Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice

Villeda SA et al. Nat Med. 2014 Jun;20(6):659-63.

Ines Ana EDERER, JC 12.10.2015

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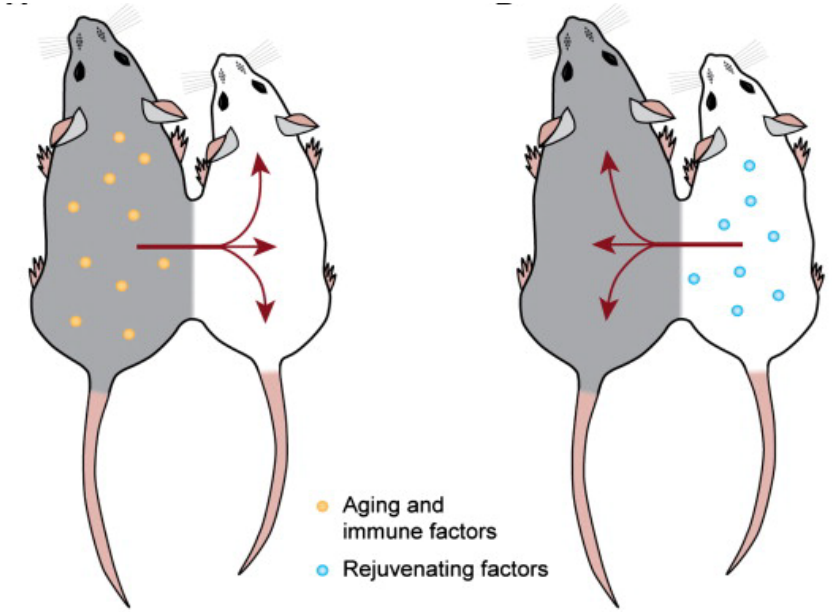
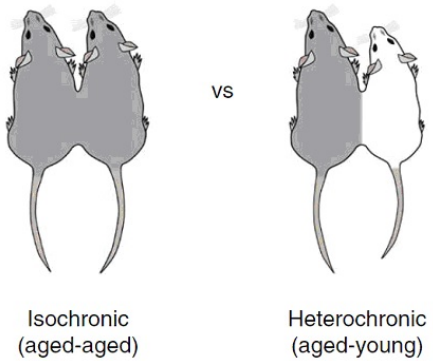
Introduction

- What are heterochronic parabionts?





Aging

- Decreased neurogenesis
- Impaired synaptic plasticity
- Impaired cognition

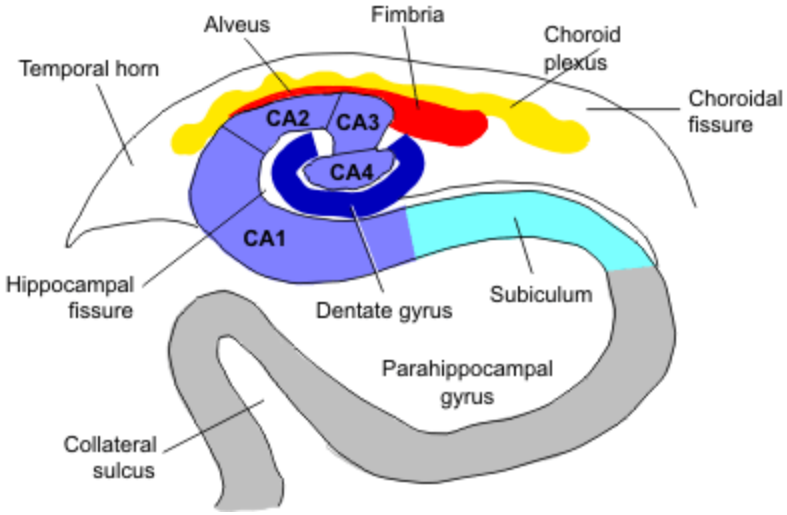
Rejuvenation

- Increased neurogenesis
- Unknown effect on synaptic plasticity?
- Unknown effect on cognition?

Introduction

Hippocampus - special vulnerability to aging

- downregulation of plasticity-related genes
- reduced spine density
- decreased synaptic plasticity
- impairments in associated cognitive functions



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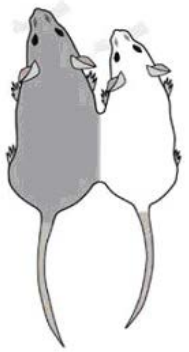
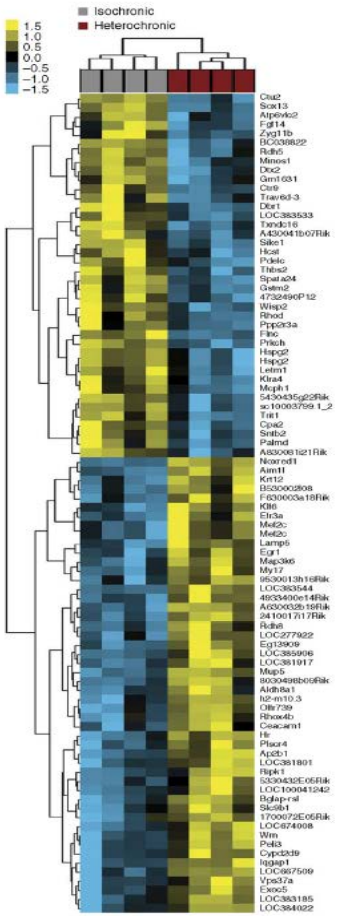
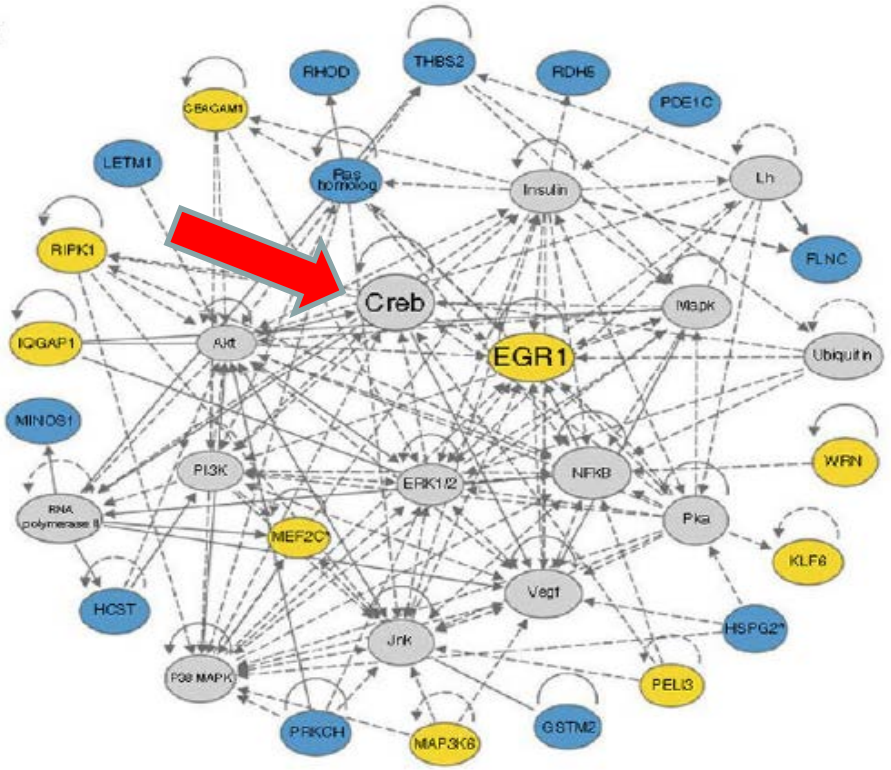
I. Molecular, structural and functional changes



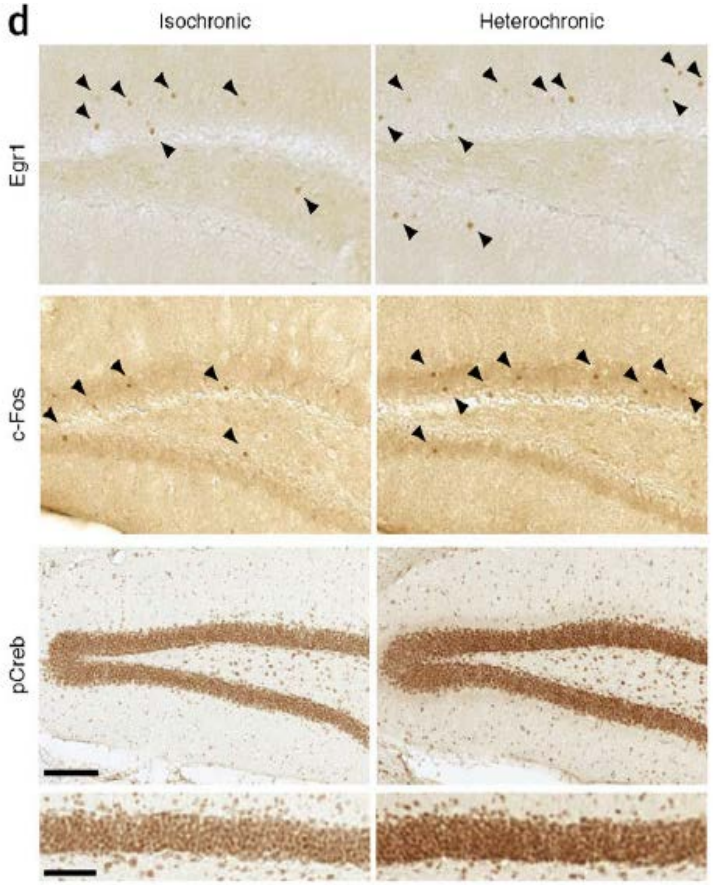
Genome-wide microarray analysis

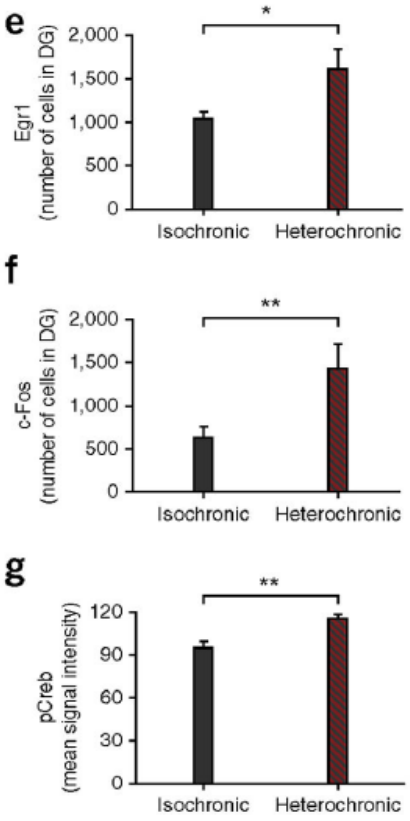




Biological pathways involved in synaptic plasticity using IPA software based on differentially expressed genes in isochronic and heterochronic parabionts.

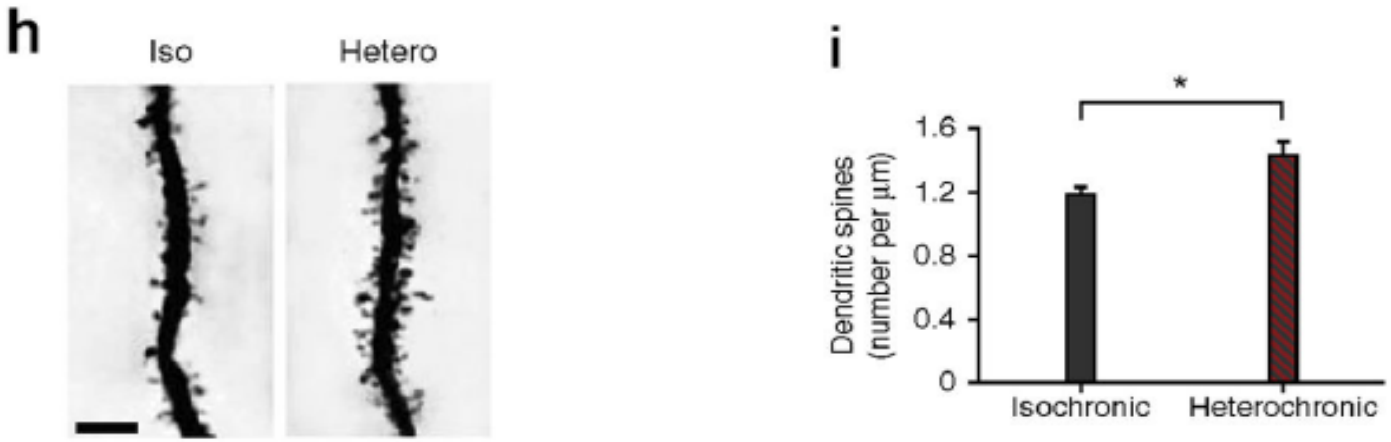
Immunohistochemical analysis of Egr1, c-Fos, and pCreb in the DG



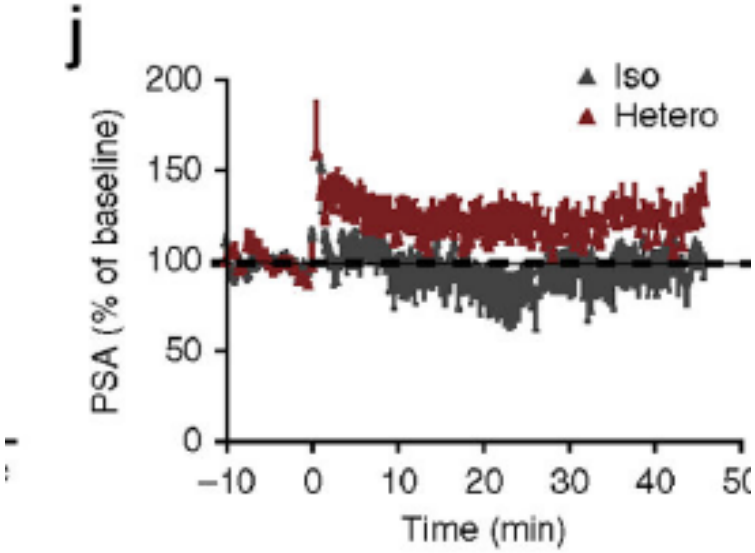


Quantification of immunostaining

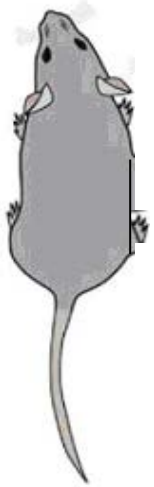
Golgi stain image and quantification of dentritic spine density in granule cell neurons in the DG



Extracellular population spike amplitude (PSA) recorded from the DG of aged parabionts – representative LTP levels for isochronic and heterochronic parabionts



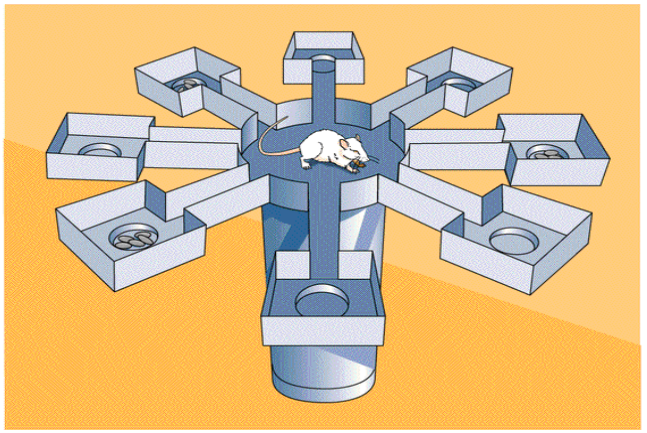
II. Cognitive changes



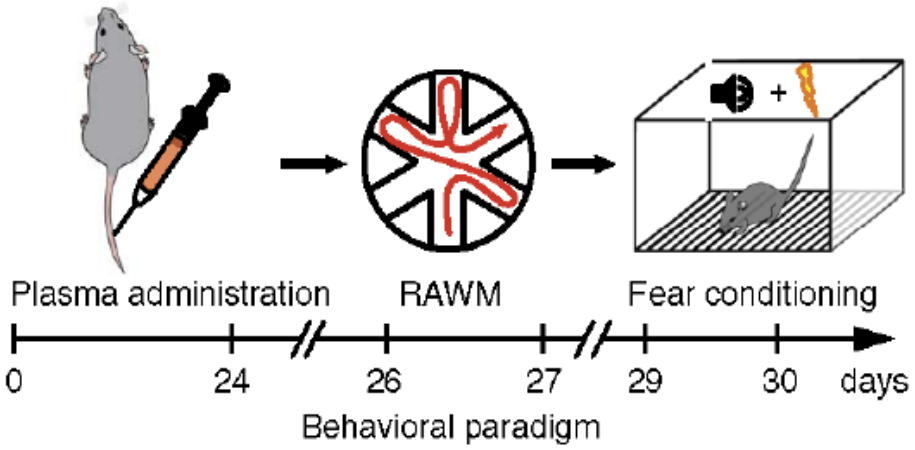
Testing hippocampal-dependent cognitive functions:

1. RAWM – spatial learning and memory

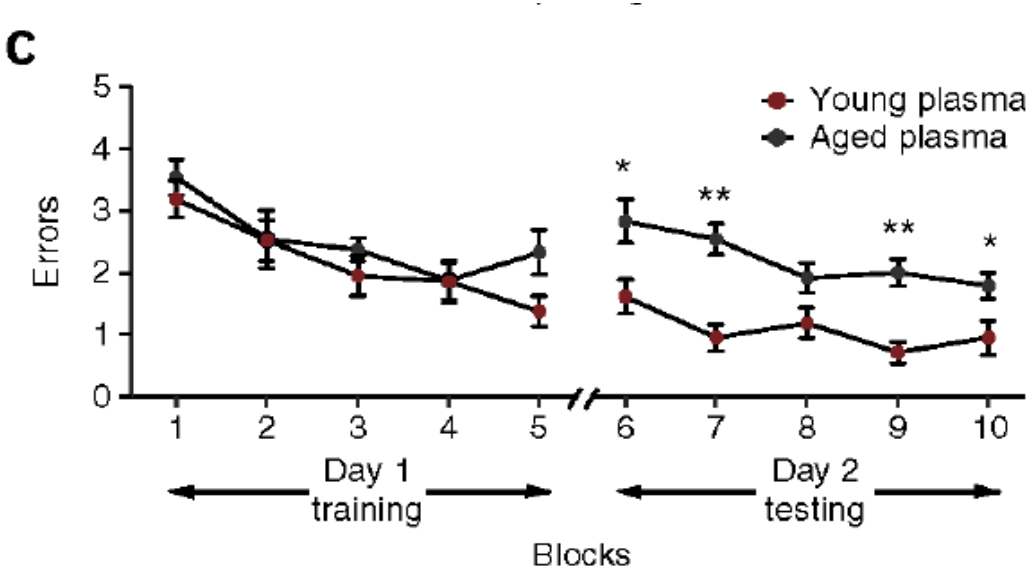
2. Contextual fear conditioning



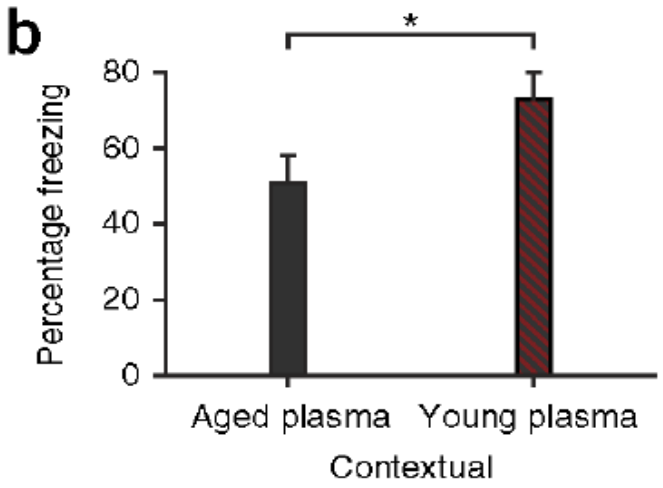
Administration of young blood plasma in aged mice (18 months), n=8/group.



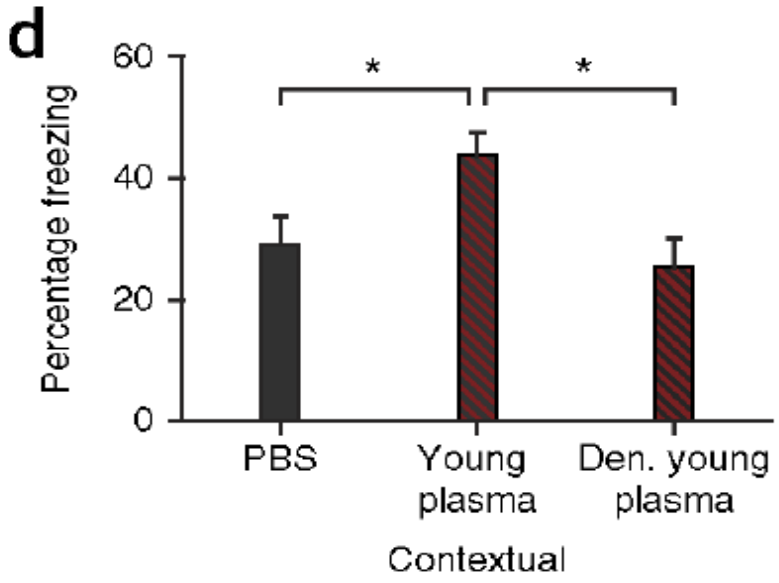
Aged mice given young plasma exhibit enhanced learning and memory for hidden platform location during testing phase.



Mice receiving young plasma demonstrate increased freezing in contextual memory testing.

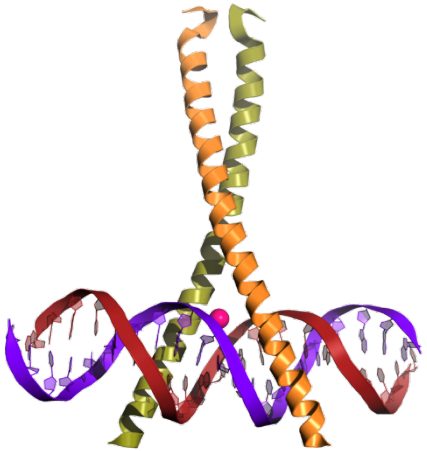


Additional fear-conditioning experiment: saline, young plasma or heat-denatured young plasma to aged animals



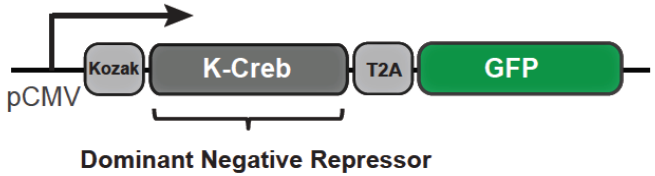
Rejuvenation through heat-labile factors?!

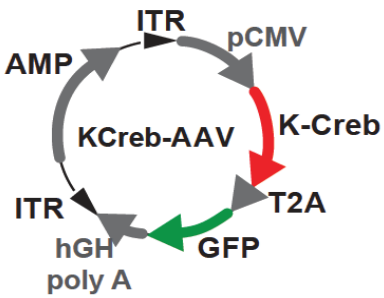
III. Creb signaling

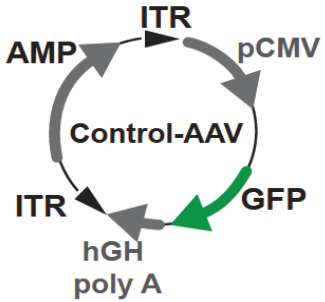


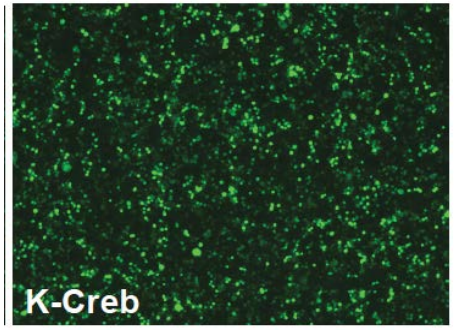
IIIa.

Adult mice infected with AAVs encoding K-Creb in tandem with GFP.

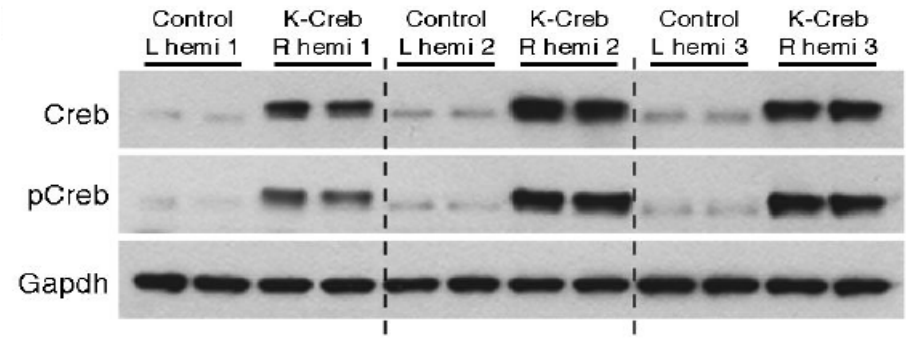


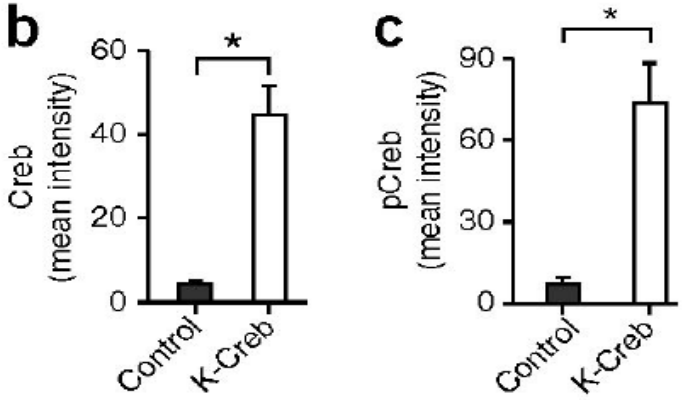






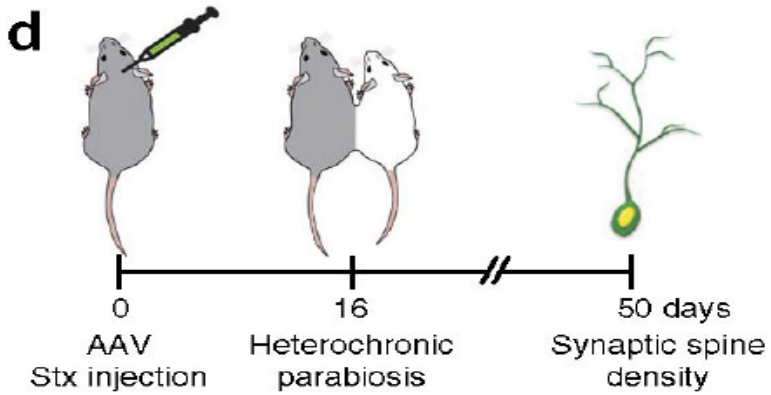
Western blot analysis of K-Creb overexpression in isolated hippocampi

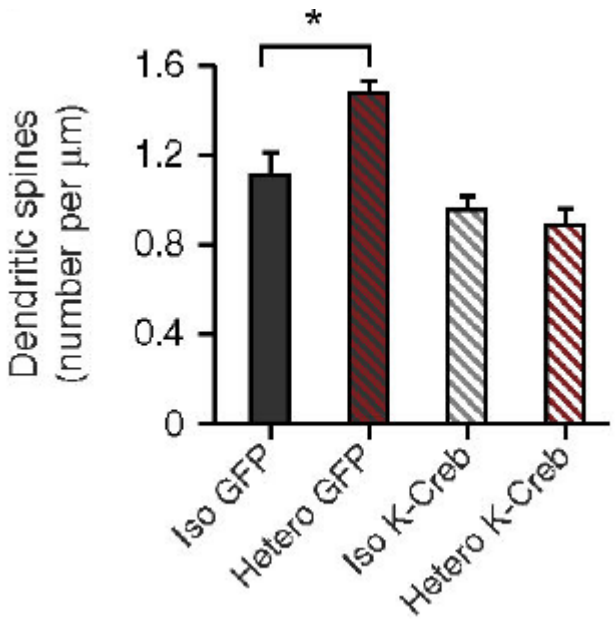




IIIb.

Dendritic spine density in DG assessed by AAV-mediated neuronal tracing

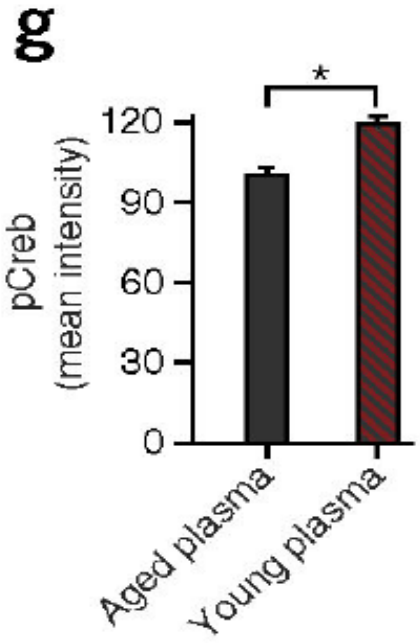


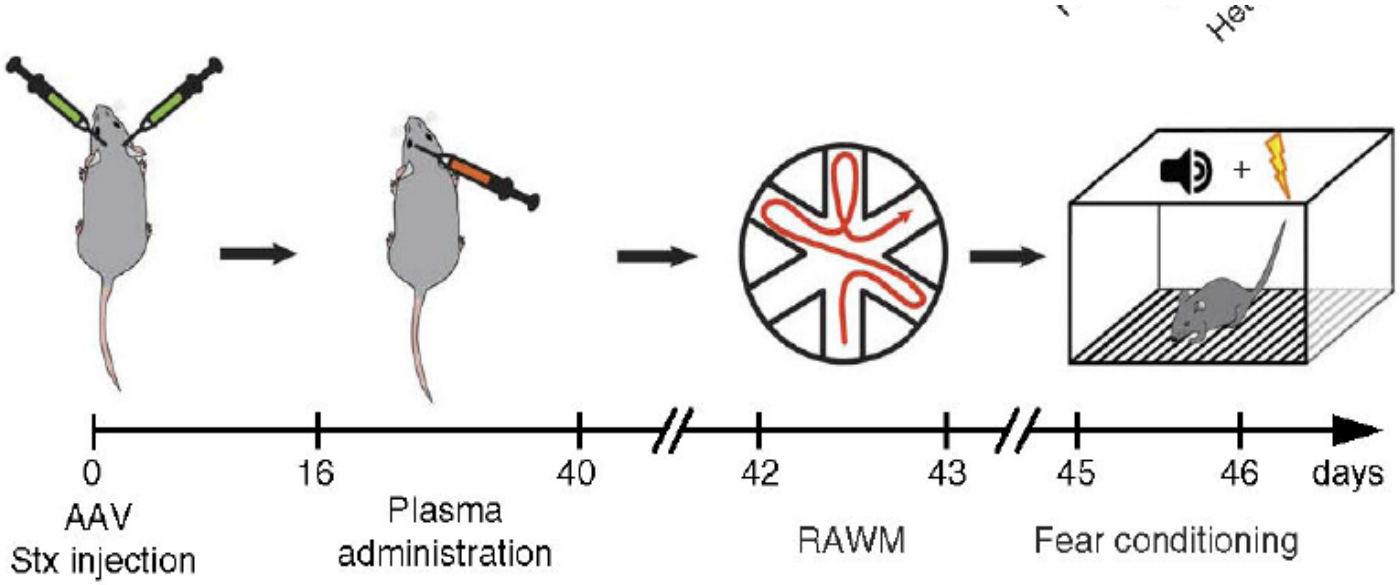


Confirmation of results through shRNAs in N2A neuronal cell line and in the hippocampi of adult mice: Consistent with previous data.

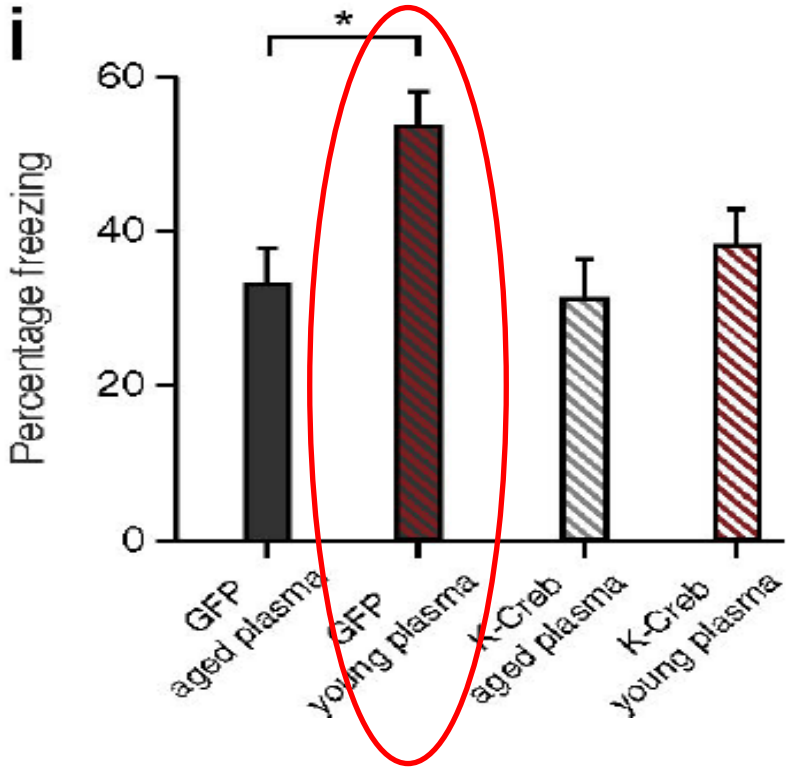
IIIc.

Phosphorylated Creb in the DG of aged animals

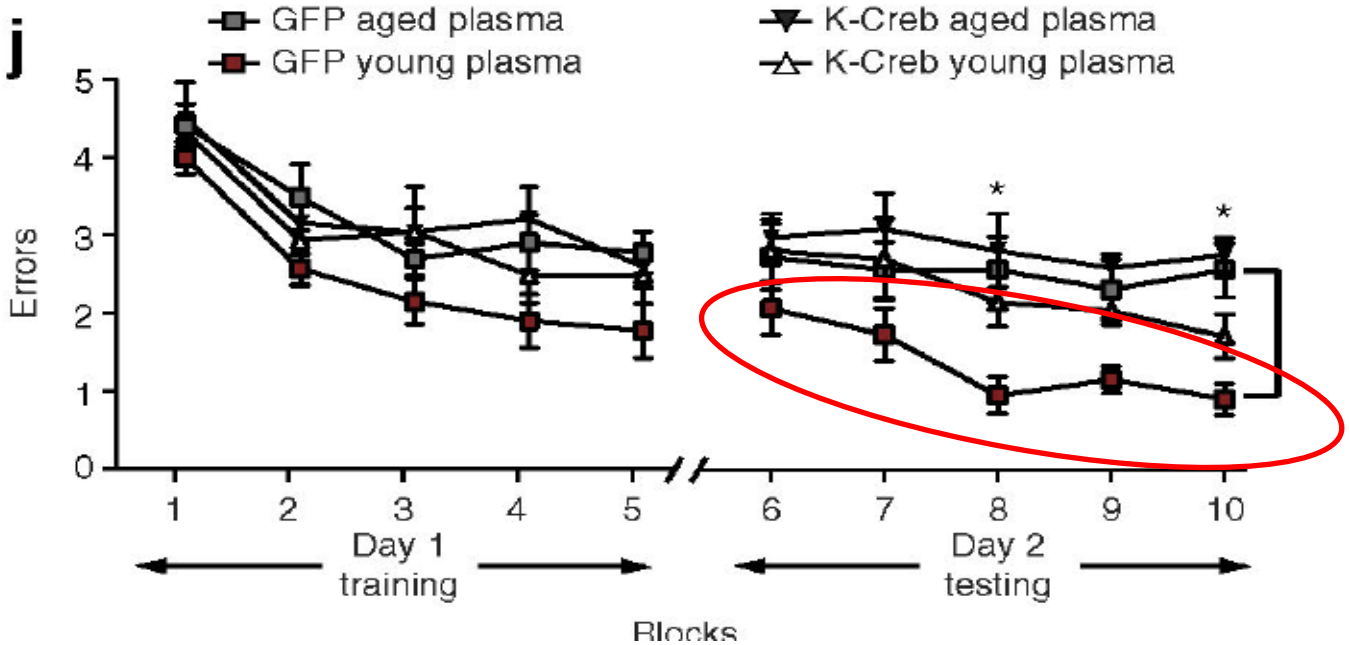


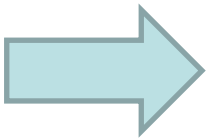

0
16
40
42
43
45
46
days
AAV
Stx injection
Plasma
administration
RAWM
Fear conditioning

Contextual fear conditioning after plasma treatment



RAWM after plasma treatment





cognitive improvements after administration of young plasma are partly mediated by Creb.

Summary

- Exposure of aged animals to young blood can counteract effects of brain-aging at the molecular, structural and cognitive level.
- Heterchronic parabiosis enhances dendritic spine density and synaptic plasticity in aged hippocampus and elicits a plasticity-related expression profile.
- Administration of young blood plasma improves hippocampal dependent cognitive functions such as spatial learning and memory.
- Creb is one member of the regulatory network underlying cognitive and structural enhancements in the aged hippocampus.

Comment/Criticism

- cued memory testing vs contextual memory testing (p 9)
- “pro-aging” vs “pro-youthful” factors (p.5)
- conflicting conclusion (p.3 Golgi, LTP)
- reproducibility (p.5)

- popular topic and complex (!) methods (many data not published – 19 pages of supplementary tables)
- transferability of results into human being – PRP?!

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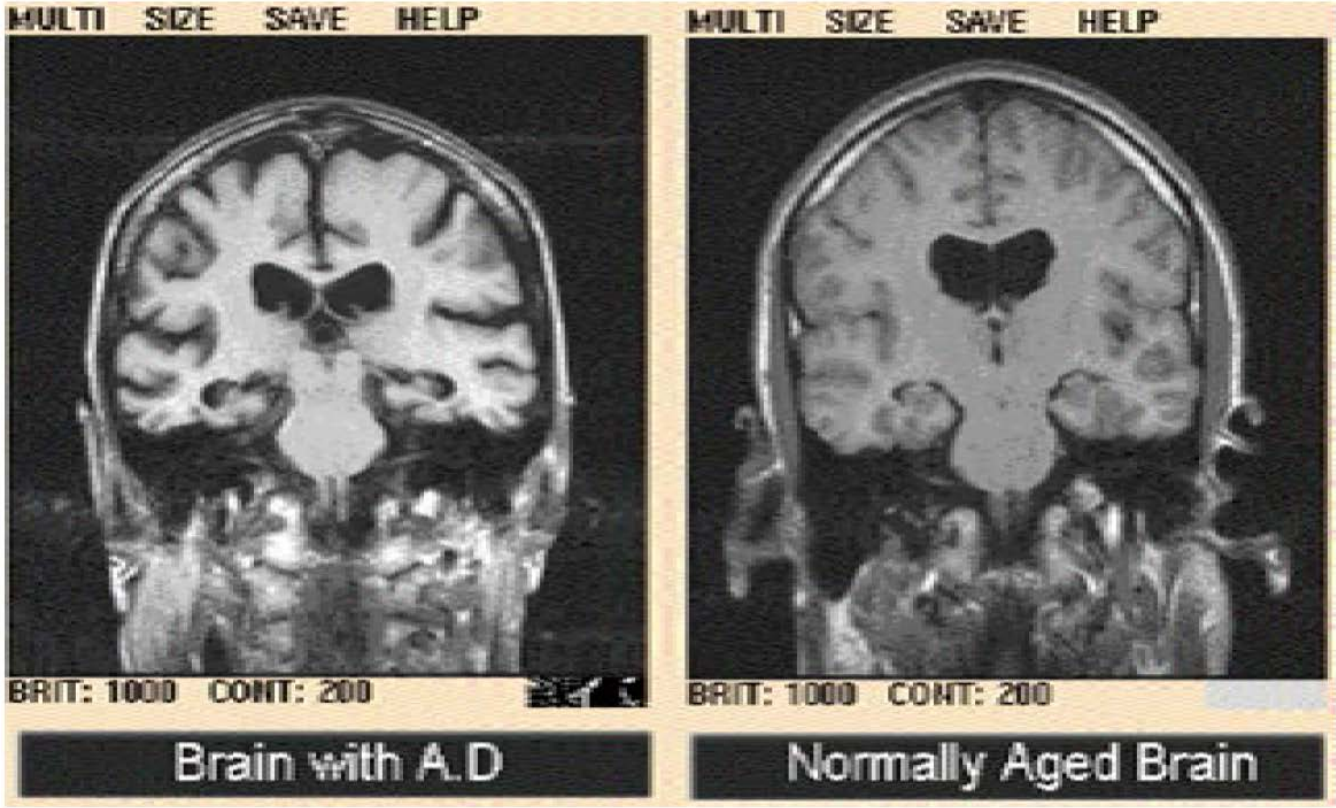
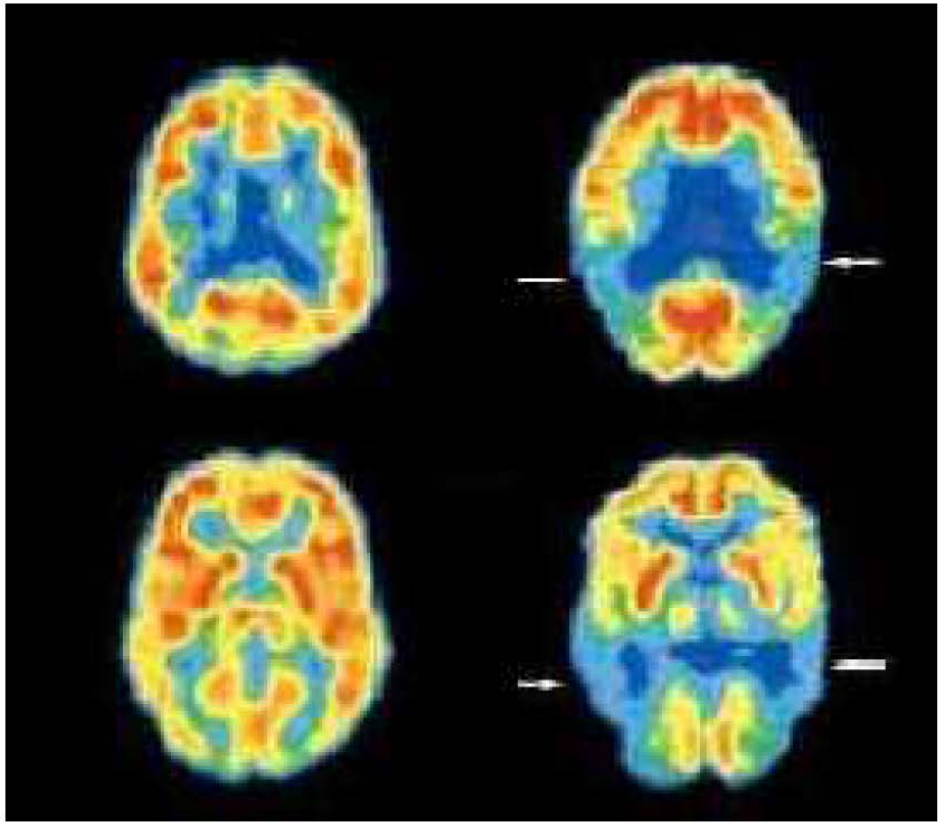
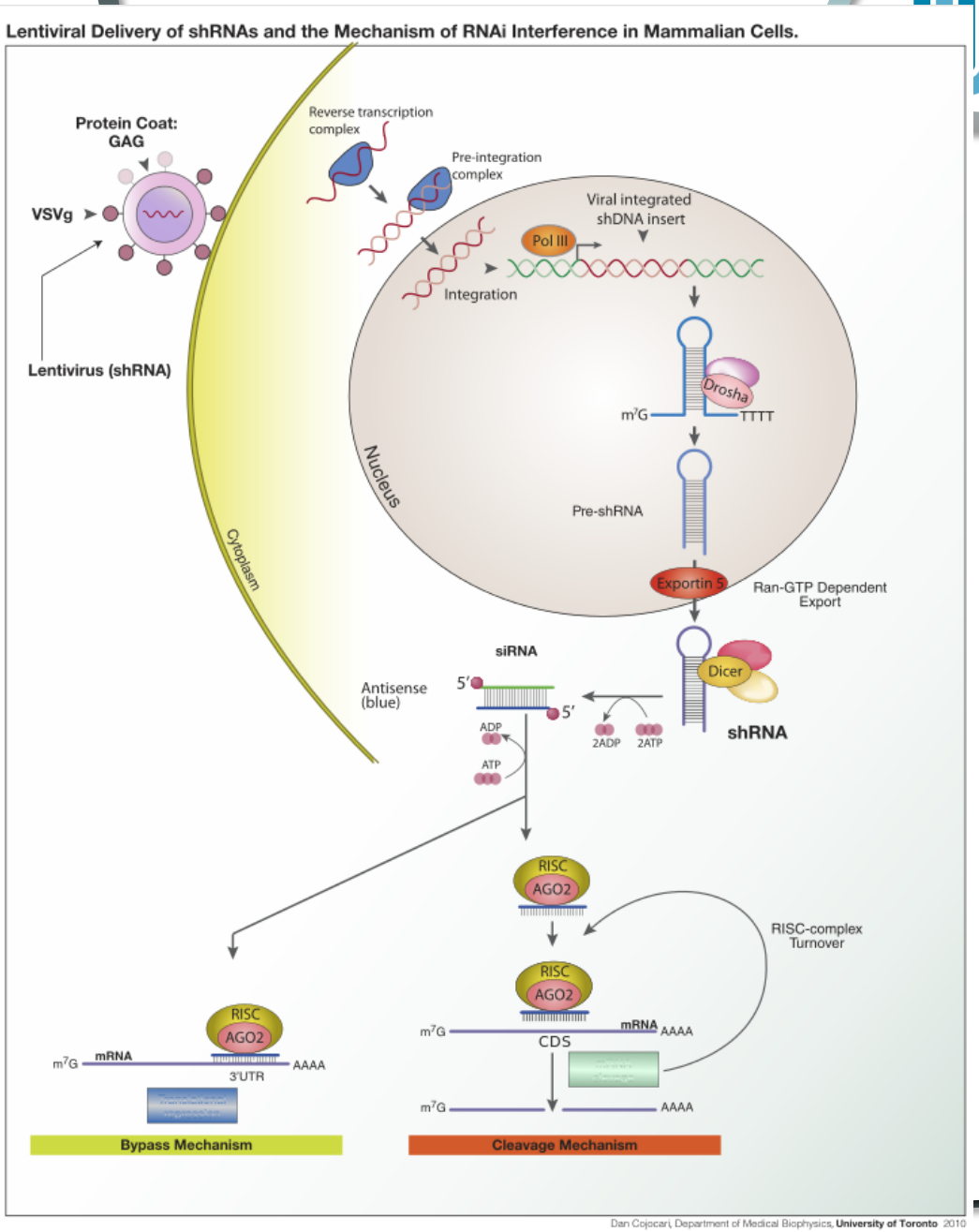
Thank you for your attention!

MRT


MULTI SIZE SAVE HELP
BRIT: 1000 CONT: 200
Brain with A.D
MULTI SIZE SAVE HELP
BRIT: 1000 CONT: 200
Normally Aged Brain

PET




Lentiviral Delivery of shRNAs and the Mechanism of RNAi Interference in Mammalian Cells.
Protein Coat: GAG
VSVg
Lentivirus (shRNA)
Reverse transcription complex
Pre-integration complex
Integration
Viral integrated shDNA insert
Pol III
Nucleus
Cytoplasm
m7G
Drosha
TTTT
Pre-shRNA
Exportin 5
Ran-GTP Dependent Export
Dicer
shRNA
siRNA
Antisense (blue)
5'
ADP
ATP
2ADP
2ATP
RISC
AGO2
RISC-complex Turnover
mRNA
AAAA
3'UTR
CDS
Bypass Mechanism
Cleavage Mechanism
Dan Cojocari, Department of Medical Biophysics, University of Toronto 2010