

MHC class I molecules and Antigen presentation

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Research In Dolan Lab

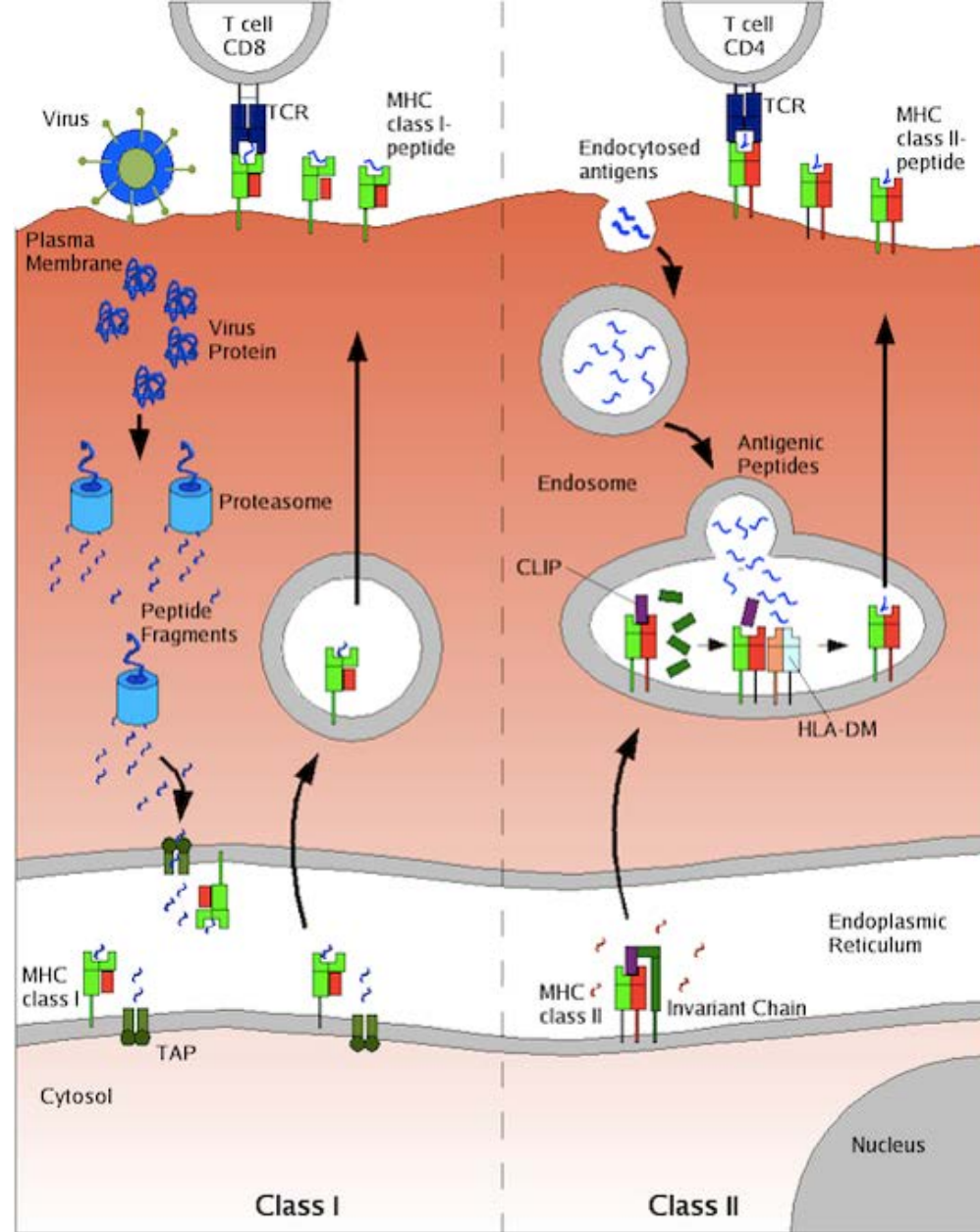
Adaptive Immune System



Major Histocompatibility Complex (MHC)



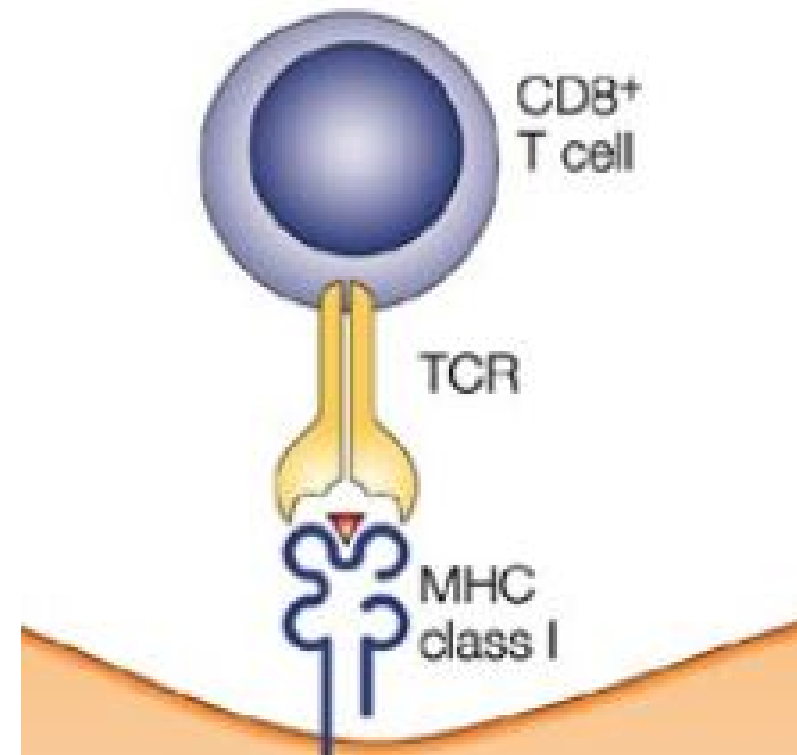
MHC Class I Antigen Presentation Pathway



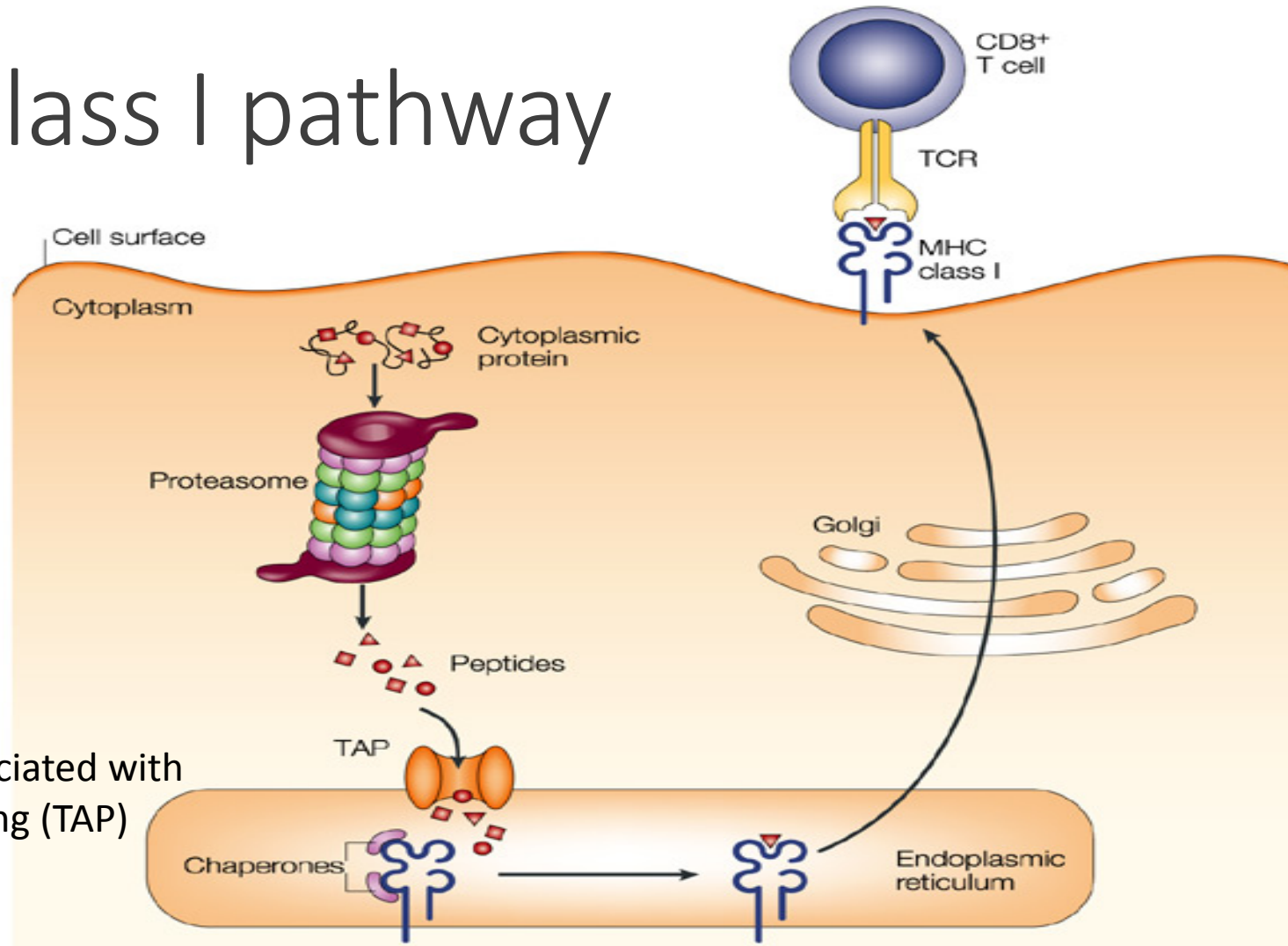
Understanding Antigen presentation

MHC Class I molecules:

- Associated with all nucleated cell type in the body
- Bind antigens that are generated within a cell “Endogenous antigens”
- Present to cytotoxic T cell lymphocytes
- recognized by **CD8⁺ T cells**.



MHC class I pathway



Transporter associated with antigen processing (TAP)

Why we care?

Understanding will allow us to better utilize and modify the system to our needs.

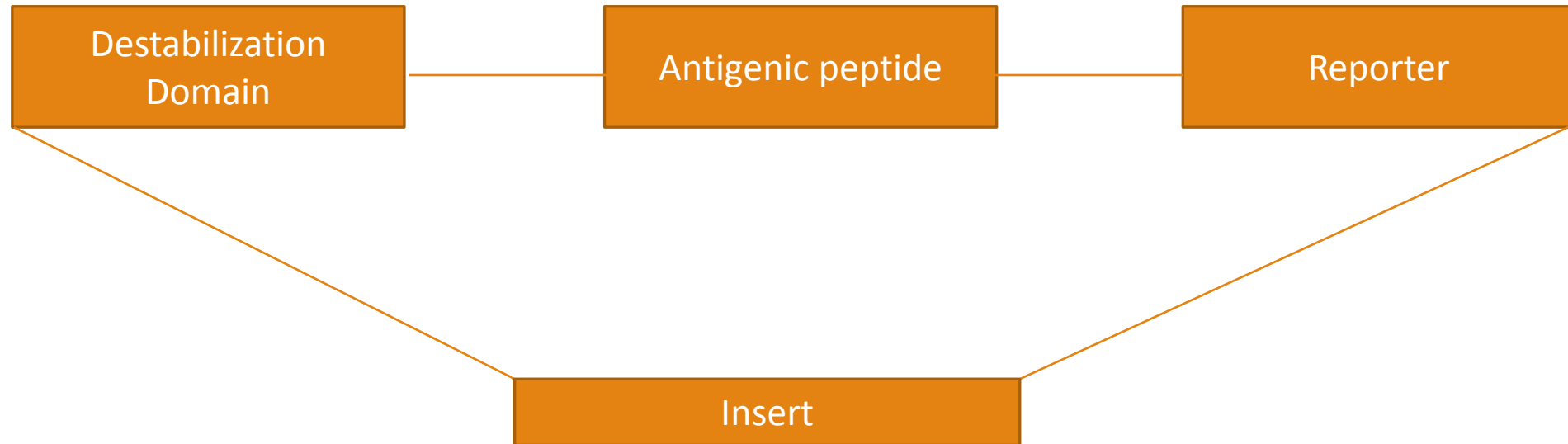
- Viral Infection, oncogenesis



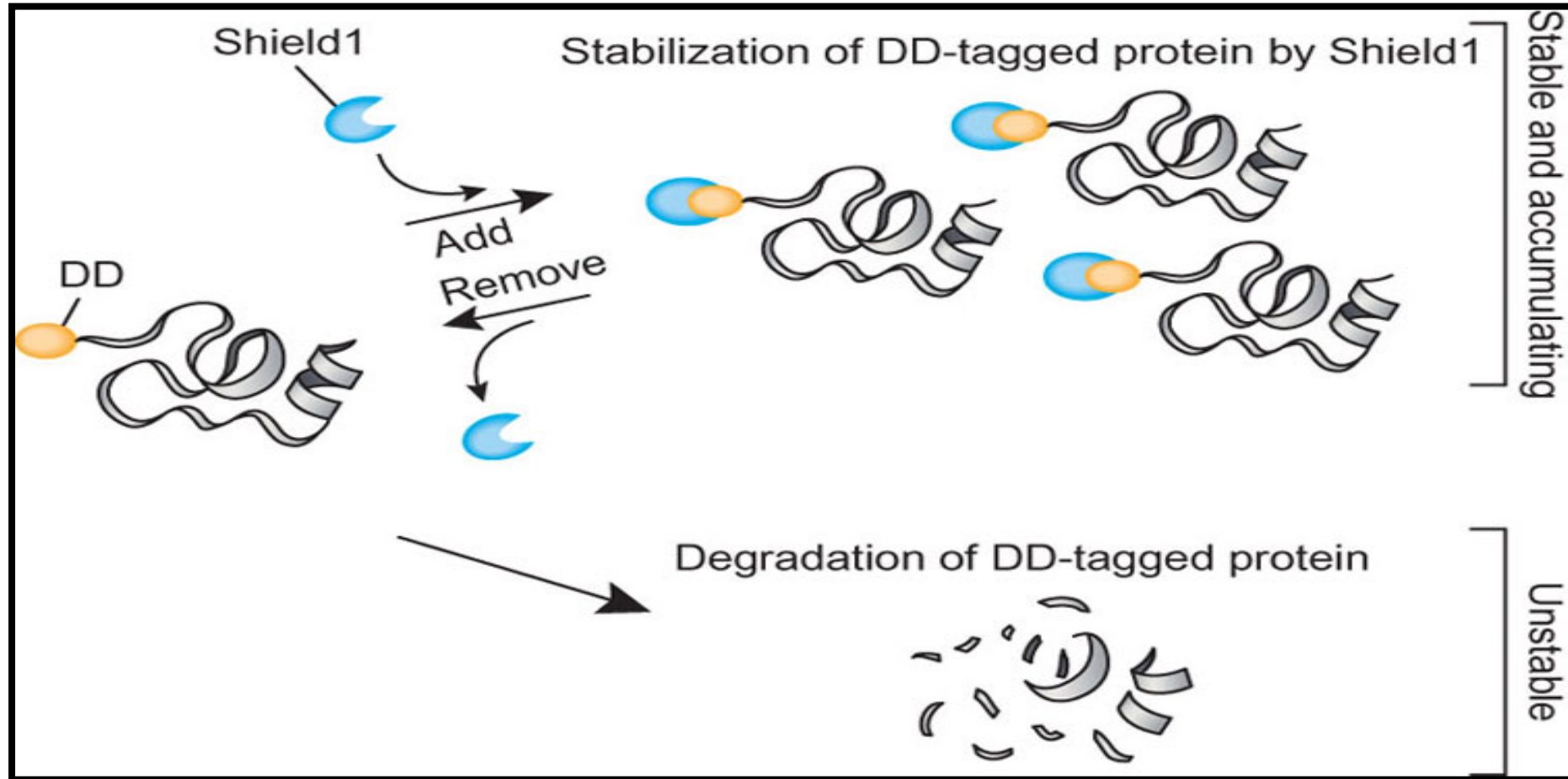
- Autoimmune disease



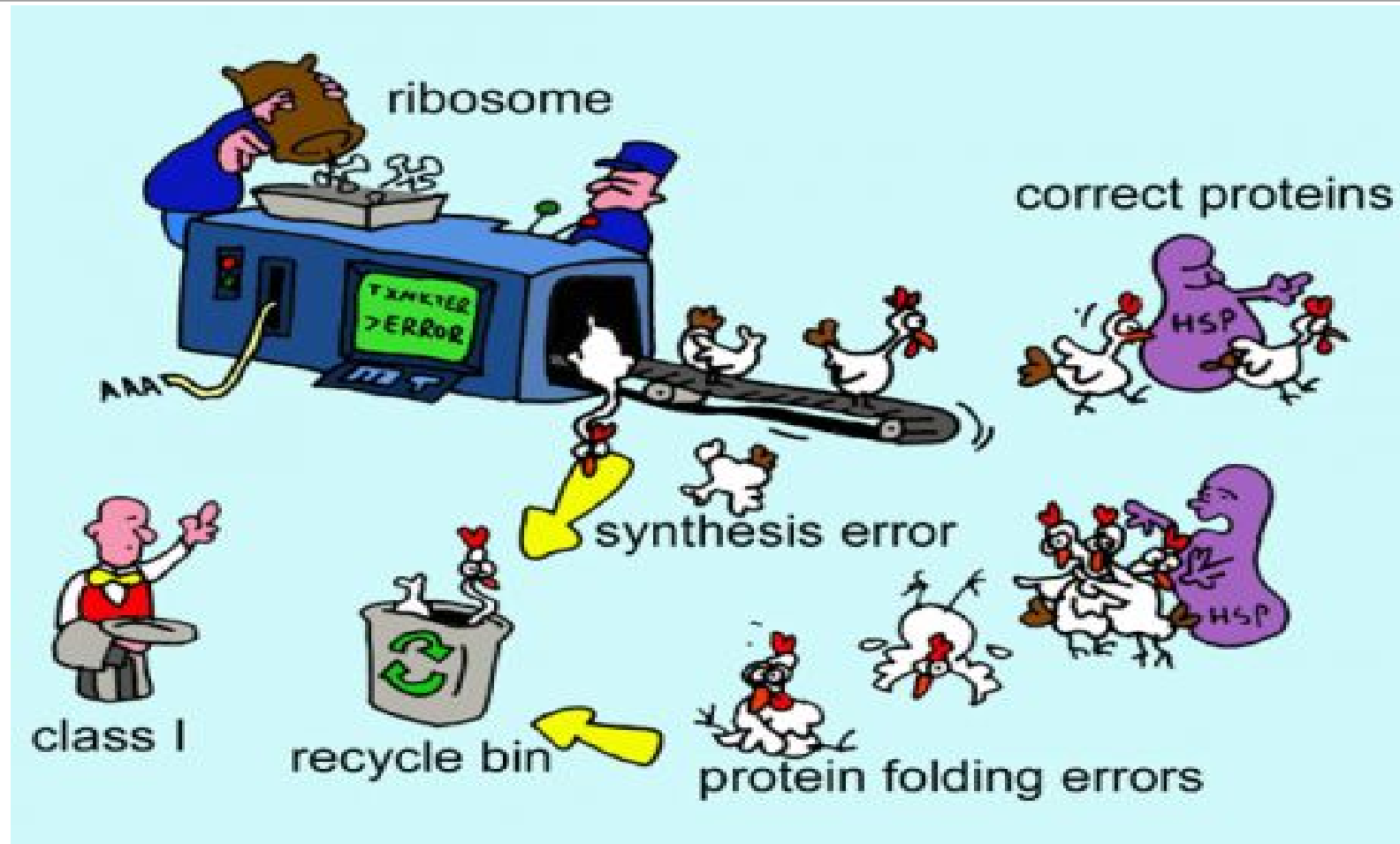
Three domain Model



Protein Stability

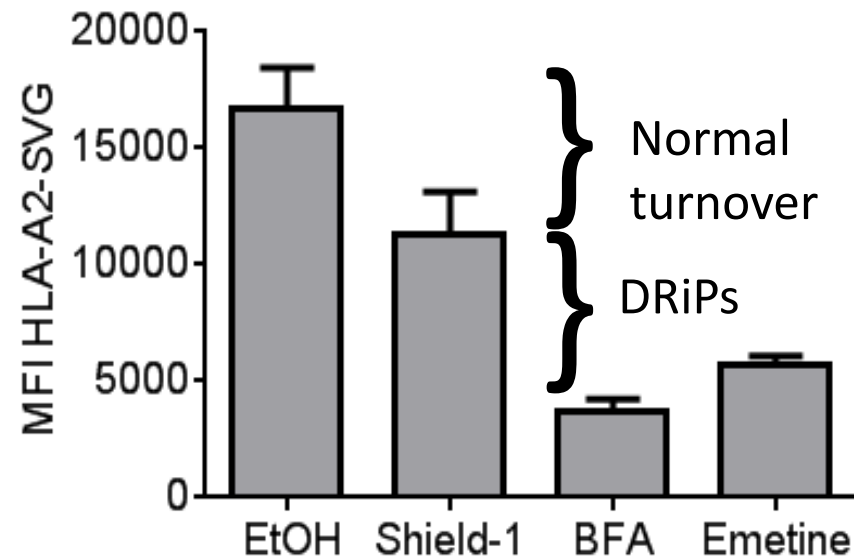


Defective ribosomal products or DRiPs



Studying DRiPs

- Restrict antigen presentation to → DRiPs only

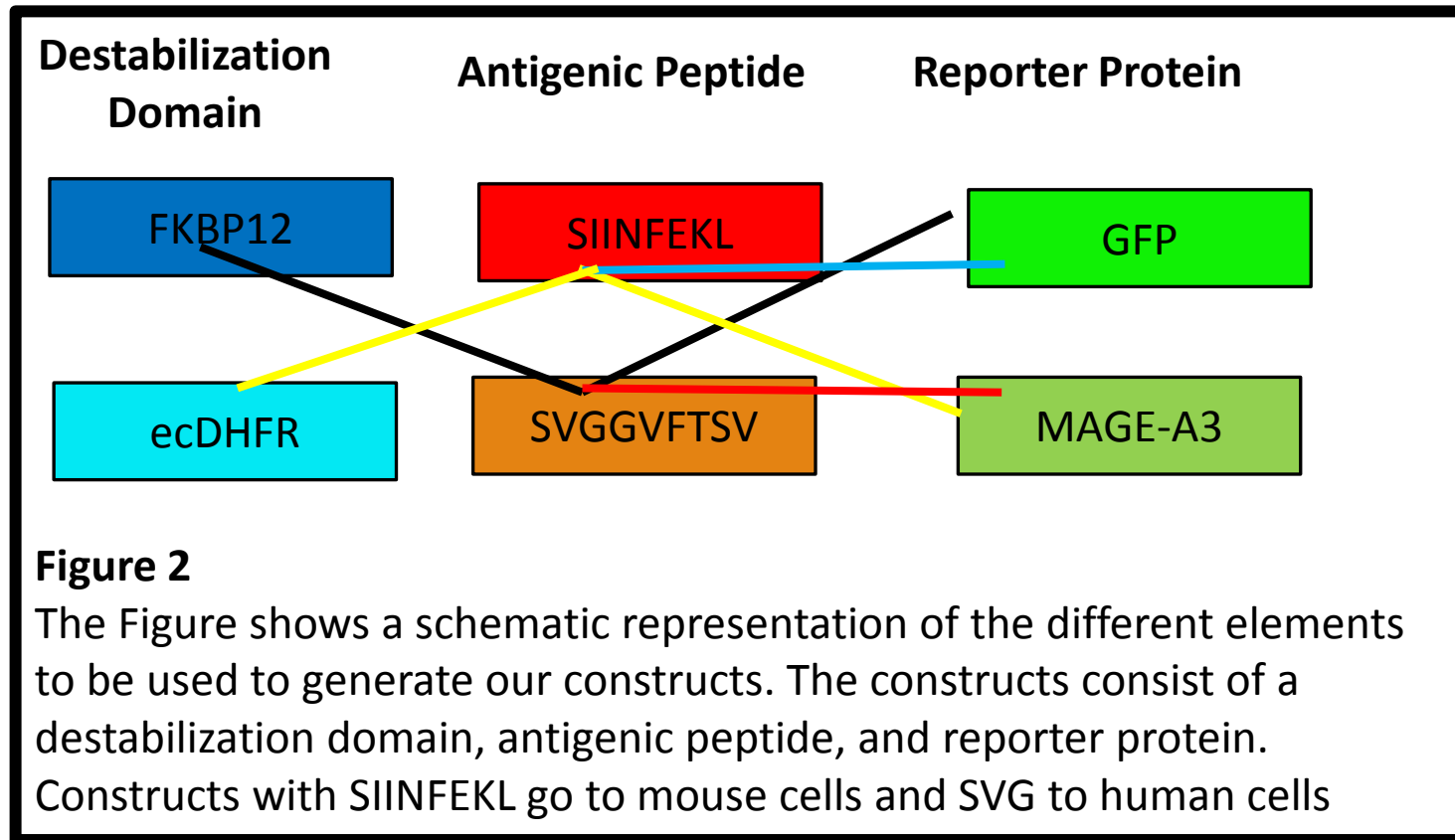


Latest Discoveries

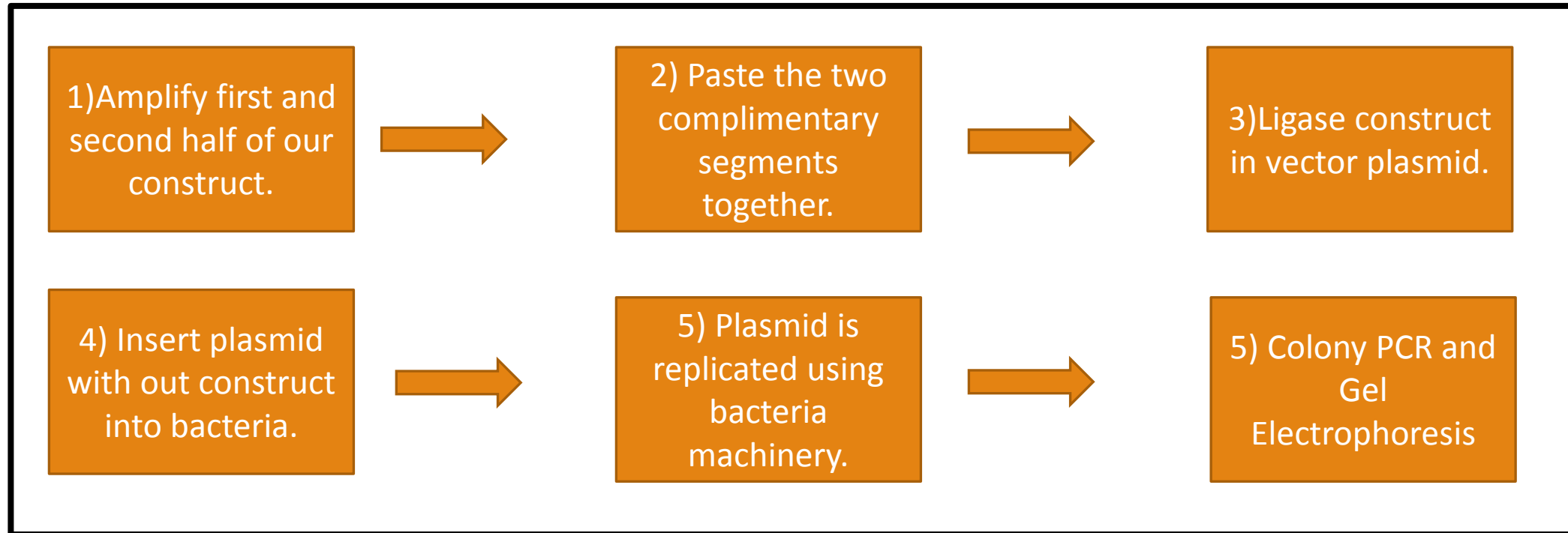
- Certain compounds diminish DRiPs presentation (eer1, CLPGA 2)
- Inhibition of the protein p97 had no effect.
- Chlamydia Infection increased DRiP synthesis and presentation
- Inhibition of USP14 decreased DRiP synthesis and presentation

SUCH DISCOVERIES MIGHT BE LIMITED TO OUR CURRENT MODEL!

Model Protein



Creating constructs: A General Outline



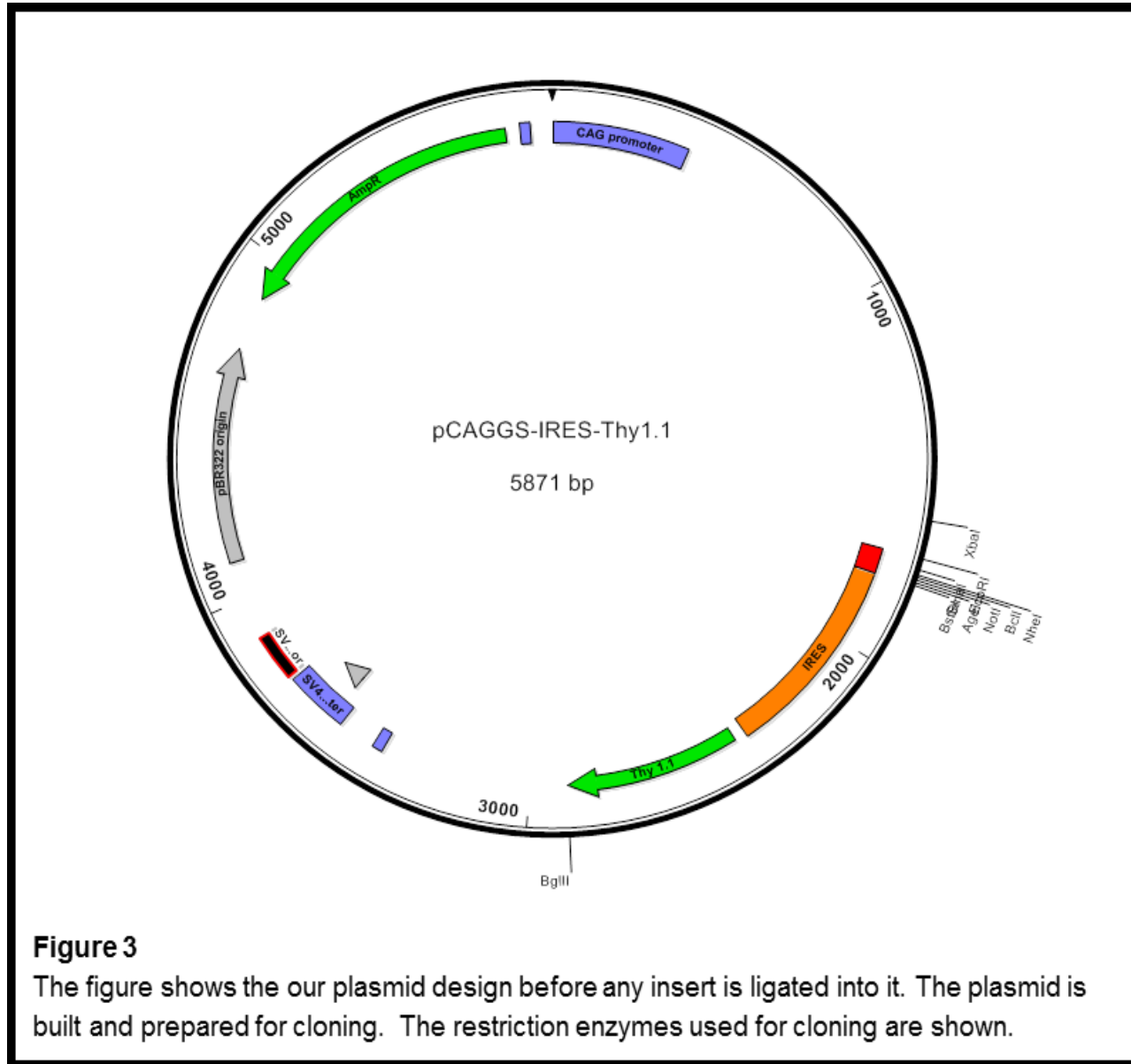


Figure 3

The figure shows the our plasmid design before any insert is ligated into it. The plasmid is built and prepared for cloning. The restriction enzymes used for cloning are shown.

Model Constructs

Destabilization domain-Antigenic Peptide- Reporter protein

FKBP12-SIINFEKL-MAGE A3

ecDHFR-SIINFEKL-MAGE A3

FKBP12-SVGGVFTSV-MAGE A3

ecDHFR-SVGGVFTSV-MAGE A3

Constructs made then what?

- Measure antigen presentation
- Determining the number of peptide–MHC class I complexes on the cell surface
- Measure the MHC class I complexes expressed by cell type and compare that to the number of T cells present and/or their activity rates
- Quantify the levels of full-length proteins by quantitative Western blotting
- Measure efficiency of antigen presentation =
$$\frac{\# \text{ antigens presented}}{\# \text{ proteins}}$$

Western Blot

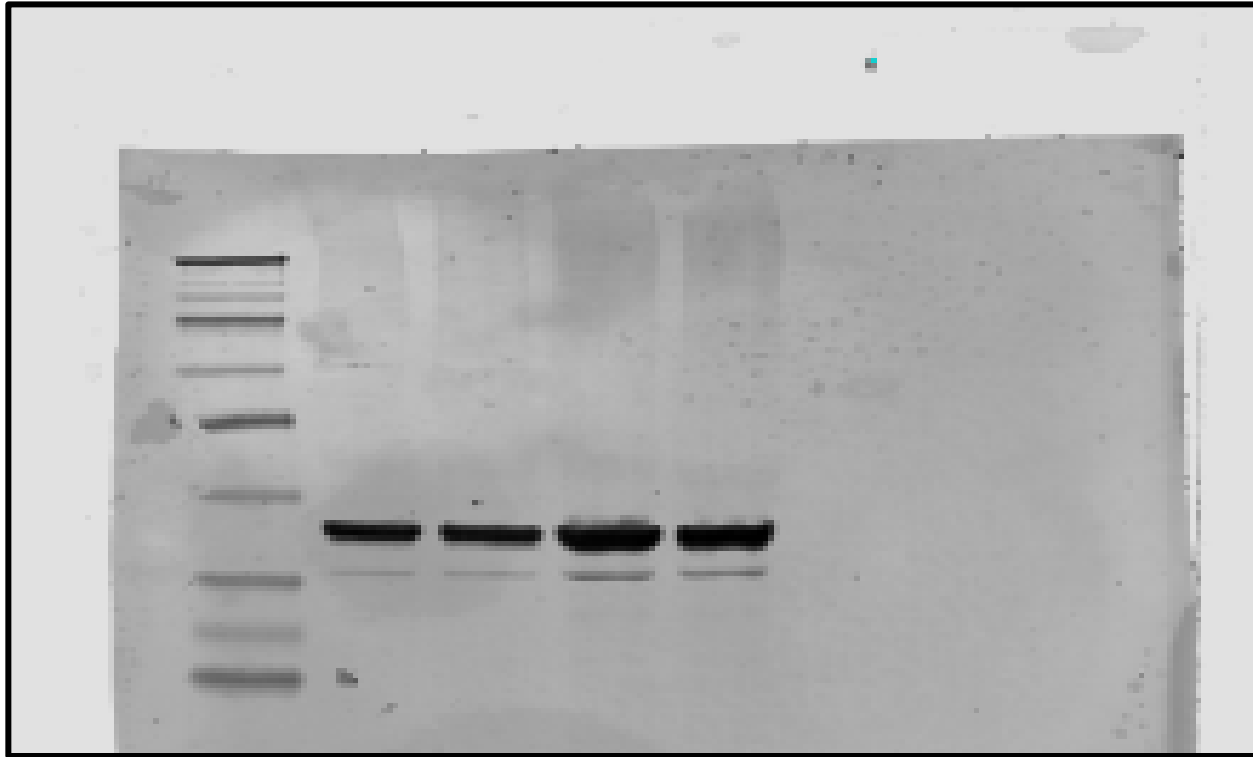


Figure 4

This image depicts western blot of the two different constructs. Starting left to right is our protein ladder, Shld-L construct, EtOH-L, Shld-K, and finally EtOH-K

Acknowledgements

- Brian Dolan
- Ryan Simmons
- Amy Palmer



References:

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Questions/Discussion
