Impaired proteodynamics in hematological and other malignancies; potential biomarkers and therapeutic targets

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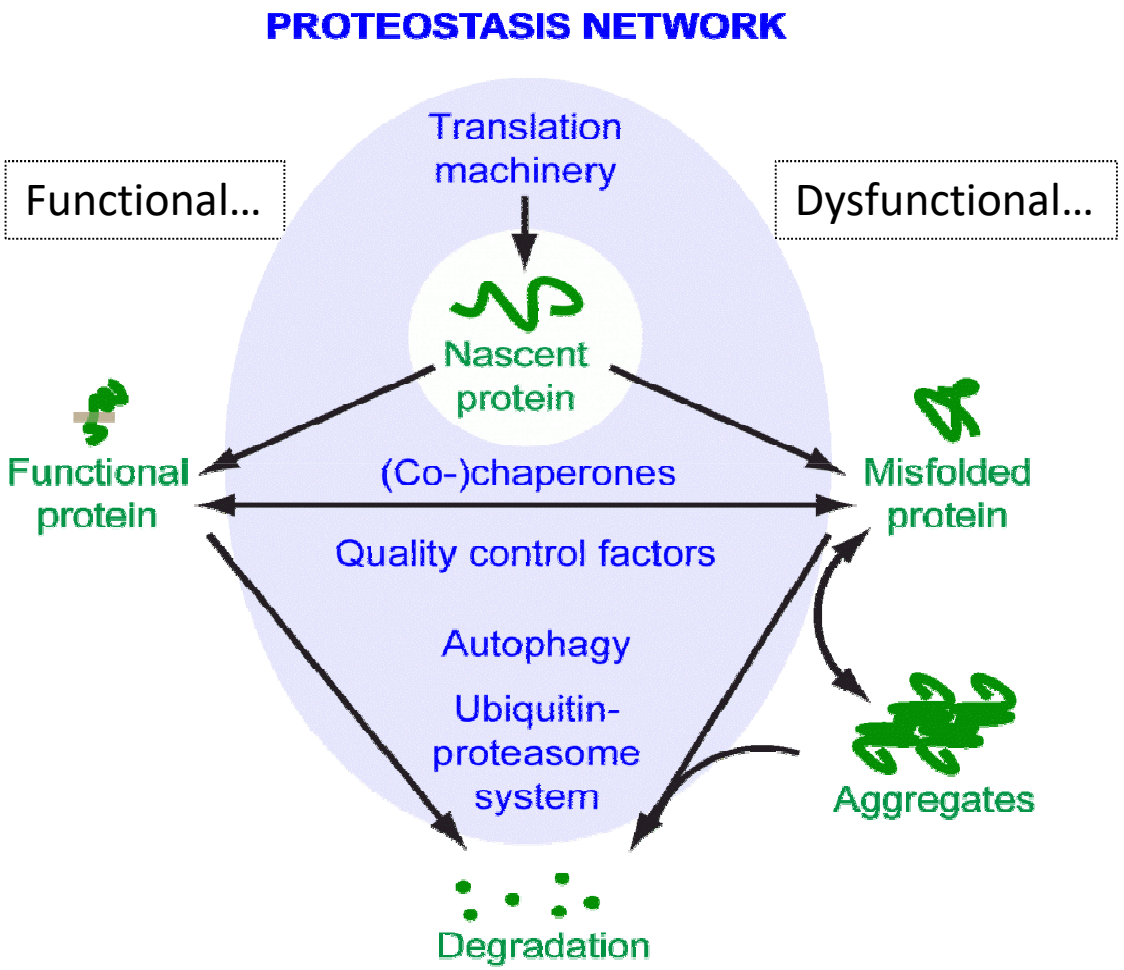
What is proteodynamics?

- **Proteostasis:** homeostasis of proteins from synthesis to degradation; *in narrow sense* mainly detection and removal (degradation by proteolysis) of misfolded proteins and aggregates

- **Proteodynamics:** everything happening to the protein during and after translation; includes proteostasis, but also multiple forms of protein ***modification*** *(including that by proteolysis);* stresses the dynamic interactions between differenc components of the proteome

Proteostasis – general



M. Kampmann Lab
University of California, San Francisco
https://kampmannlab.ucsf.edu/proteostasis-network

JCB

Ambre J. Sala et al. J Cell Biol
doi:10.1083/jcb.201612111

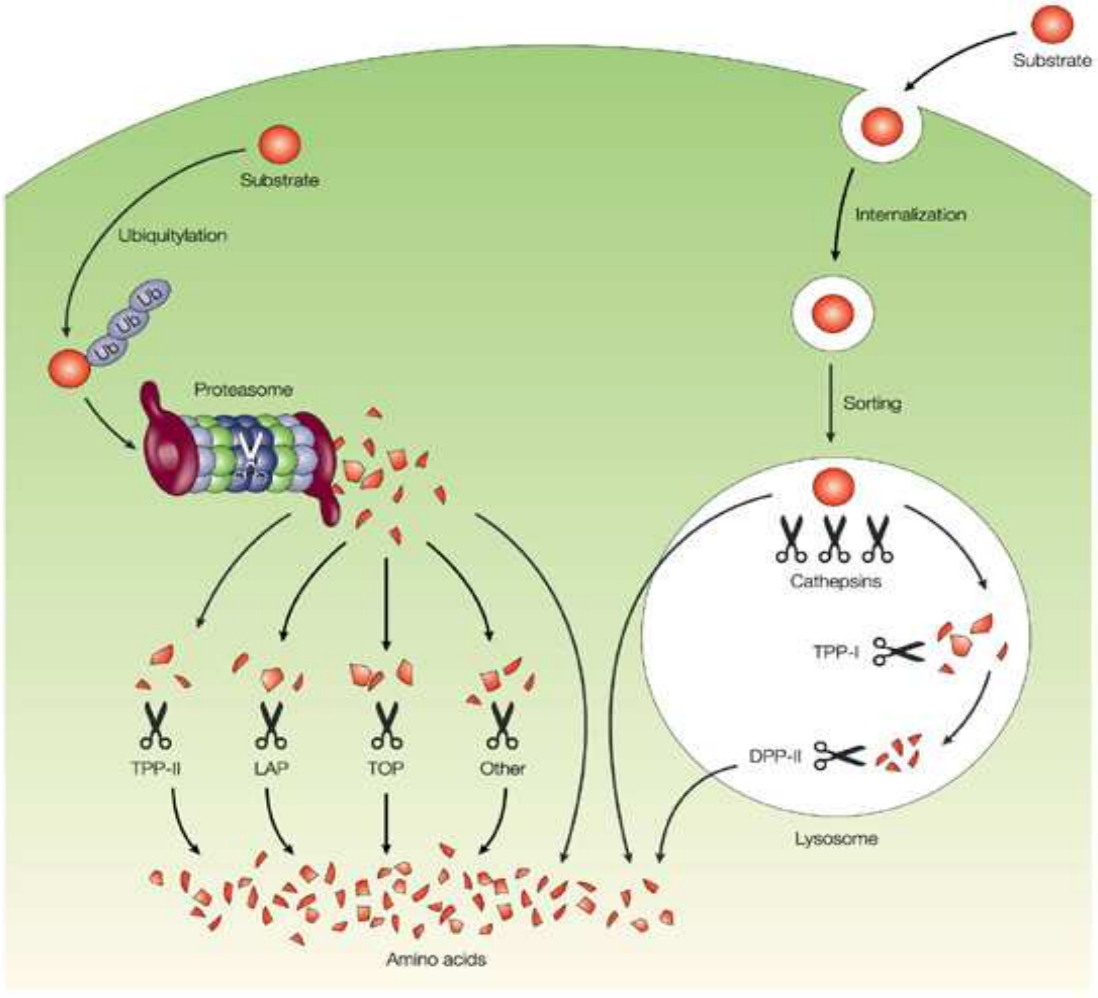
Proteolysis: degradation versus modulation

- Intracellular proteolysis can be:
  - **Degrading** (protein → peptide → aminoacids), e.g.
    - proteasome or lysosome-dependent (vide: **proteostasis**)

OR

  - **Regulatory or modifying** (protein [peptide] → modified proteins or smaller peptides with different properties), e.g.
    - immunoproteasomes, proteolytic cascade of complement, HTRA serine proteases, **calpain-calpastatin system …**

Degrading proteolysis occurs in every cell

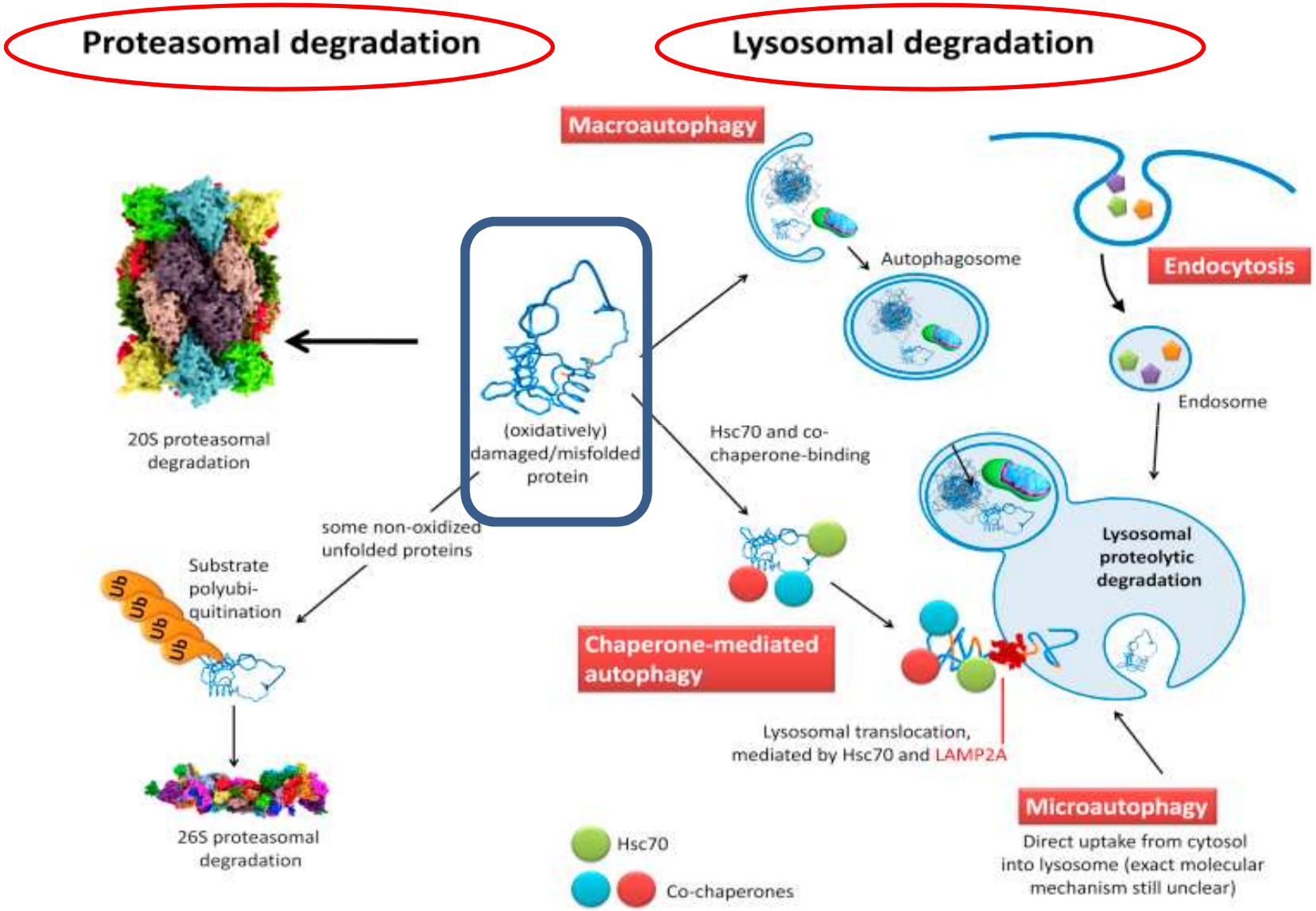


Nature Reviews | Drug Discovery

- Serves to:
  - **Remove** damaged (used up) proteins - **PROTEOSTASIS**
  - **Cleave** substrates for metabolism
  - **Protect** from extra/intracellular pathogens
  - Regulate proliferation **(cyclin and other [proto]oncogene degradation) - antineoplastic**

Proteostasis - mechanisms

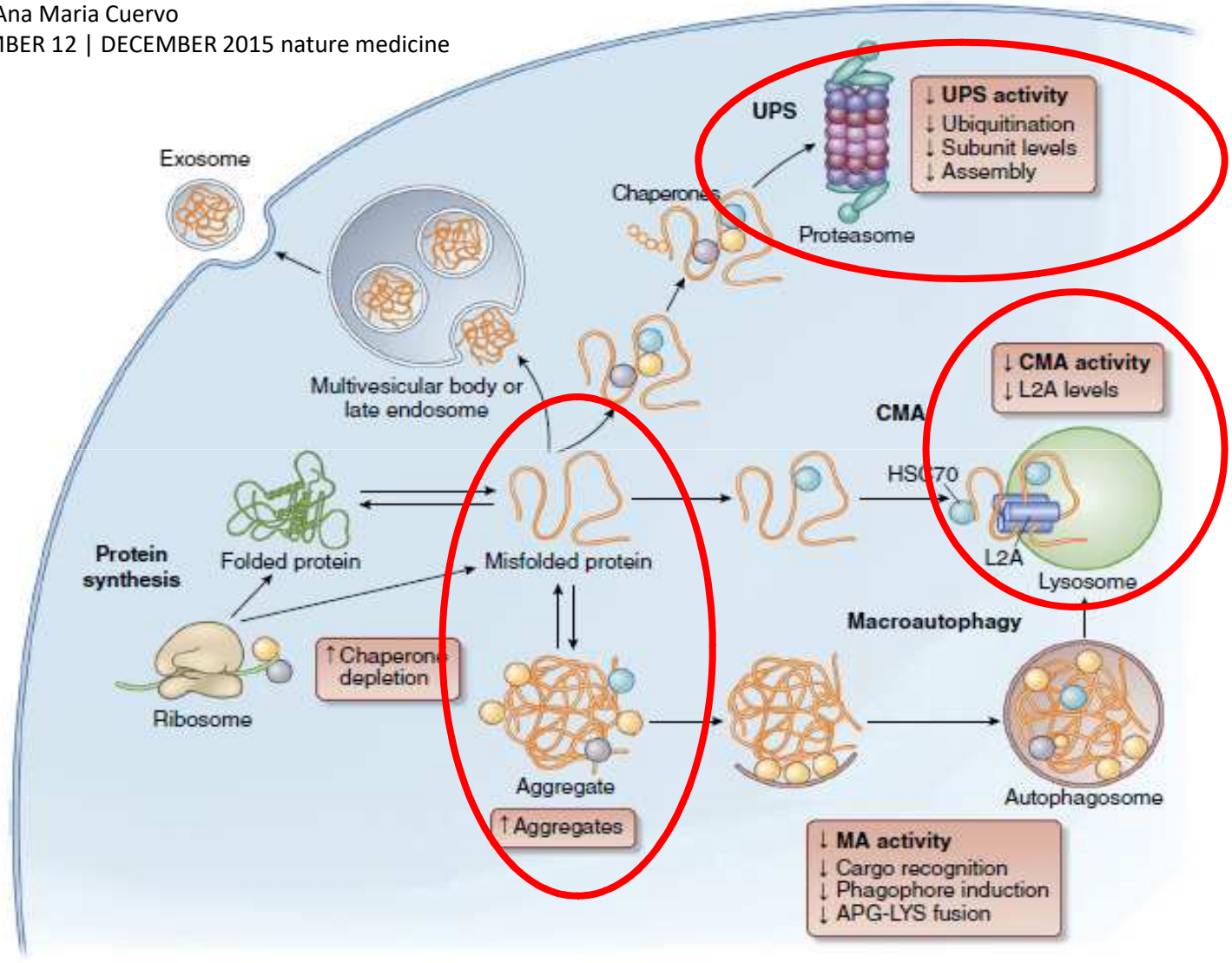
Korovila et al.,
Redox Biol. 2017 Oct; 13: 550–567



For a cell, barring mishaps and programmed death, it is either to age (become **senescent**) or to transform (become **cancerous**) [based on seed J. Campisi's works]

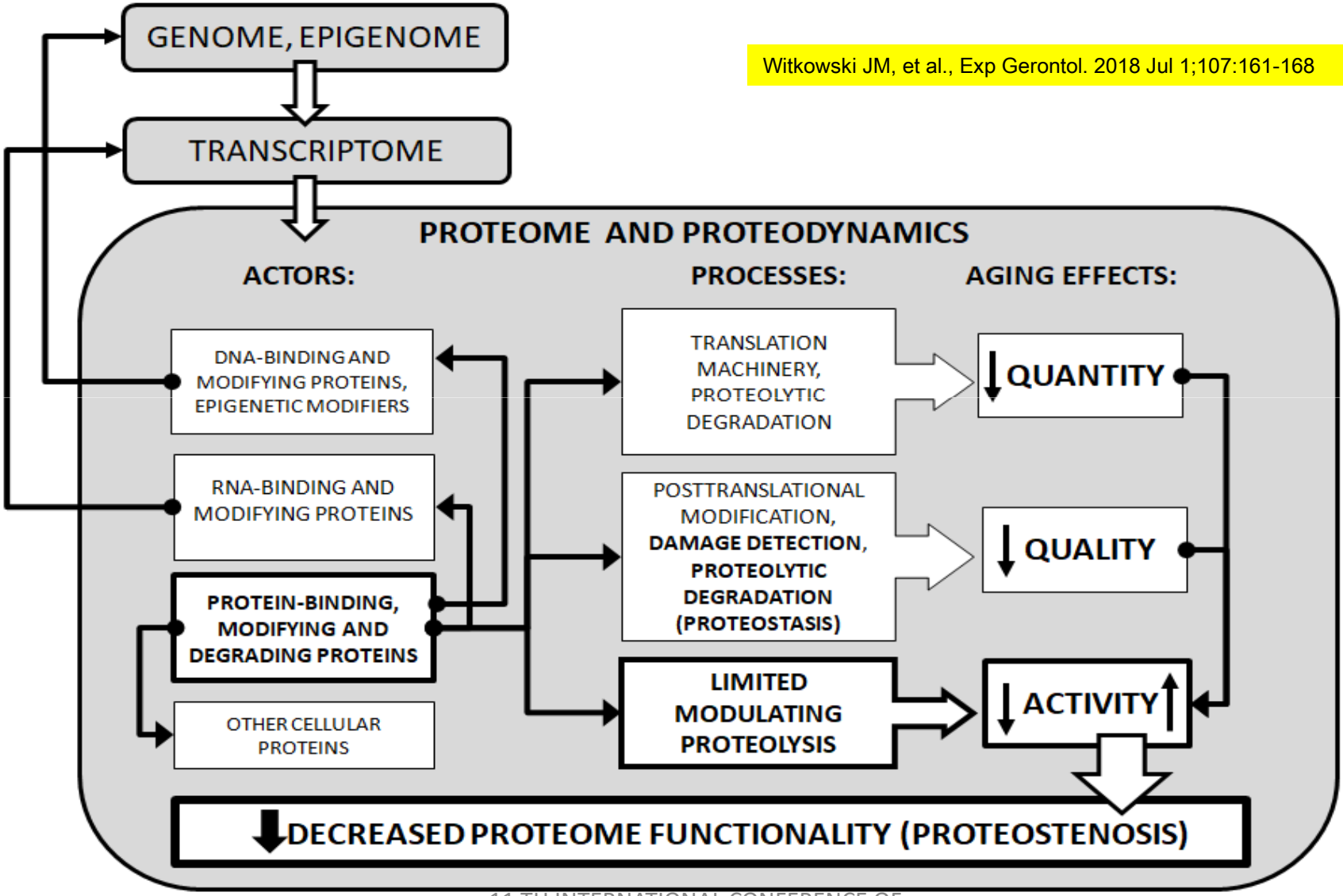
With age, proteostasis is failing…

Susmita Kaushik & Ana Maria Cuervo
VOLUME 21 | NUMBER 12 | DECEMBER 2015 nature medicine



INCREASED PROTEOTOXIC STRESS

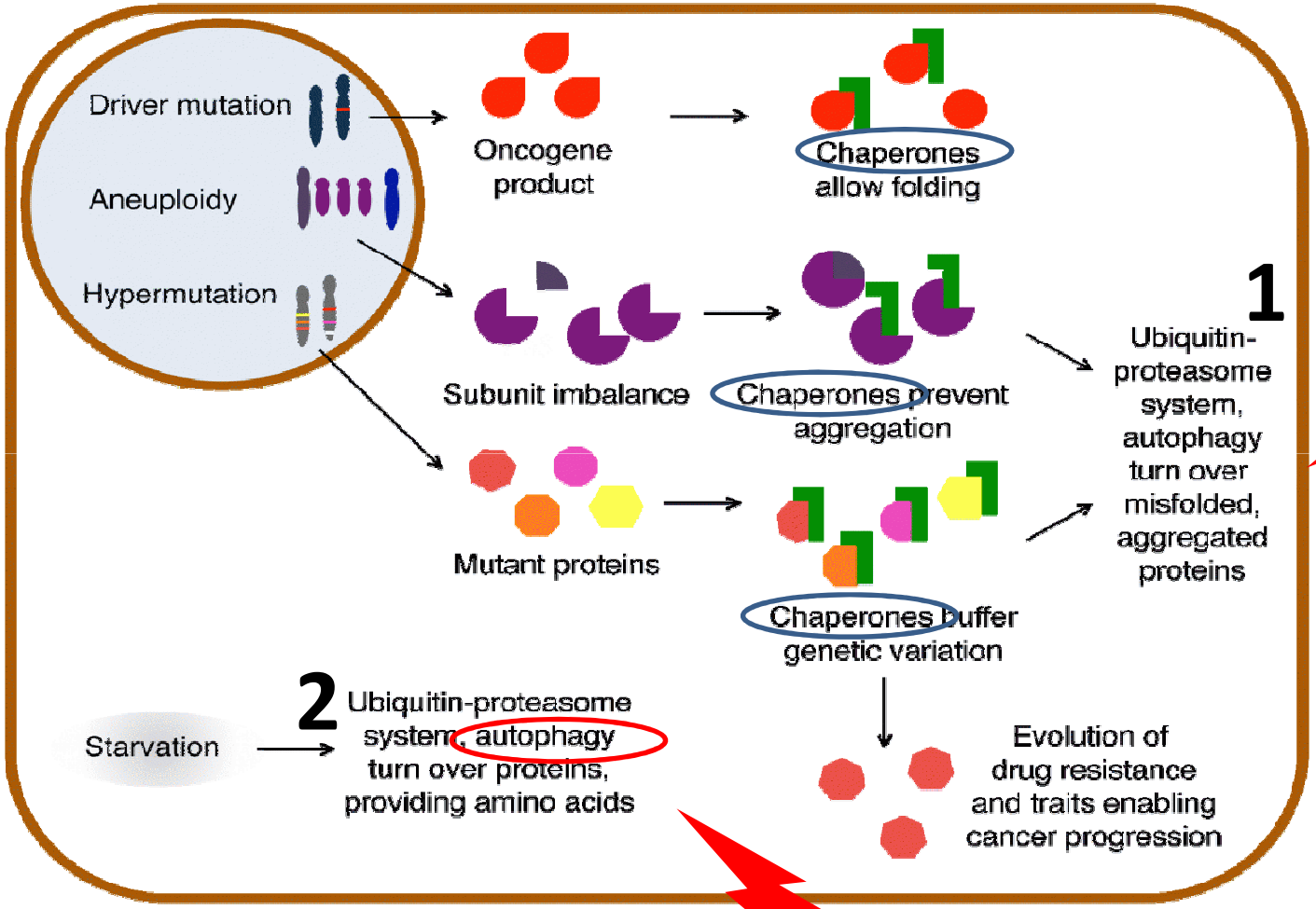
HYPOTHESIS: PROTEODYNAMICS FAILS IN AGING CELLS



Witkowski JM, et al., Exp Gerontol. 2018 Jul 1;107:161-168

On the other hand….

Cancer cells' addiction to proteostasis



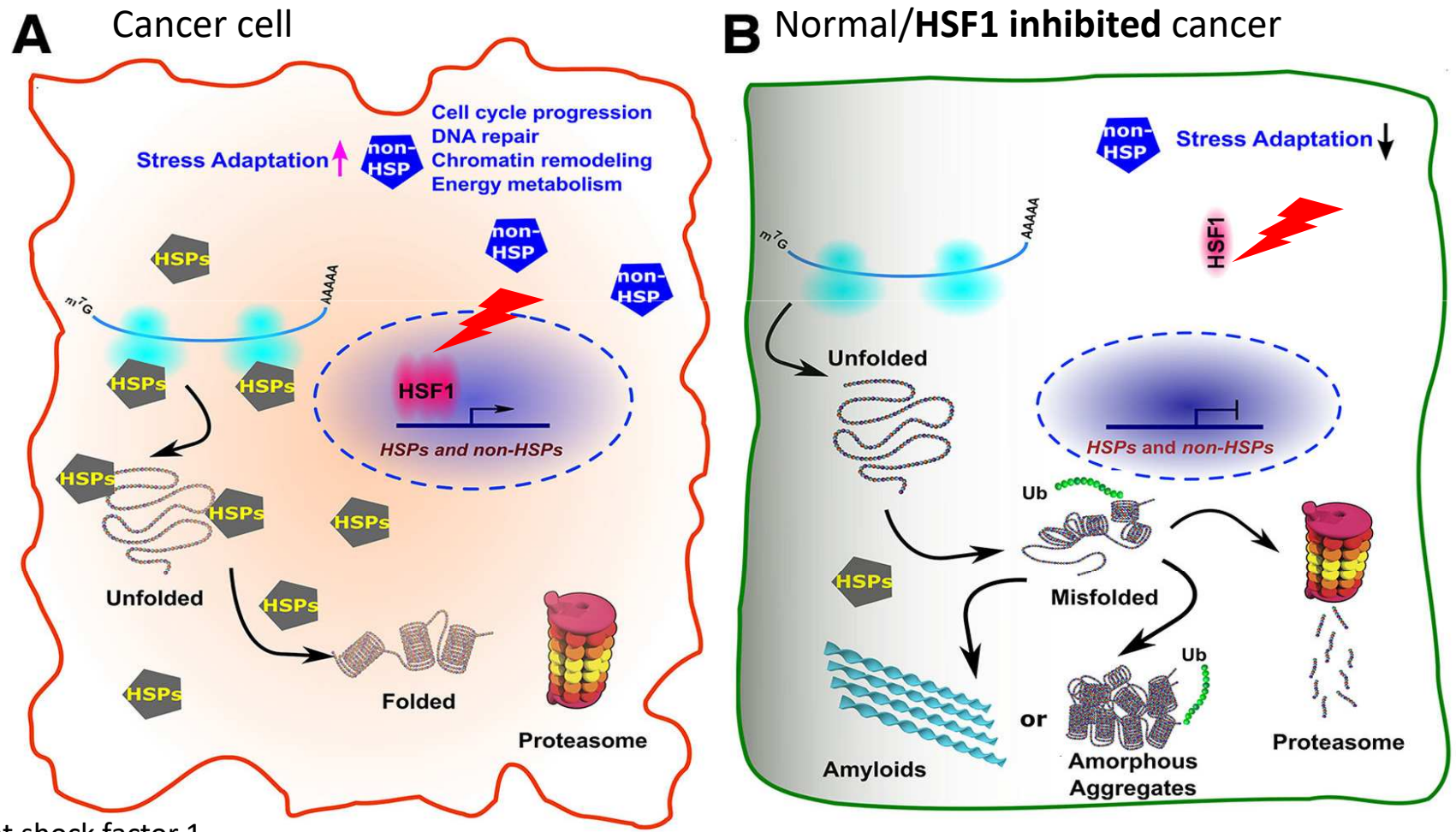
https://kampmannlab.ucsf.edu/cancer

1 – elimination of the bad proteins

2 – food provision

In cancer cells proteostasis is more robust…

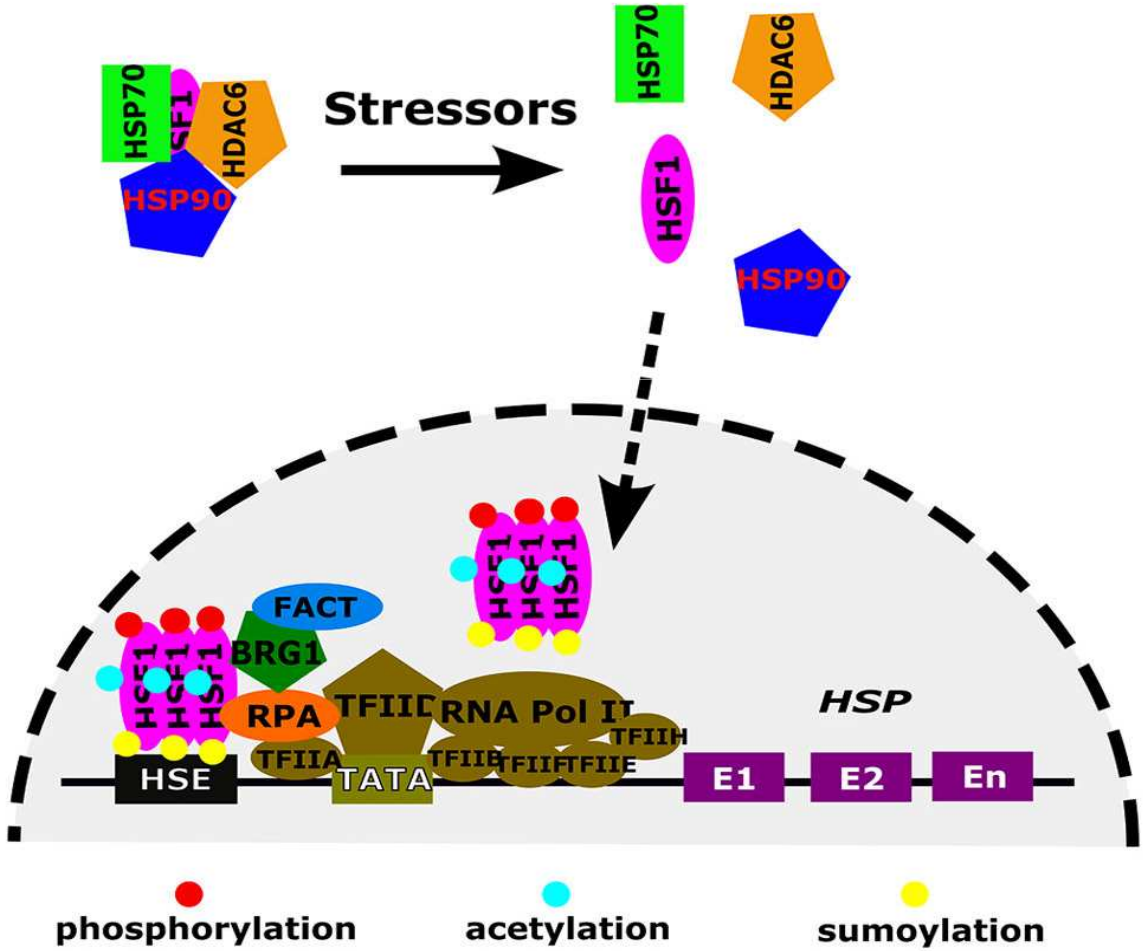
HSF1: Guardian of Proteostasis in Cancer. Dai C, Sampson SB.
Trends Cell Biol. 2016 Jan;26(1):17-28. doi: 10.1016/j.tcb.2015.10.011



HSF1 – heat shock factor 1

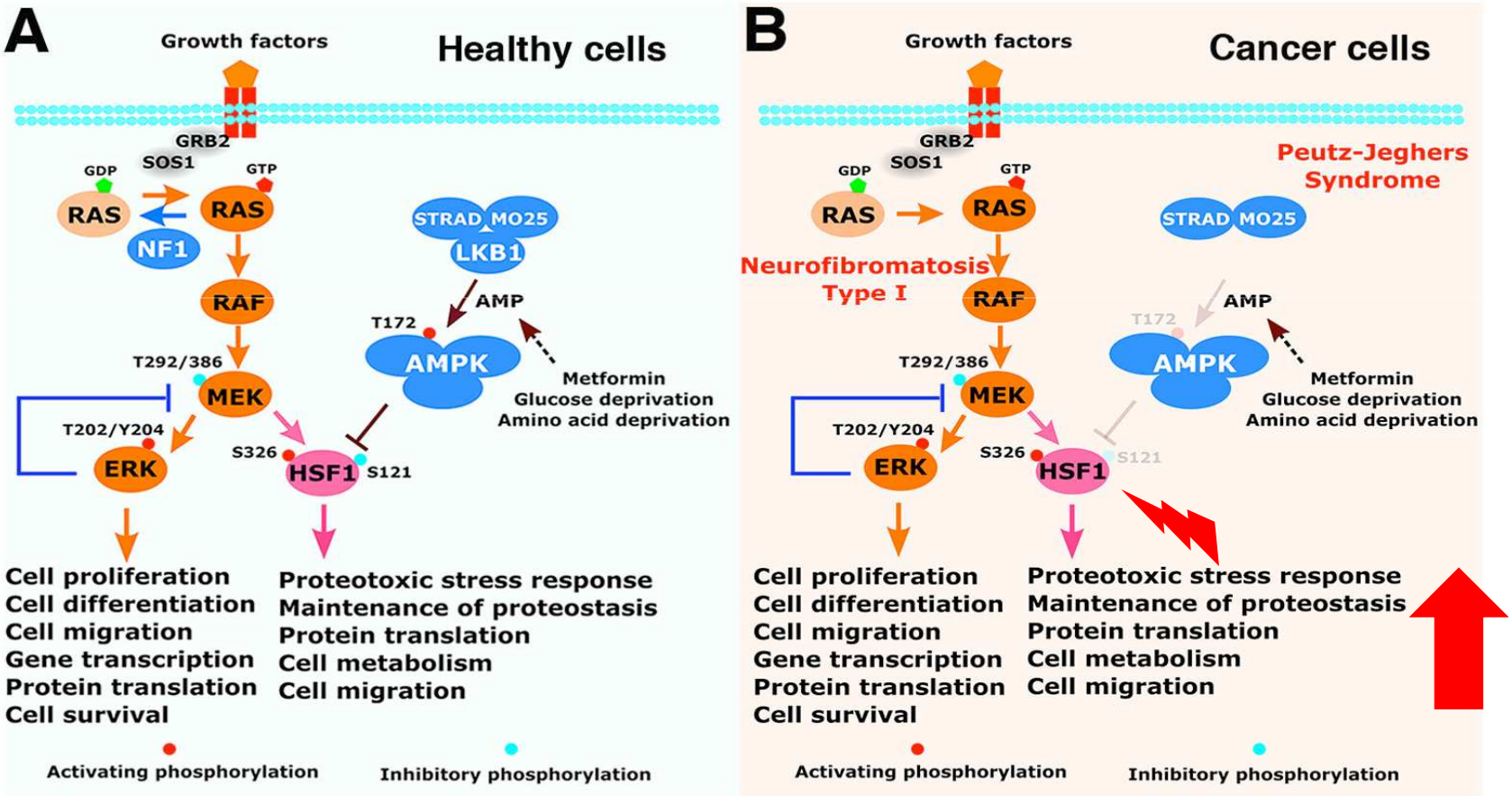
How does it work?

HSF1: Guardian of Proteostasis in Cancer. Dai C, Sampson SB.
Trends Cell Biol. 2016 Jan;26(1):17-28. doi: 10.1016/j.tcb.2015.10.011



What makes HSF1 active in cancer cells?

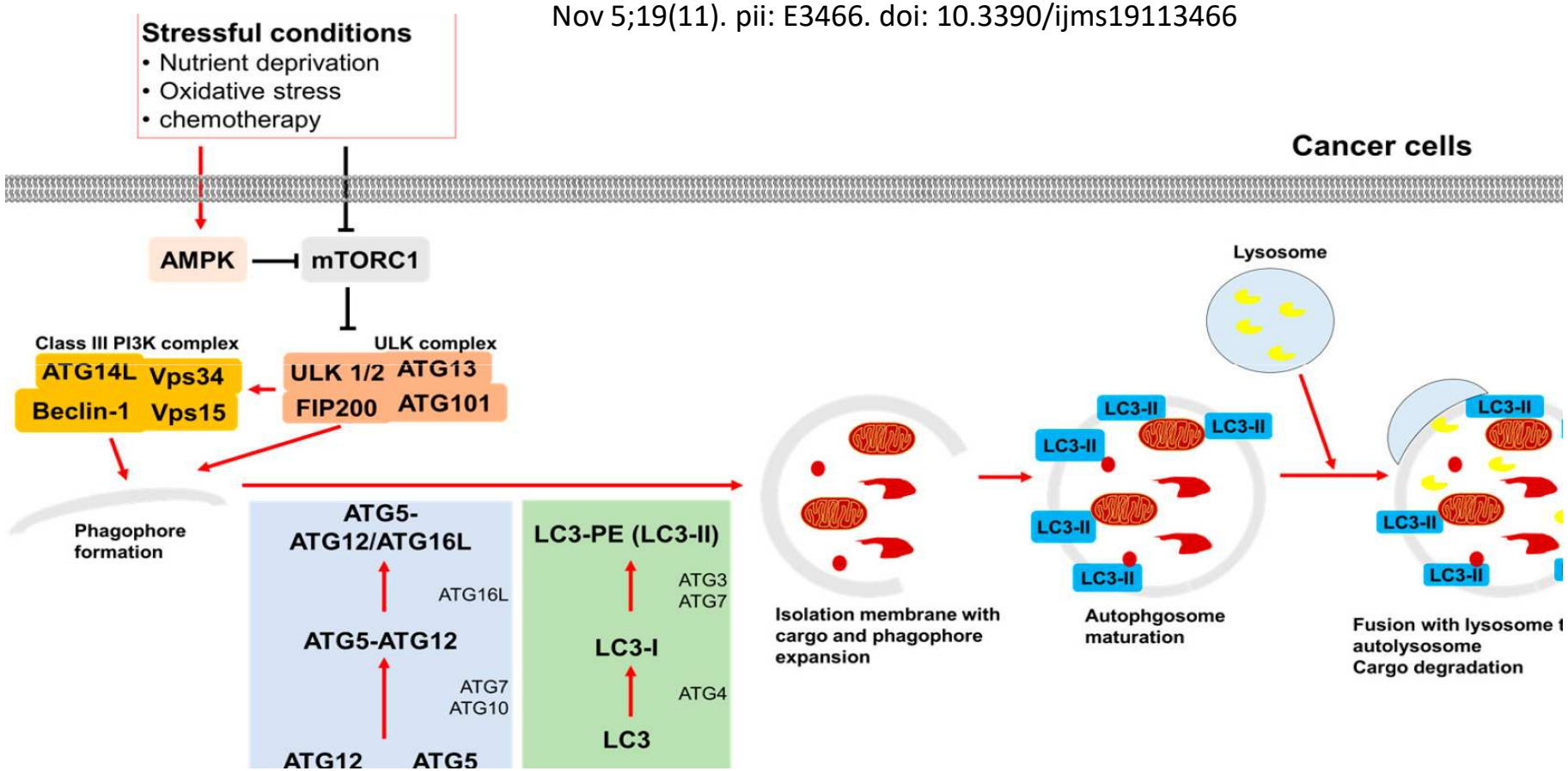
Cancerous cells suffer **chronic proteoteoxic stress** from without and within.
The HSF1-mediated **PSR is constitutively mobilized** within cancerous cells.



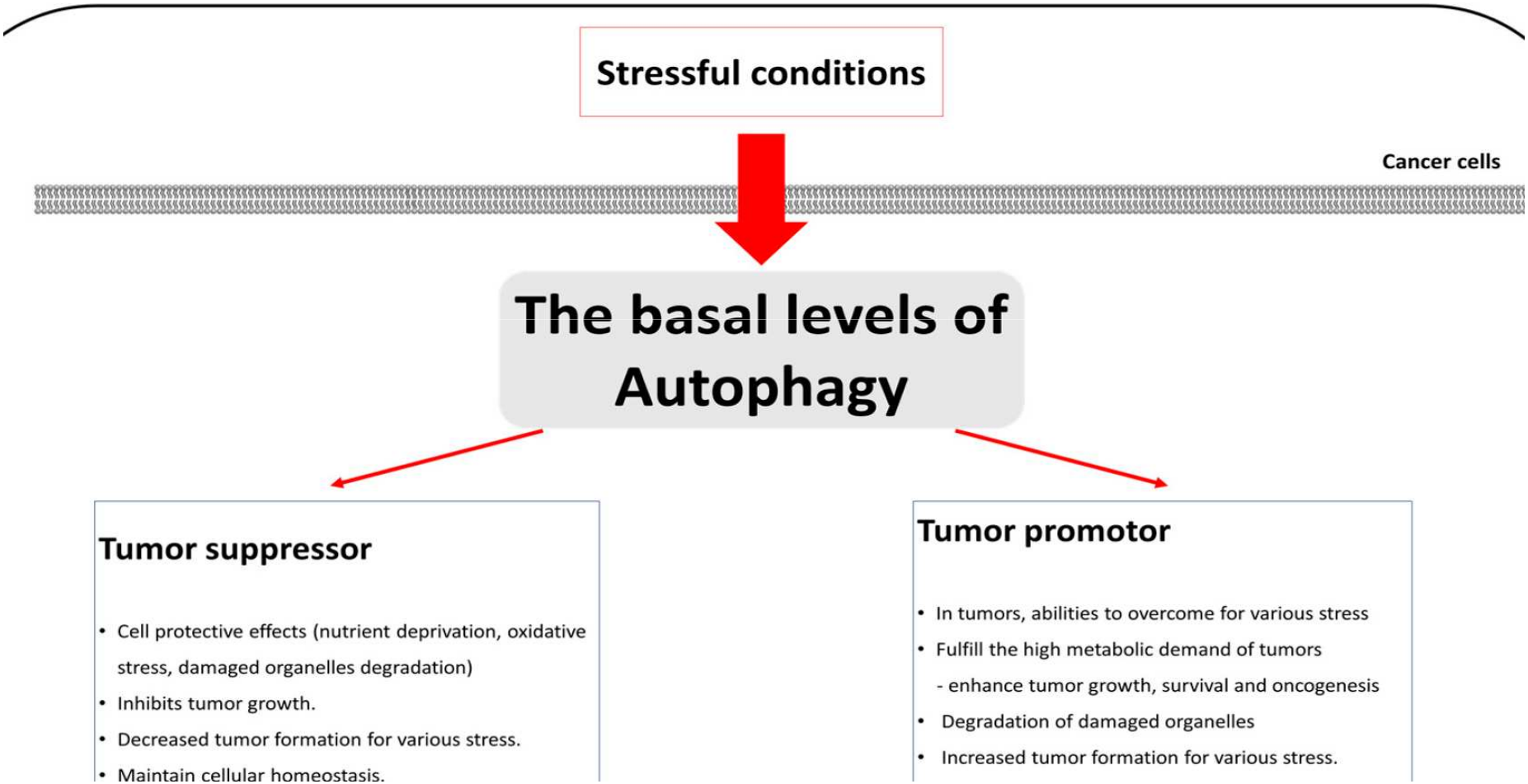
HSF1: Guardian of Proteostasis in Cancer. Dai C, Sampson SB. Trends Cell Biol. 2016 Jan;26(1):17-28. doi: 10.1016/j.tcb.2015.10.011

Autophagy and cancer cells: how does it work?

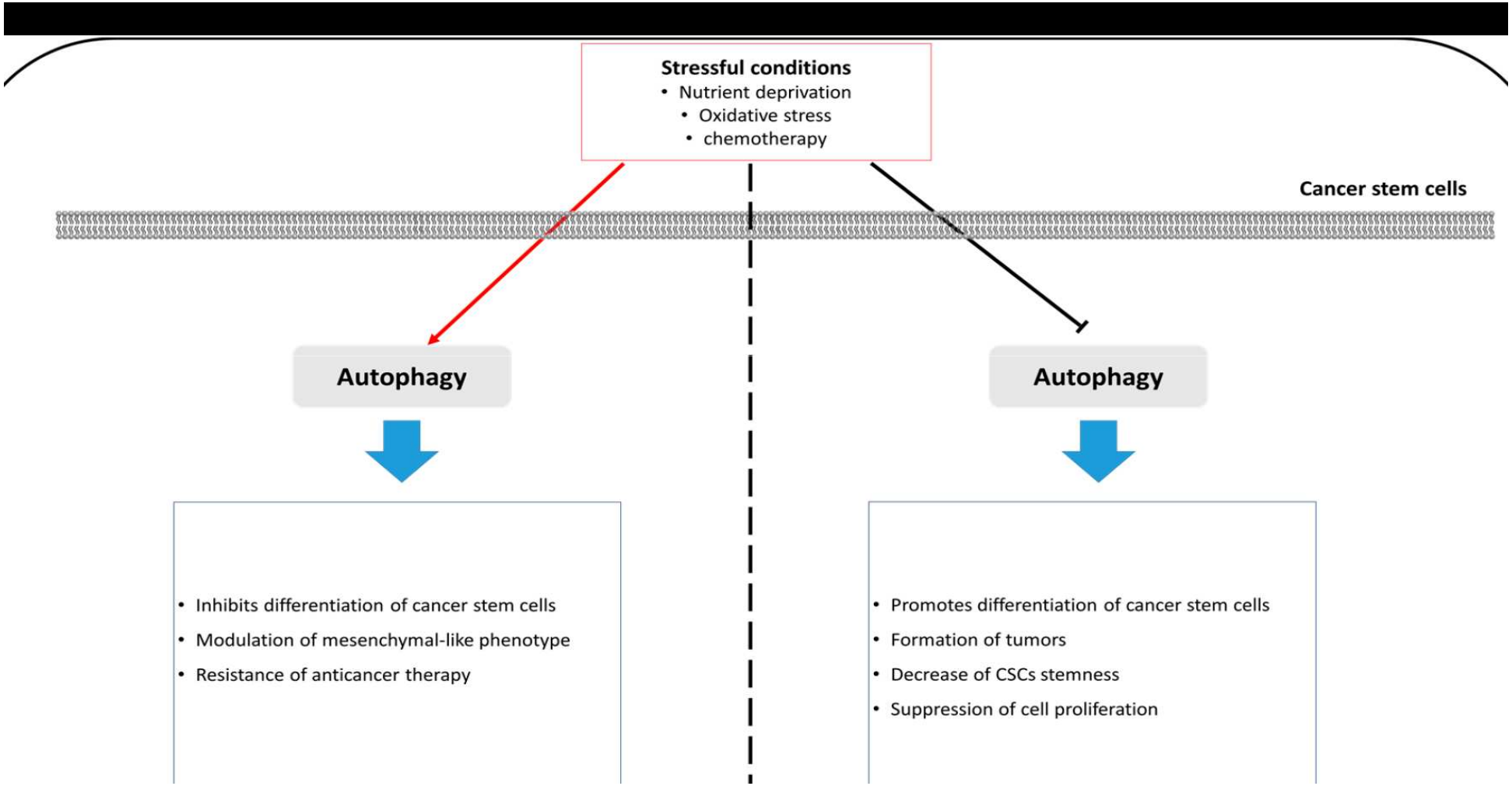
Yun CW, Lee SH., The Roles of Autophagy in cancer. Int J Mol Sci. 2018 Nov 5;19(11). pii: E3466. doi: 10.3390/ijms19113466



Autophagy and cancer cells: promotor OR suppressor



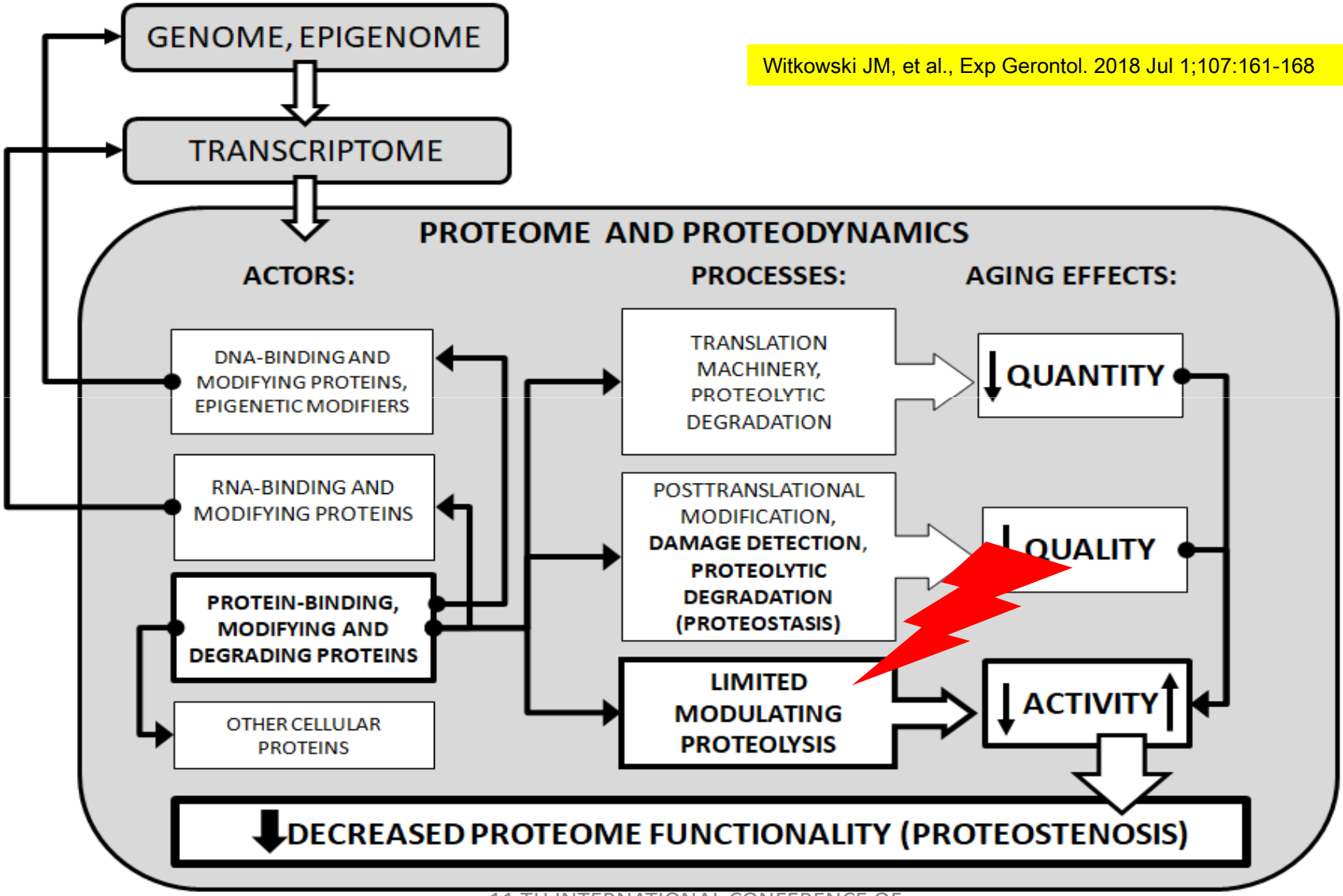
Autophagy and cancer stem cells; also duality..



Conclusions 1

- Cancer (in general neoplastic) cells survive stronger external and internal challenges and effects of different stresses by maintaining robust proteostasis
- This property is therapeutically explored (proteasome inhibitors)
- Autophagy may have different (promoting or suppressive) consequences, depending on cancer type, microenvironment etc…

Is there a role for limited, (modifying) proteolysis in cancers?



Witkowski JM, et al., Exp Gerontol. 2018 Jul 1;107:161-168

Regulatory (modifying) proteolysis: removal of part of the protein/peptide **rendering the remaining part active…. or inactive**; representative tools: **calpains**

What are calpains?

- **Cytosolic, calcium-activated, neutral cysteine proteases**
- **Activity affects cell division, apoptosis, movement, and other cell-specific functions impaired by aging… AND BY TRANSFORMATION**

UBIQUITOUS (and with an **absolute** Ca^{2+} dependency)

- **μ-calpain (CAPN-1, BM and blood cells) 1-100μM Ca^{2+}**
- **m-calpain** (**CAPNP- 2**, skeletal muscle, **PBMC**) **0,1-1mM Ca^{2+}**

} in vitro…

•In the cytoplasm, balanced STOICHOMETRICALLY with endogenous inhibitor, **calpastatin (CAST)**

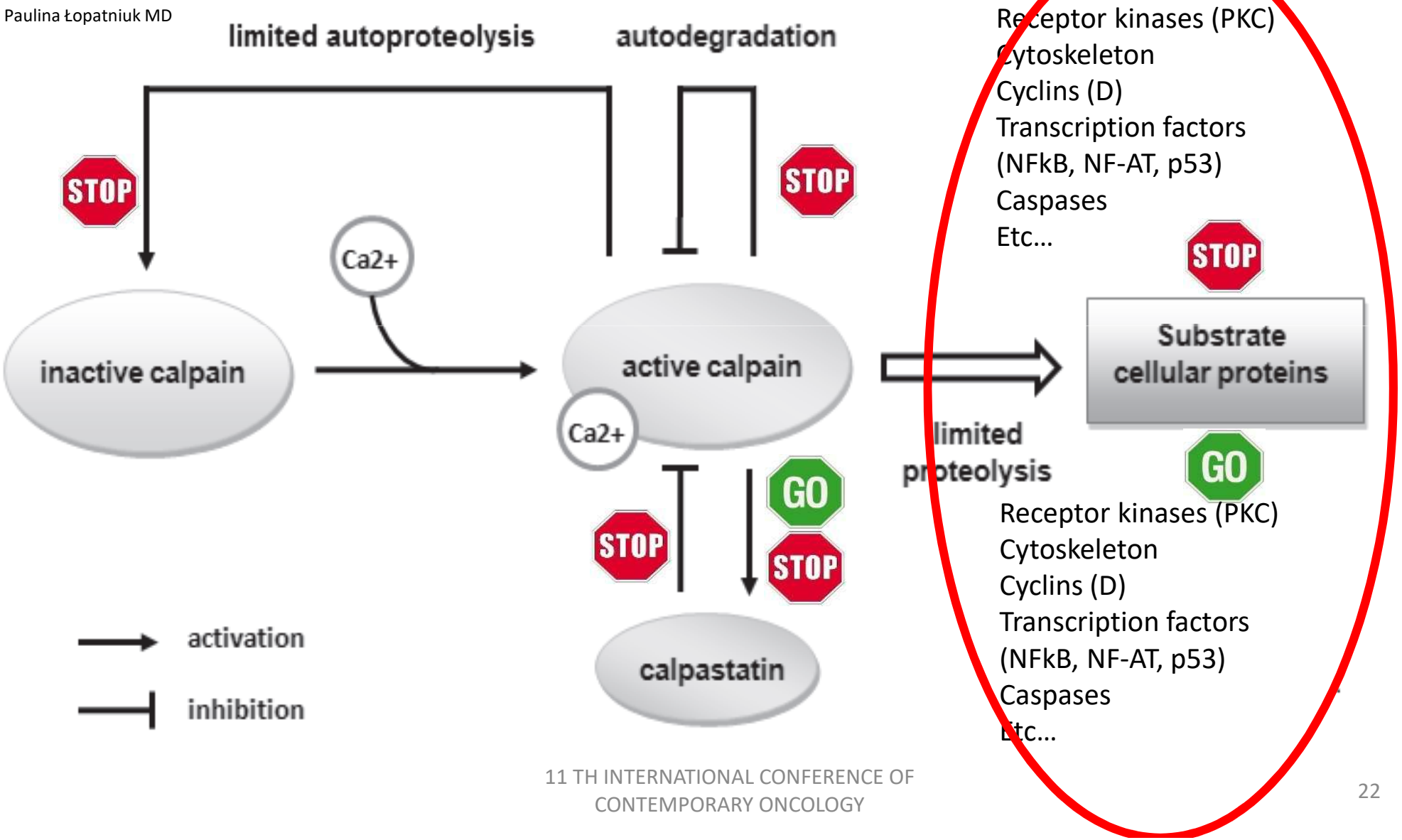
TOGETHER, they form the calpain-calpastatin system, CCS

TISSUE-SPECIFIC (variable Ca^{2+} dependency, structure and sites)

Paulina Łopatniuk MD

How does it work?

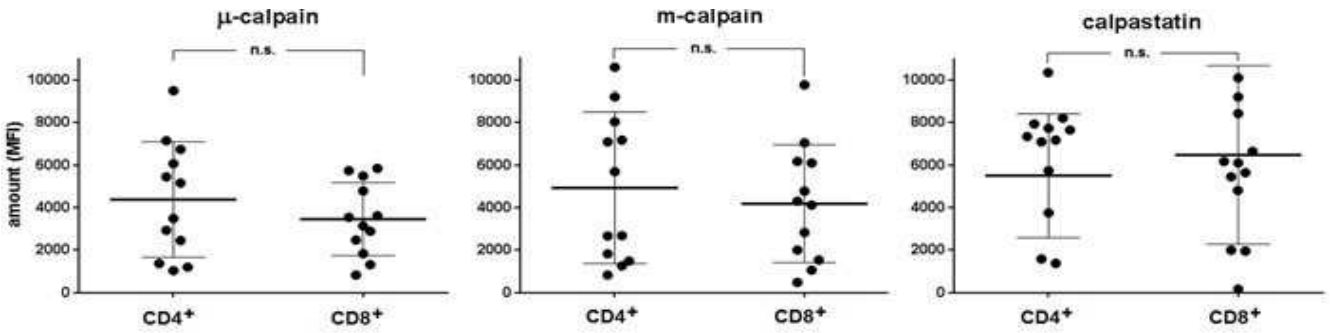
Łopatniuk & Witkowski, Acta Biochim Pol. 2011;58(3):287-96



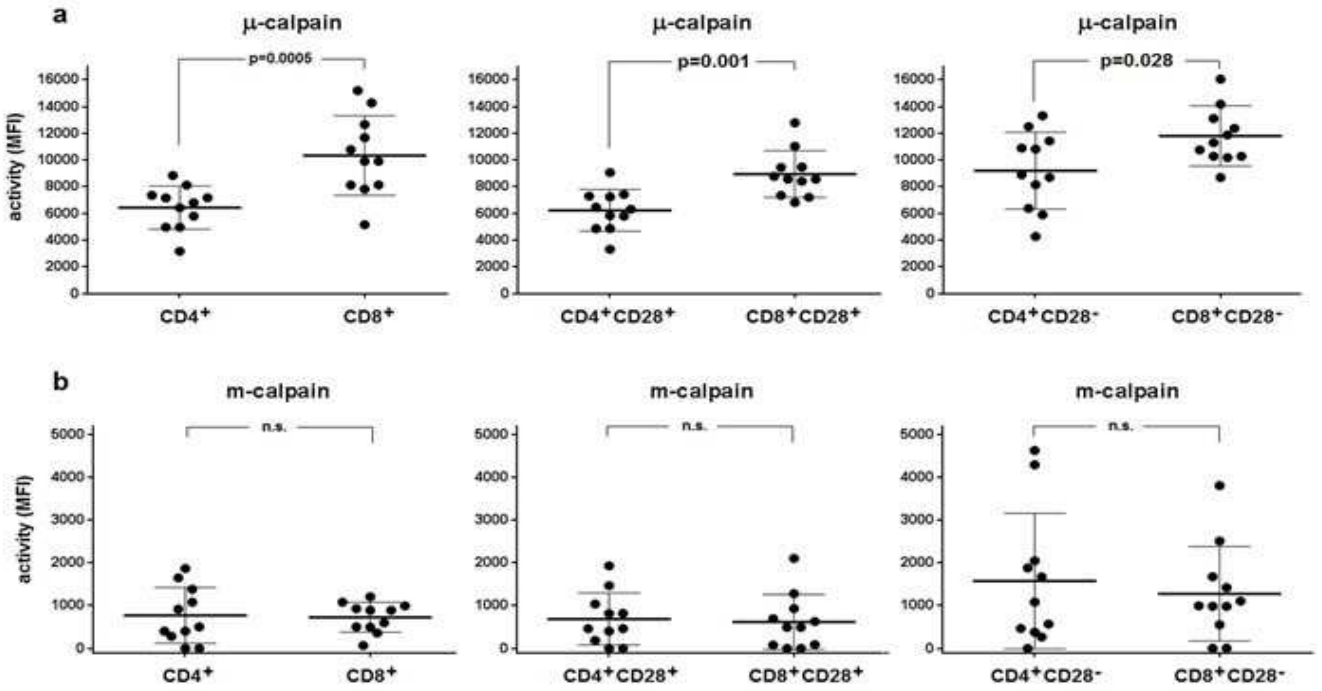
Calpains and T cell functions – **resting** amount & activity

Mikosik A, et al., Oncotarget. 2016 Nov 22;7(47):76479-76495

AMOUNT



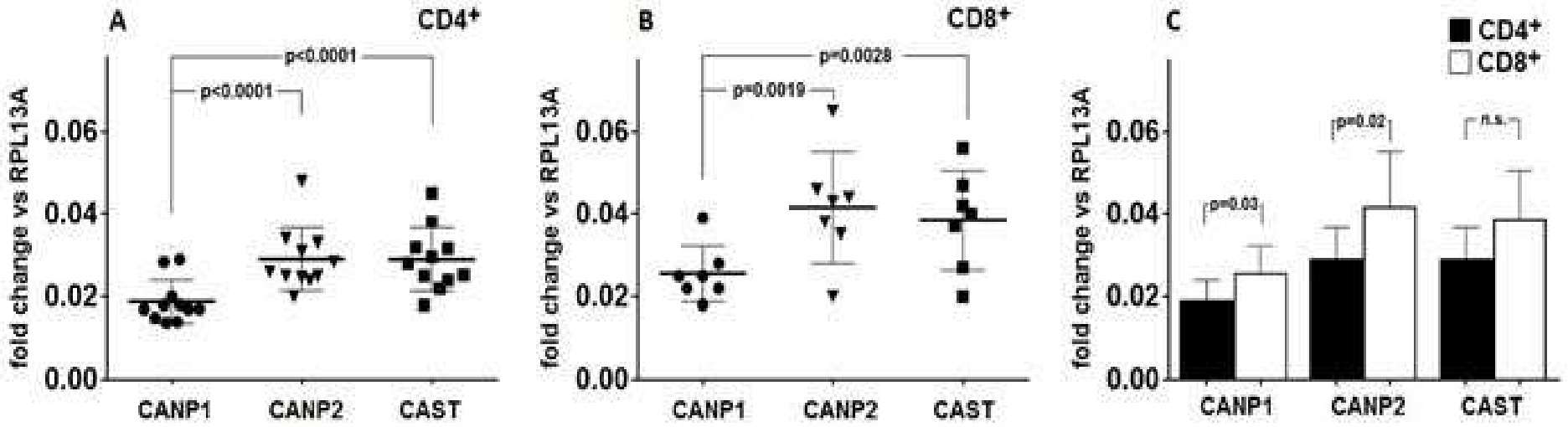
ACTIVITY



Anna Mikosik PhD

Calpains and T cell functions – **constitutive** transcription; consequence of autodegradation?

Joanna Frąckowiak MS

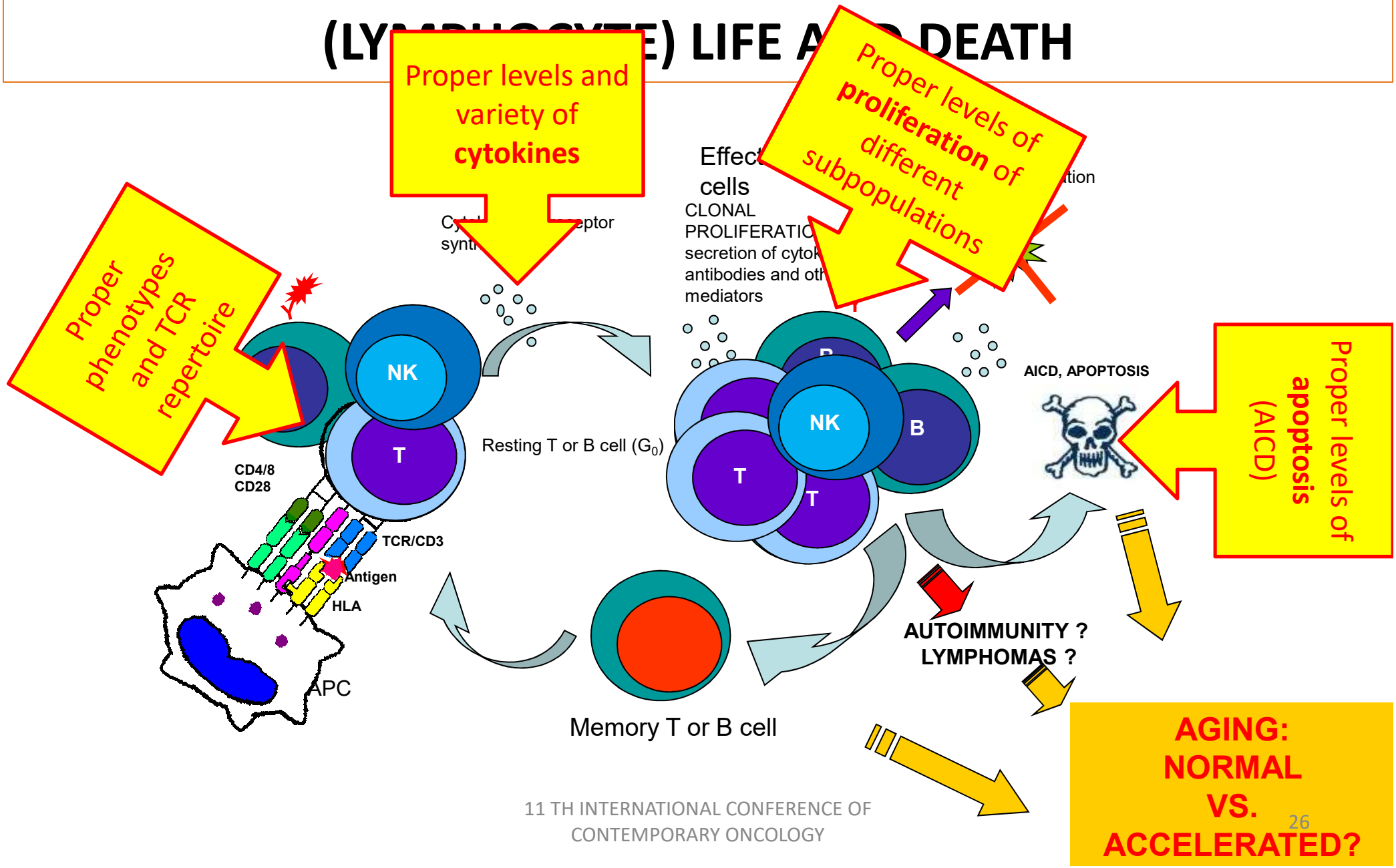


Mikosik A, et al., Oncotarget. 2016 Nov 22;7(47):76479-76495

Active calpains autodestruct.
So,if their amount and activity is maintained,
they have to be important for lymphocyte physiology…

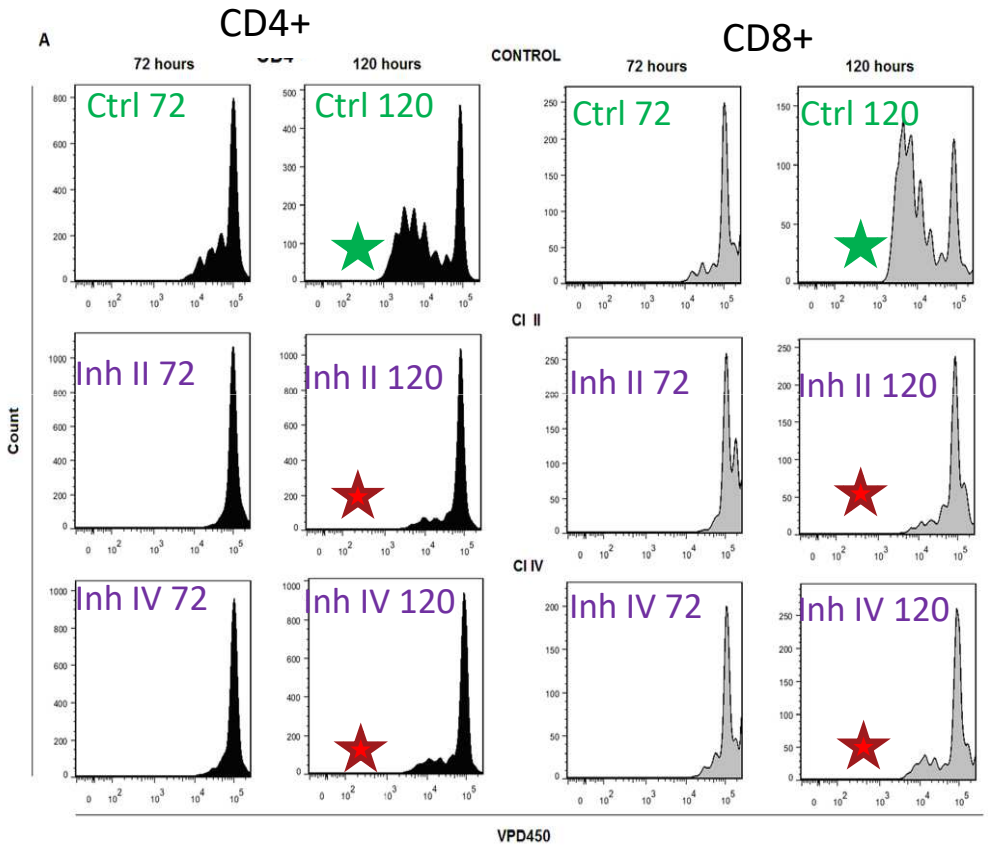
So, what do calpains control in normal and malignant lymphocytes?

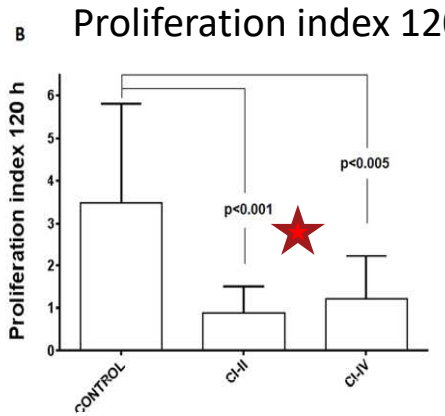
ADEQUATE IMMUNE REACTION IS A BALANCE BETWEEN (LYMPHOCYTE) LIFE AND DEATH

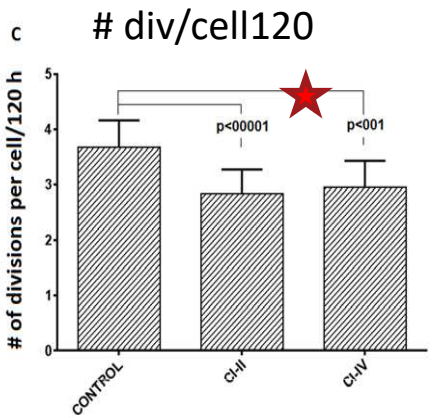


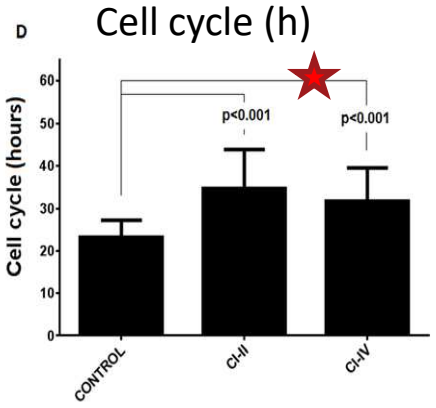
Calpain inhibition at rest - reduced T cell proliferation

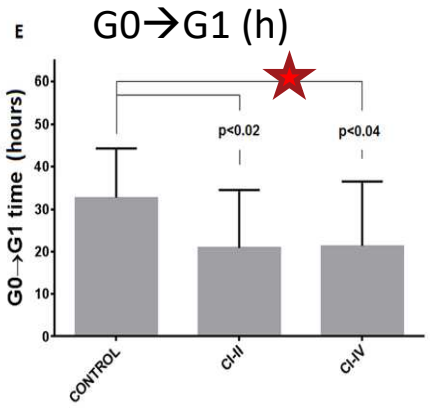
Proliferation in vitro (anti-CD3, DCT detection)







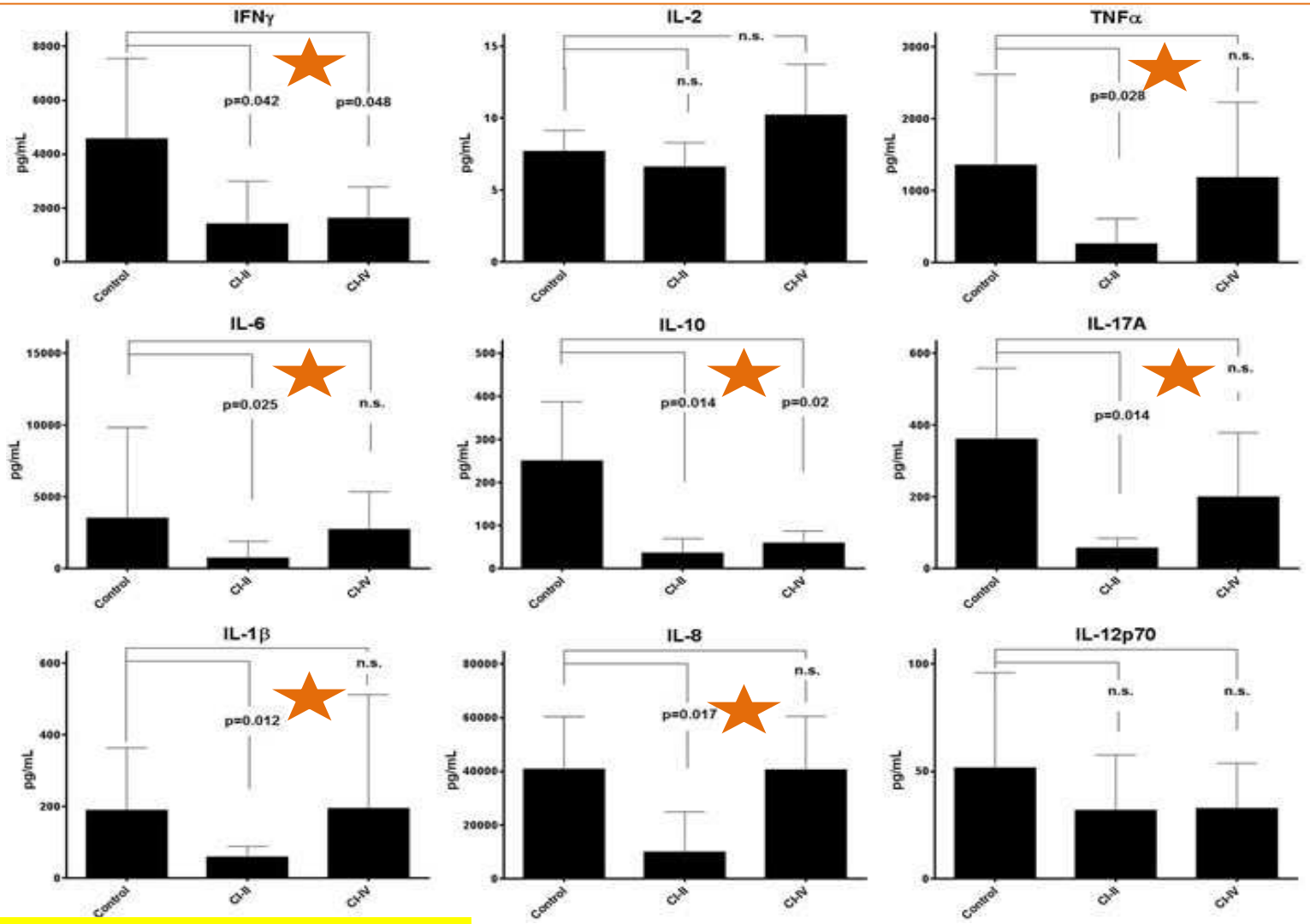




Calpain inhibitor treatment

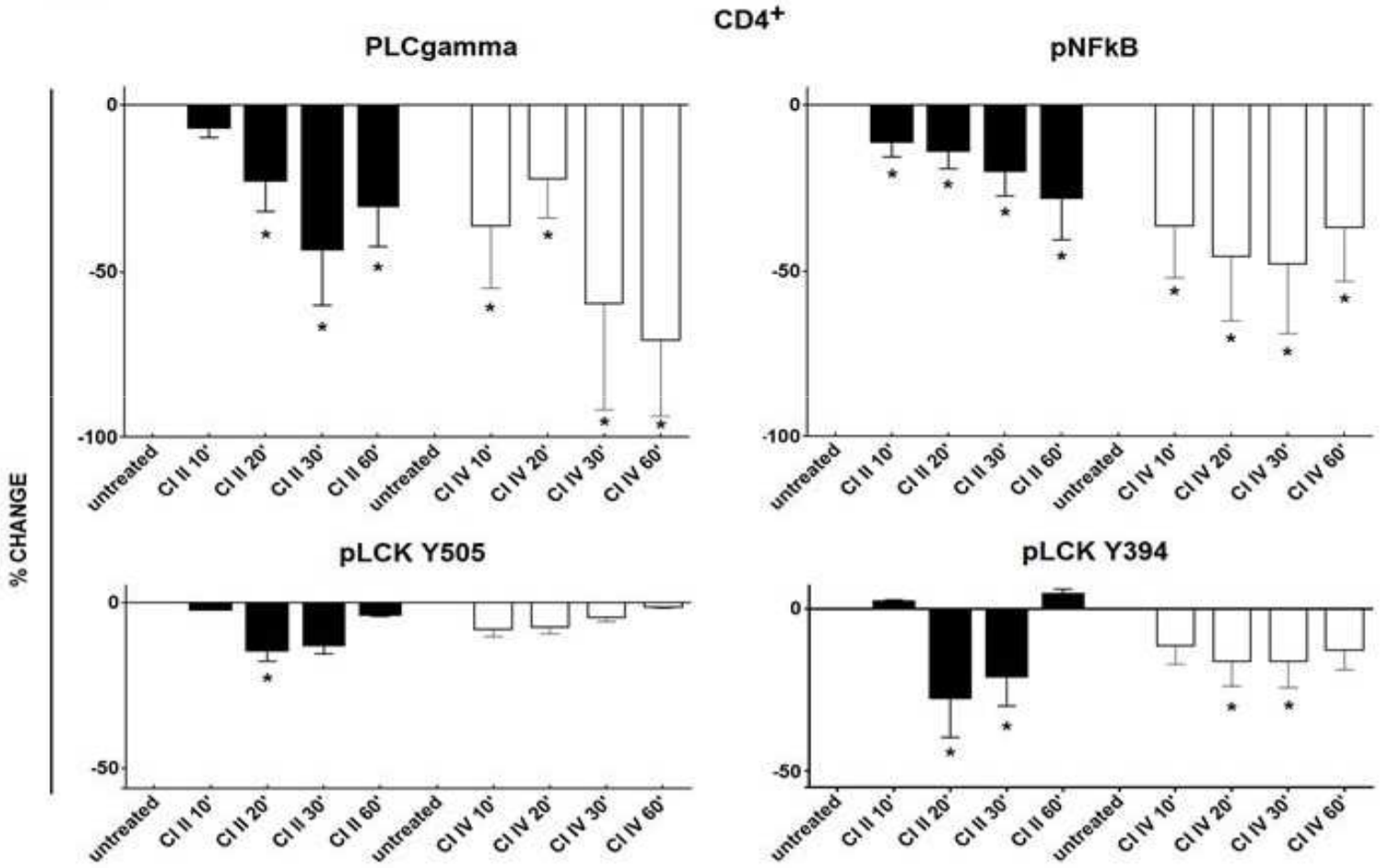
Mikosik A, et al., Oncotarget. 2016 Nov 22;7(47):76479-76495

Calpain inhibition at rest – decreased cytokine production



Mikosik A, et al., Oncotarget. 2016 Nov 22;7(47):76479-76495

Calpain inhibition at rest – less phosphorylated signal transduction molecules in resting T cells

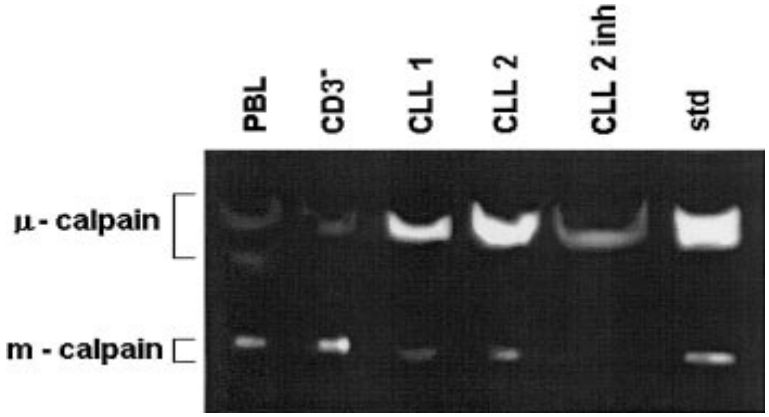


Mikosik A, et al., Oncotarget. 2016 Nov 22;7(47):76479-76495

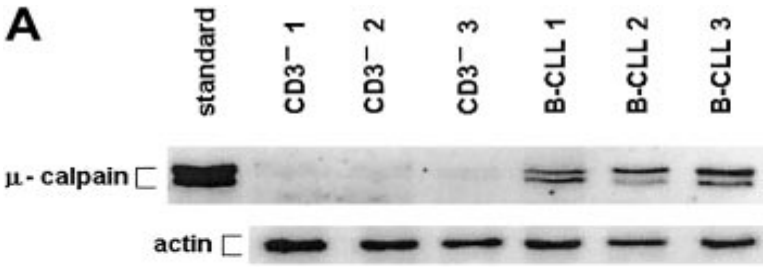
How are the calpains modified in lymphoid leukemias and what are the consequences?

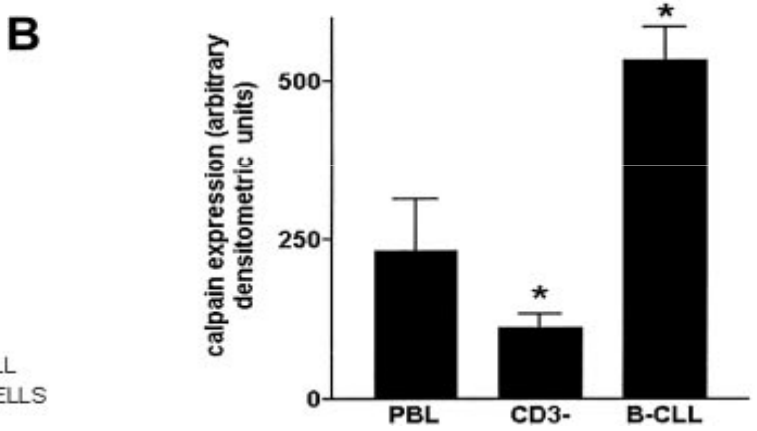
Calpains in B-CLL: more transcript, more protein, higher total available activity …

J.M. Witkowski et al., *Blood 2002,100: 1802-1809*



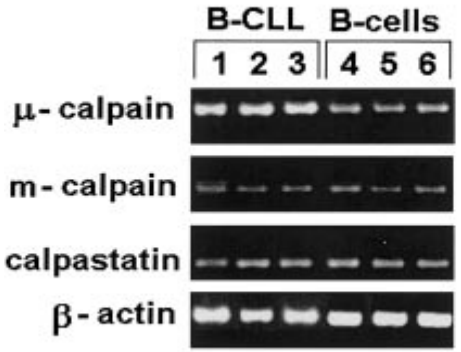
Activity: Casein zymography

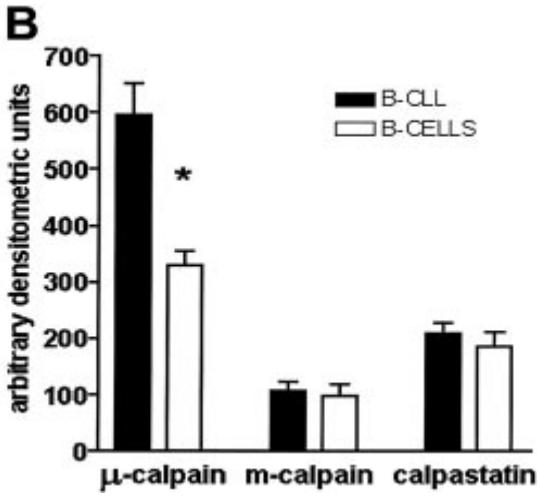




Amount: WB

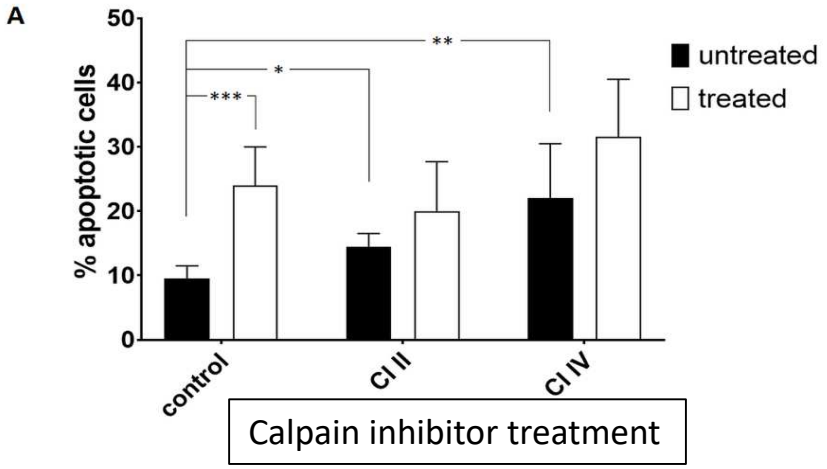
A Transcript: RT-PCR

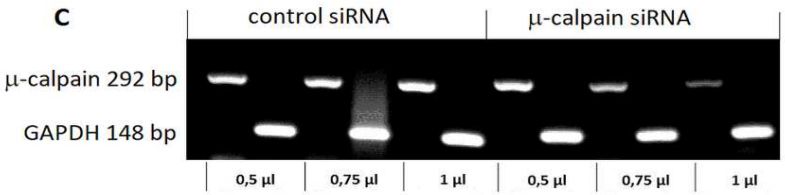


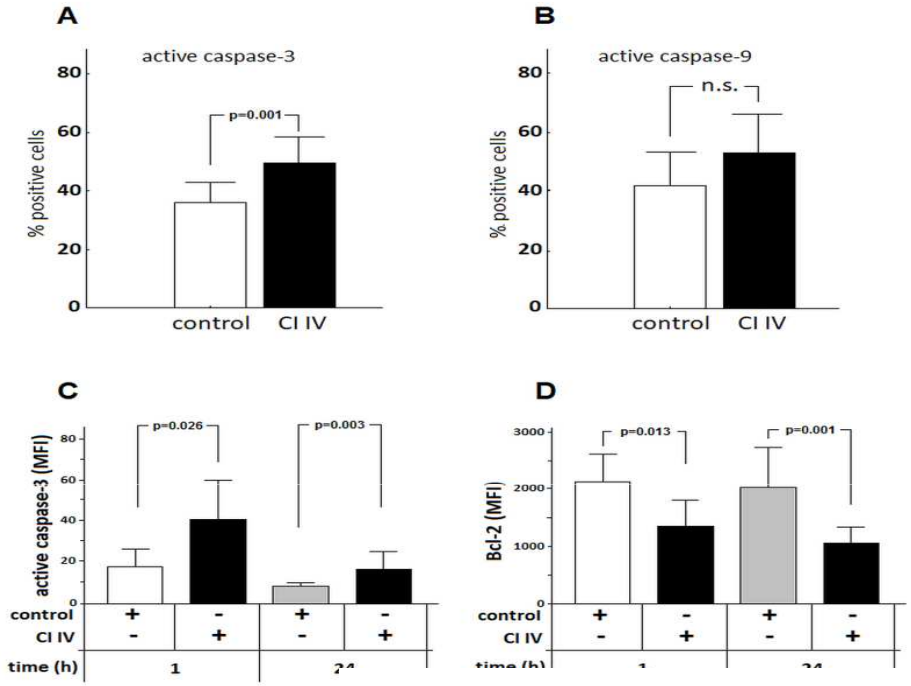


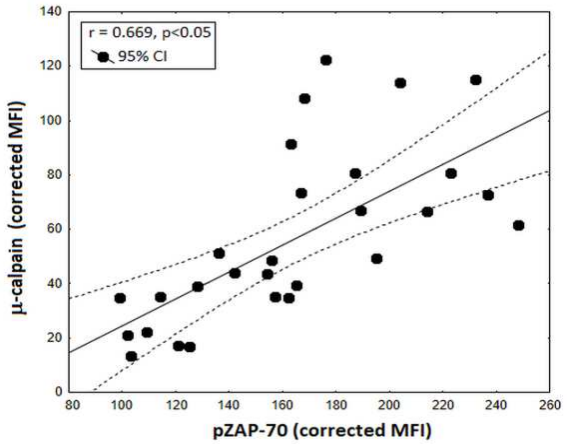
Calpains in B-CLL: pro-survival, anti-apoptotic…

Łopatniuk et al., Sci Rep 2018 (submitted)







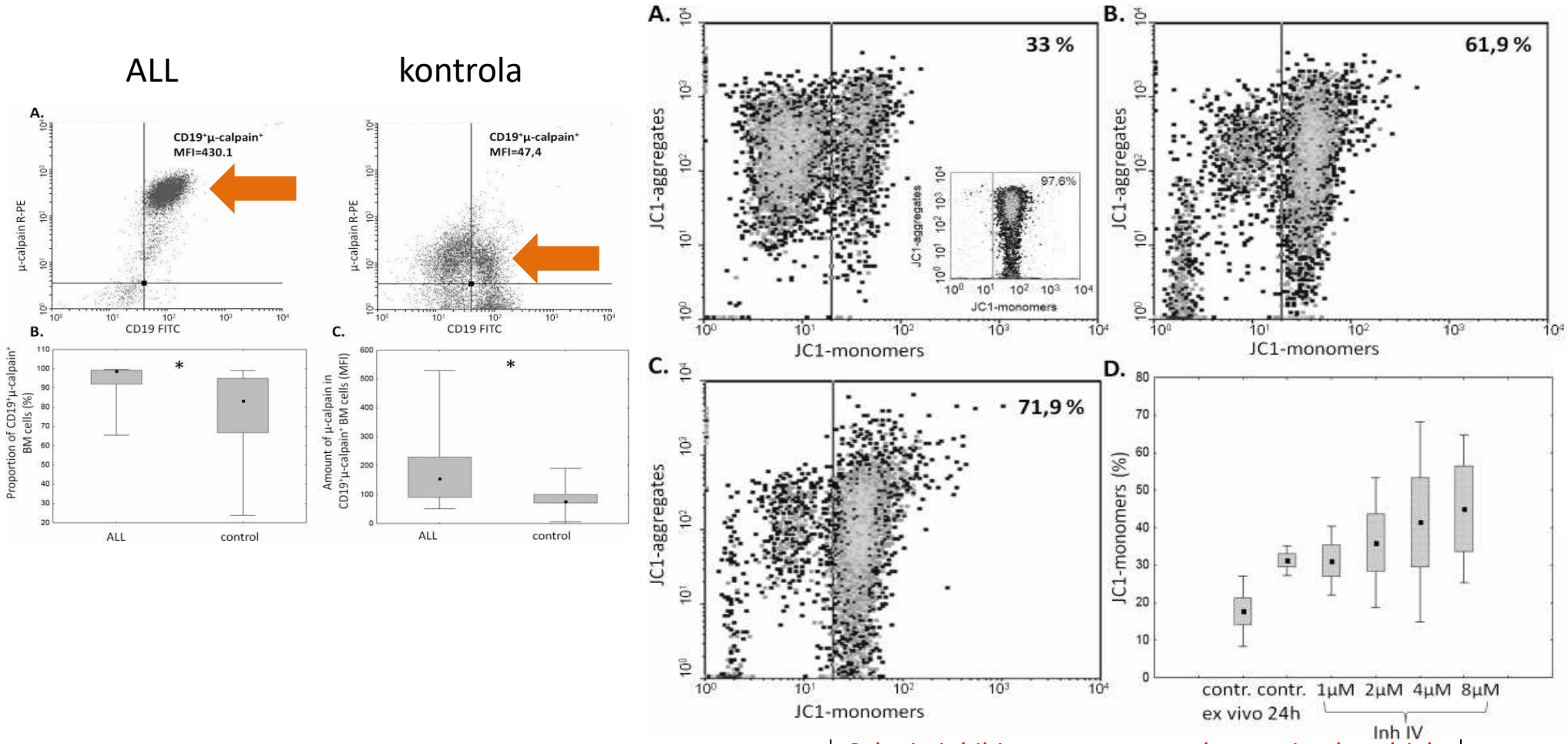


CASPASES

BCL2

ZAP70

Calpains in ALL blasts: more and also anti-apoptotic…

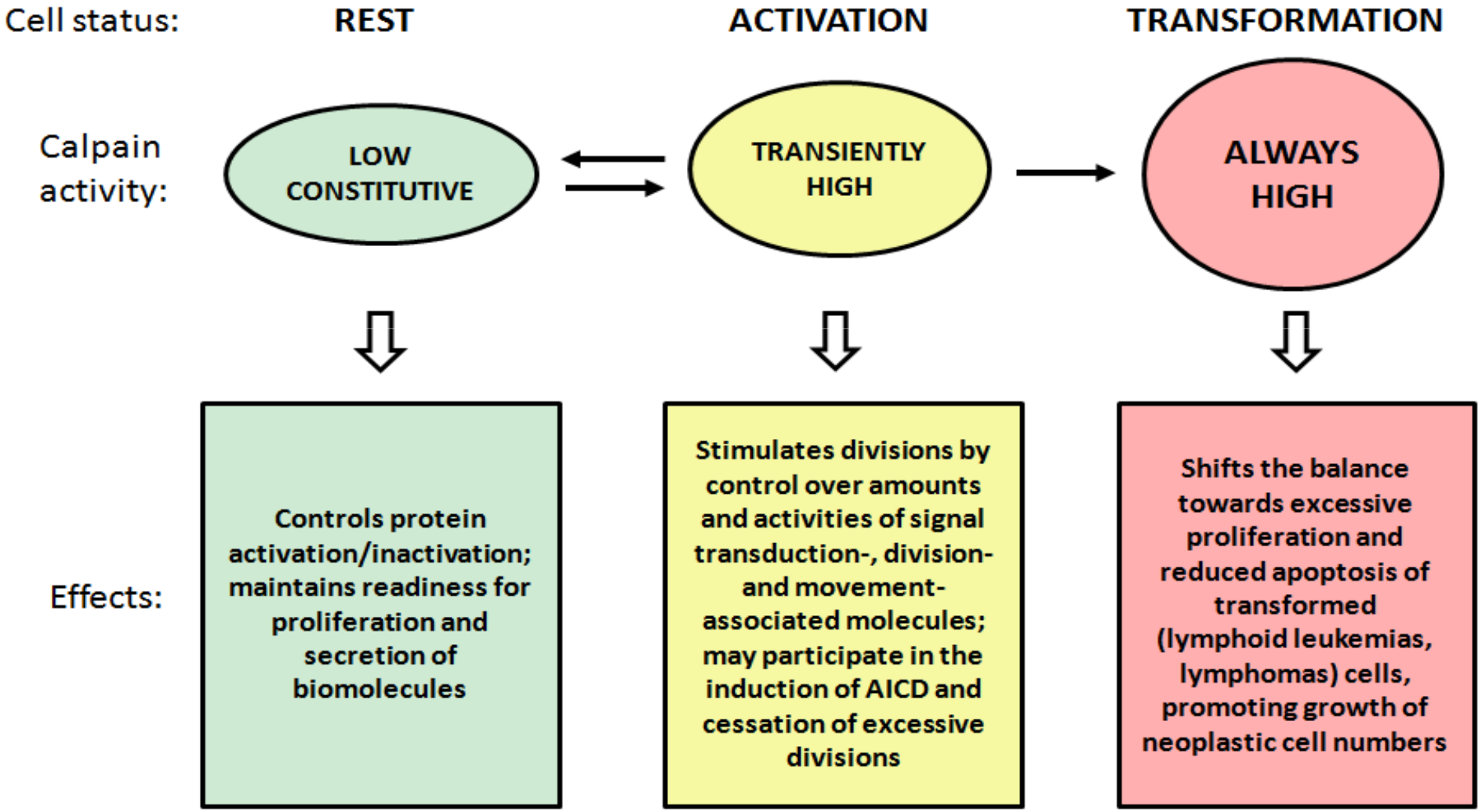


Mikosik A, et al., PLoS One. 2015 Aug 28;10(8):e0136615. doi: 10.1371/journal.pone.0136615

Calpain inhibitor treatment reduces mitochondrial electronegativity → early marker of apoptosis and reduced mitochondria function

Summary: the role of calpains in lymphocytes

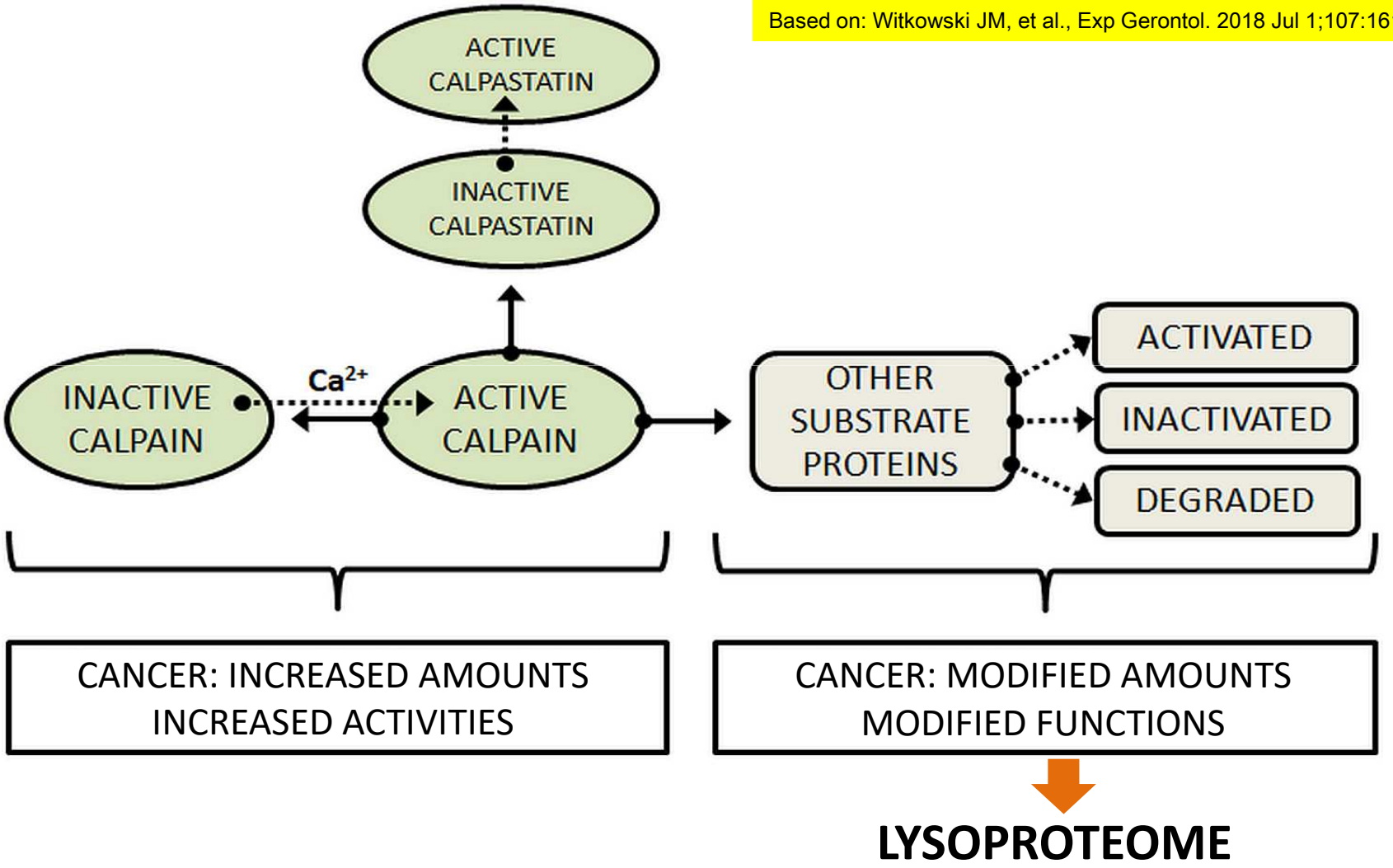
Witkowski J.M. et al.,Calpain-Calpastatin System in Lymphoid Neoplasm of the Aged.
In: Extermann M. (eds) Geriatric Oncology. Springer, 2018

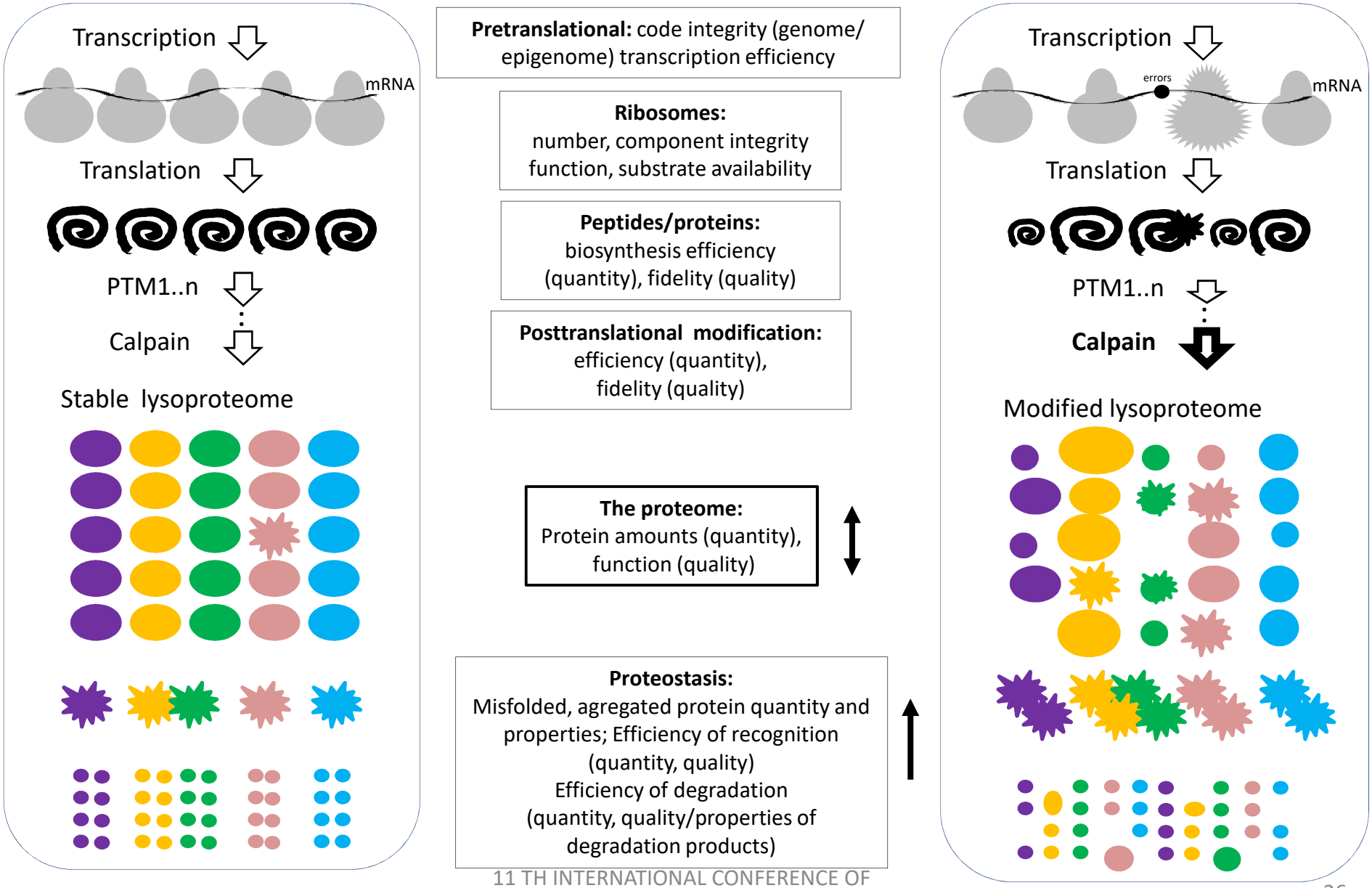


HYPOTHESIS:

calpains, cancer, proteodynamics & the lysoproteome

Based on: Witkowski JM, et al., Exp Gerontol. 2018 Jul 1;107:161-168




NORMAL CELL
BIOMARKERS
CANCER CELL
Transcription
mRNA
Translation
PTM1..n
Calpain
Stable lysoproteome
Pretranslational: code integrity (genome/ epigenome) transcription efficiency
Ribosomes: number, component integrity function, substrate availability
Peptides/proteins: biosynthesis efficiency (quantity), fidelity (quality)
Posttranslational modification: efficiency (quantity), fidelity (quality)
The proteome: Protein amounts (quantity), function (quality)
Proteostasis: Misfolded, agregated protein quantity and properties; Efficiency of recognition (quantity, quality) Efficiency of degradation (quantity, quality/properties of degradation products)
Transcription
errors
mRNA
Translation
PTM1..n
Calpain
Modified lysoproteome

Conclusions 2

- Limited, modifying proteolysis is ubiquitous and necessary in lymphoid cells, where it controls proliferation, secretion and apoptosis
- In lymphoid leukemias increased amounts and/or activities of calpains act pro-survival and anti-apoptotic, making calpains an interesting potential therapeutic target
- Apart from some data on increased calpain activities in lymphomas, not much is (yet?) known about calpain activities in other malignancies; this calls for thorough studies, which may lead to new biomarkers and therapeutic targets

Whodunnit?

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