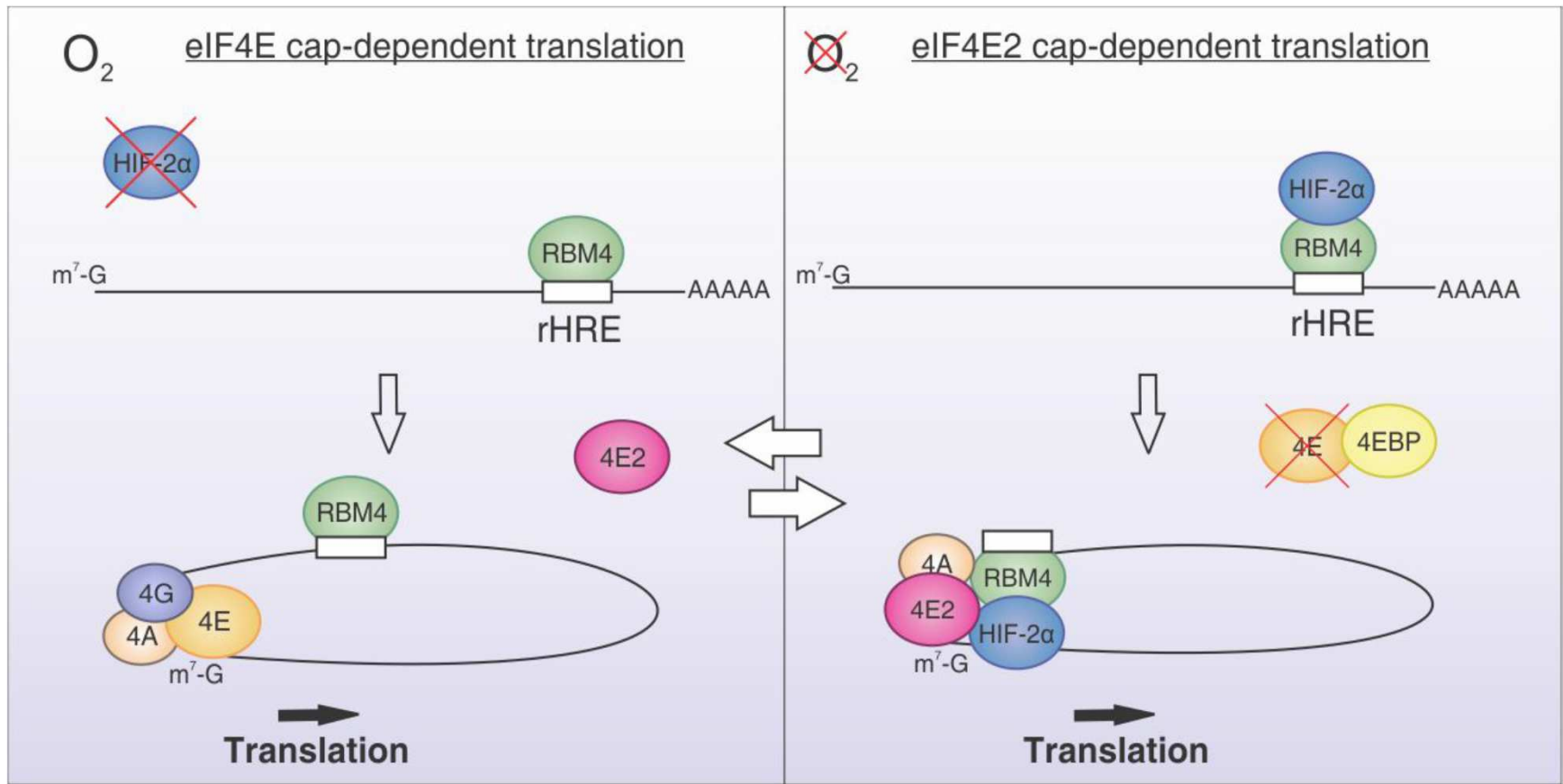
# Identification of sequences defining an RNA hypoxia response element (rHRE) in the 3' UTR

RNA hypoxia response element (rHRE)

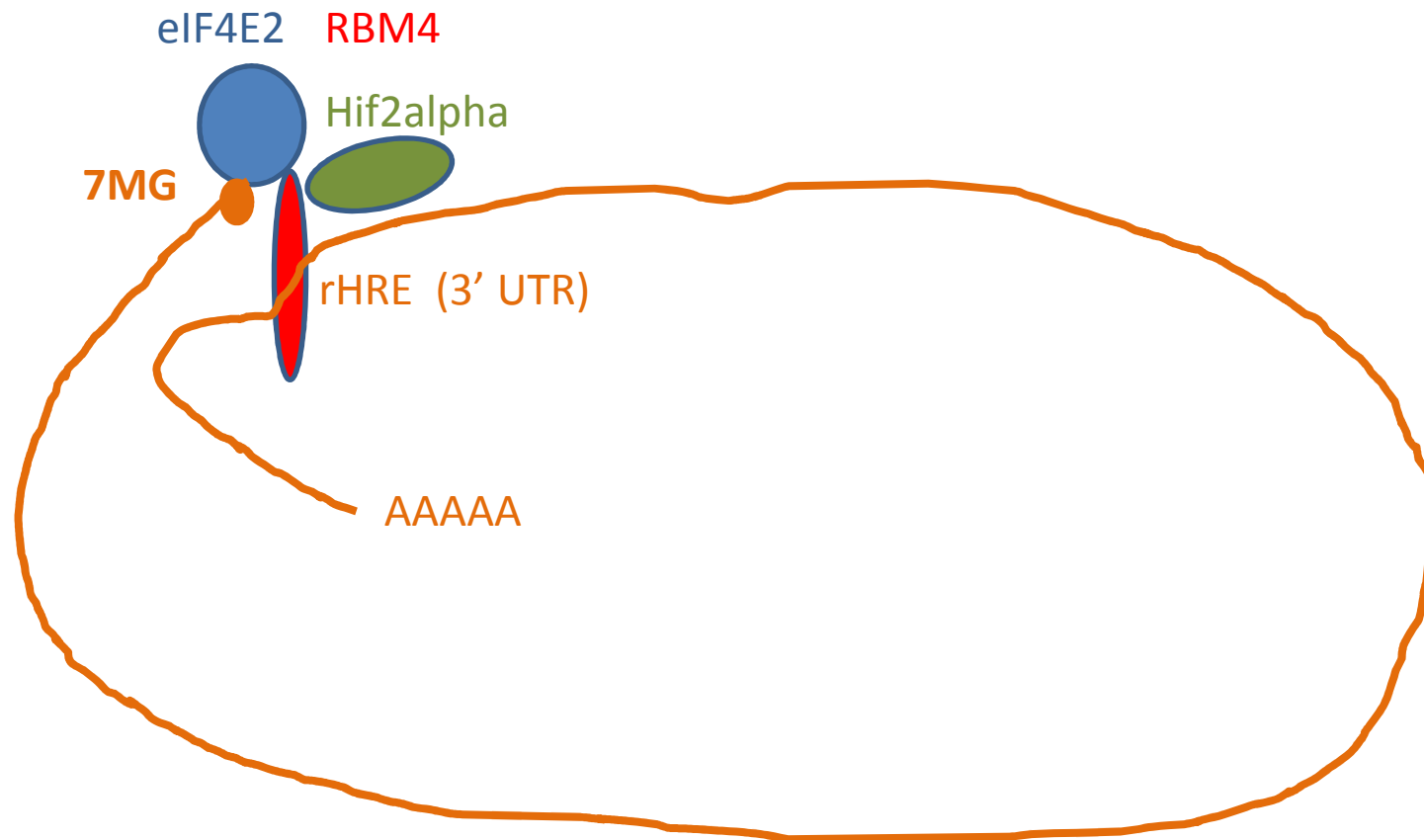


Expression of a luciferase reporter containing CGG AAA mutations near the RBM4 binding site, or in a control upstream region (uCGG).



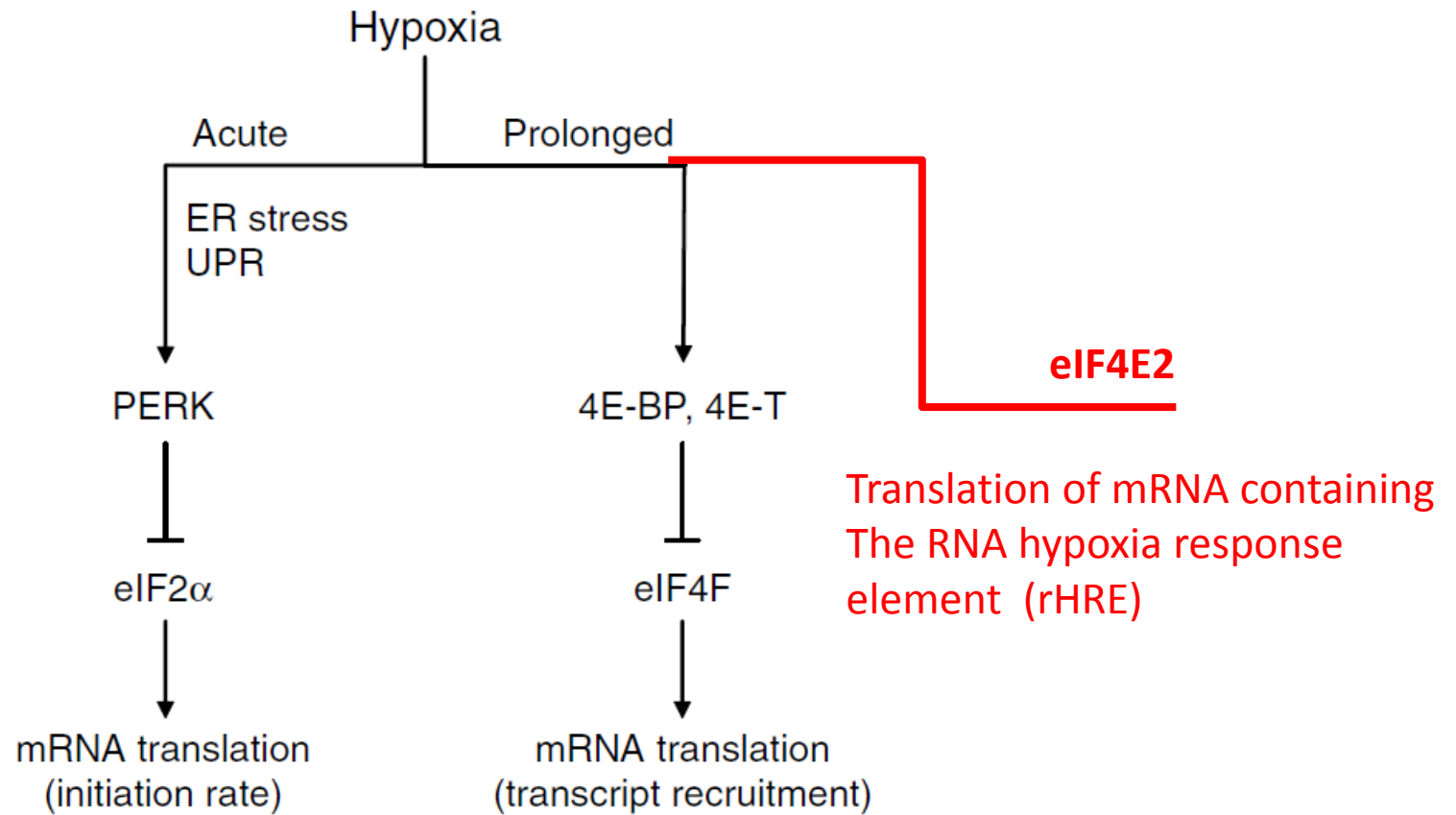


Complesso Quaternario: mRNA (rHRE), Fattore alternativo che riconosce il Cap (eIF4E2), Fattore secondo che risponde all'ipossia (hif2alpha) e proteina che lega RNA RBM4

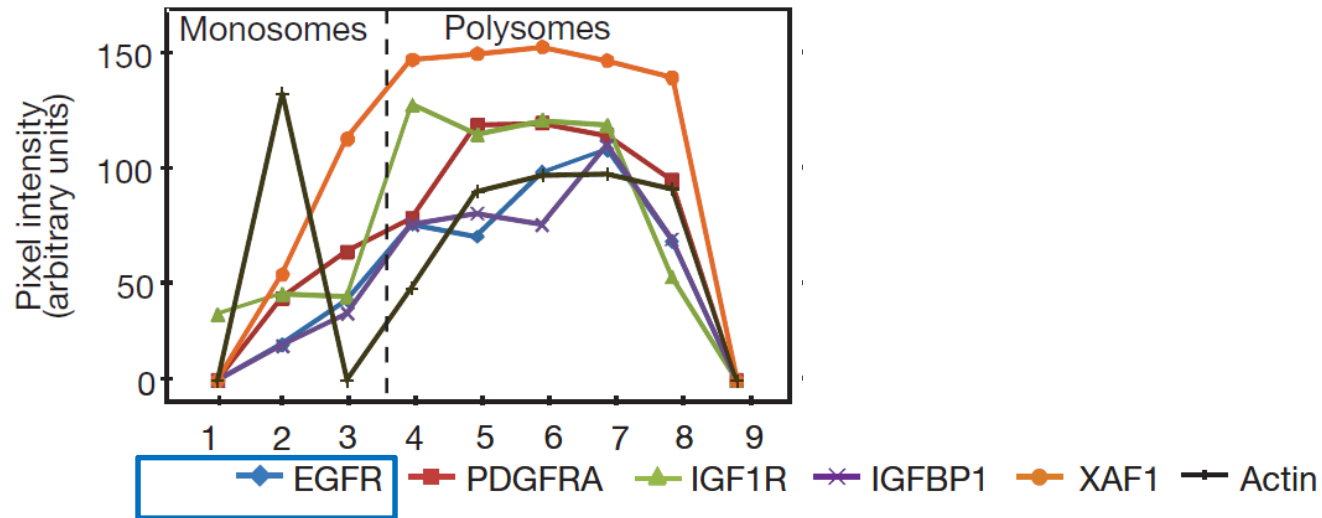




# effects of hypoxia on mRNA translation

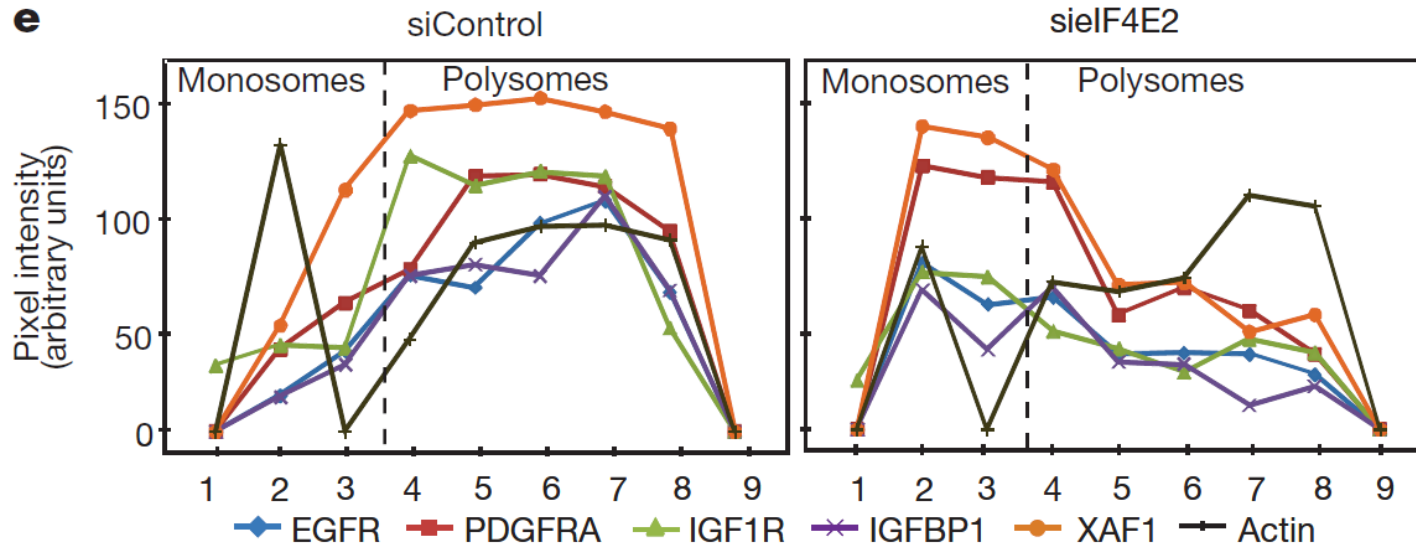


## Polysomal distribution of specific mRNA in hypoxic cells



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

## Silencing of eIF4E2 in hypoxic cells



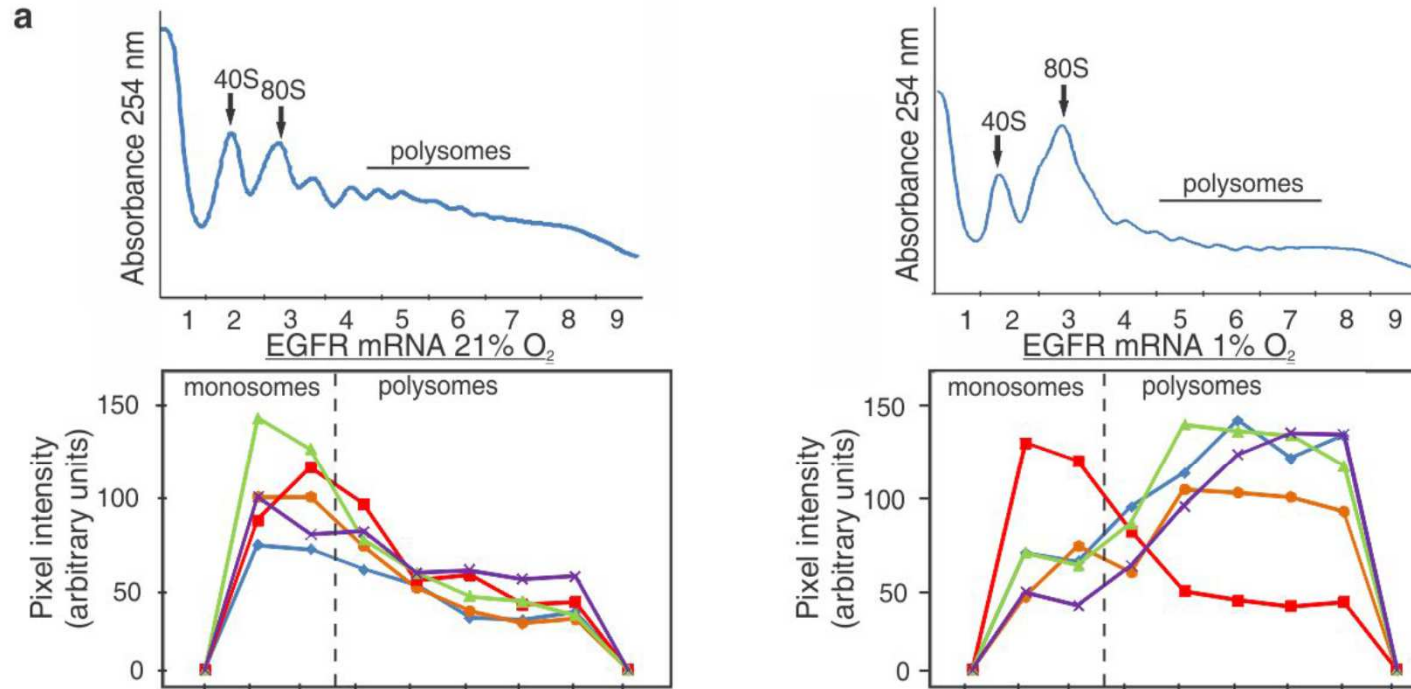
the HIF-2a-RBM4-~~eIF4E2~~ complex **DOES NOT** targets mRNAs to polysomes for active translation

- An RNA hypoxia response element (rHRE) recruits the complex that includes the oxygen-regulated hypoxia-inducible factor 2a (HIF-2a), the RNA-binding protein RBM4 and the cap-binding eIF4E2 to several mRNAs, including that encoding the epidermal growth factor receptor EGFR

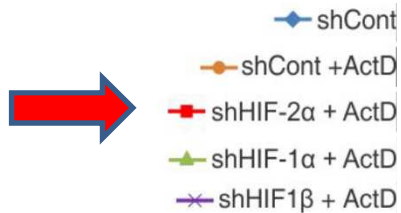
Once assembled at the rHRE, the HIF-2a–RBM4–eIF4E2 complex captures the 5' cap and targets mRNAs to **polysomes for active translation**, thereby evading hypoxia-induced repression of protein synthesis

**cells have evolved a program by which oxygen tension switches the basic translation initiation machinery**

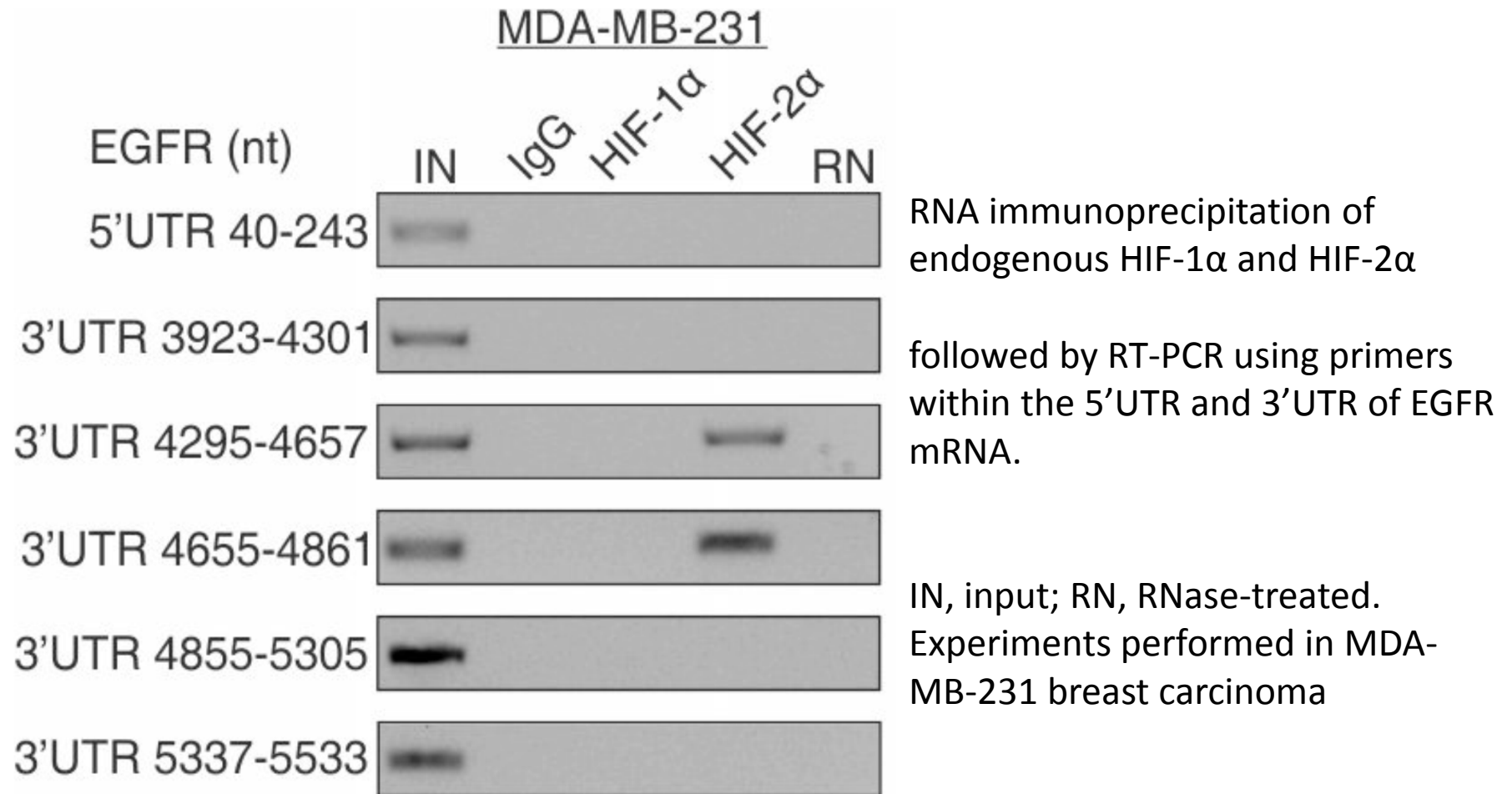
EGFR



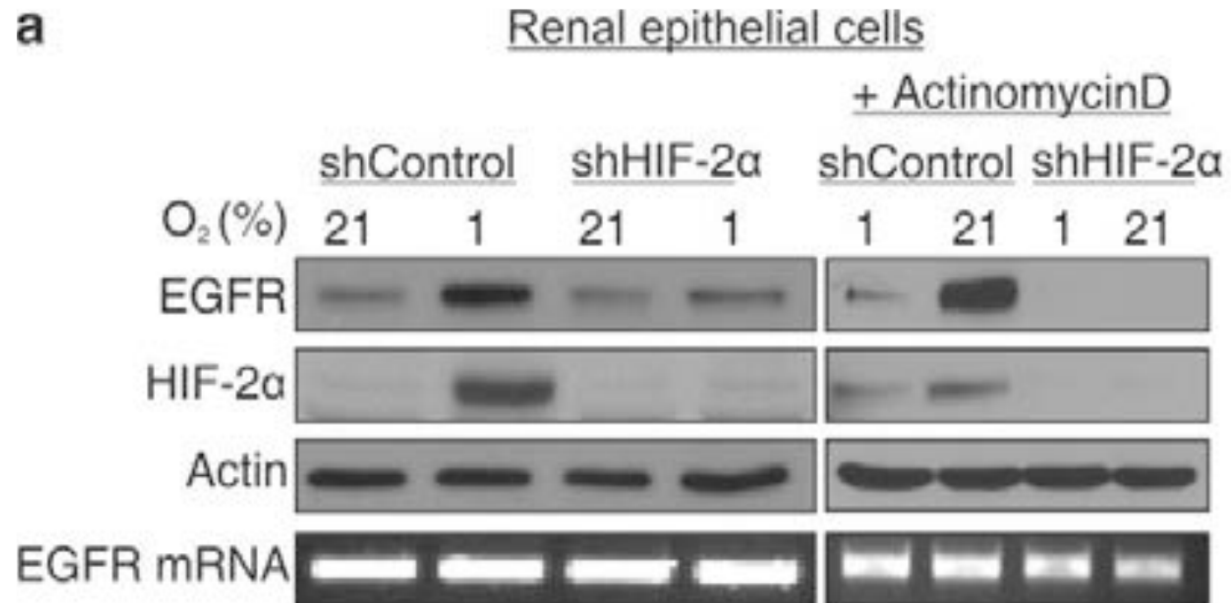
## Polysome distribution of EGFR mRNA in normoxic and hypoxic cells



## HIF-2 $\alpha$ associates with a region of the EGFR mRNA 3'UTR



# EGFR levels in hypoxic cells





Complesso Quaternario: mRNA (rHRE di EGFR),  
Fattore alternativo che riconosce il Cap (eIF4E2),  
Fattore secondo che risponde all'ipossia  
(hif2alpha) e proteina che lega RNA RBM4

