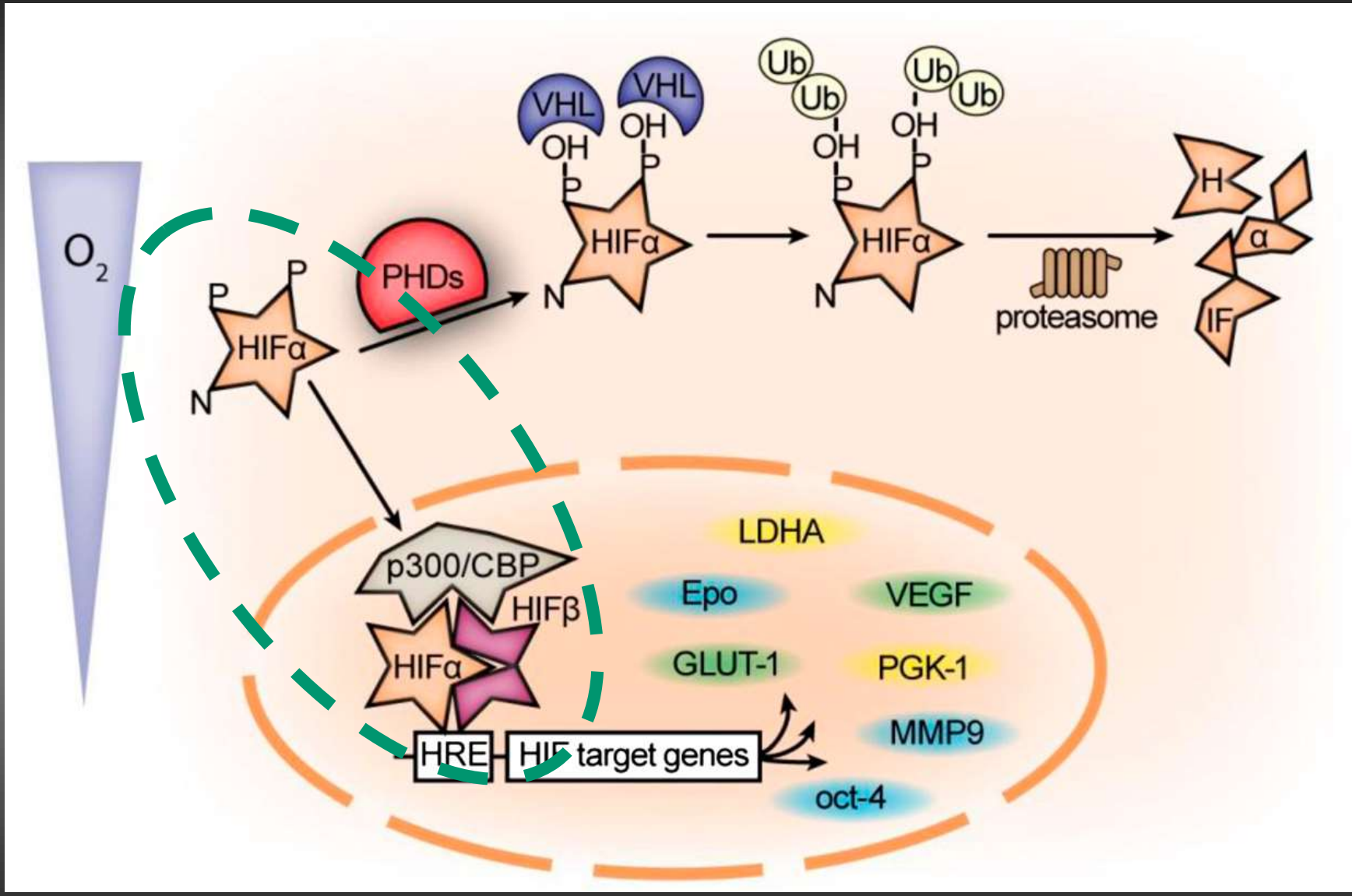
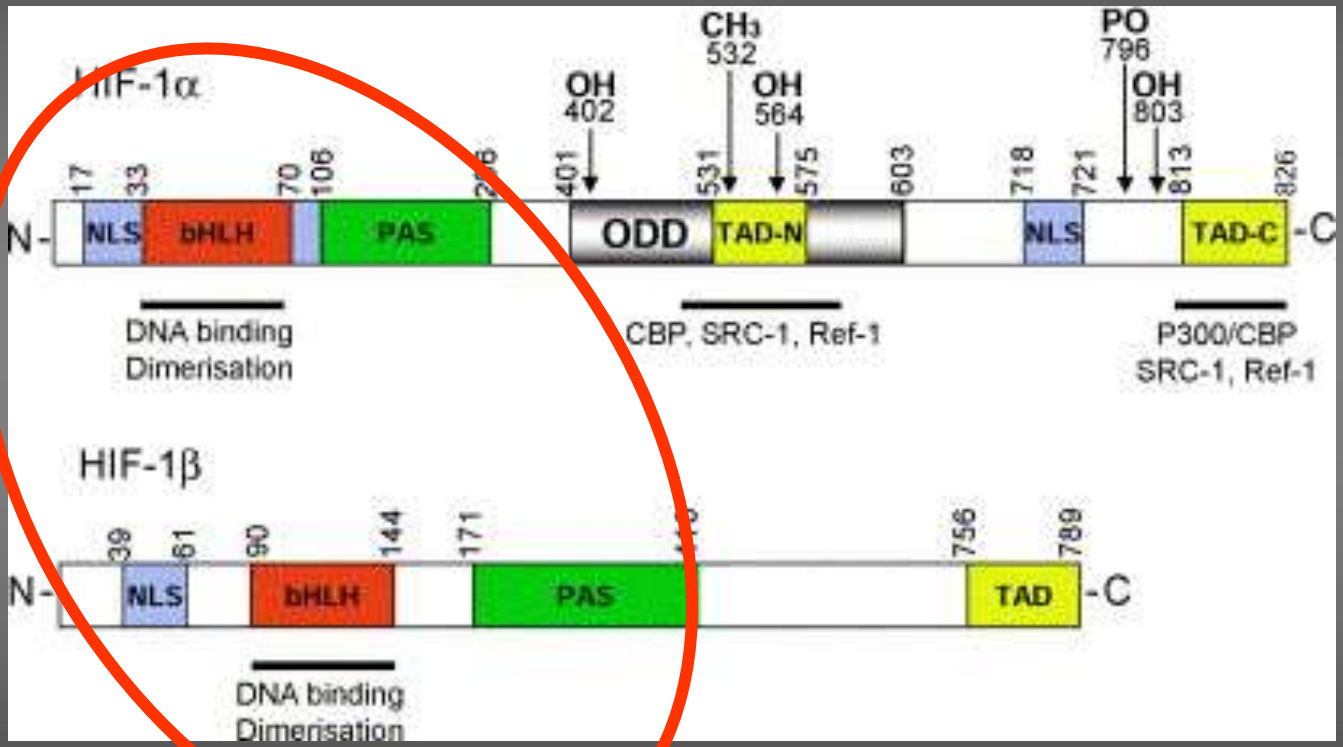
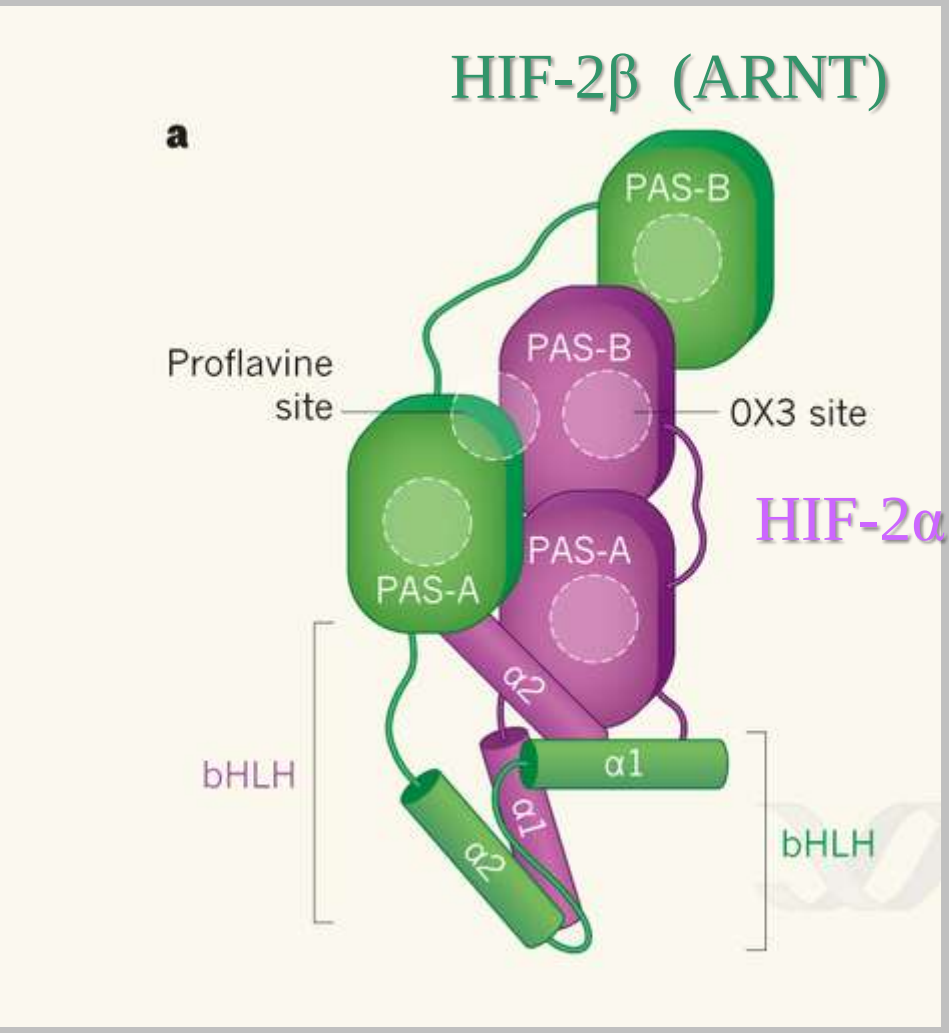

O2
PHDs
P
P
HIFα
N
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
proteasome
H
α
IF
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

Struttura HIF

Struttura di HIF1 alpha/beta

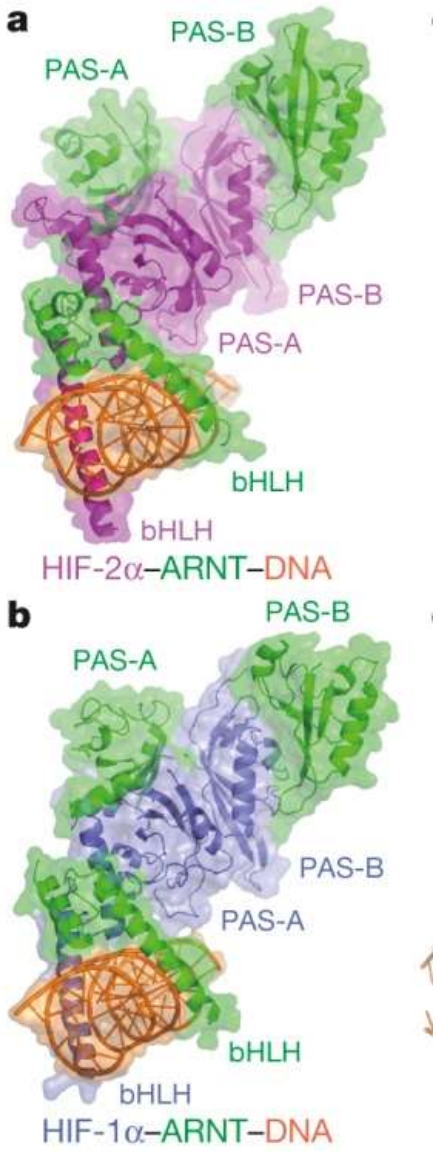




PAS,PAS-B (interaction domains)

bHLH (DNA Binding domain)

DNA-bound HIF-α–ARNT structures.



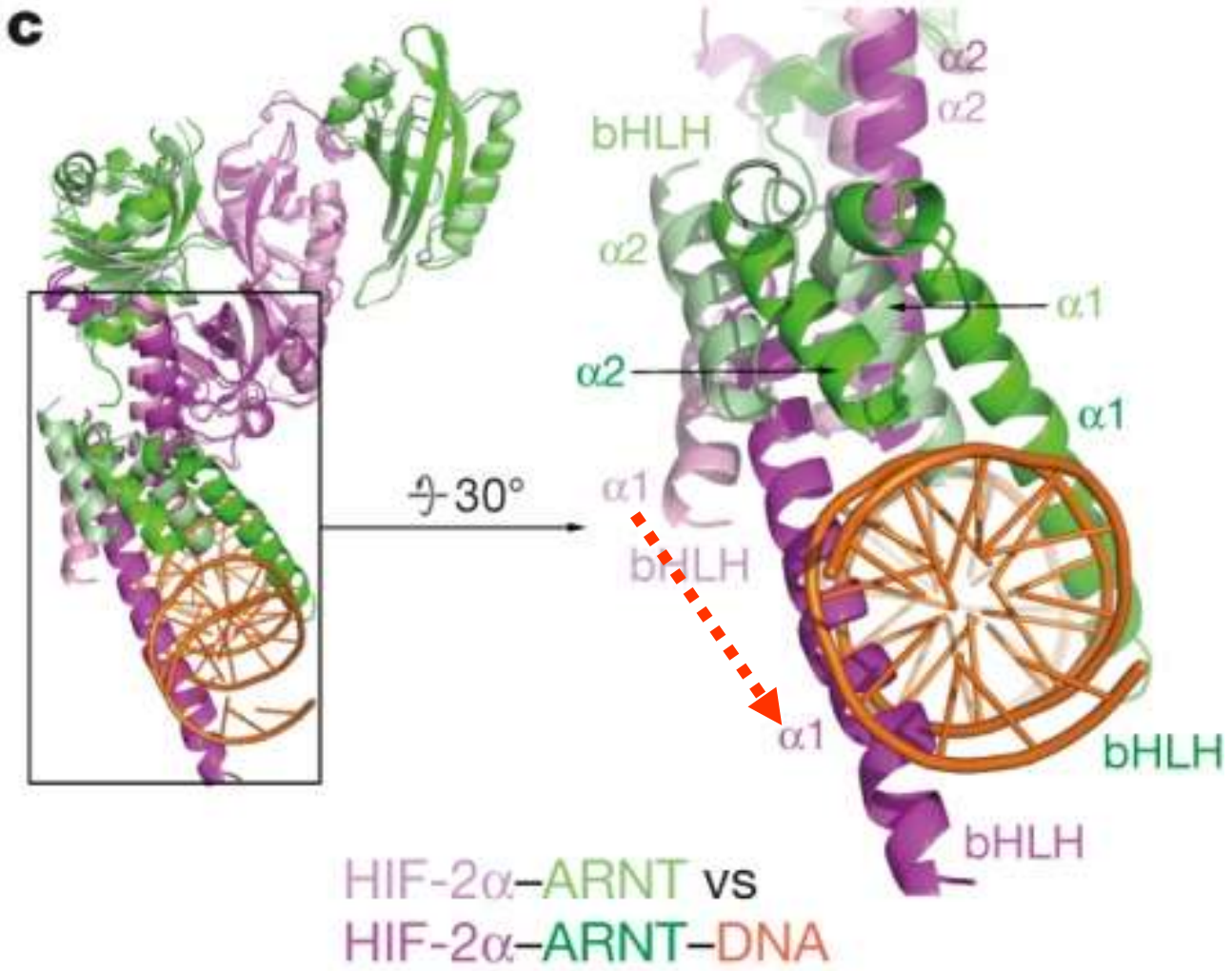
HIF-2α–ARNT–DNA (a) and

HIF-1α–ARNT–DNA (b) complexes

nature

D Wu *et al. Nature* **000**, 1-6 (2015) doi:10.1038/nature14883

DNA-bound HIF-α–ARNT structures.



dramatic conformational changes at the bHLH domains

nature

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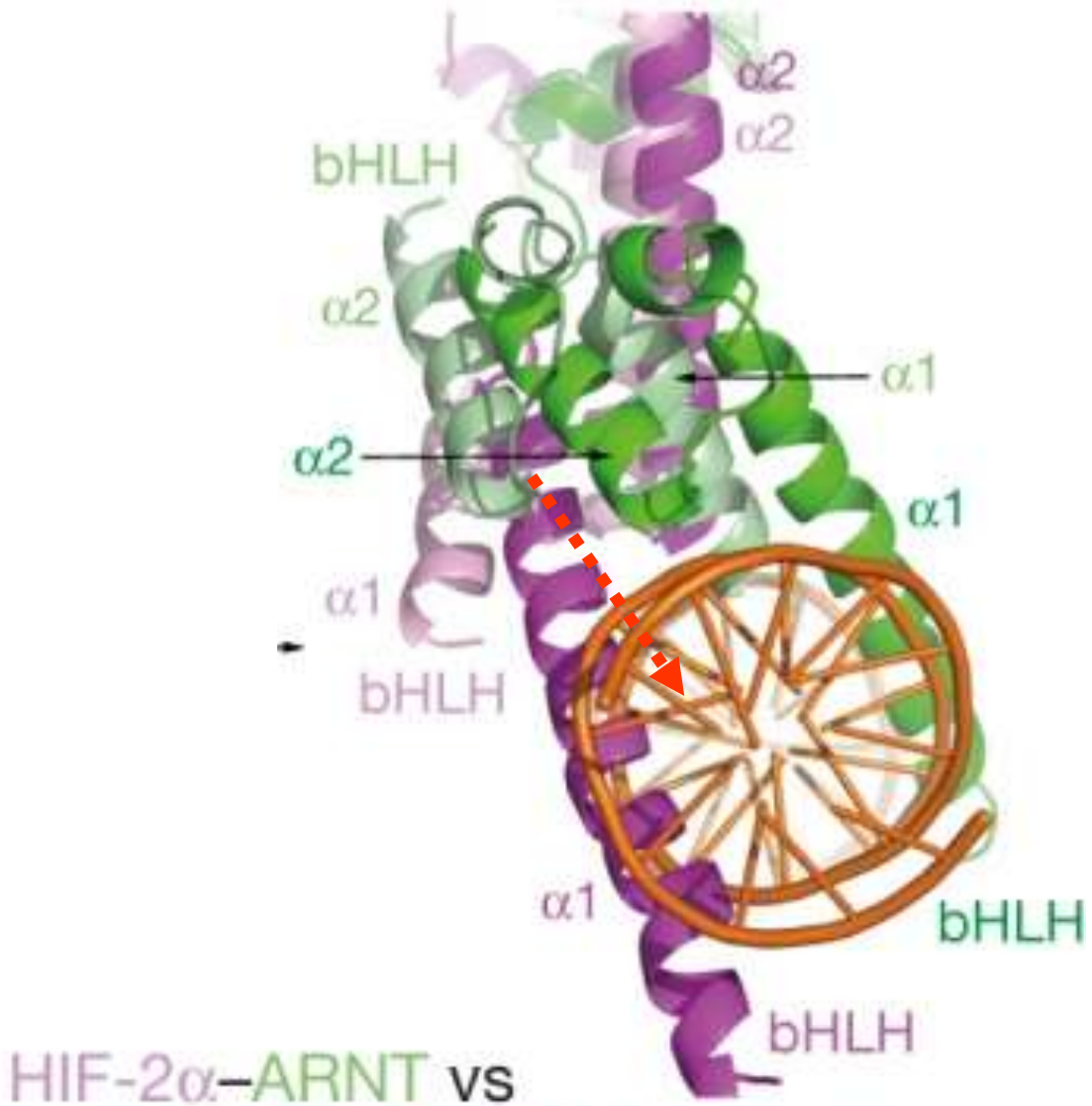
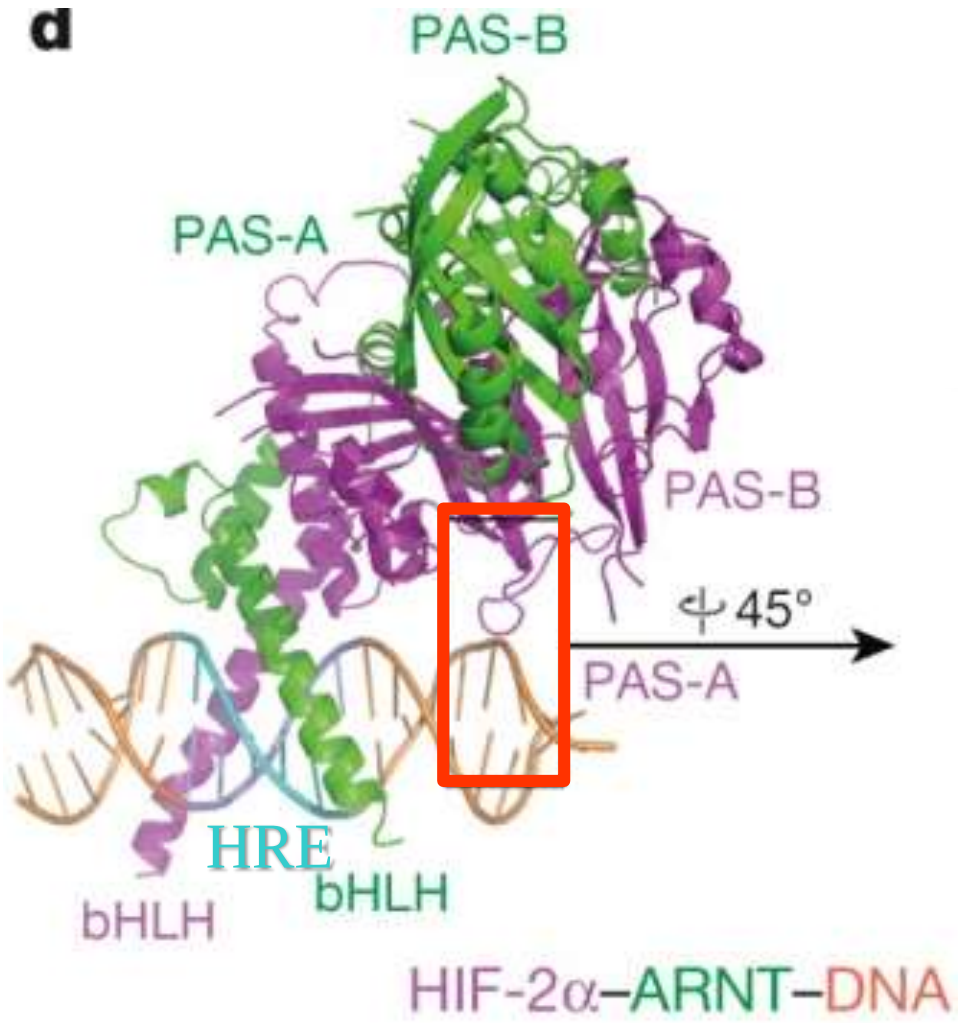
DNA-bound HIF-α–ARNT structures.

dramatic conformational changes at the bHLH domains



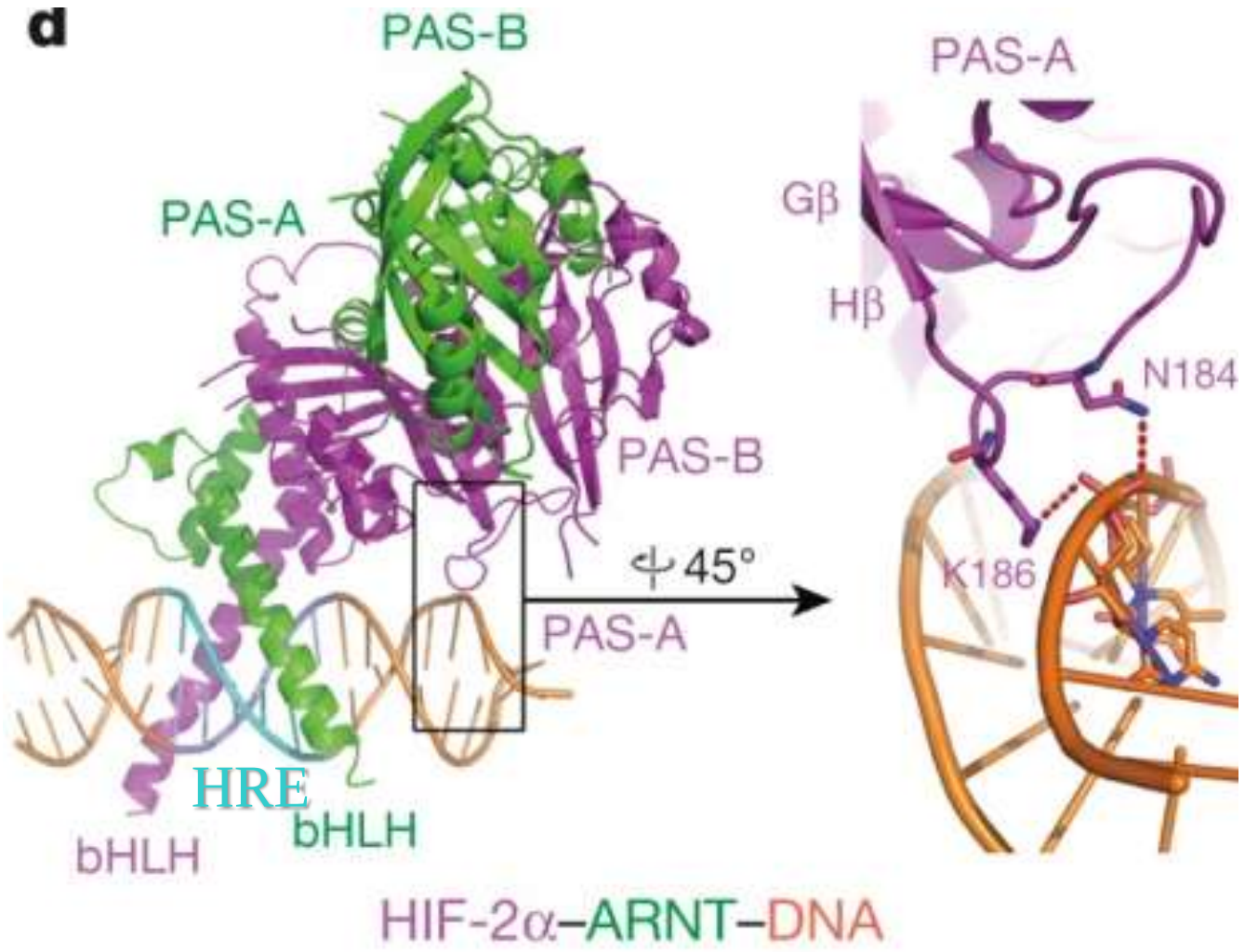
DNA (HRE)-bound HIF-α–ARNT structures.



nature

D Wu *et al. Nature* **000**, 1-6 (2015) doi:10.1038/nature14883

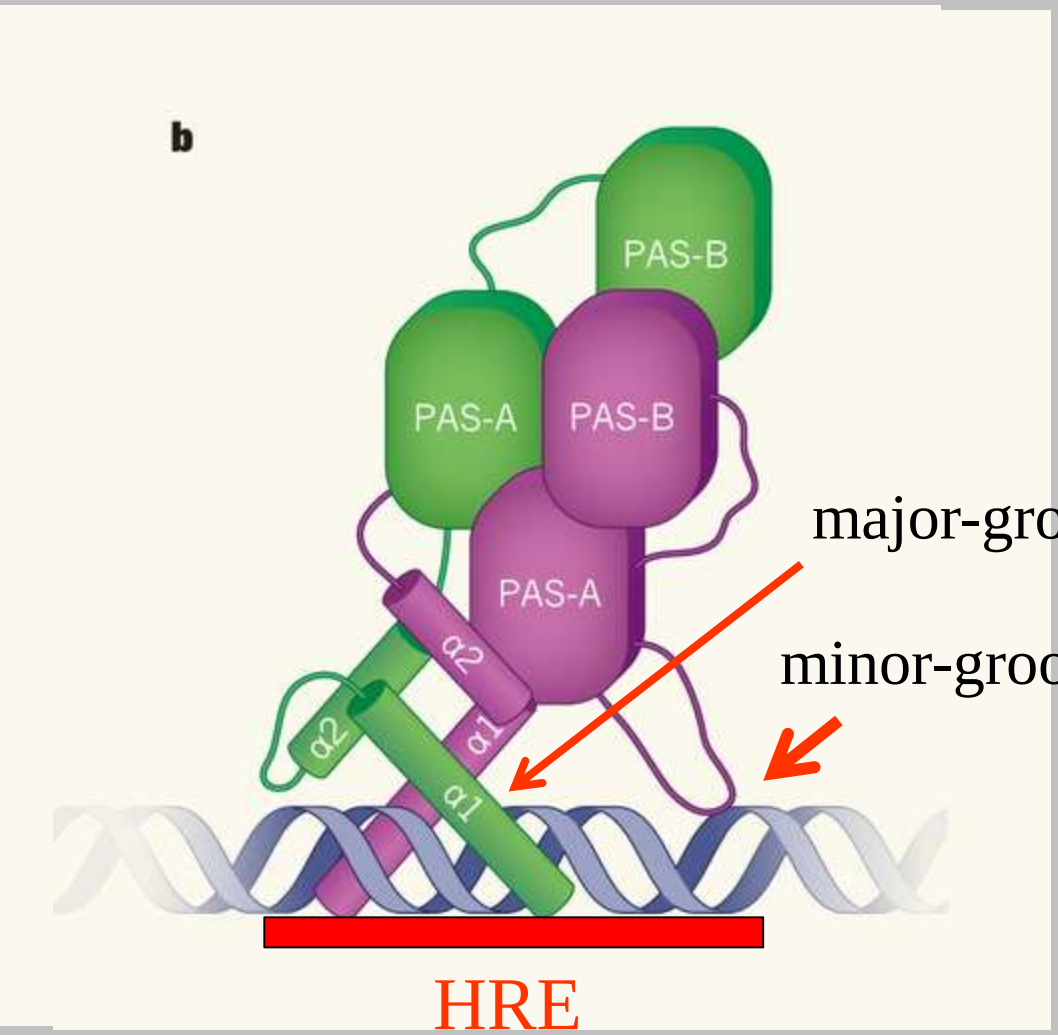
DNA (HRE)-bound HIF-α–ARNT structures.



nature

D Wu *et al. Nature* **000**, 1-6 (2015) doi:10.1038/nature14883

bHLH (DNA Binding domain
PAS,PAS-B (interaction domains



The hypoxia-responsive element (HRE) is a regulatory element mediating transactivation by the hypoxia-inducible factor (HIF).

The data from over 70 genes suggest that endogenous HREs are composite regulatory elements comprising

a) the conserved HIF-binding site with an **A/GCGTG** core sequence
b) a **highly variable flanking sequence**.

the flanking sequence provides binding sites for additional transcription factors required to amplify the hypoxic response/ make the HRE tissue-specific

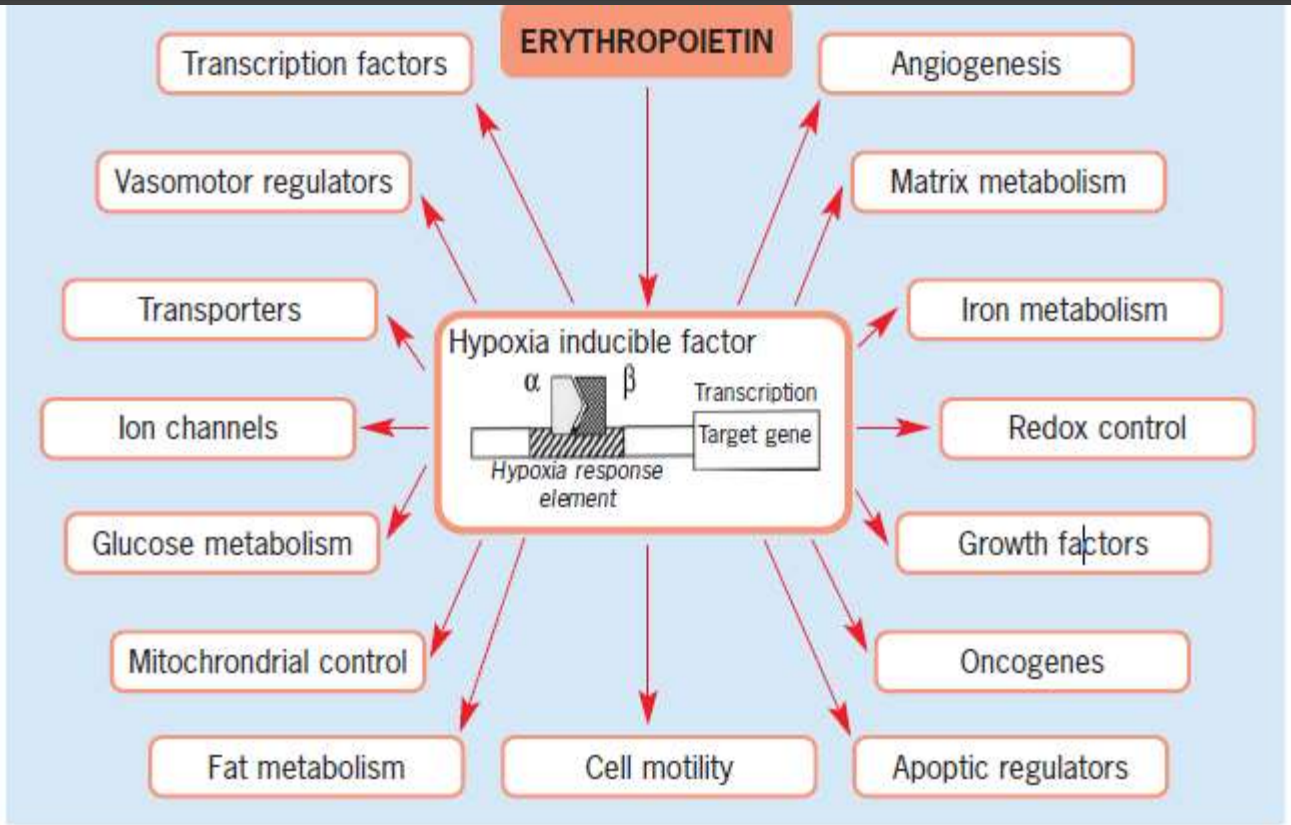
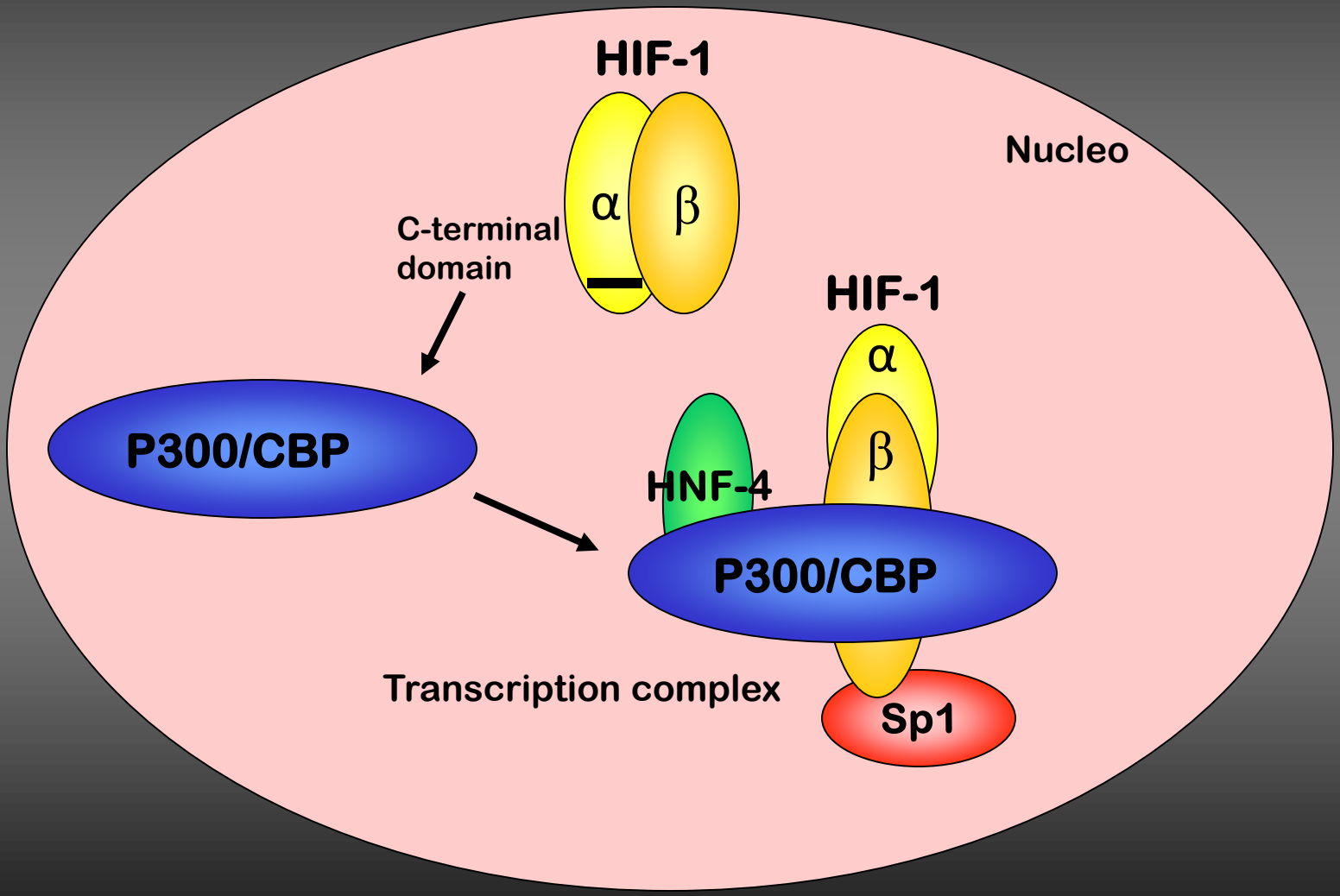


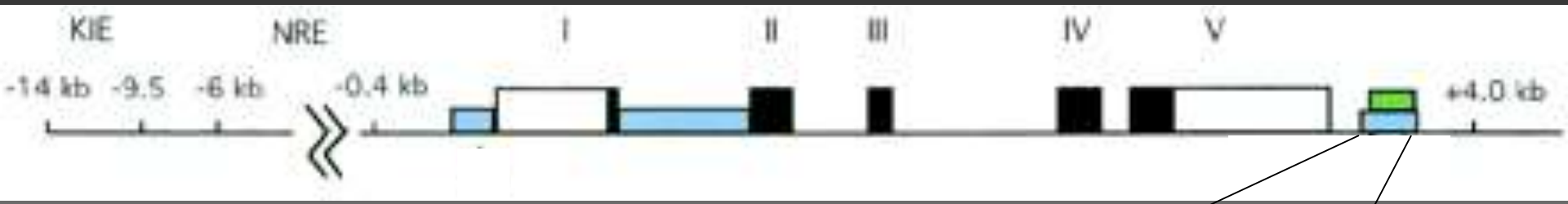
Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.

REGOLAZIONE DELL'ESPRESSIONE DELL'ERITROPOIETINA DA PARTE DELL'IPOSSIA

Regolazione del gene Epo da parte dell'ipossia

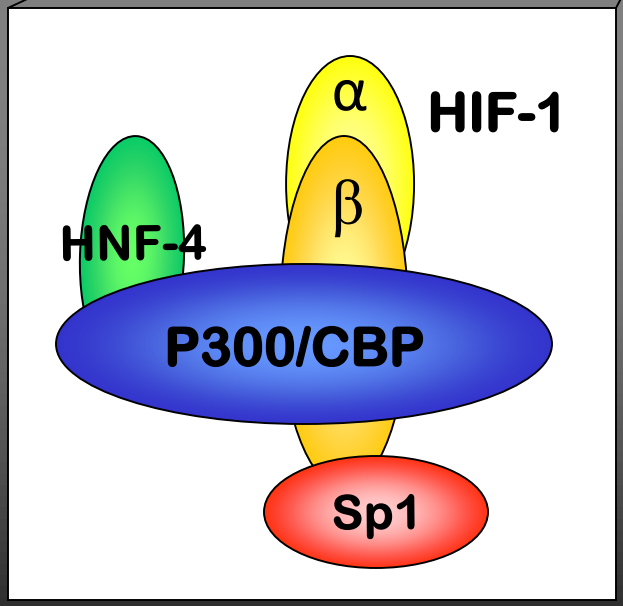


Regolazione del gene Epo da parte dell'ipossia

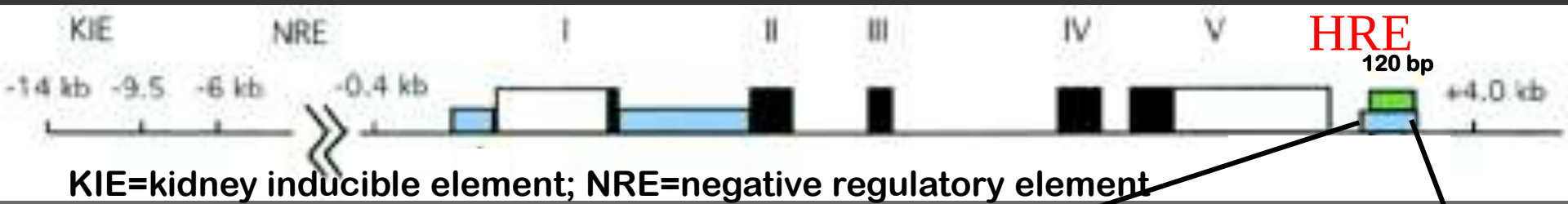


Promotore

Transcription complex



Regolazione del gene Epo da parte dell'ipossia



KIE=kidney inducible element; NRE=negative regulatory element

50 pb nella Regione 3'

HRE

GGCCCTACGTGCTGTCTCACACAGCCTGTCTGACCTCTCGACCTACCG

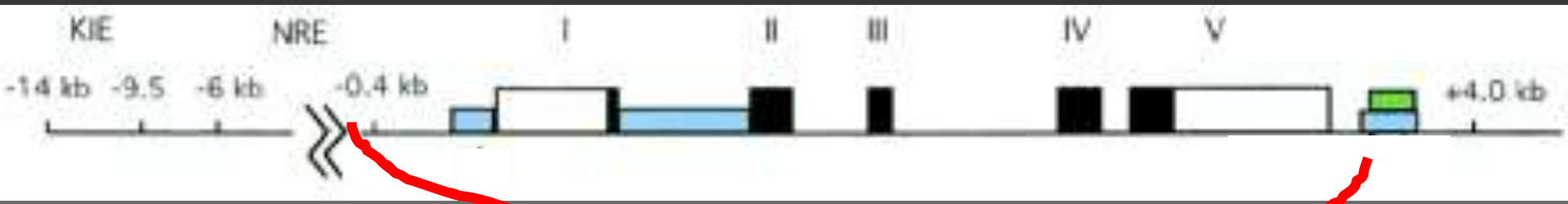
Sequenza di legame per ***HIF-1***

Sequenza di legame per ***HNF-4***

HNF-4 (Hepatocyte nuclear factor 4) fattore di trascrizione espresso nel cortex renale e nel fegato come Epo → contribuisce alla regolazione tessuto-specifica

Mutazioni a carico di una di queste sequenze inibiscono l'induzione di Epo da parte dell'ipossia

Regolazione tessuto-specifica del gene Epo

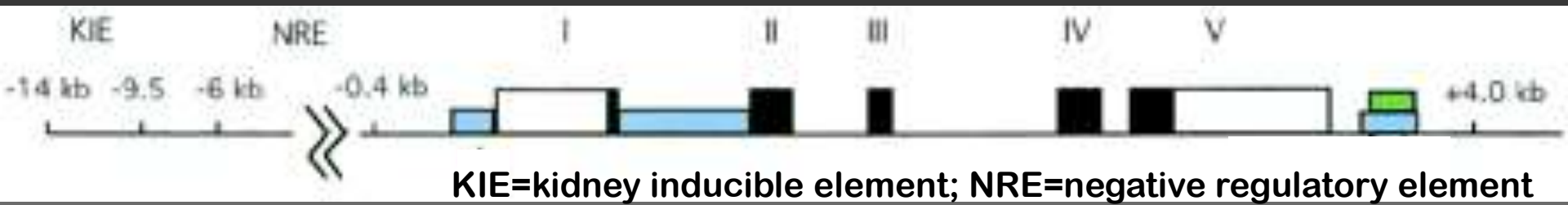


Promotore

Sp1: fattore di trascrizione ubiquitario

- Regione –61 –45: contribuisce alla regolazione da parte dell'ipossia → sequenza di legame per ***Sp1***
- Sito di legame per GATA: inibizione dell'espressione di Epo
- Sito CACCC: sequenza stimolatrice dell'espressione di Epo

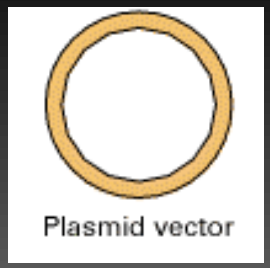
Regolazione tessuto-specifica del gene Epo



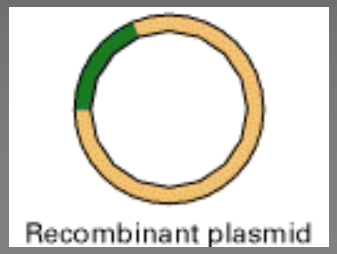
KIE=kidney inducible element; NRE=negative regulatory element

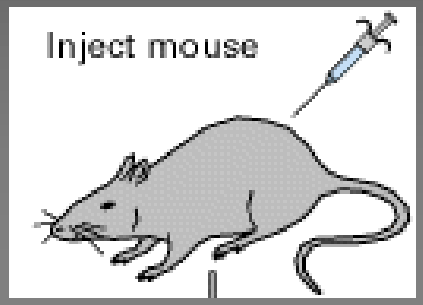
Regione 5'

- 9.5-14 kb → Sequenza richiesta per l'espressione nel rene KIE
- Entro le 9.5 kb → Sequenza richiesta per l'espressione nel fegato
- 0.4-6 kb → Sequenza regolatoria negativa NRE che inibisce l'espressione di Epo nei tessuti che non producono Epo



+

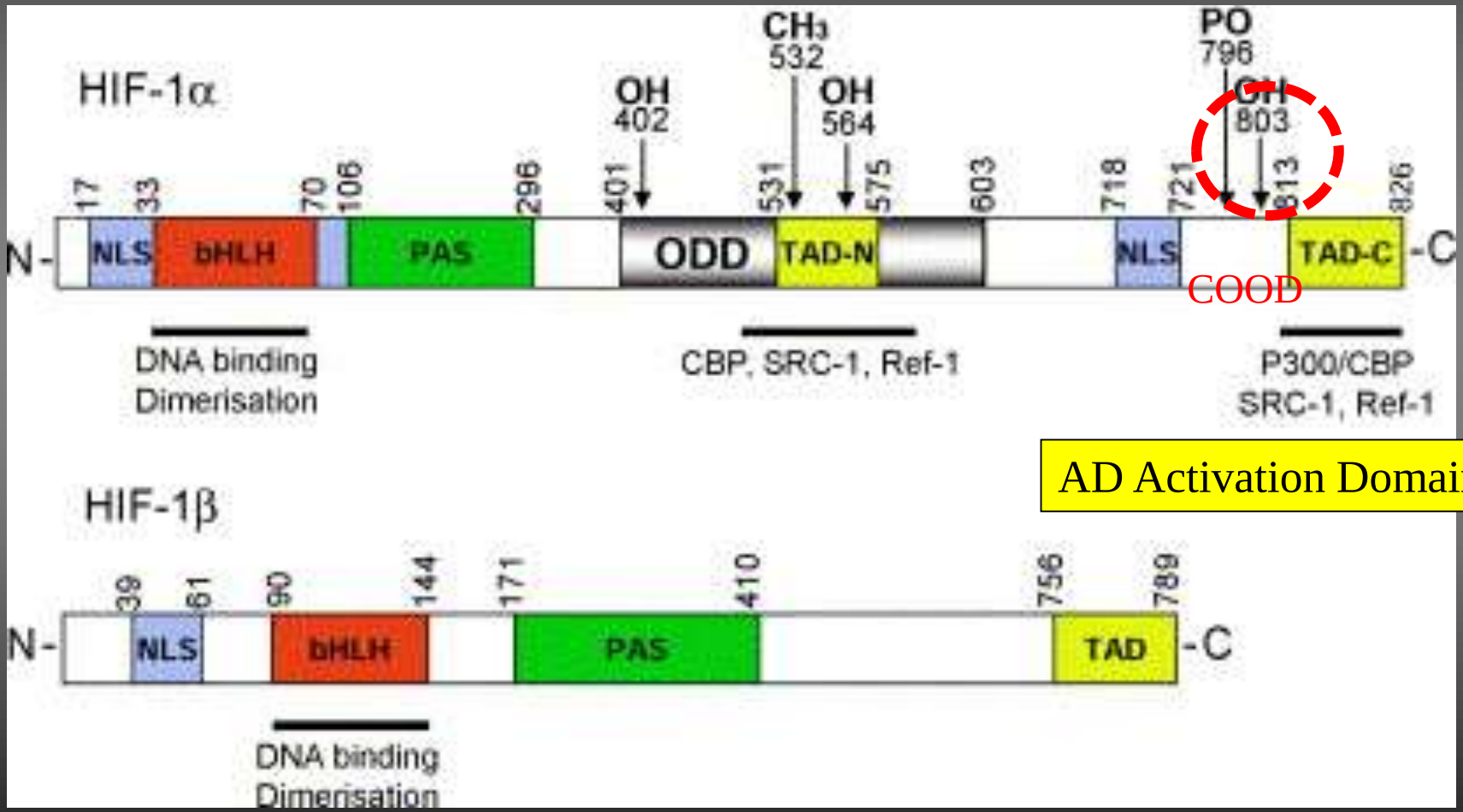


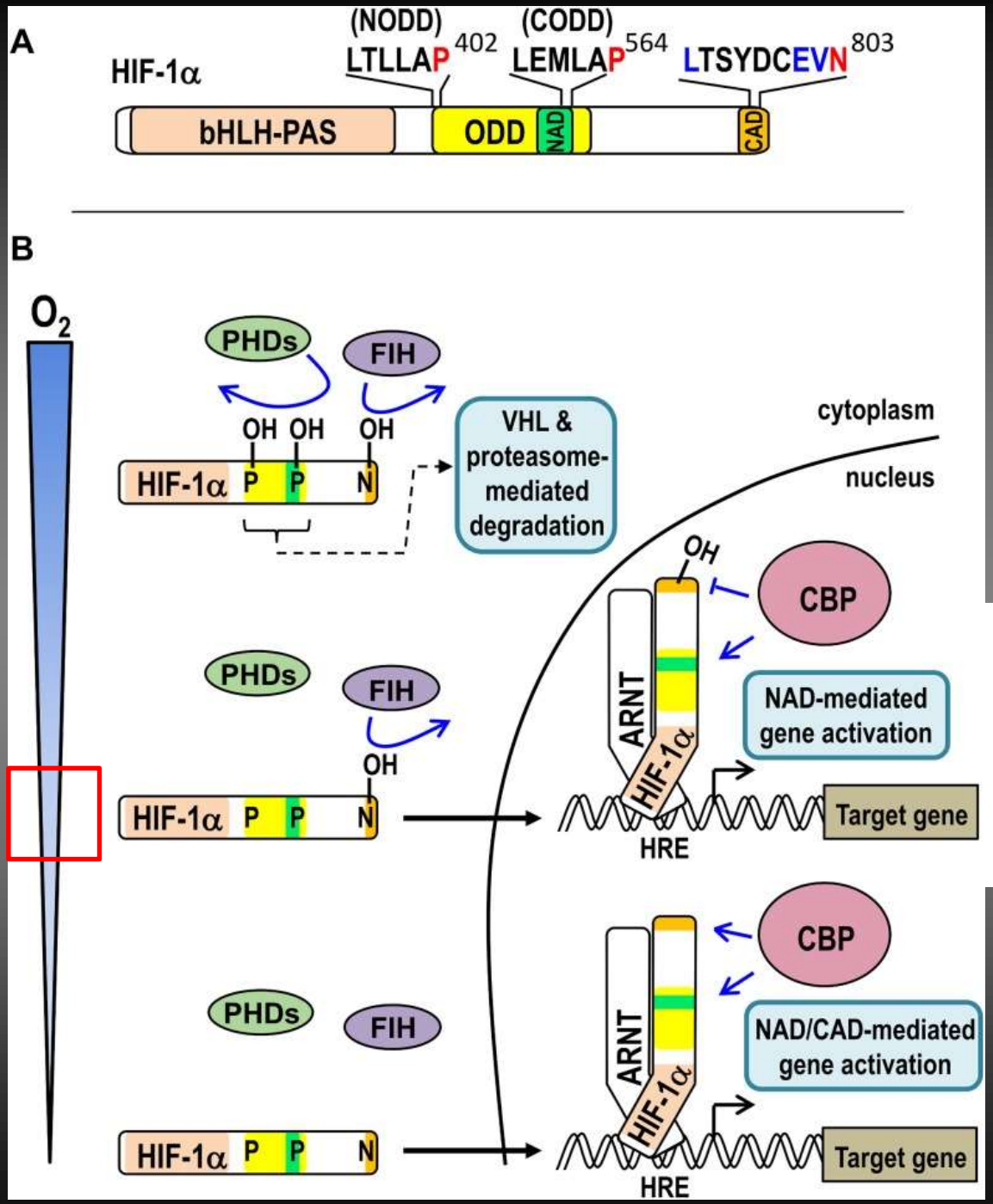


1. Epo espressa in fegato, rene e tessuti che normalmente non esprimono Epo
2. Epo espresso nel fegato ma non nel rene → 400 bp-6 kb: sequenze regolatorie negative
3. Epo espresso nel fegato ma non nel rene → Entro le 9.5 kb: sequenze per l'espressione nel fegato
4. Epo espresso nel rene → 9.5-14 kb: sequenze per l'espressione nel rene

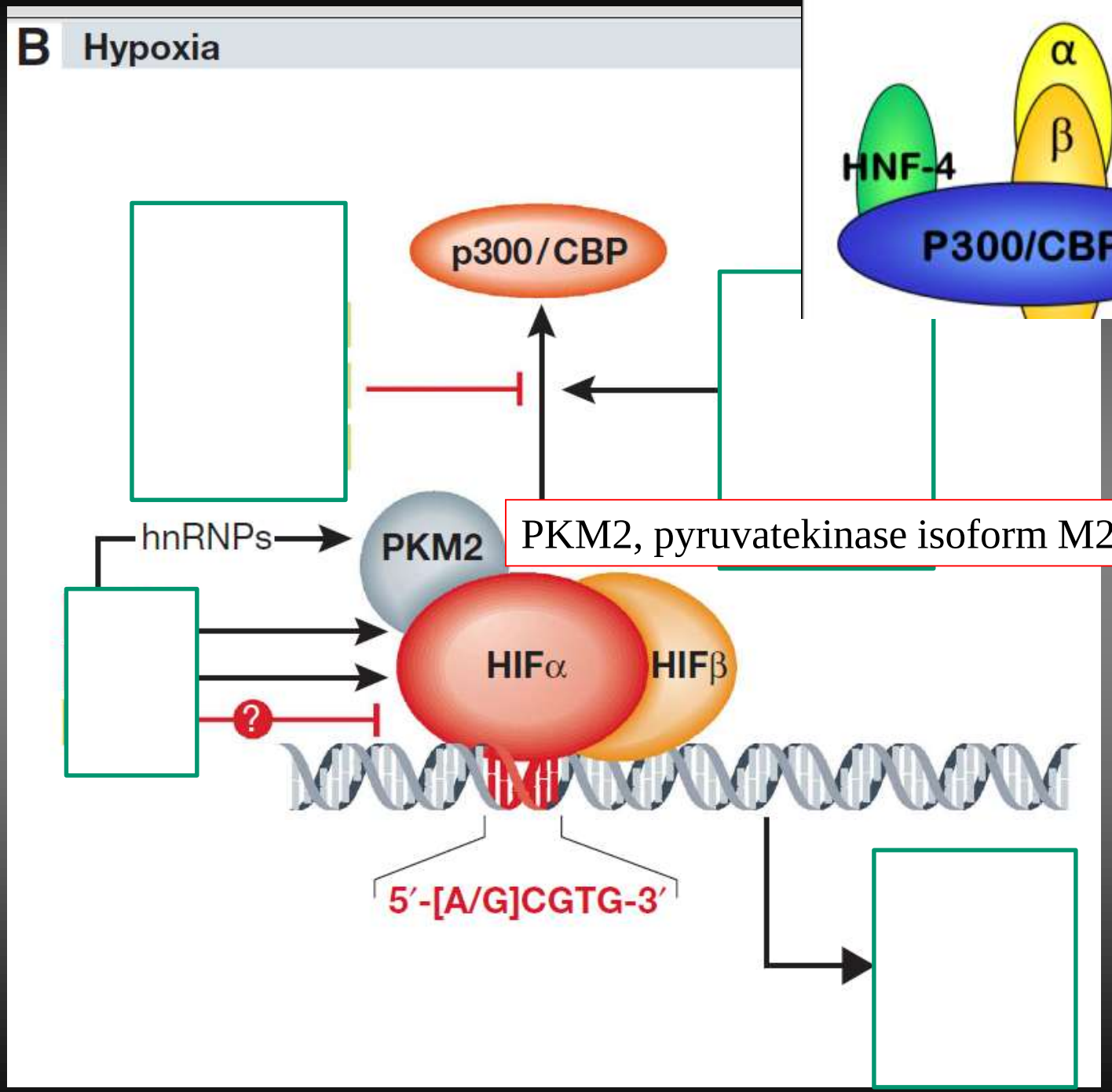
Semenza et al. PNAS 1989; Semenza et al., Mol Cell Biol. 1990; Semenza et al., PNAS 1991

Asparaginyl hydroxylation (Asn N 803, CAD) by Factor Inhibiting HIF (FIH)





Asparaginyl hydroxylation of N803 by FIH inhibits binding of the HIF1a CAD to coactivator p300/CBP



PKM2, pyruvatekinase isoform M2

Pyruvate kinase (PK)

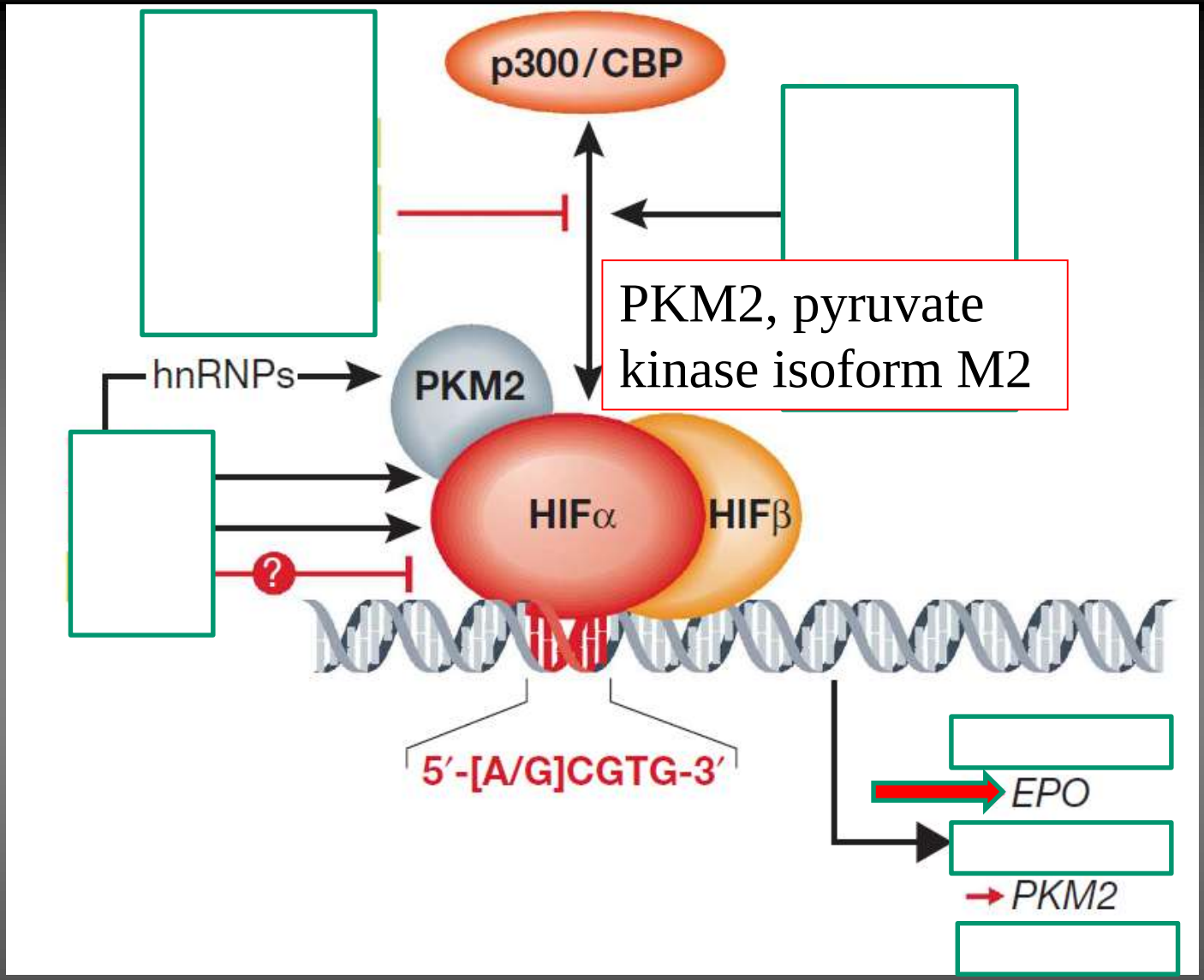
Enzima glicolitico e Fattore di trascrizione

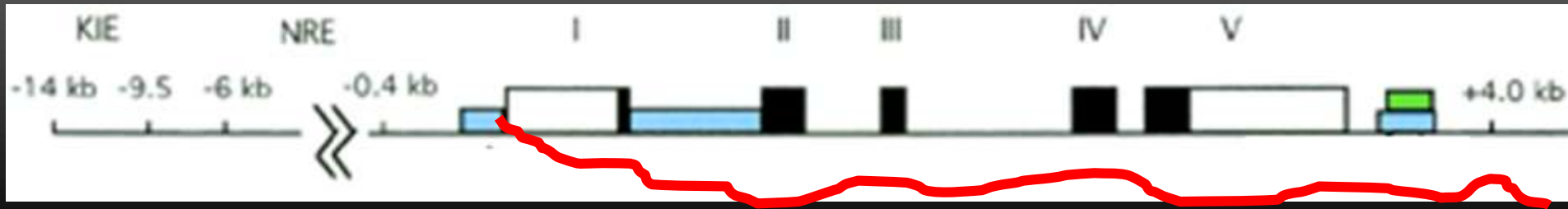
The primary function of pyruvate kinase (PK) enzyme is to catalyzes the transfer of a phosphate group (phosphoenolpyruvate PEP +ADP to Pyruvate +ATP)

There are four pyruvate kinase isoenzymes: **PKM2** is produced by alternative splicing of the PKM pre-mRNA, by **exon 10 inclusion**

PKM2 also interacts with HIF-1α in the nuclei of hypoxic cells and enhances the recruitment of p300 to HRE sites and transactivation of HIF-1α target genes!

HIF-1α binds hypoxia response elements (HRE) within the first intron of human PKM2 that contains a HIF-1-binding site


p300/CBP
PKM2, pyruvate kinase isoform M2
hnRNPs
PKM2
HIFα
HIFβ
?
5′-[A/G]CGTG-3′
EPO
PKM2

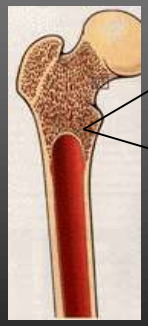

KIE
NRE
I
II
III
IV
V
-14 kb
-9.5
-6 kb
-0.4 kb
+4.0 kb

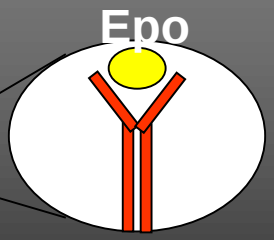
ERITROPOIETINA (Epo)

- ✓ Ormone glicoproteico di 34 kDa (165 aa)
- ✓ Struttura a 4 α-eliche (A,B,C,D)
- ✓ Funzione: stimola l'eritropoiesi

SINTESI *LEGAME CON IL RECETTORE*

Reni

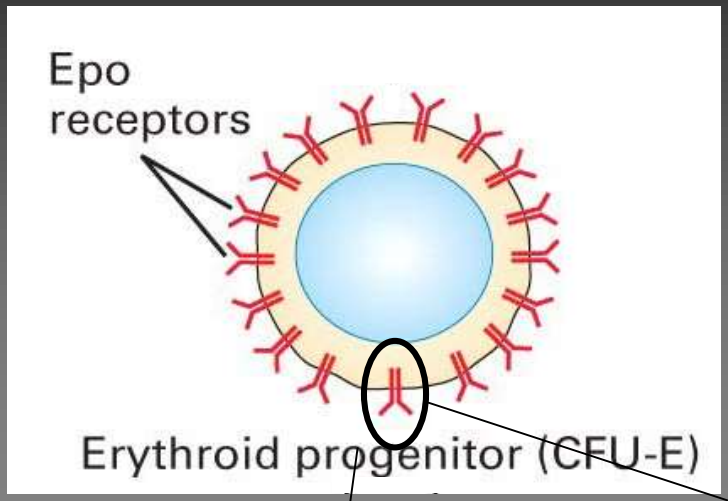




Midollo osseo

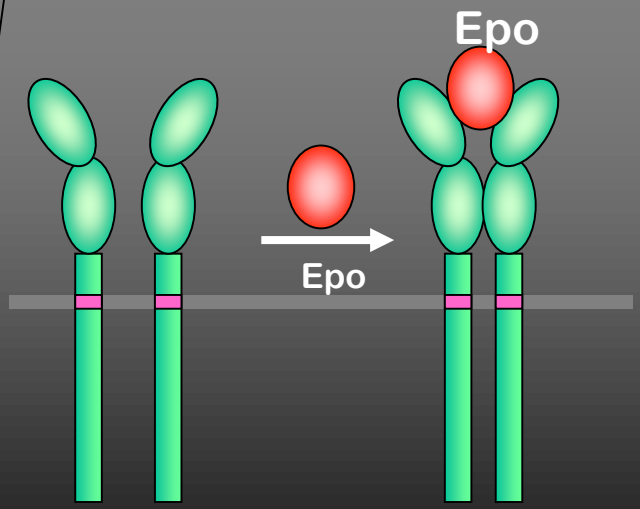
PRODUZIONE DI ERITROCITI

Il recettore dell'Epo (EpoR)



Glicoproteina transmembrana

Monomero: 66 kDa (507 aa)



Famiglia dei recettori delle citochine:

Legame del ligando

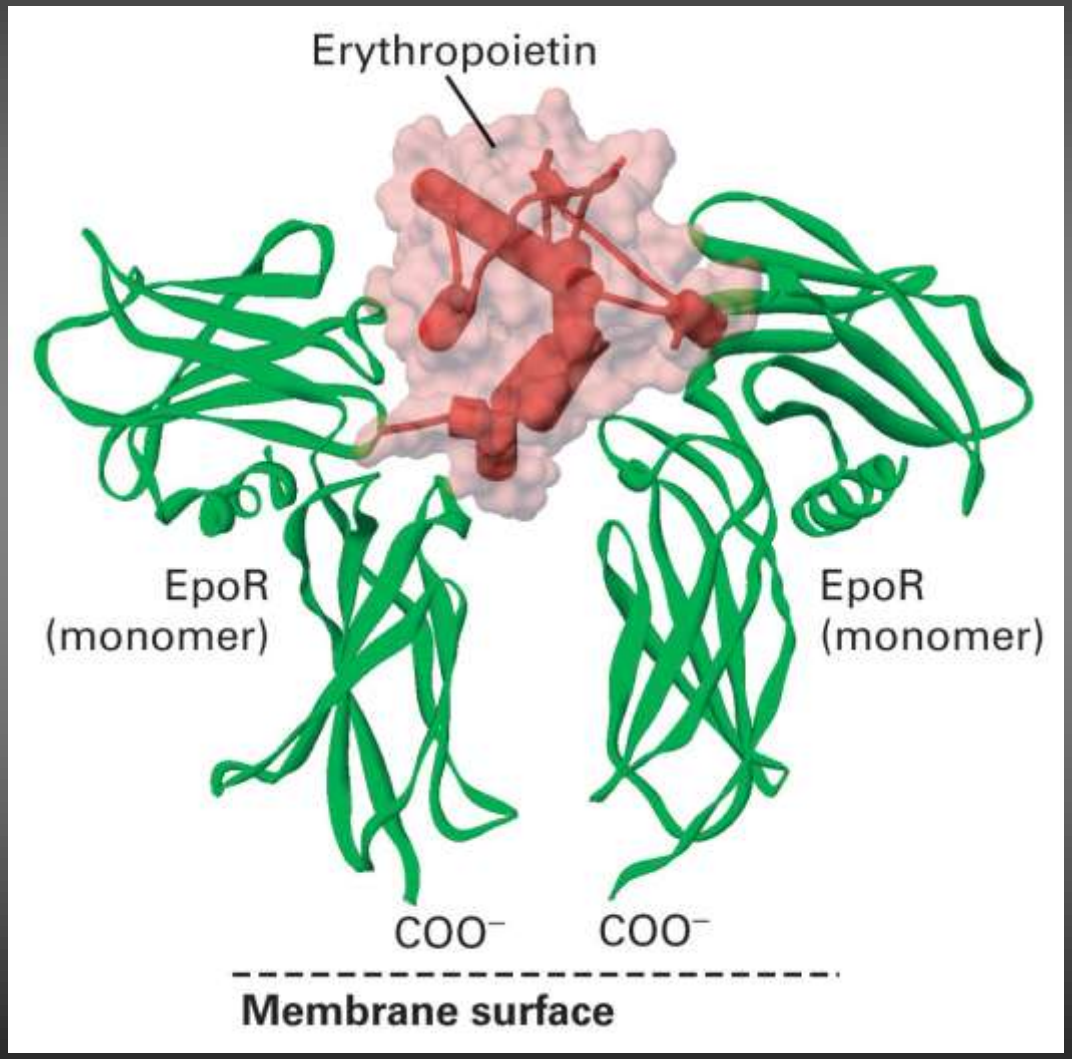
↓

Dimerizzazione

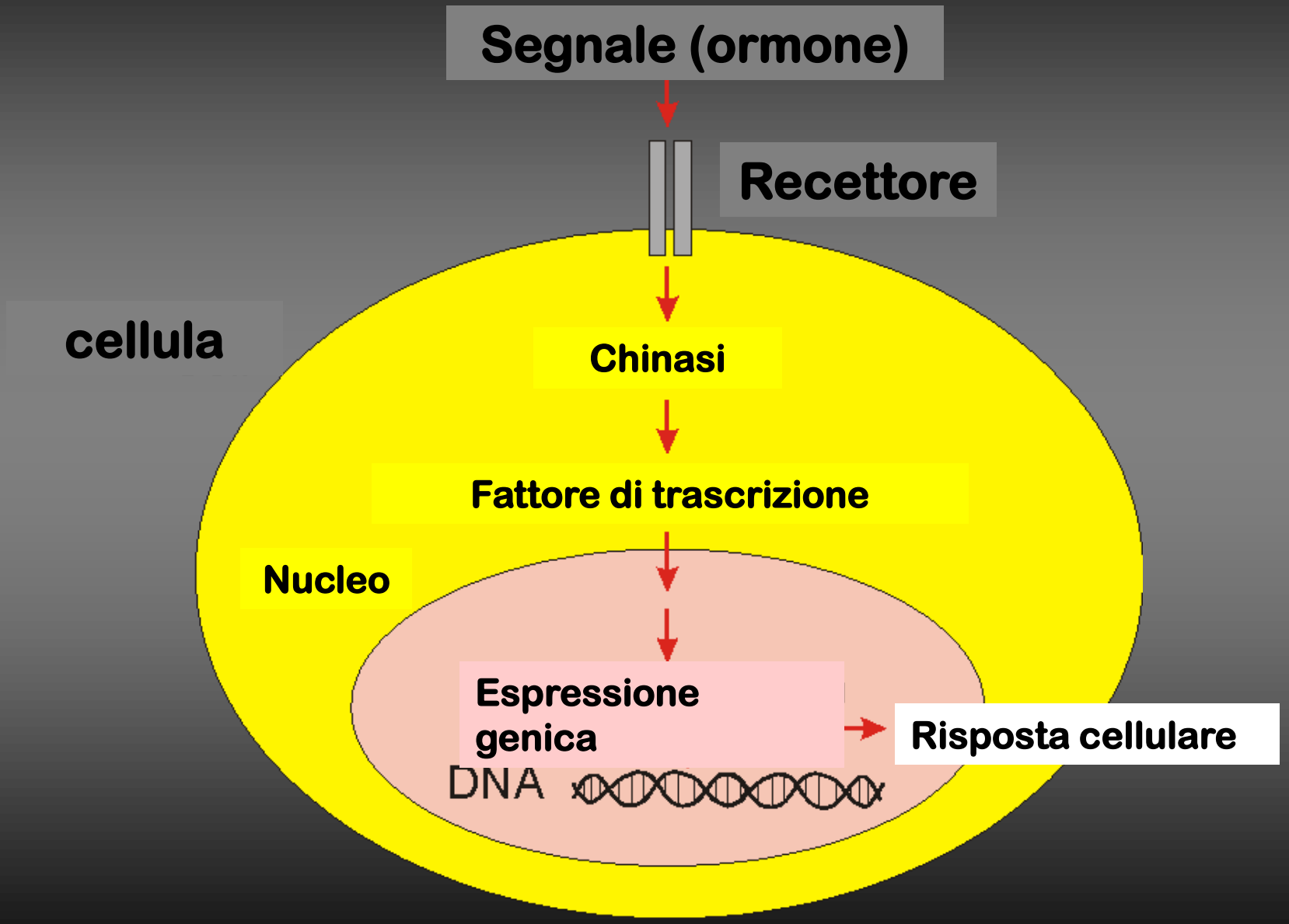
↓

Attivazione del recettore

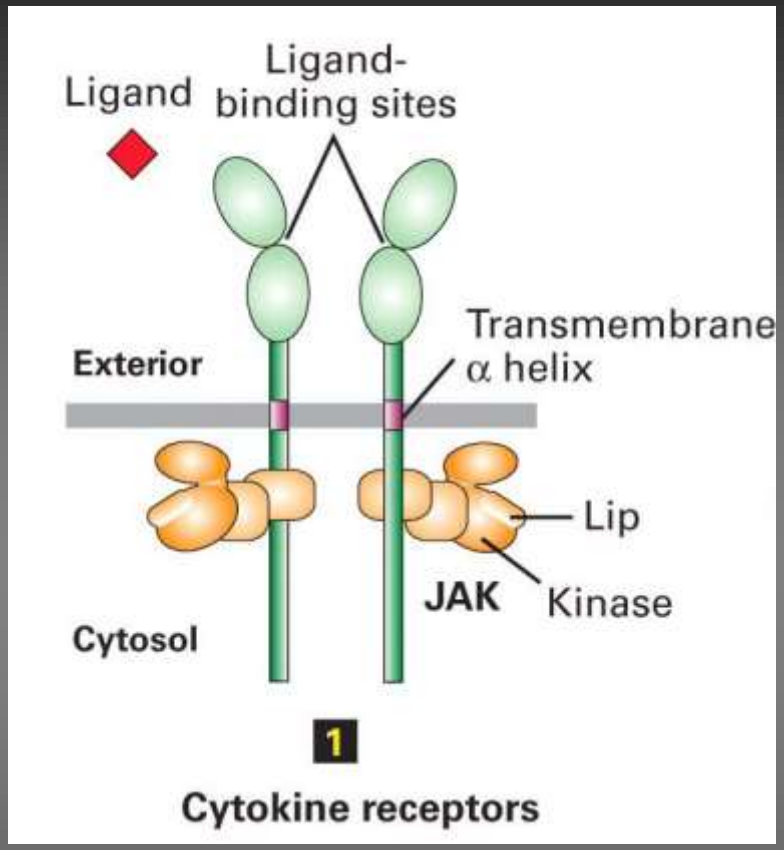
Erythropoietin-Epo Receptor complex



TRASDUZIONE DEL SEGNALE

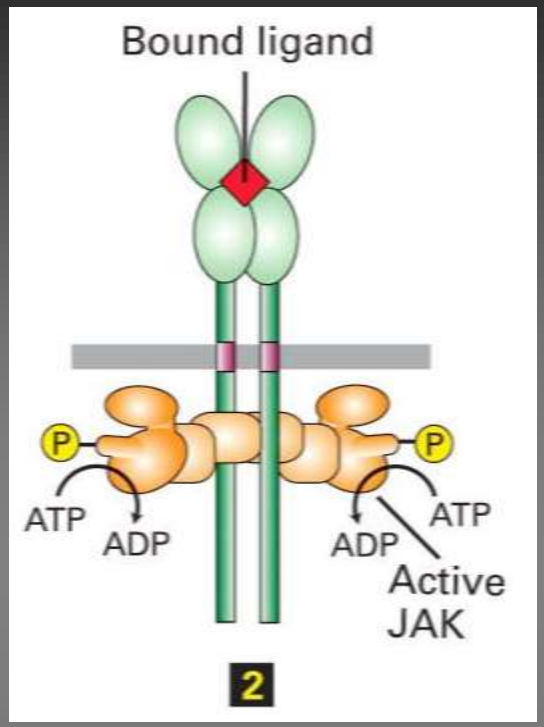


Trasduzione del segnale

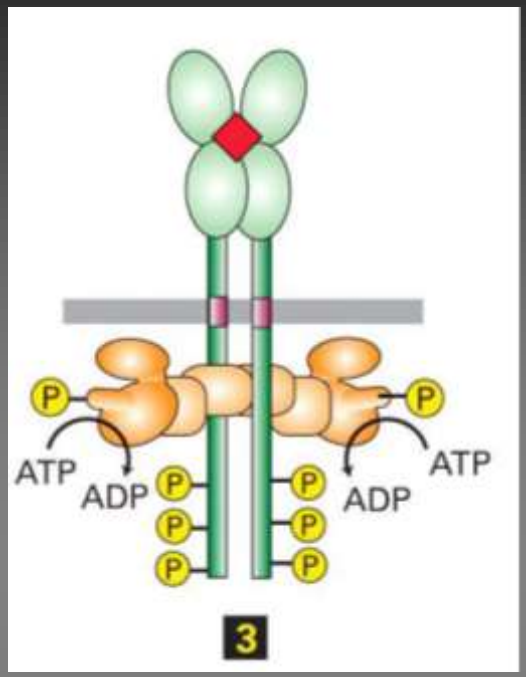


Dominio intracellulare privo di attività catalitica

Una JAK chinasi è associata al dominio citosolico di EpoR

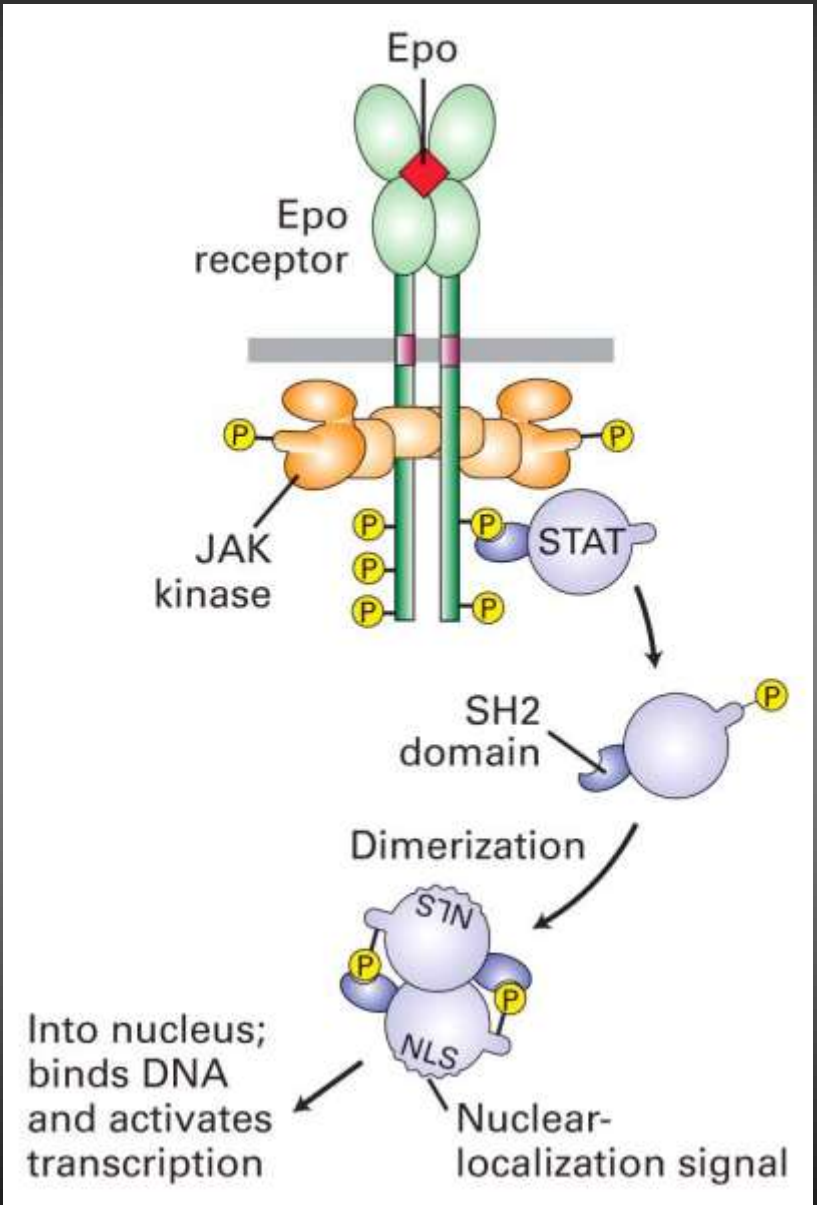


Dimerizzazione di EpoR
Fosforilazione di JAK e attivazione di JAK chinasi



JAK fosforila i residui di Tyr del dominio intracellulare di EpoR

Trasduzione del segnale



4) Legame di STATai residui di fosfo-Tyr di EpoR, mediante il dominio SH2 di STAT

5) Fosforilazione di STAT (fattore di trascrizione)

6) Dissociazione di STAT da EpoR e dimerizzazione di STAT

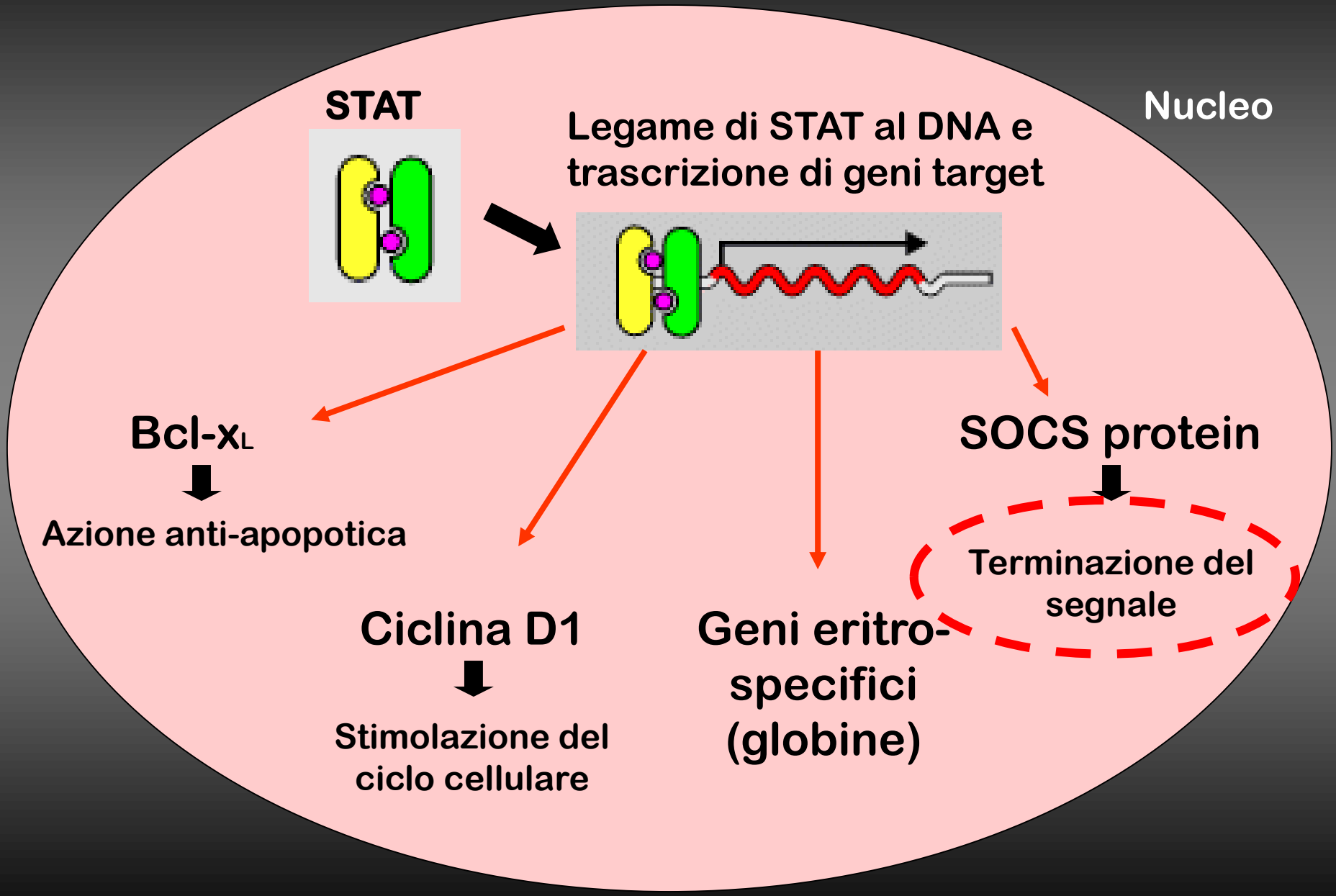
⇩

Esposizione di NLS (nuclear-localization signal)

⇩

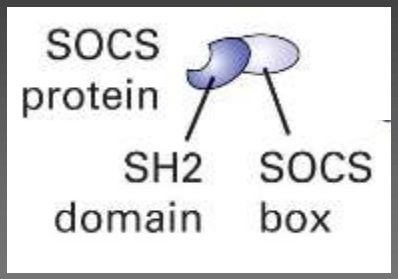
Spostamento di STAT al nucleo e legame a sequenze enhancer specifiche

Trascrizione di geni target



Terminazione del segnale

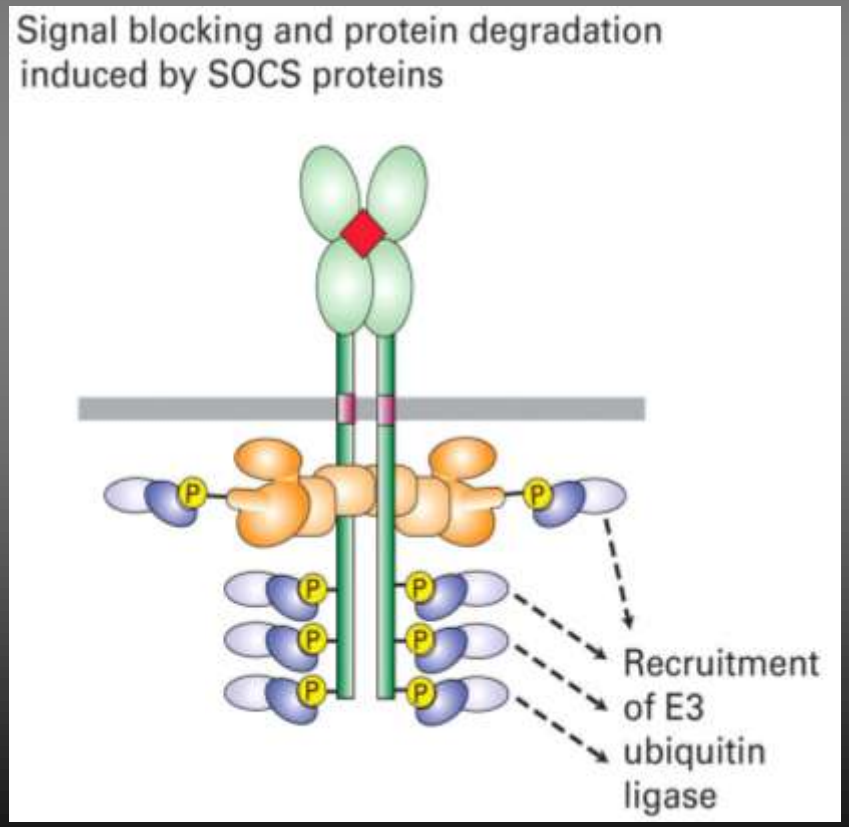
A lungo termine: *SOCS proteins*



Struttura:

-1 dominio SH2

-1 dominio SOCS (SOCS box) → richiama E3 ubiquitina ligasi



Meccanismo d'azione:

a) Il dominio SH2 si lega alle fosfo-Tyr del recettore: impedisce il legame di STAT

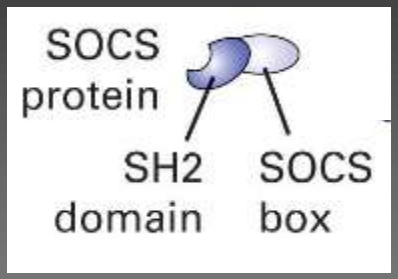
b) Il dominio SOCS richiama E3

⇩

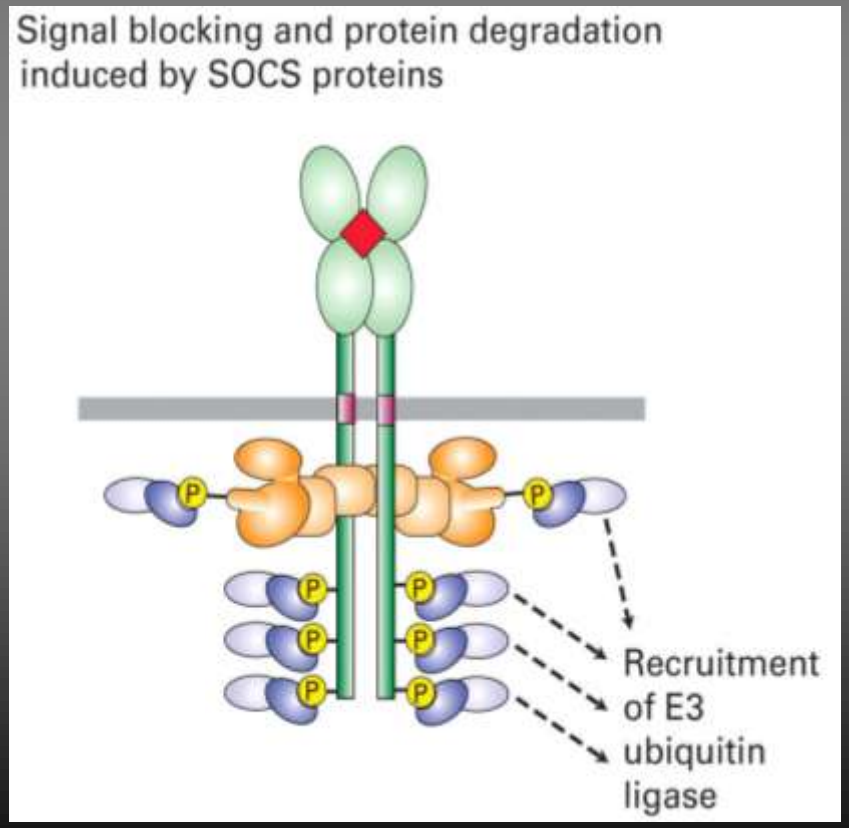
Ubiquinizzazione e degradazione proteosomica di JAK

Terminazione del segnale

A lungo termine: *SOCS proteins*



Struttura:
-1 dominio SH2
-1 dominio SOCS (SOCS box) → richiama E3 ubiquitina ligasi

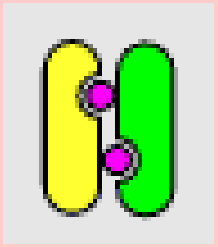


SOCS Box
40-amino acid motif, which recruits an E3 ubiquitin ligase complex consisting of
the adapter elongins B and C
Rbx2
the scaffold protein Cullin5

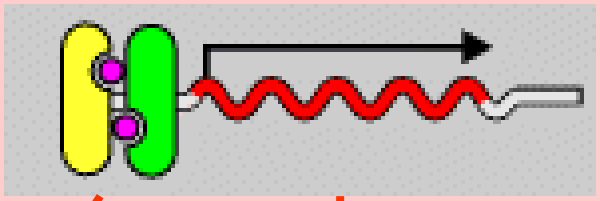
Trascrizione di geni target

Nucleo

STAT



Legame di STAT al DNA e trascrizione di geni target



Bcl-x_L

⬇

Azione anti-apopotica

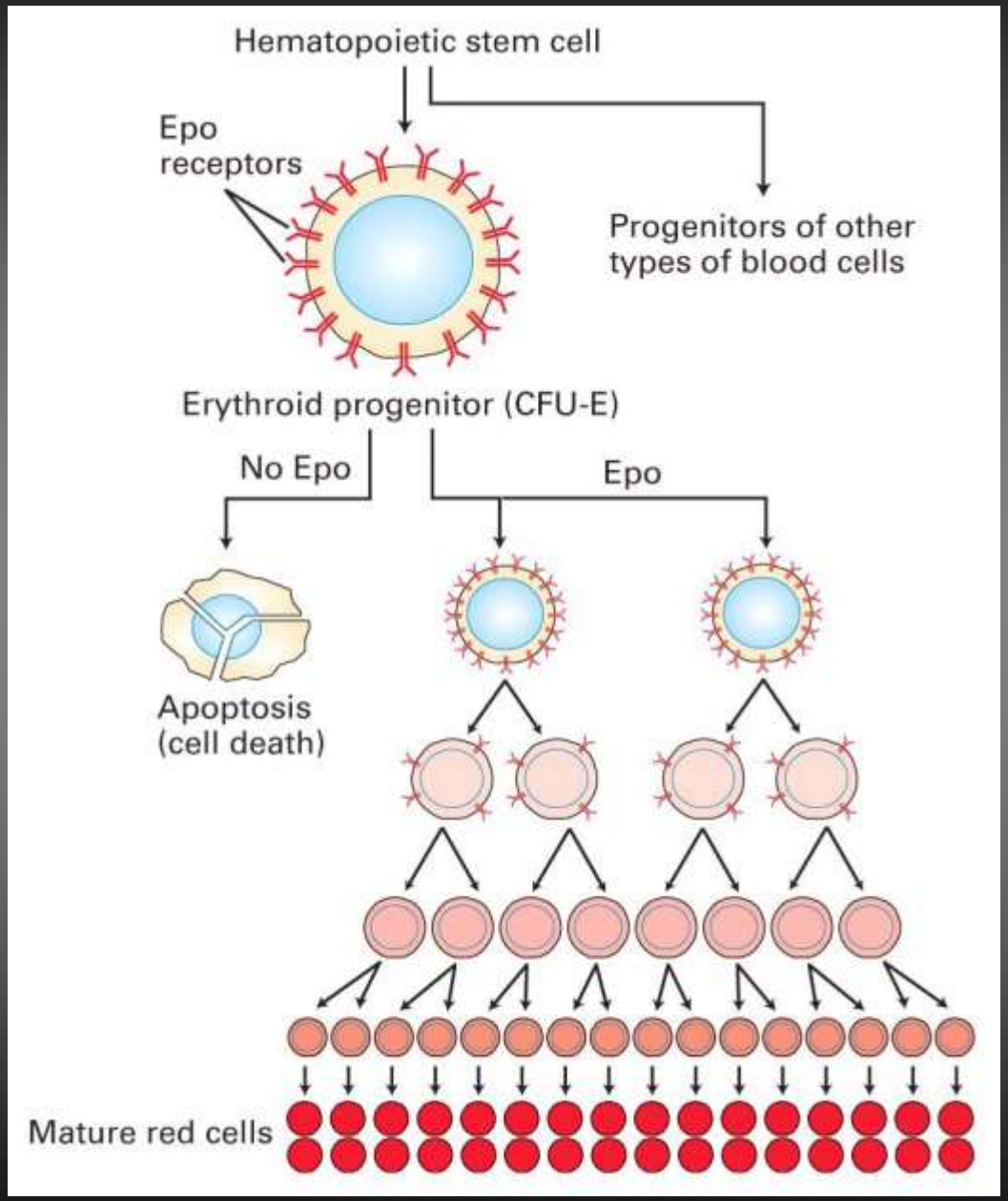
Ciclina D1

⬇

Stimolazione del ciclo cellulare

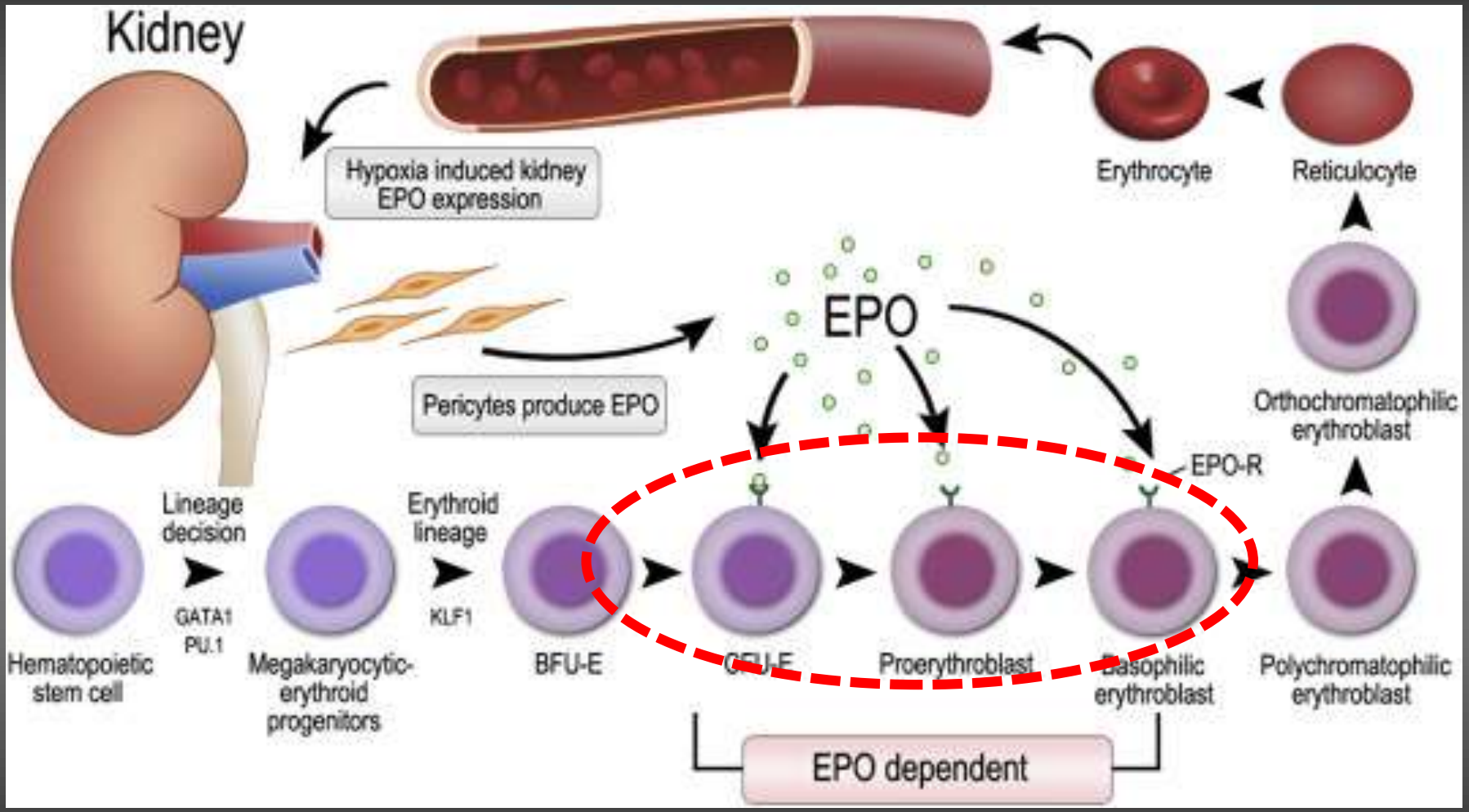
Geni eritro-specifici (globine)

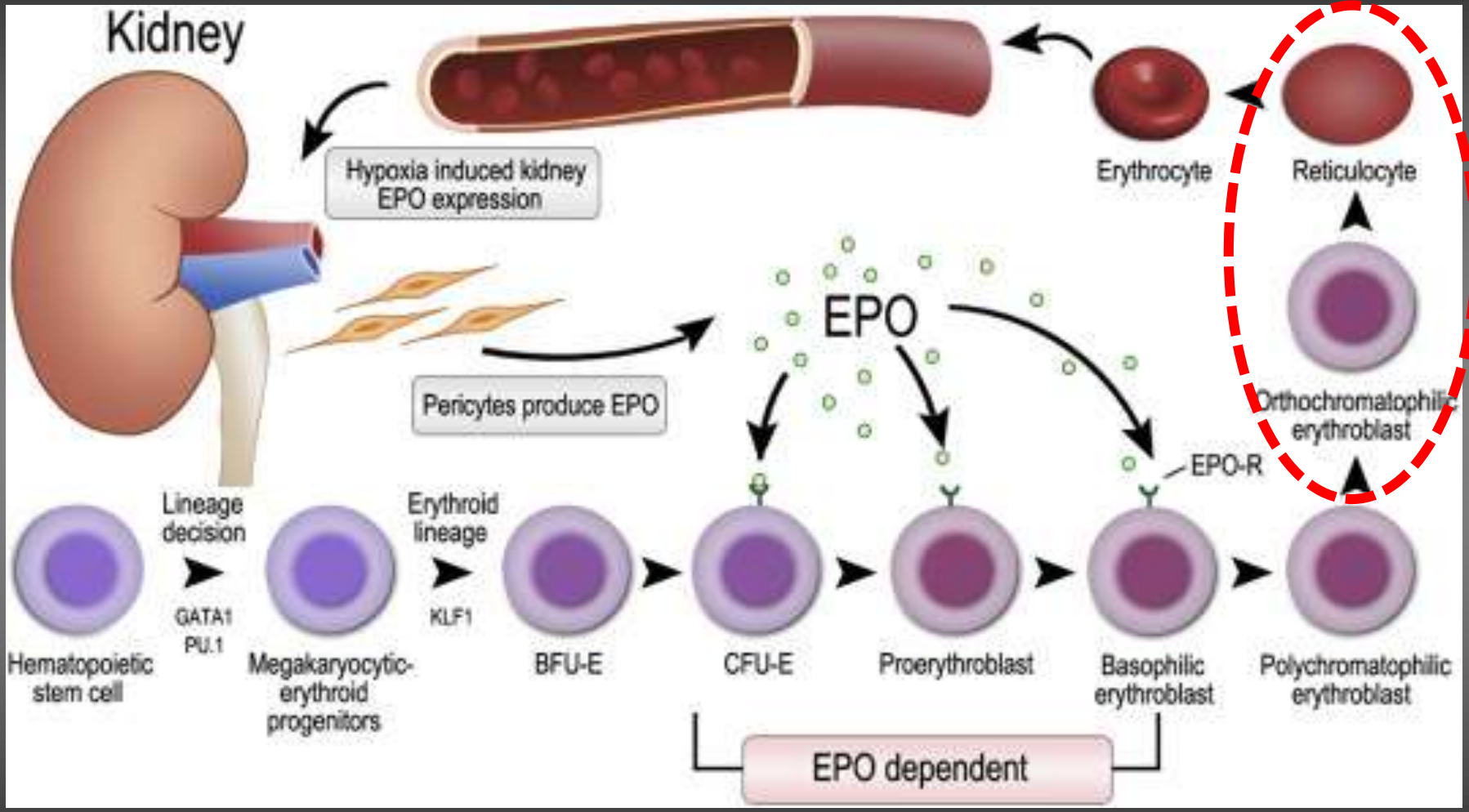
Ruolo dell'Epo nell'eritropoiesi



EpoR è espresso sulla superficie delle cellule eritroidi (massima espressione sulle CFU-E, diminuita sugli stadi più differenziati)

Epo agisce "salvando" dall' apoptosi le cellule progenitrici eritroidi, e stimolandone la maturazione


Kidney
Hypoxia induced kidney EPO expression
Erythrocyte
Reticulocyte
EPO
Pericytes produce EPO
Orthochromatophilic erythroblast
EPO-R
Lineage decision
GATA1
PU.1
Erythroid lineage
KLF1
Hematopoietic stem cell
Megakaryocytic-erythroid progenitors
BFU-E
CFU-E
Proerythroblast
Basophilic erythroblast
Polychromatophilic erythroblast
EPO dependent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Polychromatophilic erythroblast
EPO dependent

Epo

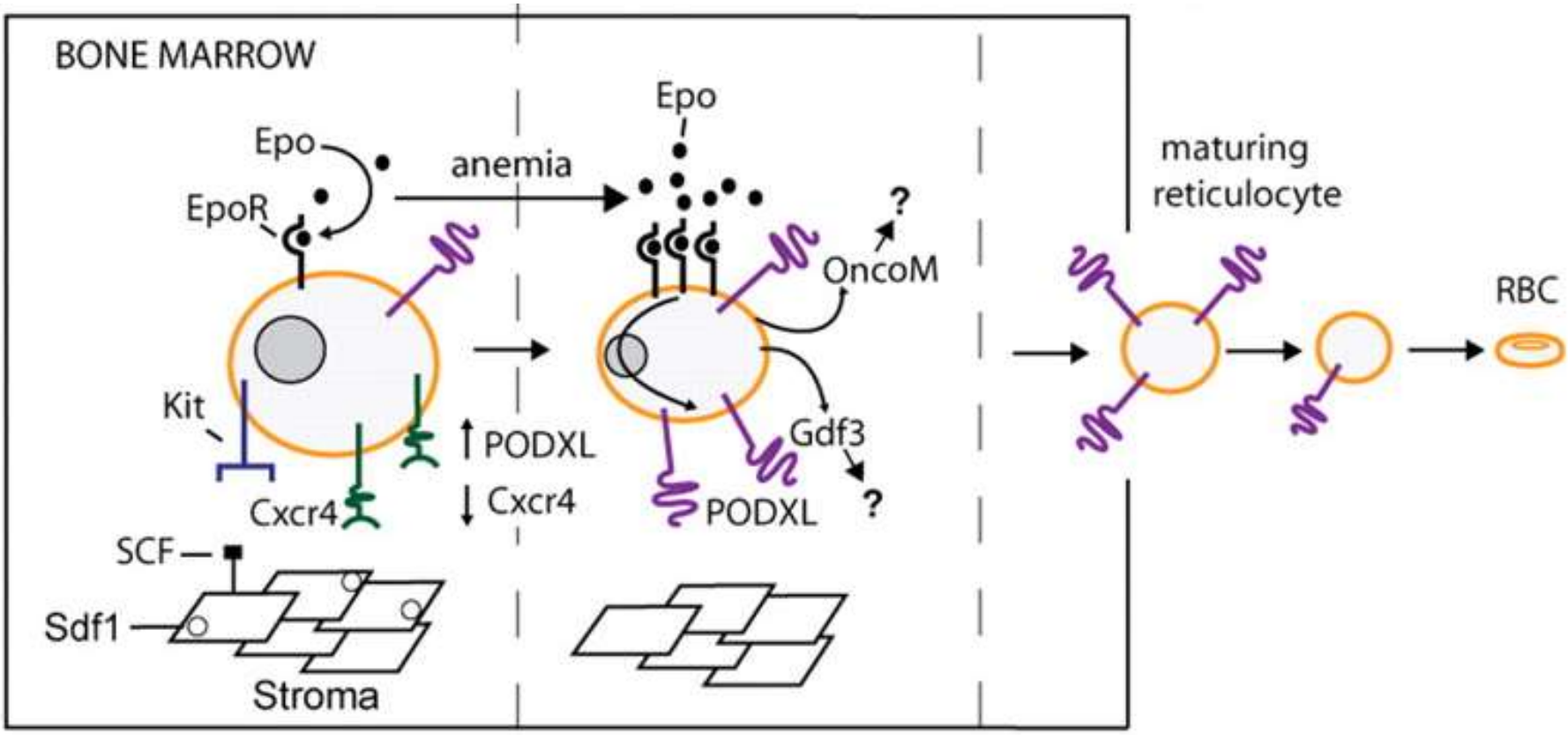
down-modulates adhesion factors:

Cxcr4 Chemokine receptor

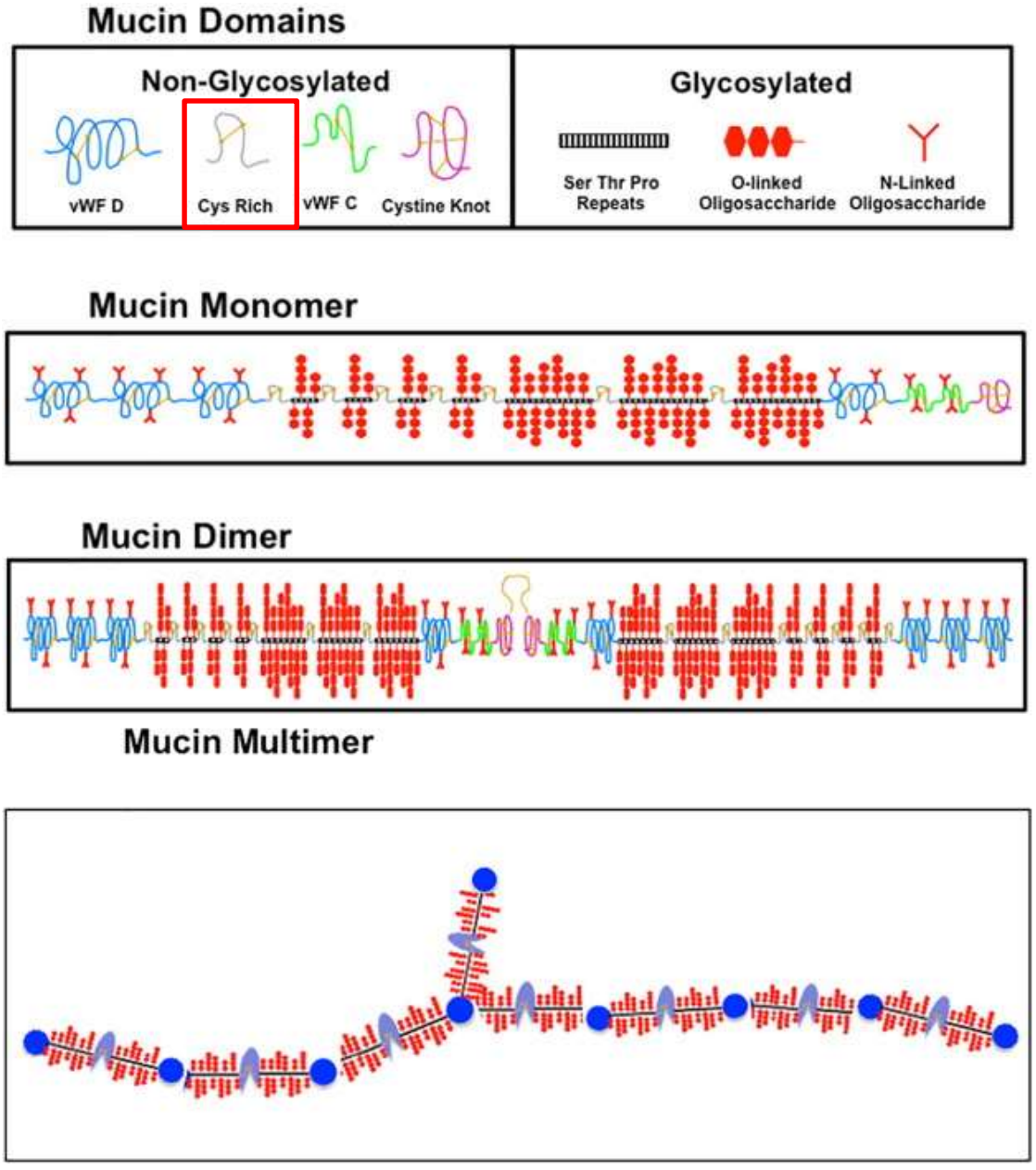
up-modulates “releasing” factors:

Podocalyxin like-1 (PODXL)

Erythropoietin modulation of podocalyxin and a proposed erythroblast niche



PODXL is a sulphated sialomucin, *antiadhesive*

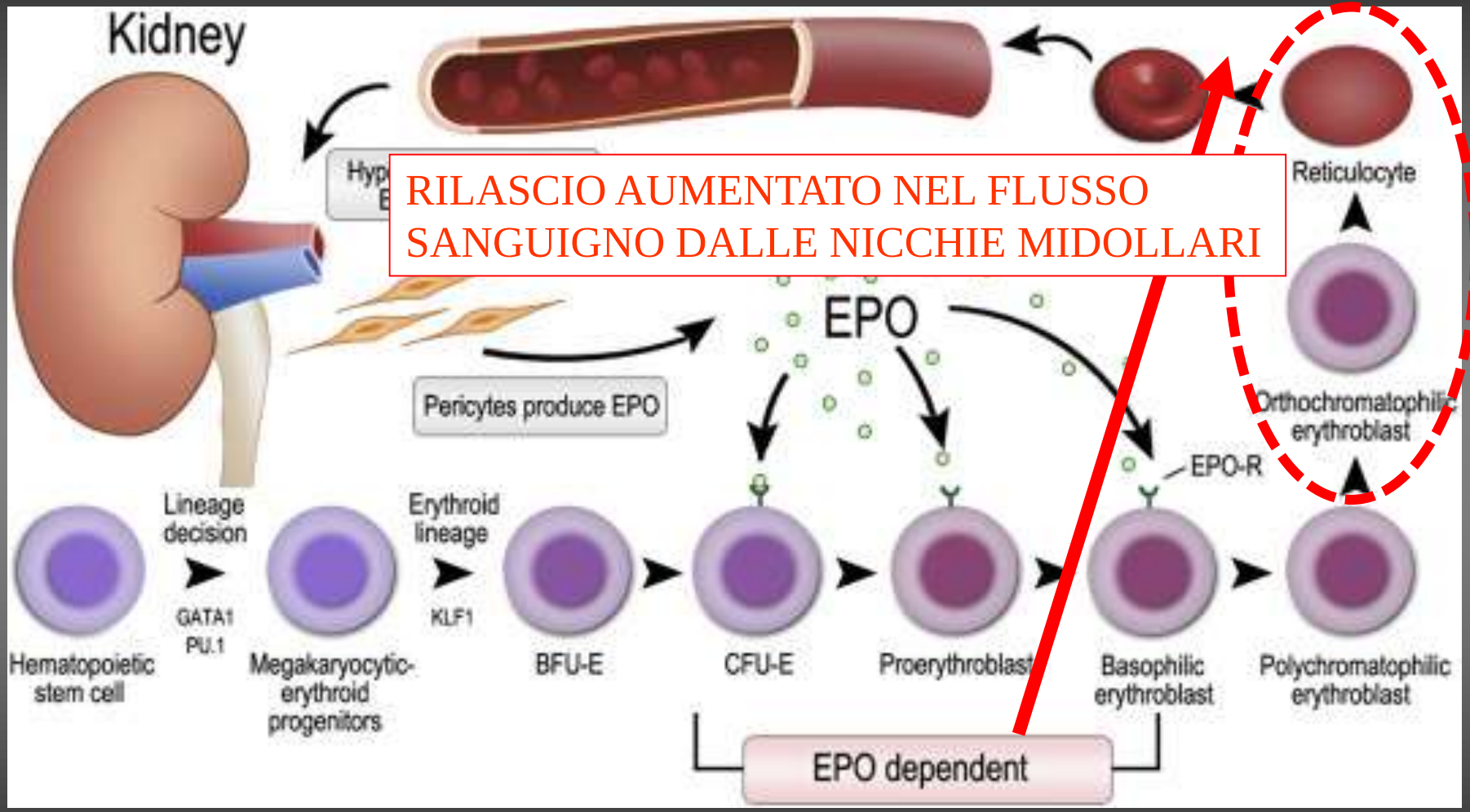


Mucins

Central region - repeats aa Ser Thr – hundreds of O-linked oligosaccharides

The cys residues participate in establishing disulfide linkages within and among mucin monomers.

Sialomucin - acid mucopolysaccharide containing sialic acid


Kidney
RILASCIO AUMENTATO NEL FLUSSO SANGUIGNO DALLE NICCHIE MIDOLLARI
Reticulocyte
Orthochromatophilic erythroblast
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