Calreticulin upregulation in renal fibrosis

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Fibrosis

Fibrosis is defined as the accumulation of extracellular matrix leading to structural and functional alterations of several organs such as lung, heart, kidney, pancreas, liver.

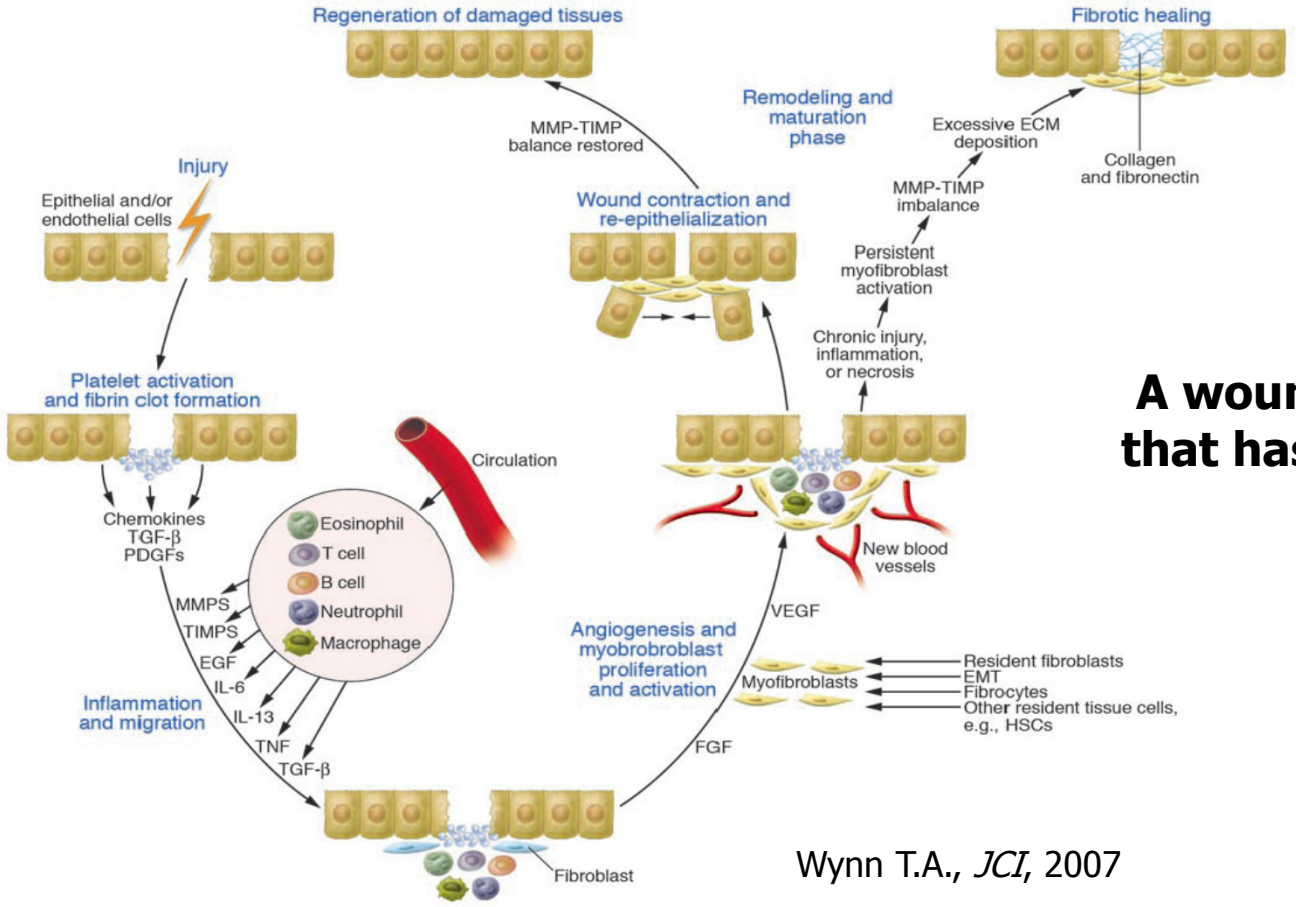
Kidney Fibrosis

- is a common feature of Chronic Kidney Diseases [CKD] (which affect 10-12% of the population) and is characterized by progressive loss of kidney function and relentless accumulation and deposition of ECM
- is considered to be an irreversible process that leads to end-stage renal failure and requires expensive and life-long treatments with dialysis or transplantation
- is increasing at a rate of approximately 7% per year among the world population

Causes of kidney fibrosis

diabetes, hypertension, infection, inflammation of renal blood vessels and glomeruli, kidney stones, cysts, genetic mutations.

Why do organs in the body develop fibrosis?

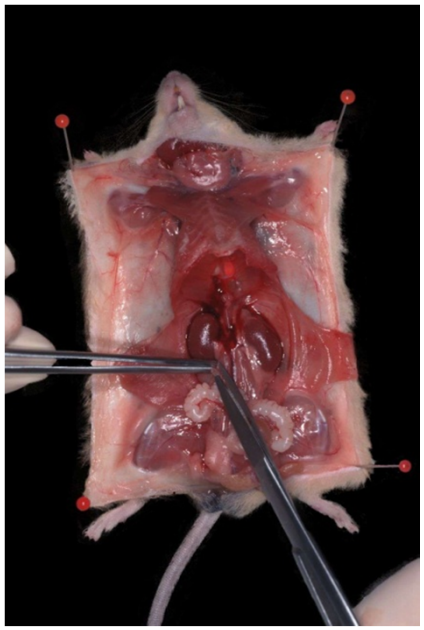


Wynn T.A., *JCI*, 2007

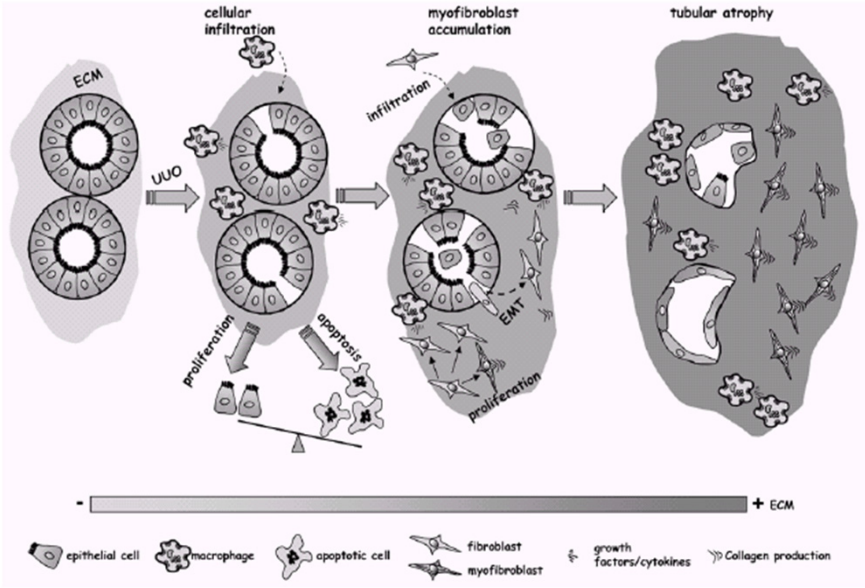
A wound-healing response that has gone out of control

45% of all the deaths in the developed world can be attributed to some form of fibroproliferative disease

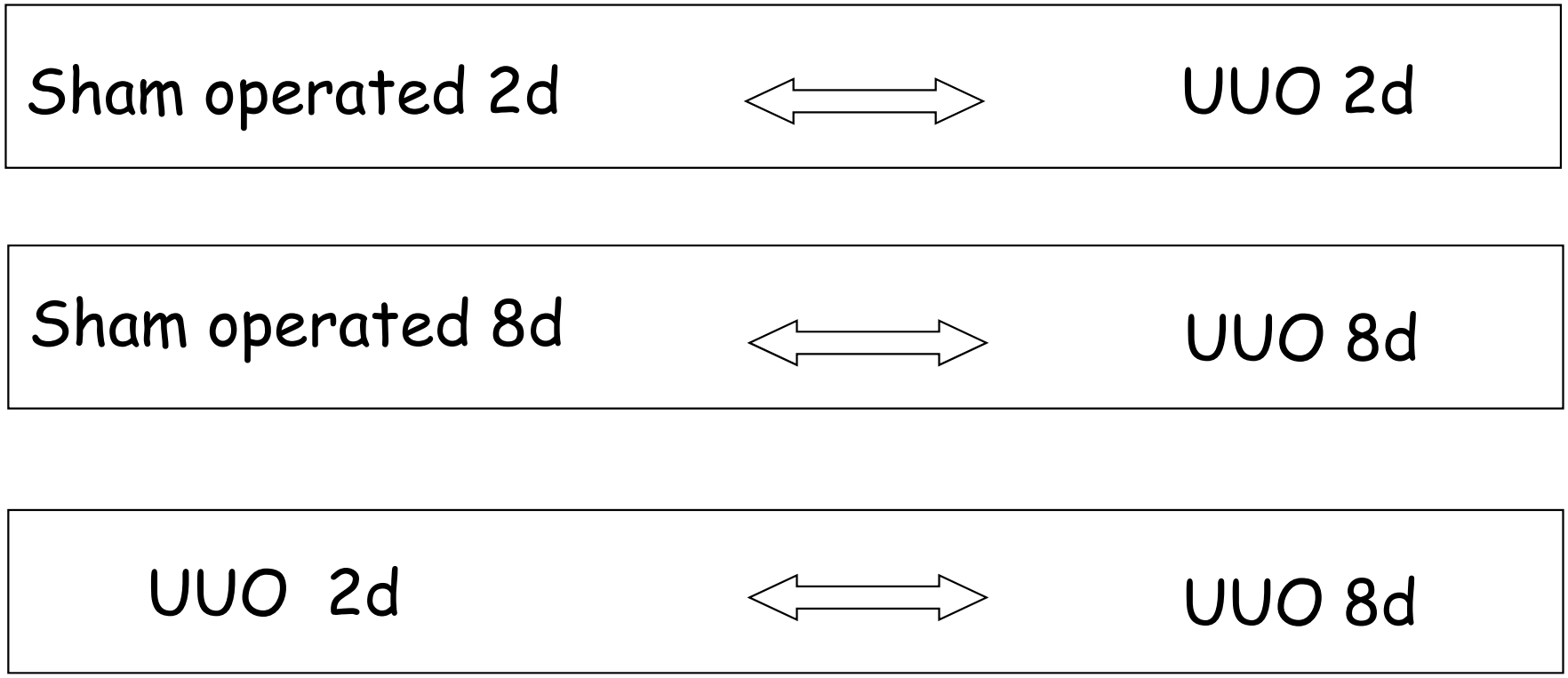
The Unilateral Ureteral Obstruction (UUO) model of kidney fibrosis



- in vivo model
- encompasses many aspects of other models of kidney fibrosis
- there are features that occur within 1 week
- mimics in a short time a situation that can take years in humans
- leaves one kidney intact
- there is evidence that animal models with UUO are reflective of the molecular changes in human situations



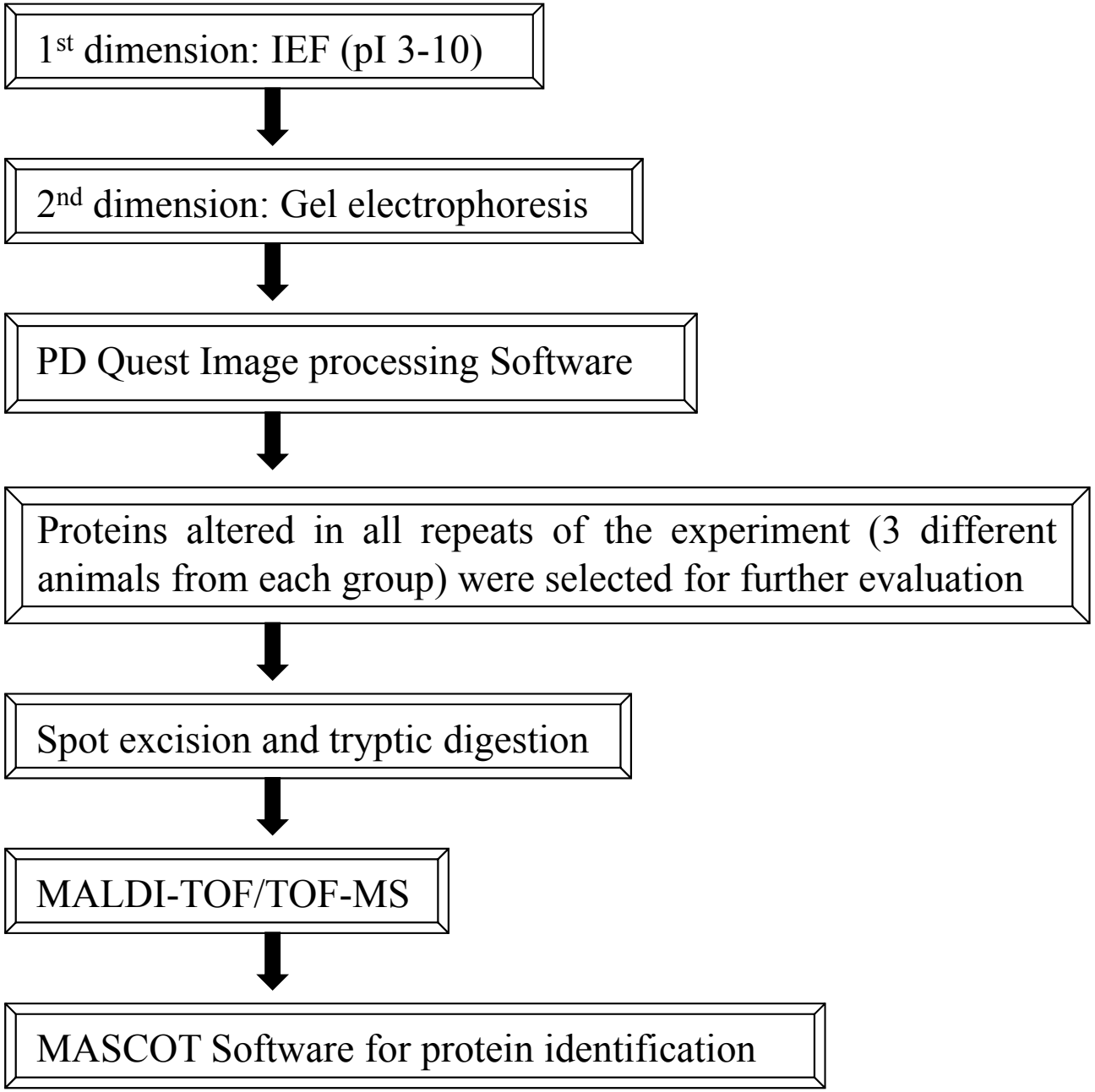
Proteomic analysis



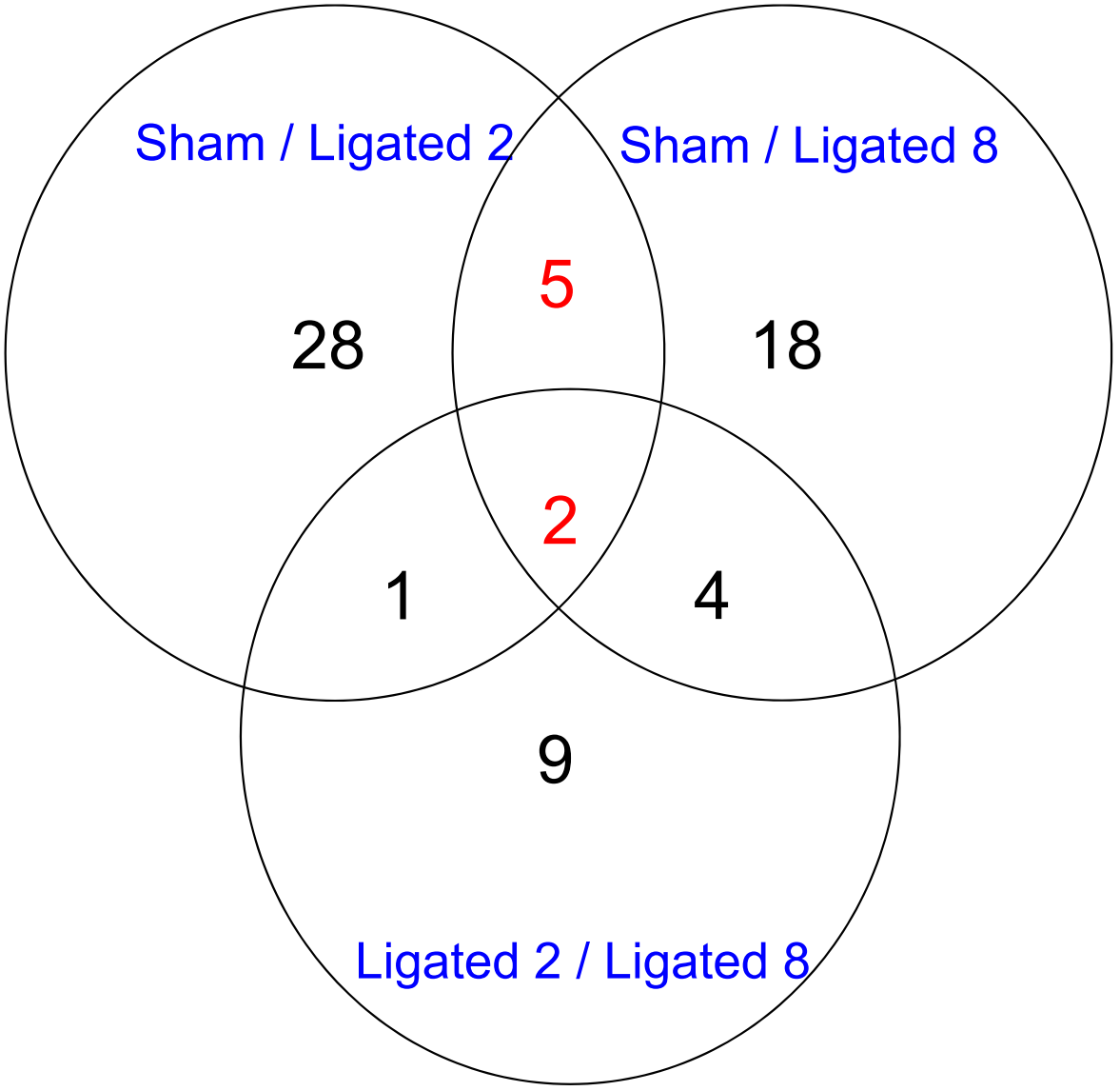
Kypreou K. *et al.*, *Proteomics*, 2008

Proteomic Approach

Classical 2DE

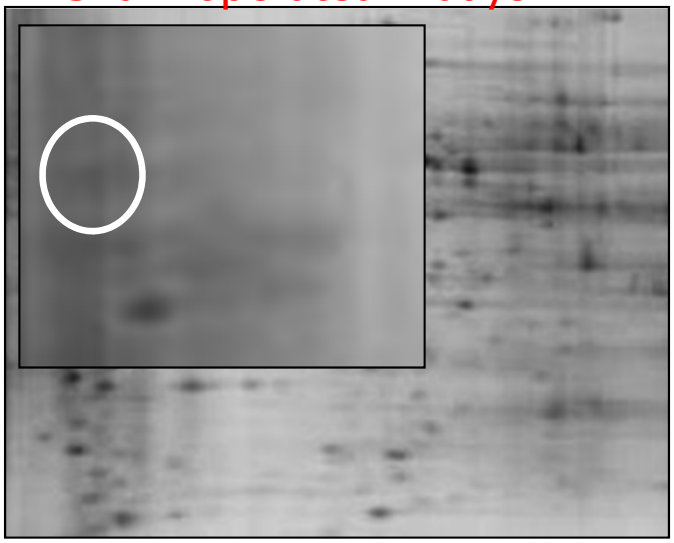


Venn diagram of identified proteins

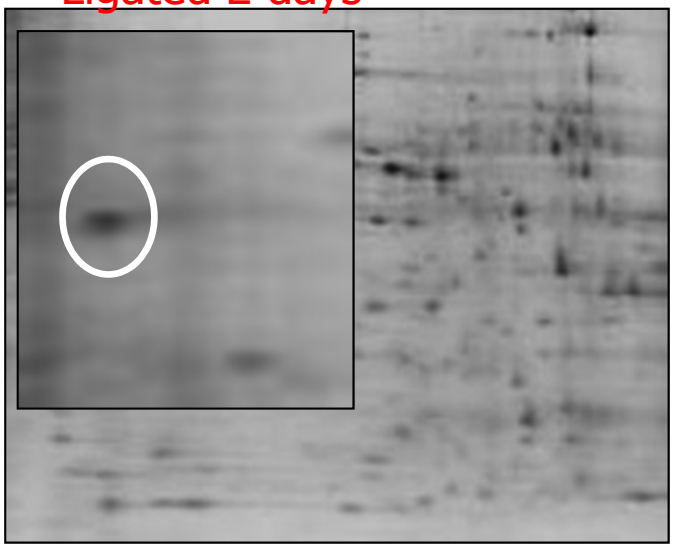


CALRETICULIN

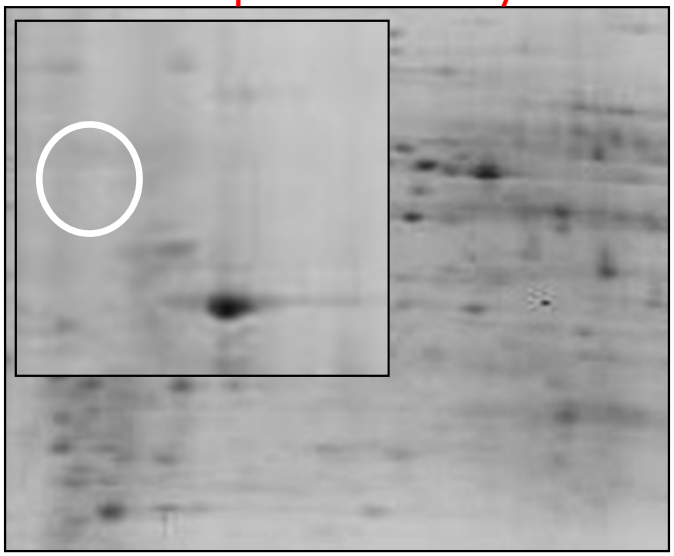
Sham operated 2 days



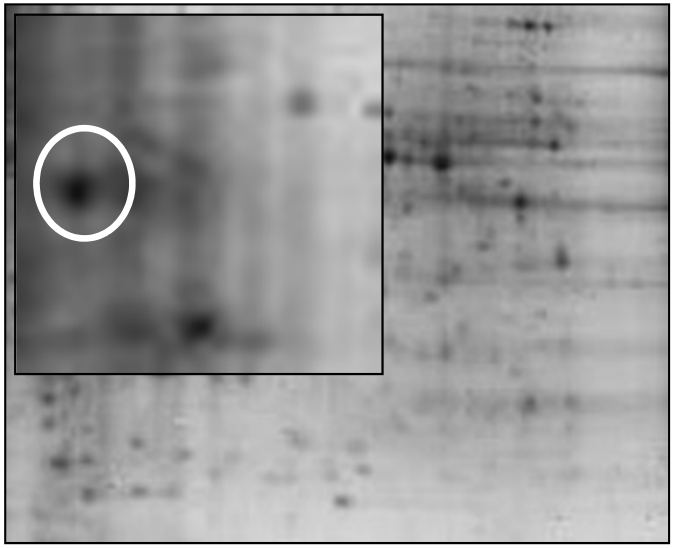
Ligated 2 days



Sham operated 8 days



Ligated 8 days



Calreticulin is a multifunctional protein

- First isolated in 1974 as a high-affinity Ca^{2+}-binding protein of the ER

Table 1. Effects of altered cellular expression of calreticulin[a]

CRT cellular expression level	Refs
Calreticulin upregulation	
Increased Ca^{2+} storage capacity of ER	14
Modulation of cell adhesiveness	19,36
Modulation of store-operated Ca^{2+} influx	14
Increased sensitivity to apoptosis	–[b]
Modulation of steroid-sensitive gene expression	18,19
Appearance of surface CRT	45
Modulation of SERCA2 function	32
Calreticulin deficiency	
Embryonic lethal at E14.5	28,29
Impaired cardiac development	28
Changes in cell adhesiveness	29,34
Increased resistance to apoptosis	76
Accumulation of misfolded proteins	–[b]
Modulation of Ca^{2+}-dependent gene transcription	28
Inhibition of agonist-dependent Ca^{2+} release from ER stores	28

[a]Abbreviations: CRT, calreticulin; E, embryonic day; ER, endoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.
[b]I. Ahsan, R. Knee and M. Michalak (unpublished).

Box 1. Selected cellular functions attributed to calreticulin

Adhesion
- Acrosome function and sperm motility
- Activates integrins
- Affects cell migration
- Control of cellular adhesiveness
- Inhibits angiogenesis
- Initiates cell spreading
- Regulates expression of vinculin
- Upregulates expression of N-CAM
- Wound healing

Blood function
- Anti-thrombotic activity
- Autoantigen
- Binds to complement C1q (C1q receptor?)
- Component of lytic granules in CTLs and NKs
- Component of tick saliva
- Inhibits perforin-dependent killing
- Interacts with perforin
- Modulates platelet activation

Development
- Affects cardiac development
- Affects neuronal development
- Essential for mouse embryogenesis
- Induces complete cardiac block
- Oocyte fertilization
- Regulates bone cell function

ER functions
- 'Ca^{2+} sensor' in the ER lumen
- Binds to Mg^{2+}-ATP
- Ca^{2+} binding and storage
- ER chaperone
- Essential for glycoprotein maturation
- Important for MHC class I assembly
- Modulates inositol-(1,4,5)-trisphosphate-dependent Ca^{2+} release
- Modulates SERCA2b function
- Regulation of store-operated Ca^{2+} influx
- Zn^{2+} binding and storage

Gene expression
- Androgen-sensitive gene in prostate cancer
- Control of Rubella virus replication
- Control of steroid-sensitive gene expression
- Marker of viral infection
- Modulates vitamin D3 signal transduction
- Participates in host response to tumor

Others
- Affects phosphotyrosine level
- Important for cellular proliferation
- Increases sensitivity to apoptosis
- Induces NO formation in endothelial cells
- Intracellular iron transport
- Longterm memory molecule in *Aplysia*
- Mediates mitogenic effects of fibrinogen
- Stress protein

Abbreviations: CTL, cytotoxic T-lymphocyte; ER endoplasmic reticulum; MHC, major histo-compatibility complex; N-CAM, neural cell-adhesion molecule; NK, natural killer cell; NO, nitric oxide; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

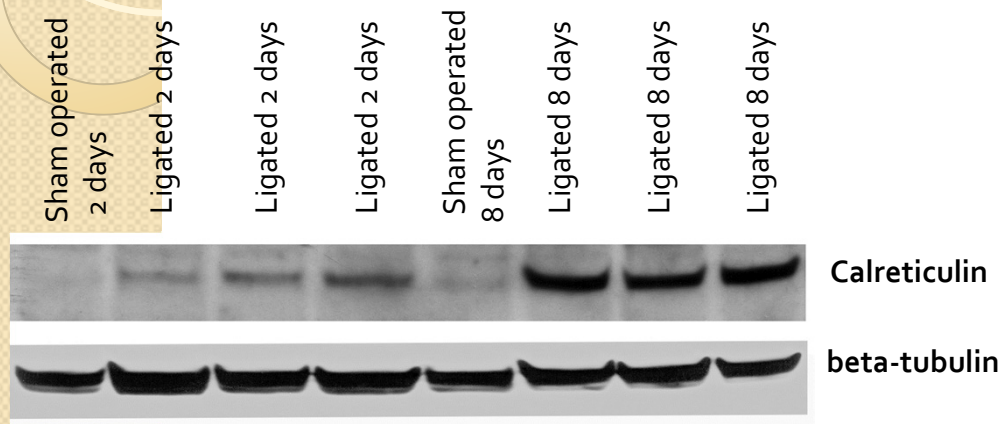
For further details, see review articles in Refs a–d and references therein.

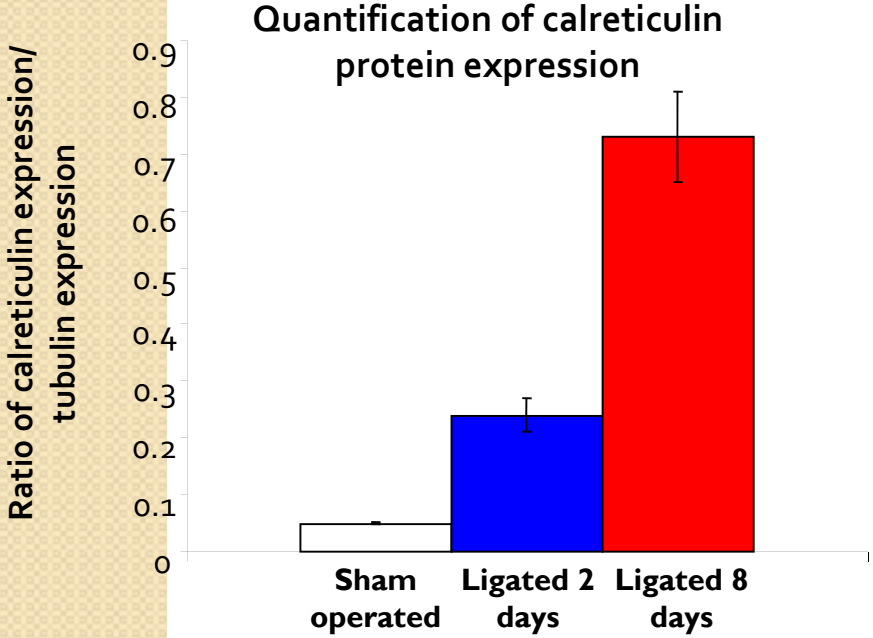
References
a Eggleton, P. *et al.* (1997) Clinical relevance of calreticulin in systemic lupus erythematosus. *Lupus* 6, 564–571
b Crofts, A.J. and Denecke, J. (1998) Calreticulin and calnexin in plants. *Trends Plant Sci.* 3, 396–399
c Nakhasi, H.L. *et al.* (1998) Implications of calreticulin function in parasite biology. *Parasitol. Today* 14, 157–160
d Michalak, M. *et al.* (1999) Calreticulin: one protein, one gene, many functions. *Biochem. J.* 344, 281–292

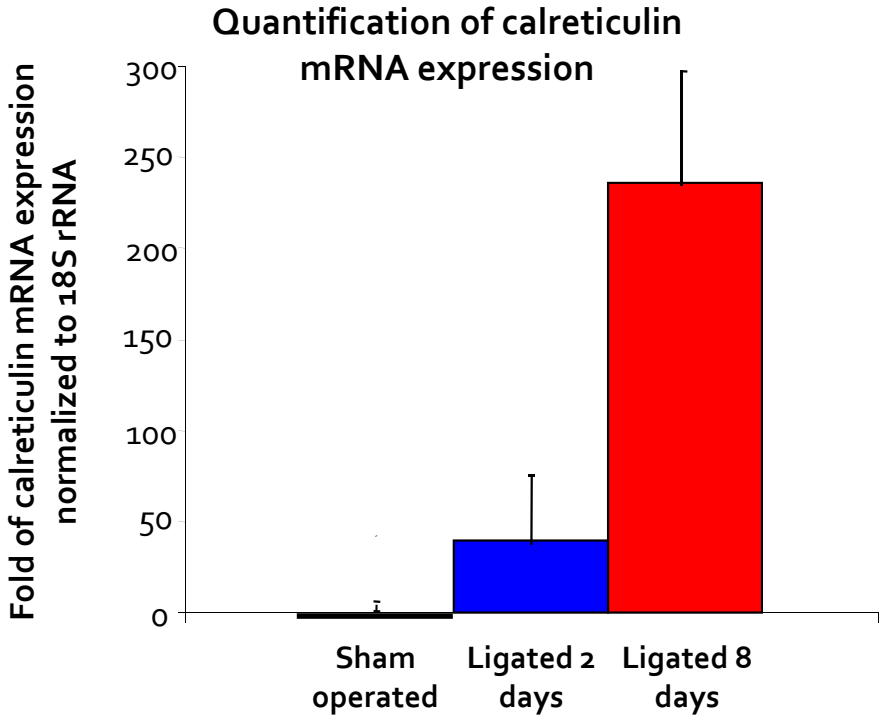
TRENDS in Cell Biology, Vol.11, 2001

There is no correlation with fibrotic processes yet !

Confirmation of Calreticulin upregulation in fibrotic samples







Kypreou K. *et al.*, *Proteomics*, 2008

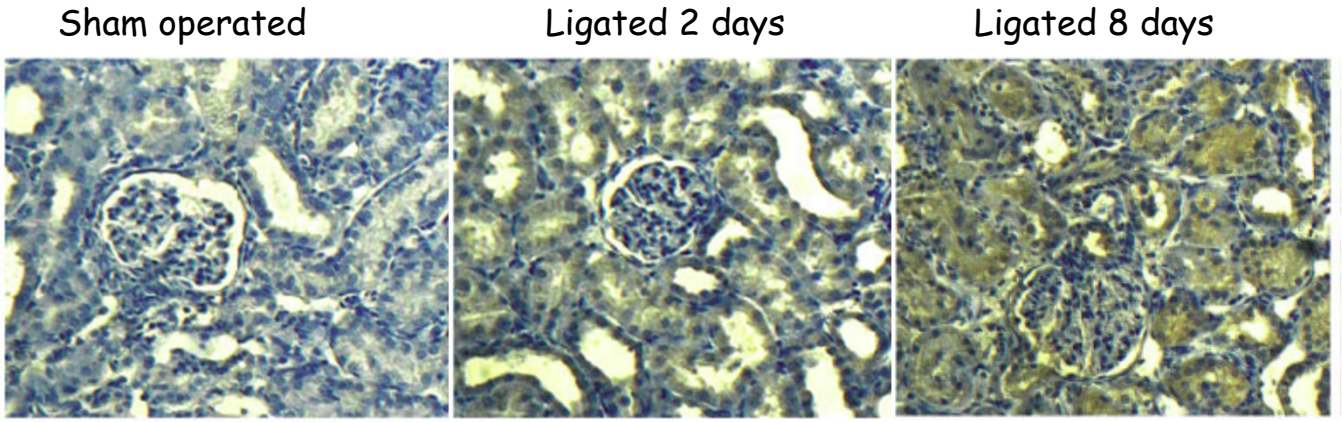
Why is Calreticulin upregulated in kidney fibrosis?

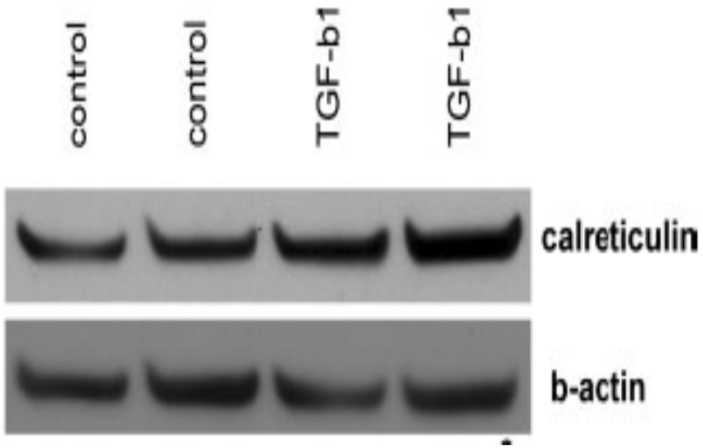
- Which is the mechanism of Calreticulin upregulation?
- What is the role of Calreticulin in fibrosis?

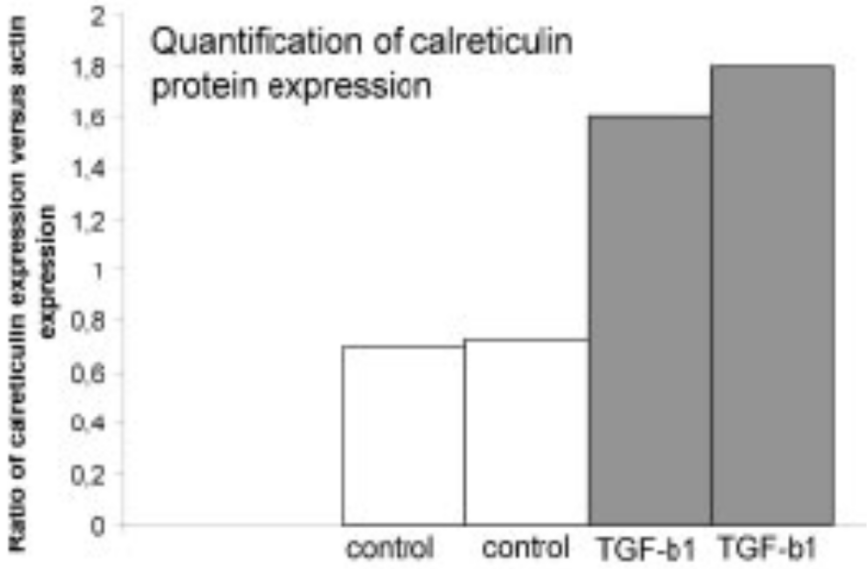
1. WHICH IS THE MECHANISM OF CALRETICULIN UPREGULATION?

Transforming Growth Factor-β (TGF-β) induces fibroblasts to synthesize ECM and has long been considered as a central mediator of the fibrotic response

Transforming Growth Factor-β (TGF-β) induces HK-2 cells to produce Calreticulin

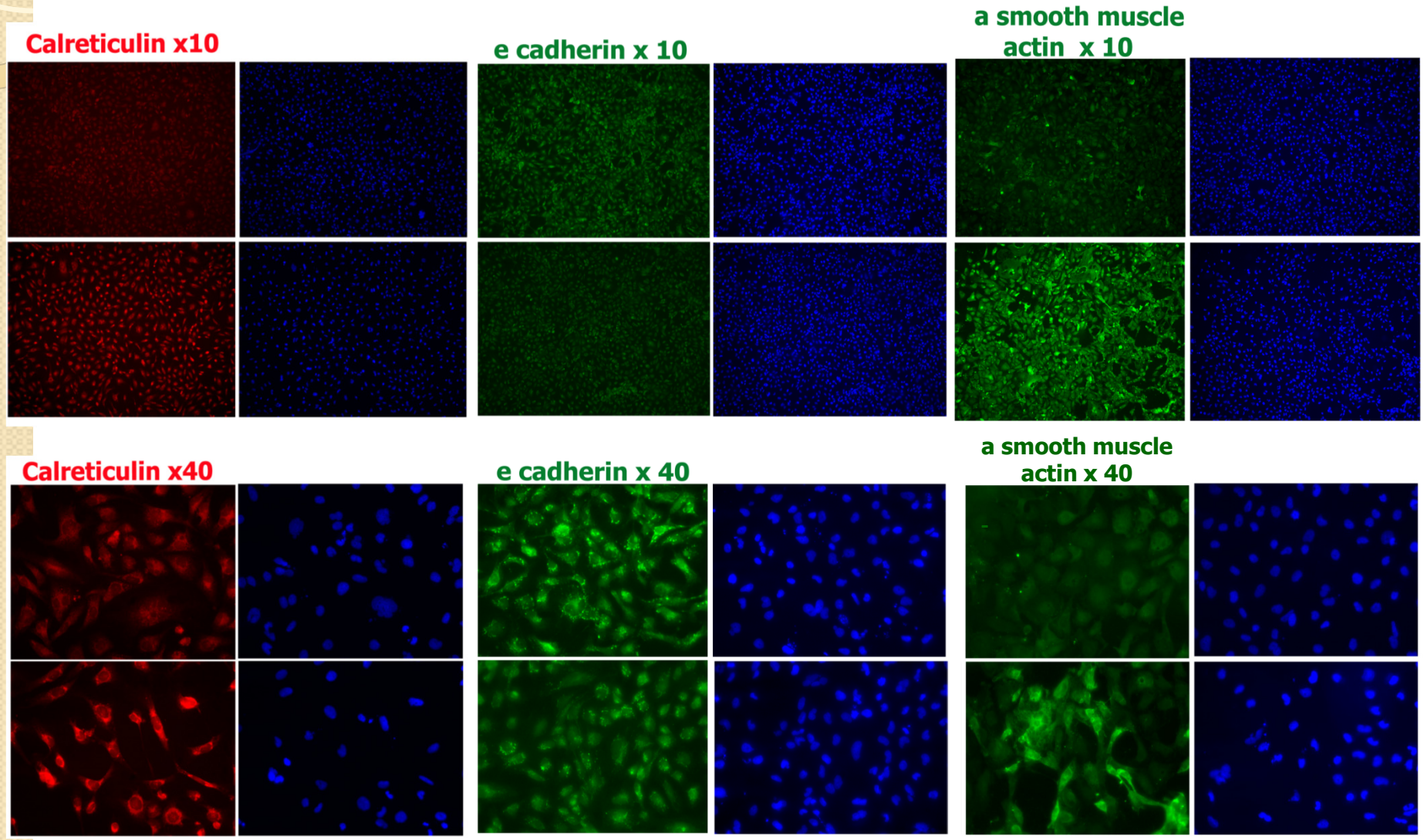




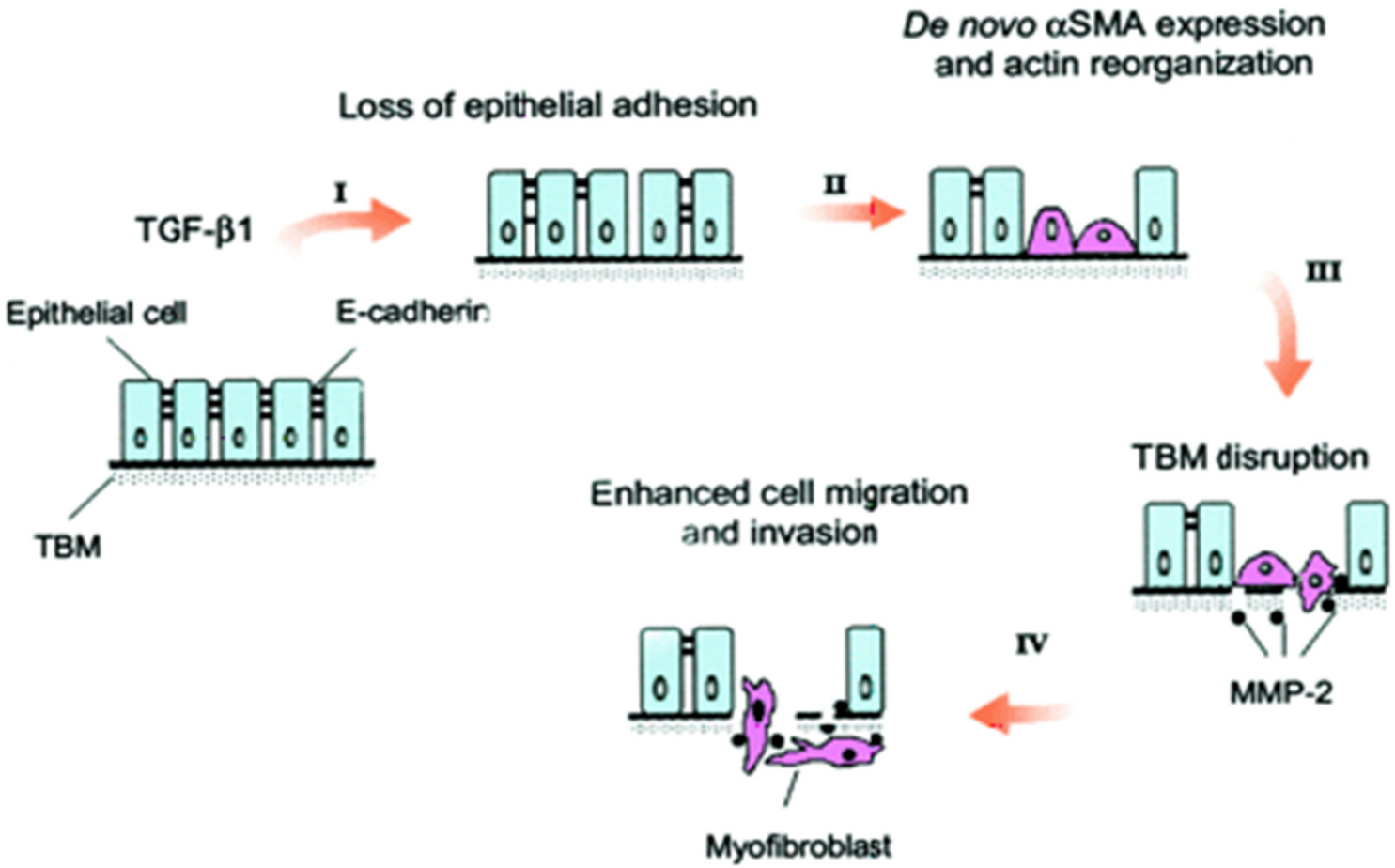


Kypreou K. *et al.*, *Proteomics*, 2008

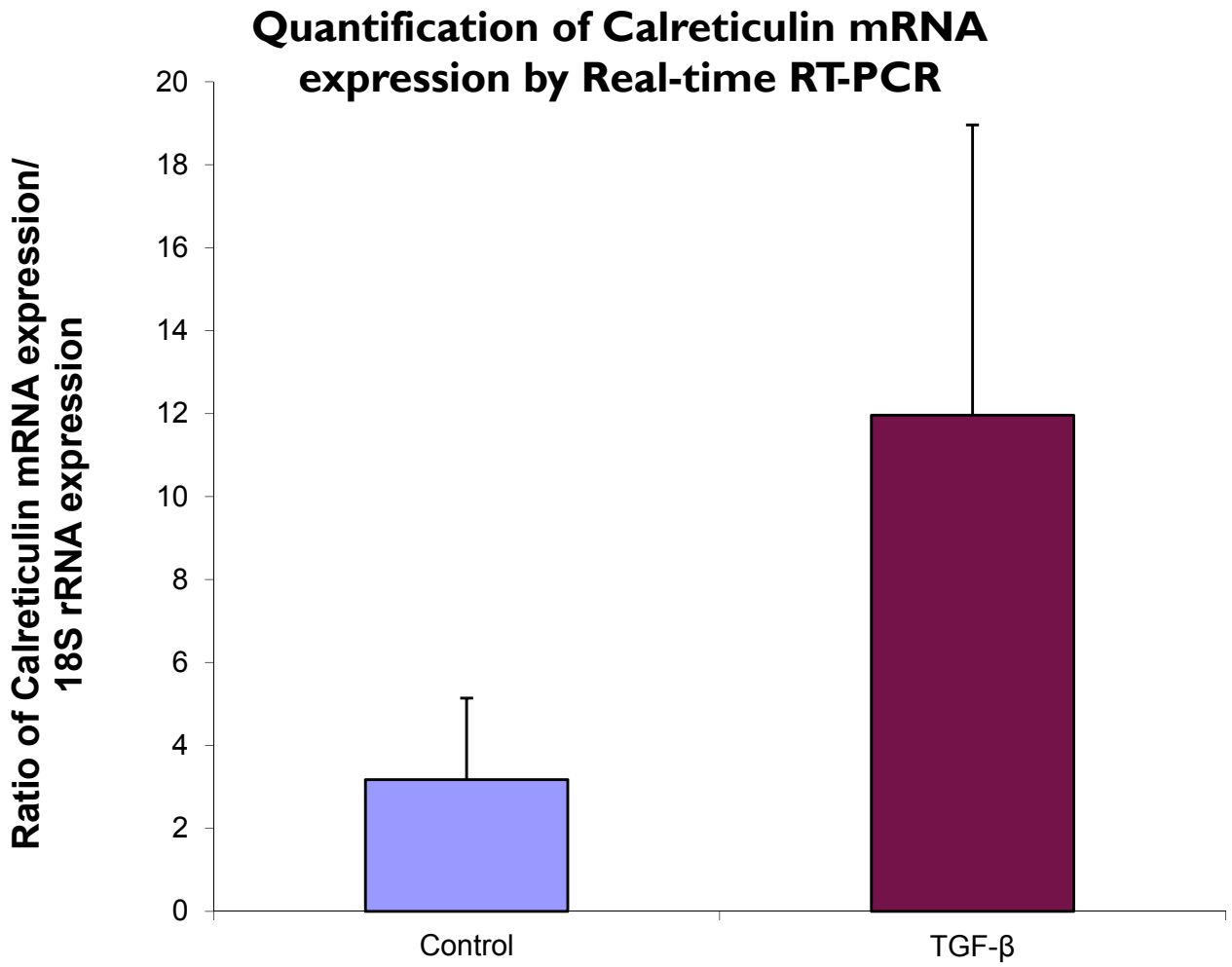
TGF-β-mediated Calreticulin induction is accompanied by Epithelial to Mesenchymal Transition in HK-2 cells



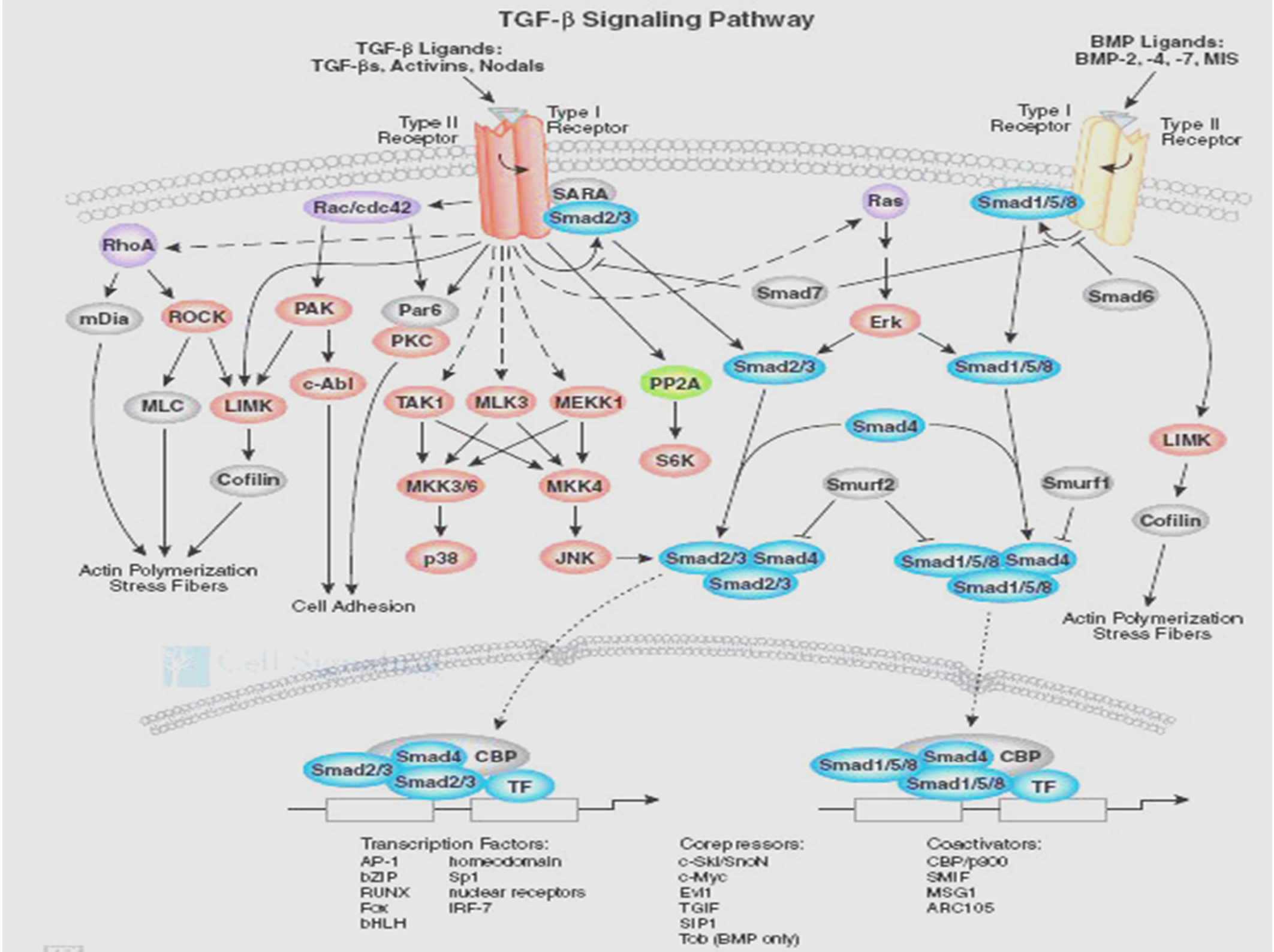
Epithelial to Mesenchymal Transition



TGF-β induces Calreticulin mRNA expression in HK-2 cells



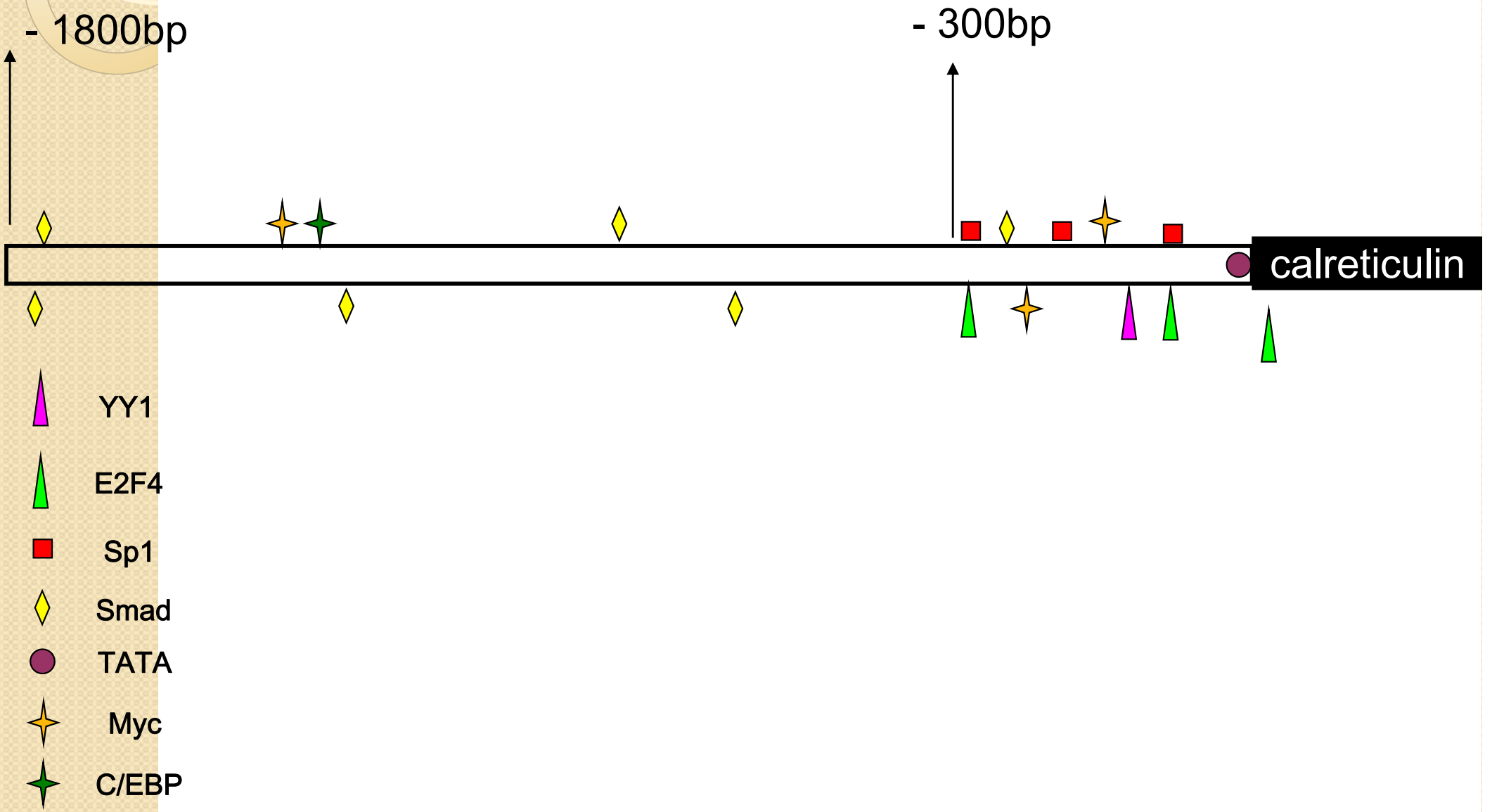
What is upstream of this induction?


TGF-β Signaling Pathway
TGF-β Ligands:
TGF-βs, Activins, Nodals
BMP Ligands:
BMP-2, -4, -7, MIS
Type II Receptor
Type I Receptor
Type I Receptor
Type II Receptor
SARA
Smad2/3
Rac/cdc42
Ras
Smad1/5/8
RhoA
Smad7
Smad6
mDia
ROCK
PAK
Par6
PKC
Erk
Smad2/3
Smad1/5/8
c-Abl
PP2A
MLC
LIMK
TAK1
MLK3
MEKK1
Smad4
LIMK
S6K
Cofilin
MKK3/6
MKK4
Smurf2
Smurf1
Cofilin
p38
JNK
Smad2/3 Smad4
Smad2/3
Smad1/5/8 Smad4
Smad1/5/8
Actin Polymerization
Stress Fibers
Cell Adhesion
Actin Polymerization
Stress Fibers
Smad4 CBP
Smad2/3
Smad2/3
TF
Smad1/5/8 Smad4 CBP
Smad1/5/8
Smad1/5/8
TF
Transcription Factors:
AP-1
bZIP
RUNX
Fox
bHLH
homeodomain
Sp1
nuclear receptors
IRF-7
Corepressors:
c-Ski/SnoN
c-Myc
Evi1
TGIF
SIP1
Tob (BMP only)
Coactivators:
CBP/p300
SMIF
MSG1
ARC105

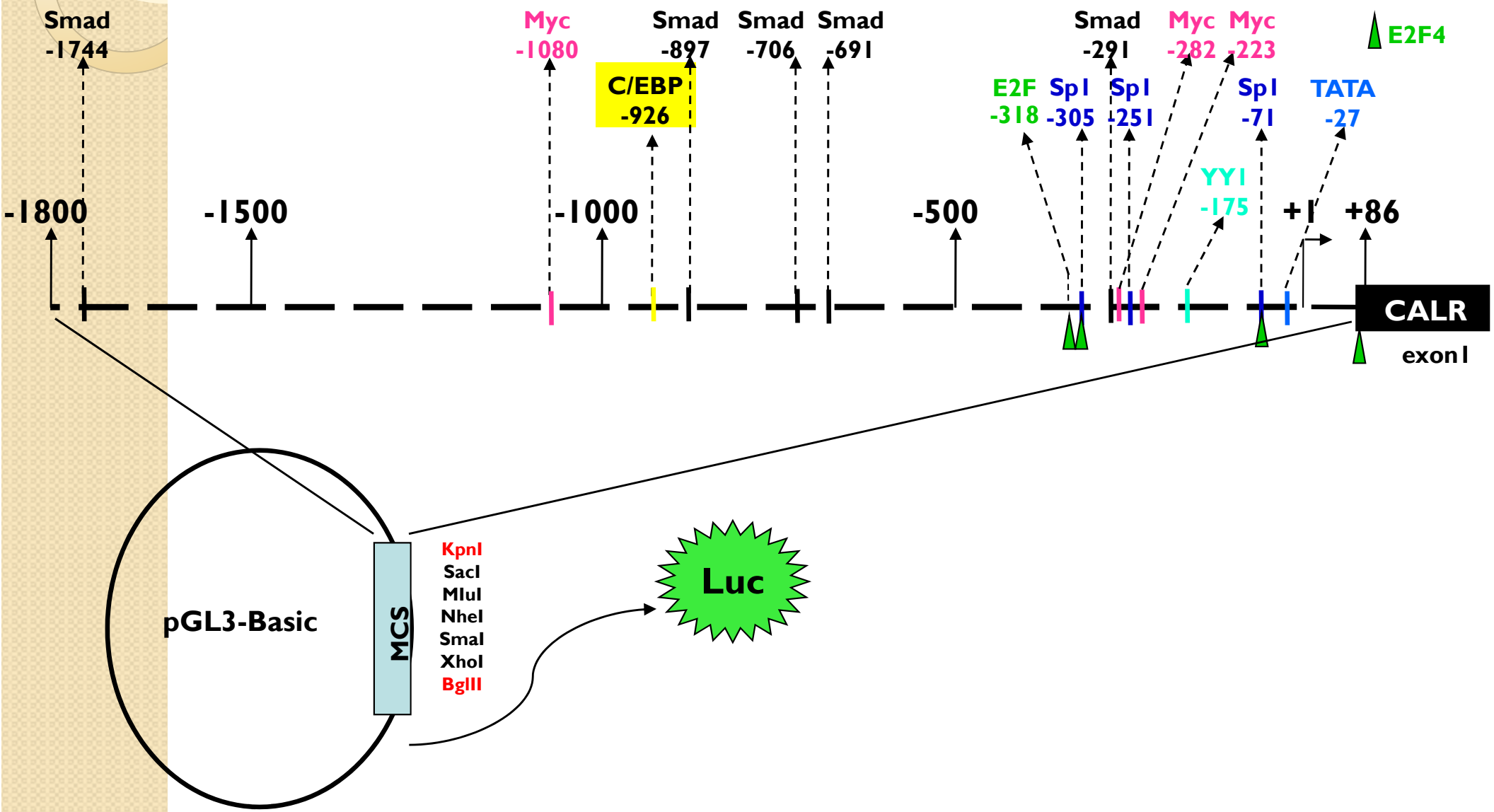
Bioinformatics analysis on Calreticulin promoter

- www.genomatix.de: Gene2Promoter
- 4 organisms: 
- Promoter sequence: 2000bp upstream and 1000bp downstream the TSS

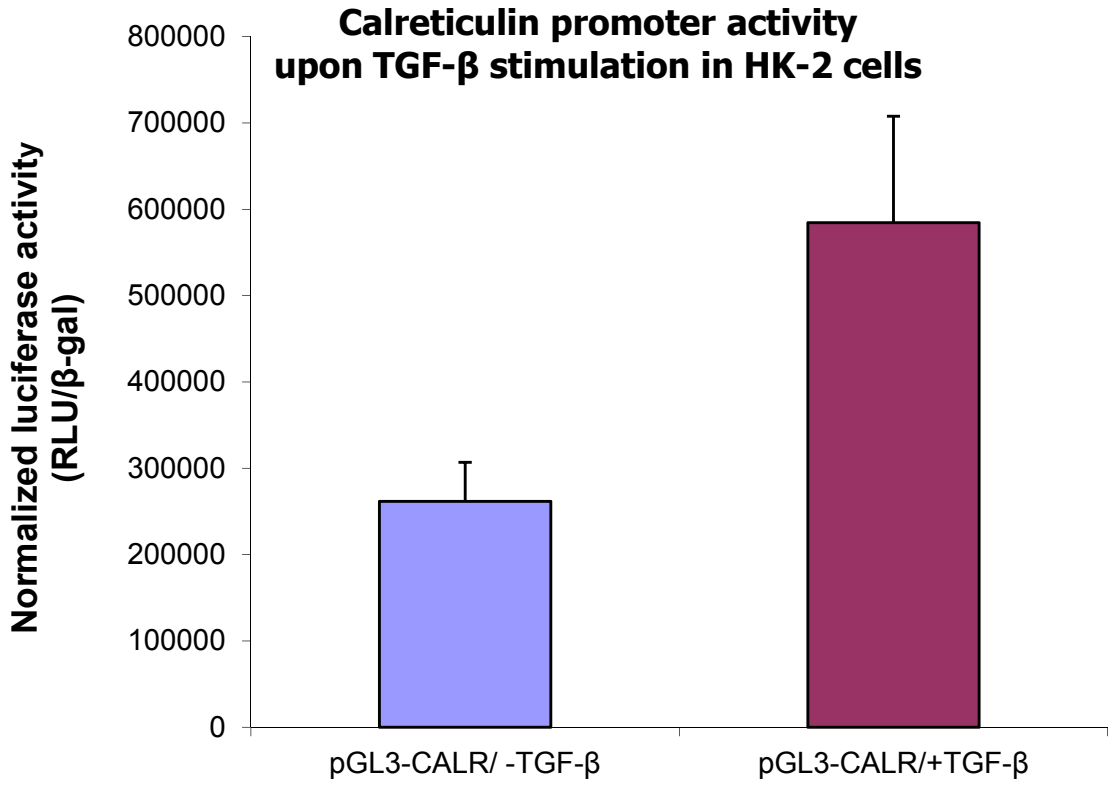
Calreticulin promoter sequence contains TF binding sites that act downstream of the TGF-β signaling



Cloning of the human Calreticulin promoter sequence

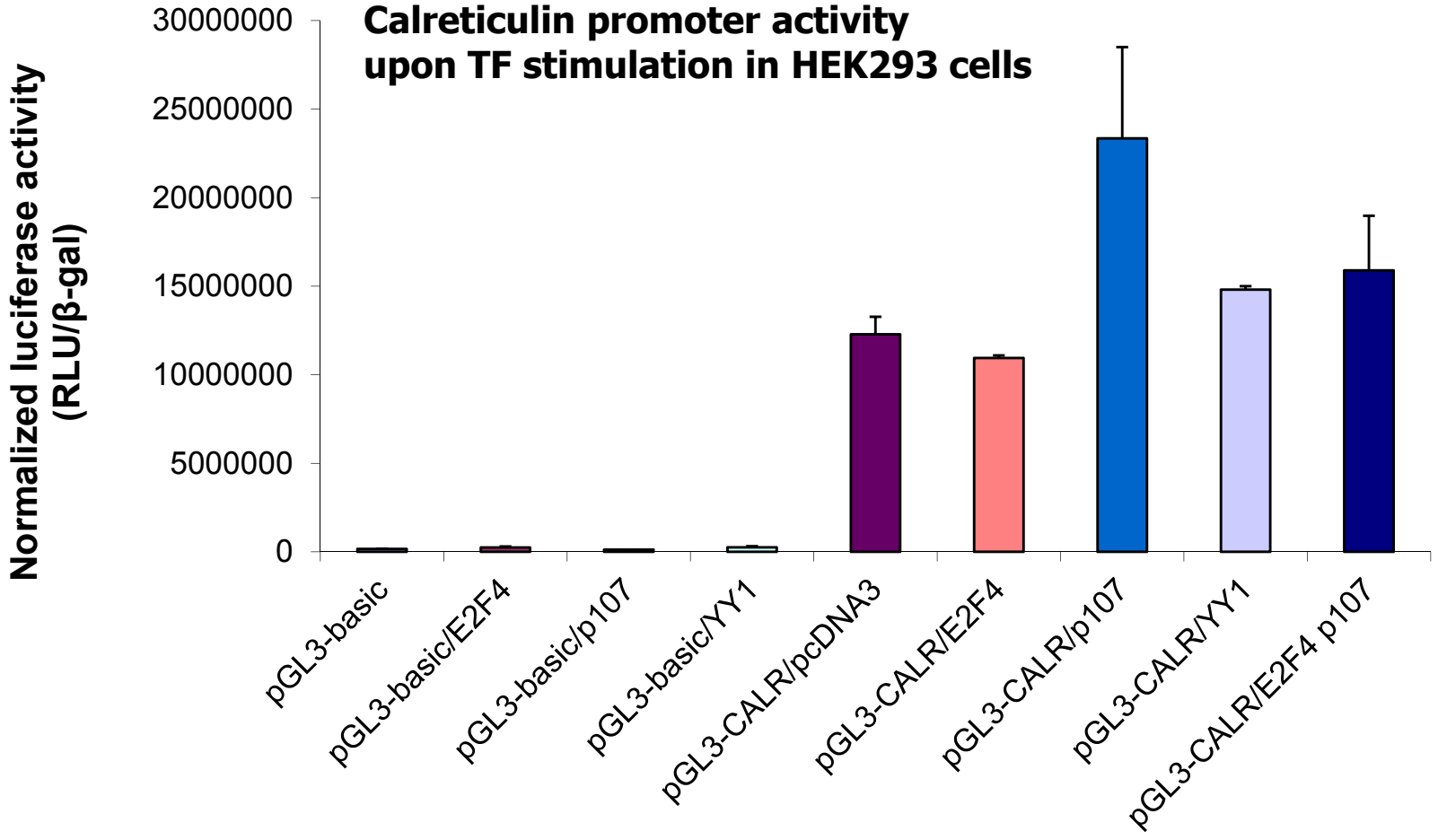


TGF-β induces Calreticulin promoter activity in HK-2 cells



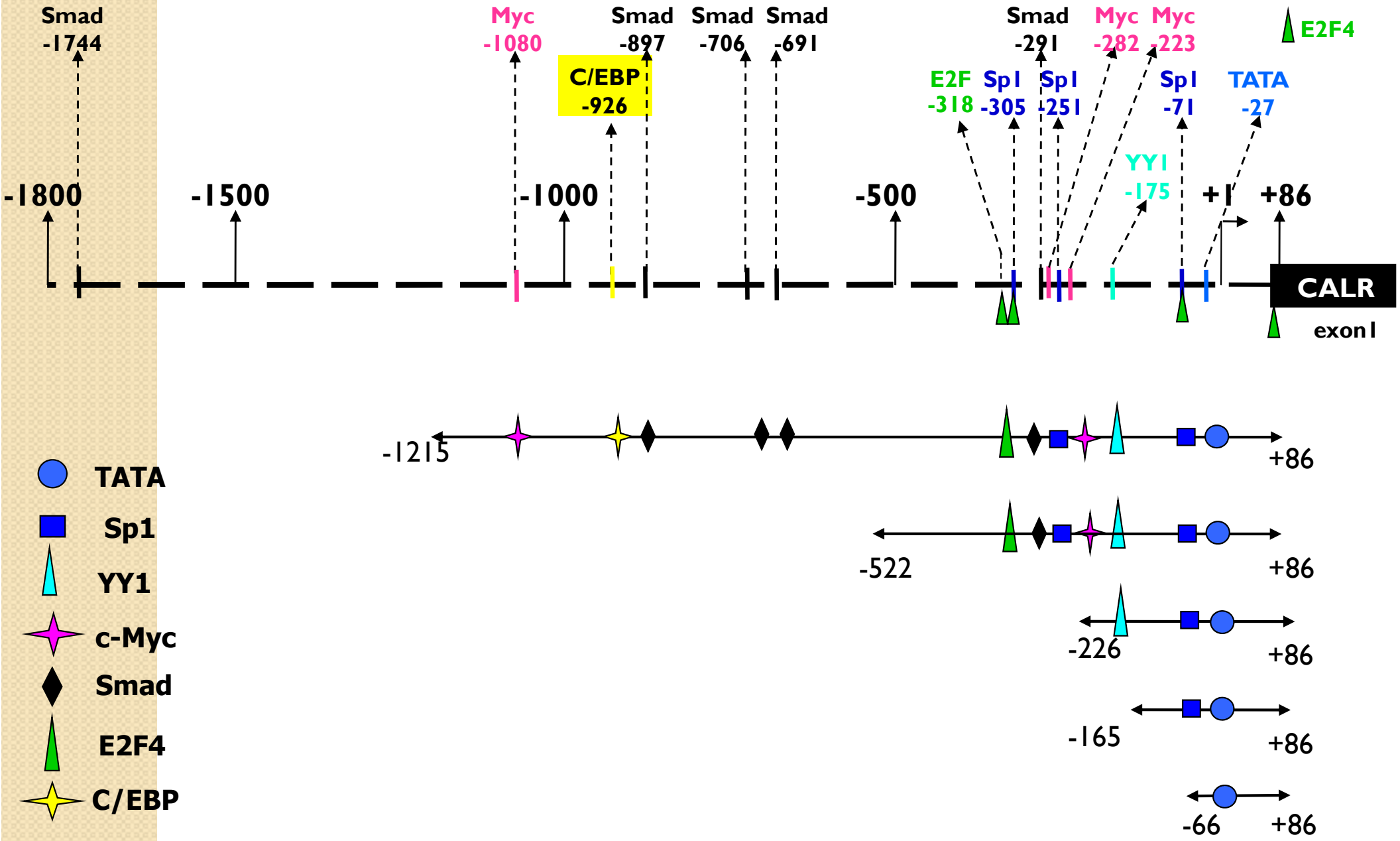
TGF-β regulates Calreticulin at the level of transcriptional activation

Calreticulin promoter is regulated by transcription factors E2F4 and p107

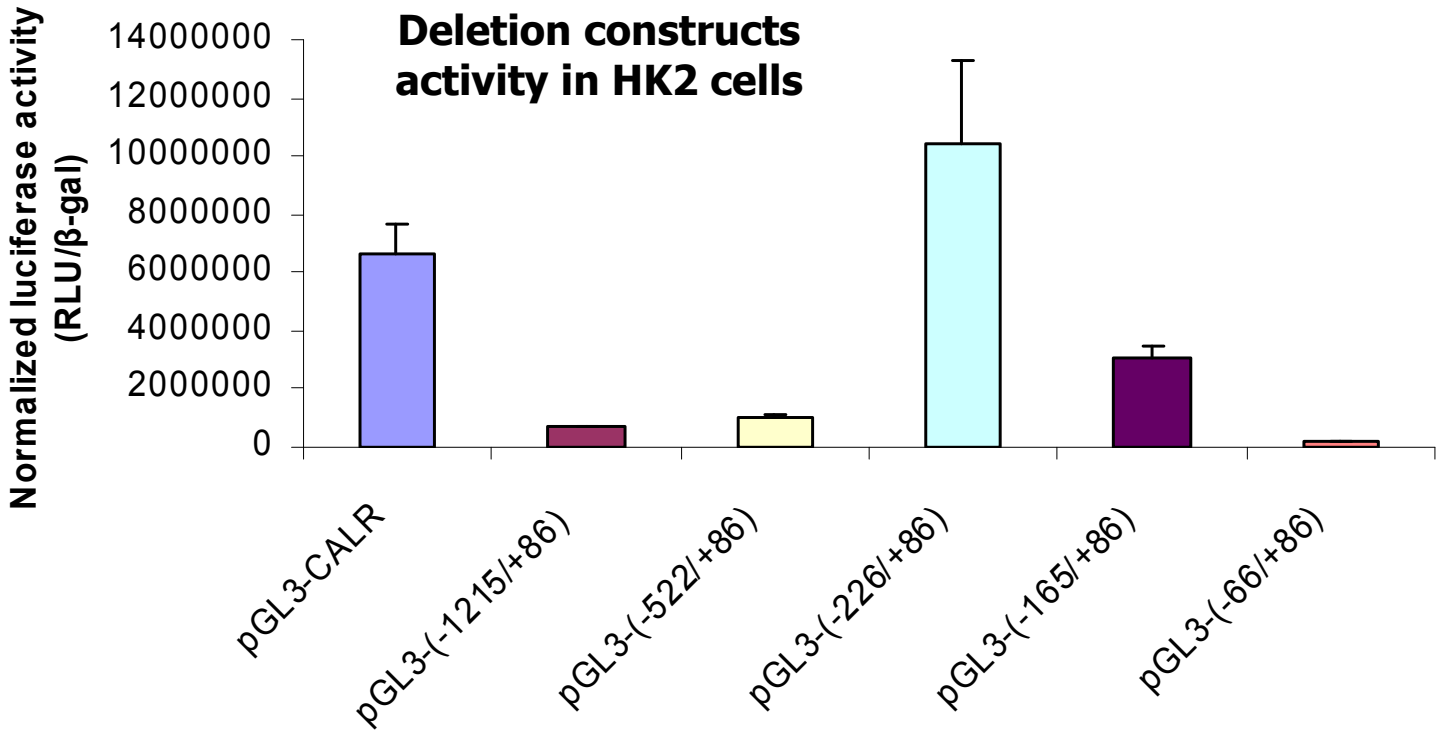


- E2F4 and p107 act downstream the TGF-β signaling pathway
- E2F4 has potential binding sites on Calreticulin promoter
- E2F4 and its cytoplasmic binding partner p107 are cell cycle regulators

Calreticulin promoter deletion constructs

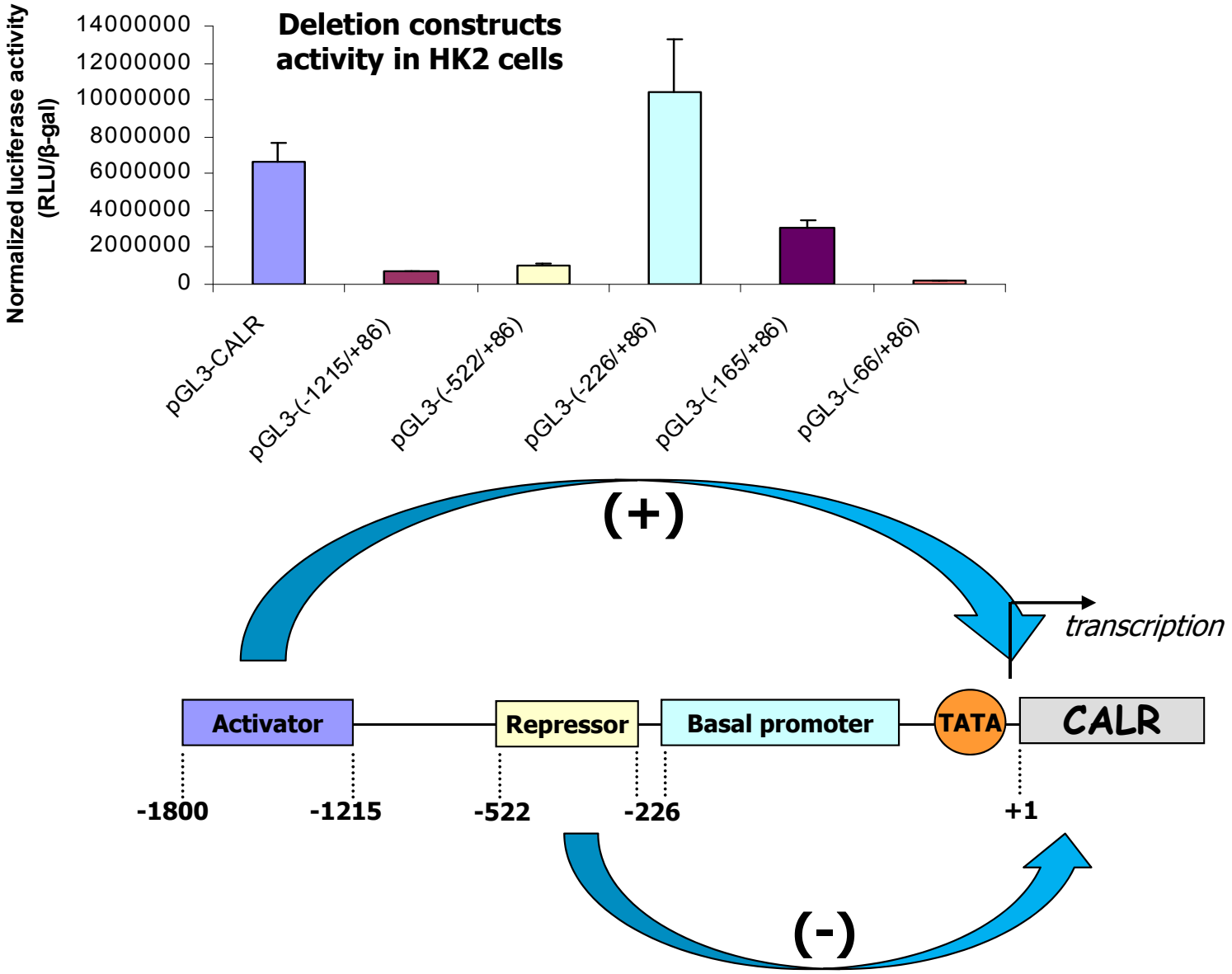


Calreticulin promoter includes activator and repressor elements

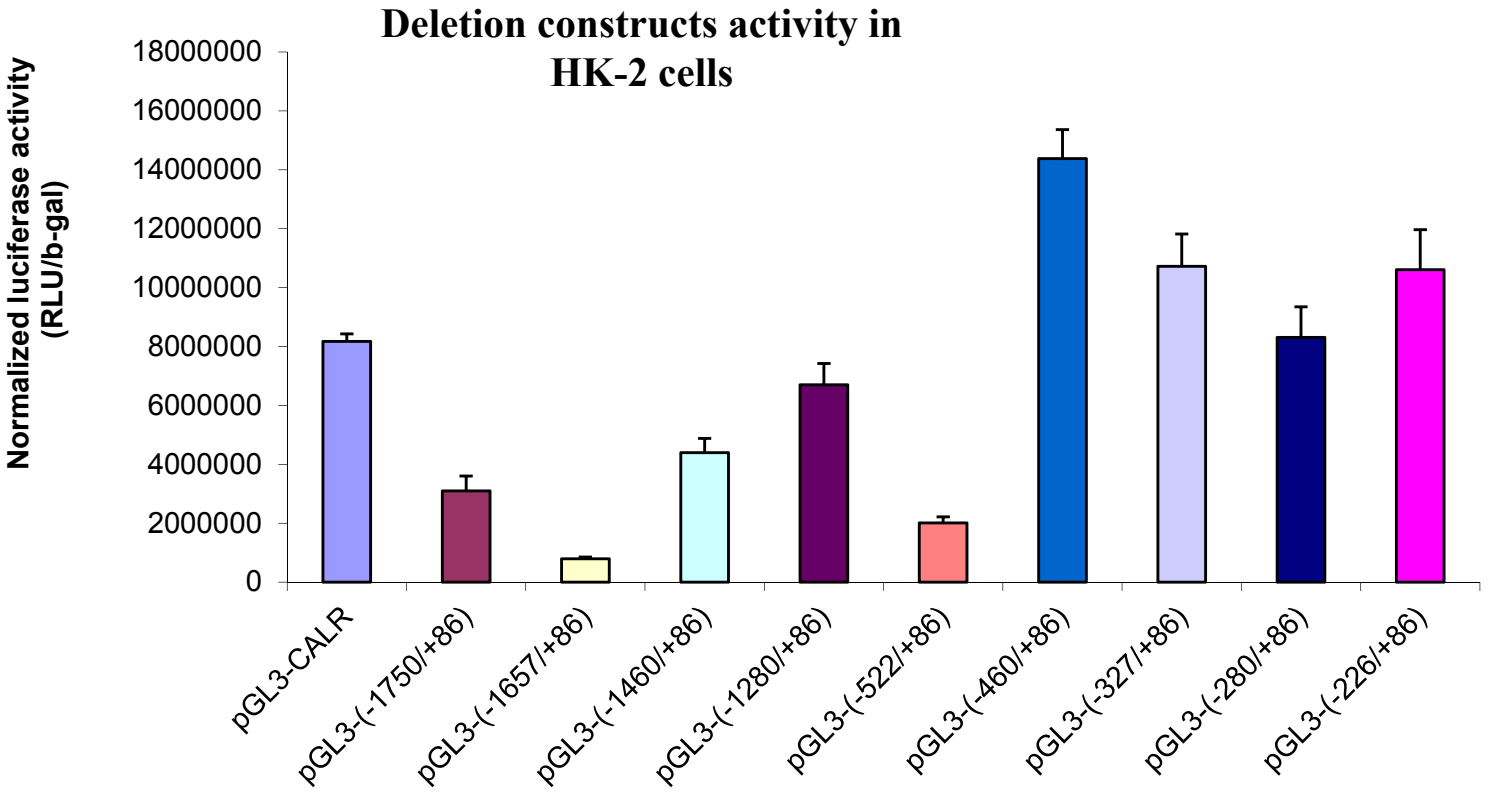


- The region from -1215 to -1800 contains transcriptional activator element(s)
- The region from -226 to -522 contains transcriptional repressor element(s)
- The fragment -226/+86 harbors the basal/core promoter sequence whose activity is altered by upstream activator and repressor elements

Calreticulin promoter includes activator and repressor elements



Calreticulin gene regulation is even more complicated…



…representing the multiplicity of Calreticulin functions

Which is the mechanism of Calreticulin upregulation in fibrosis?

- **TGF-β is likely to play a role.**

- **Other factors should also play a role, taking into consideration the complex nature of both fibrosis and Calreticulin regulation.**

- **More signaling pathways and transcription factors that upregulate Calreticulin gene expression need to be studied, and corresponded to specific regulatory elements on Calreticulin promoter.**

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