Nascent Peptide SILAC: A Proteomic Approach to Studying Translational Repression

Jamie Bates
Patrick Brown's lab
Stanford Mass Spectrometry Users' Meeting
August 21 2008

Outline

Introduction to SILAC

How SILAC has been used in the miRNA field

How I intend to use SILAC

Mass Spectrometry is not Quantitative

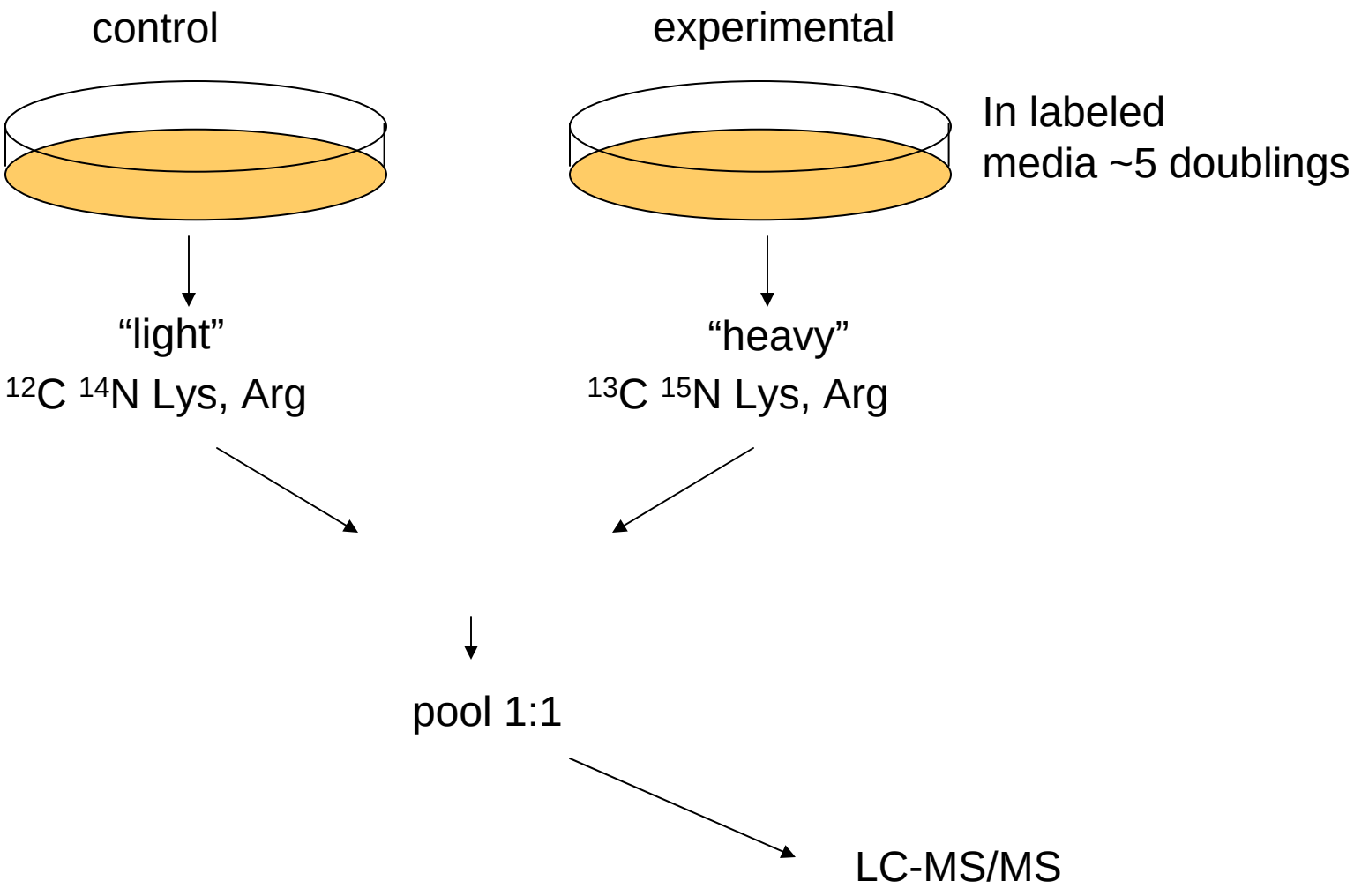
Traditional Mass Spectrometry is inherently not quantitative

- proteins protealize differently
- differ in solubilization
- differ in ability to be ionized

Quantification is necessary to measure changes in protein levels within cells or organelles

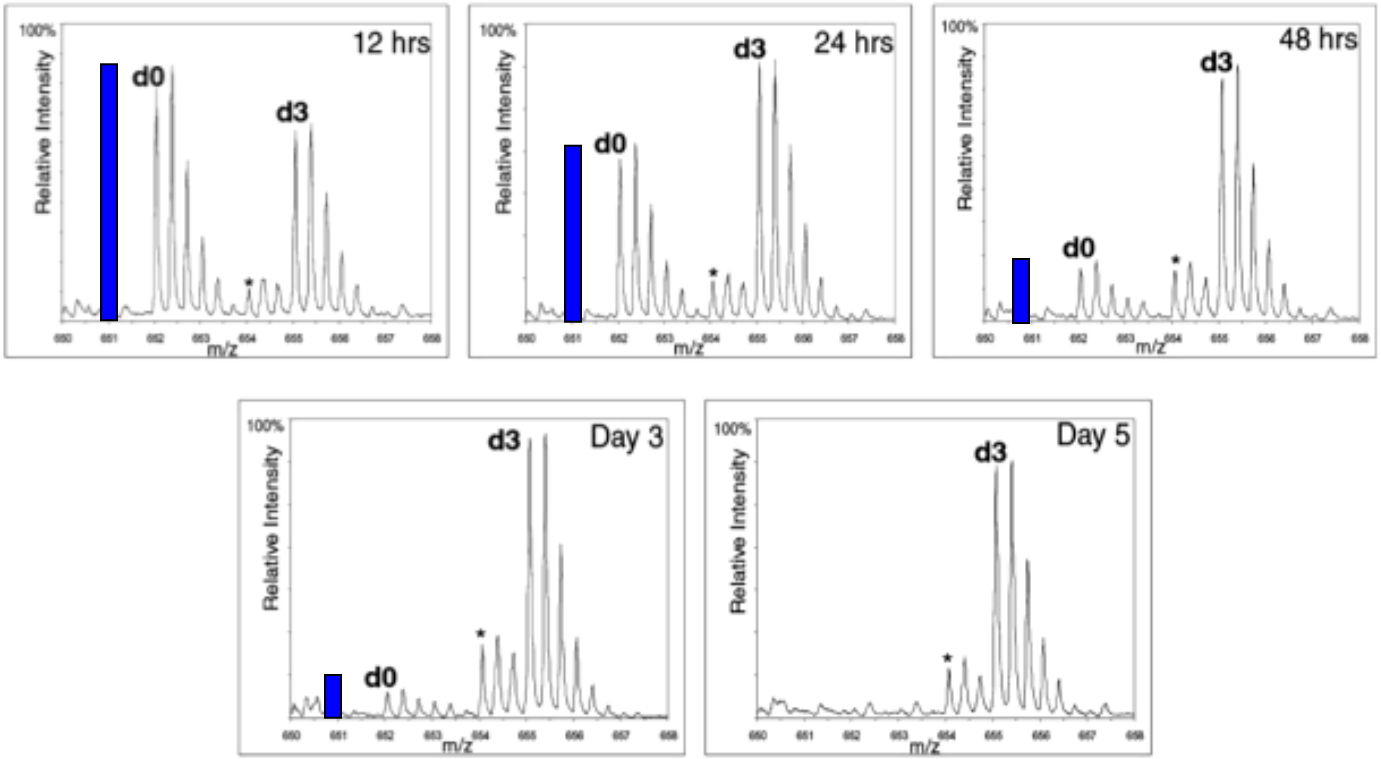
SILAC allows relative quantitation of peptides

SILAC:
Stable Isotope Labeling of Amino Acids in Cell Culture



Matthias Mann (2002) Mol. Cell. Proteomics

SILAC:
Stable Isotope Labeling of Amino Acids in Cell Culture

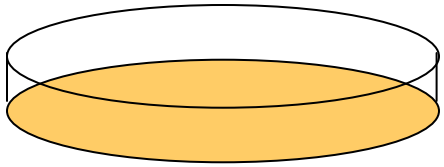


Depends on protein half life and doubling rate of the cells

Ong, S.-E. (2002) Mol. Cell. Proteomics 1: 376-386

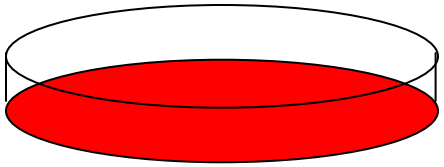
SILAC: For more than one condition

control



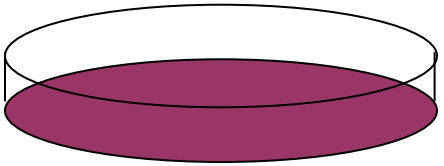
$^{12}C_6$ $^{14}N_4$-Arg (Arg 0)

Experimental 1



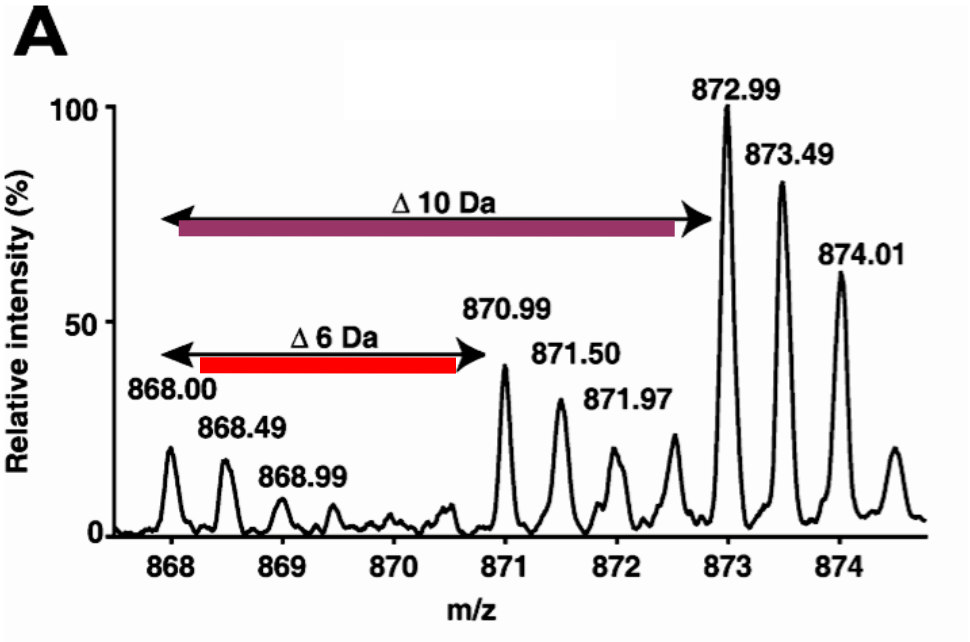
$^{13}C_6$ $^{14}N_4$-Arg (Arg 6)

Experimental 2

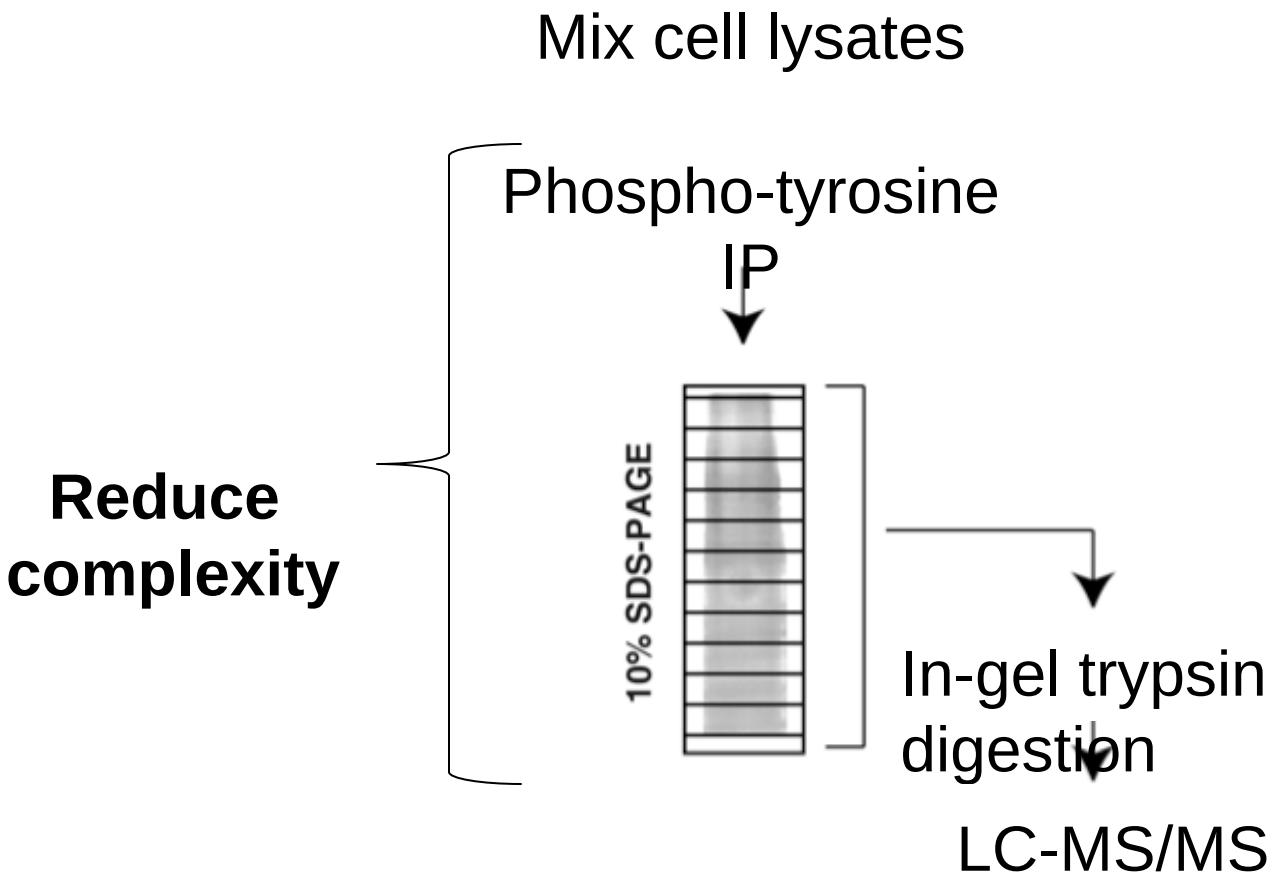


$^{13}C_6$ $^{15}N_4$-Arg (Arg10)

Red bar
Blue bar



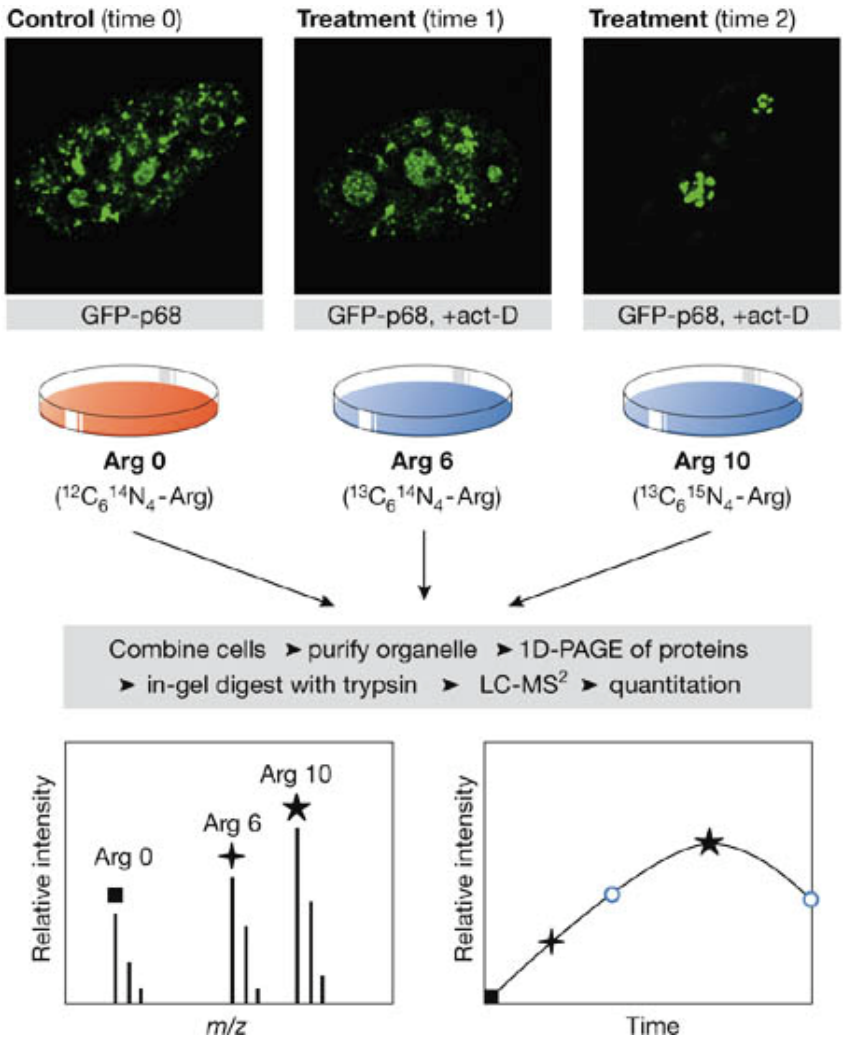
SILAC
Enriched for subsets of proteins

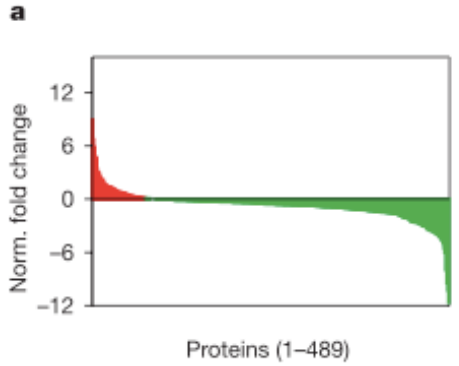


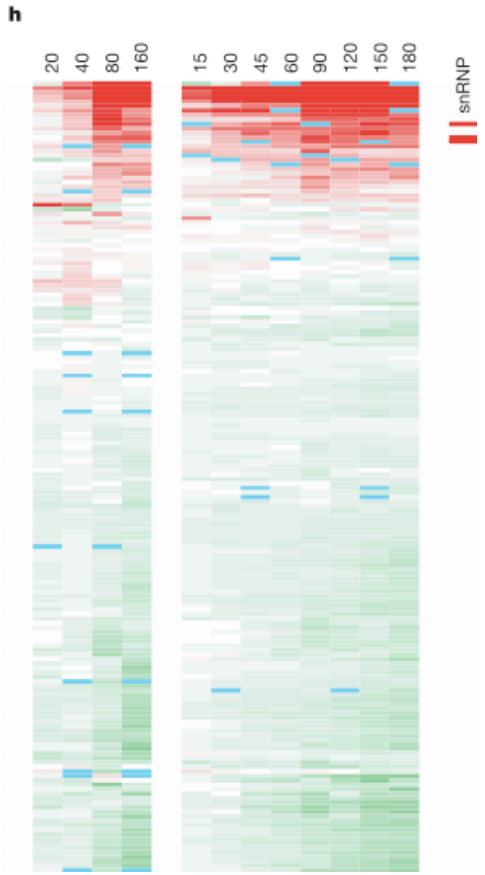
Akhilesh Pandey, (2008) Journal of Proteome Research

Nucleolar proteome dynamics

Jens S. Andersen[1]†, Yun W. Lam[2]†, Anthony K. L. Leung[2]*, Shao-En Ong[1], Carol E. Lyon[2], Angus I. Lamond[2] & Matthias Mann[1]







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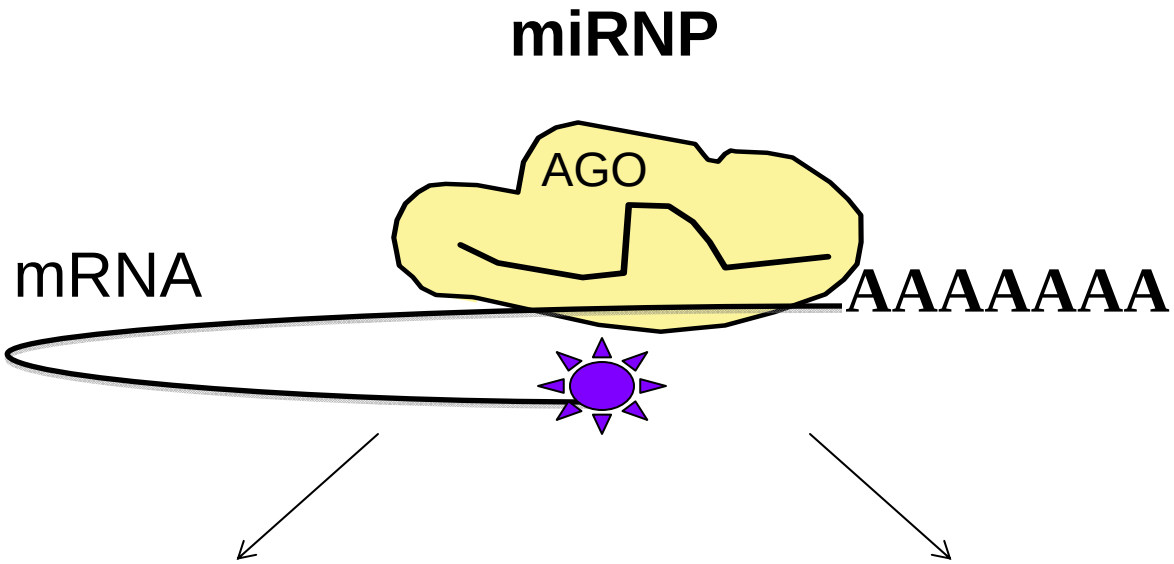
MicroRNAs Target mRNAs Resulting in Diminished Protein Levels



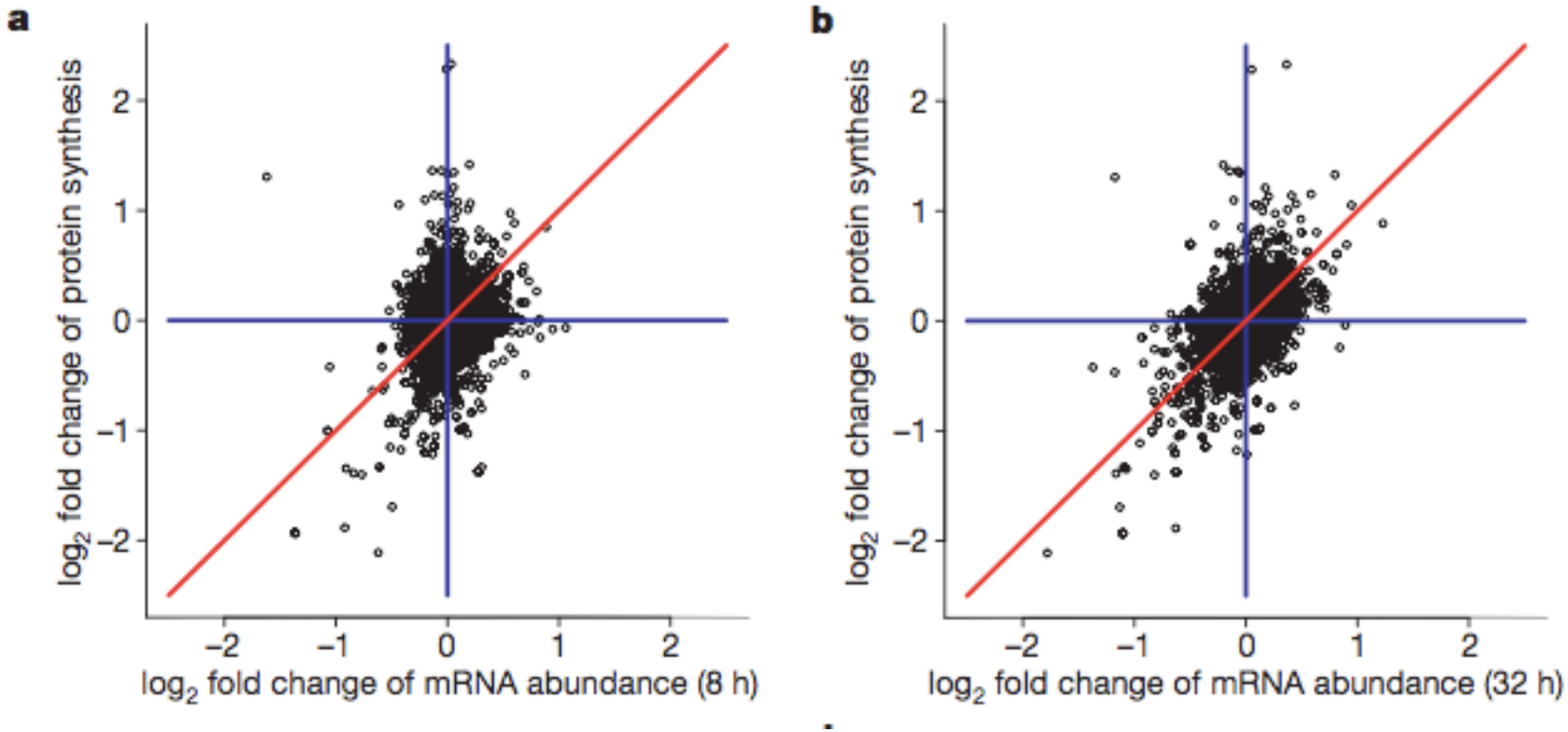
Translation inhibition

Less protein production and subsequent mRNA destruction

mRNA degradation

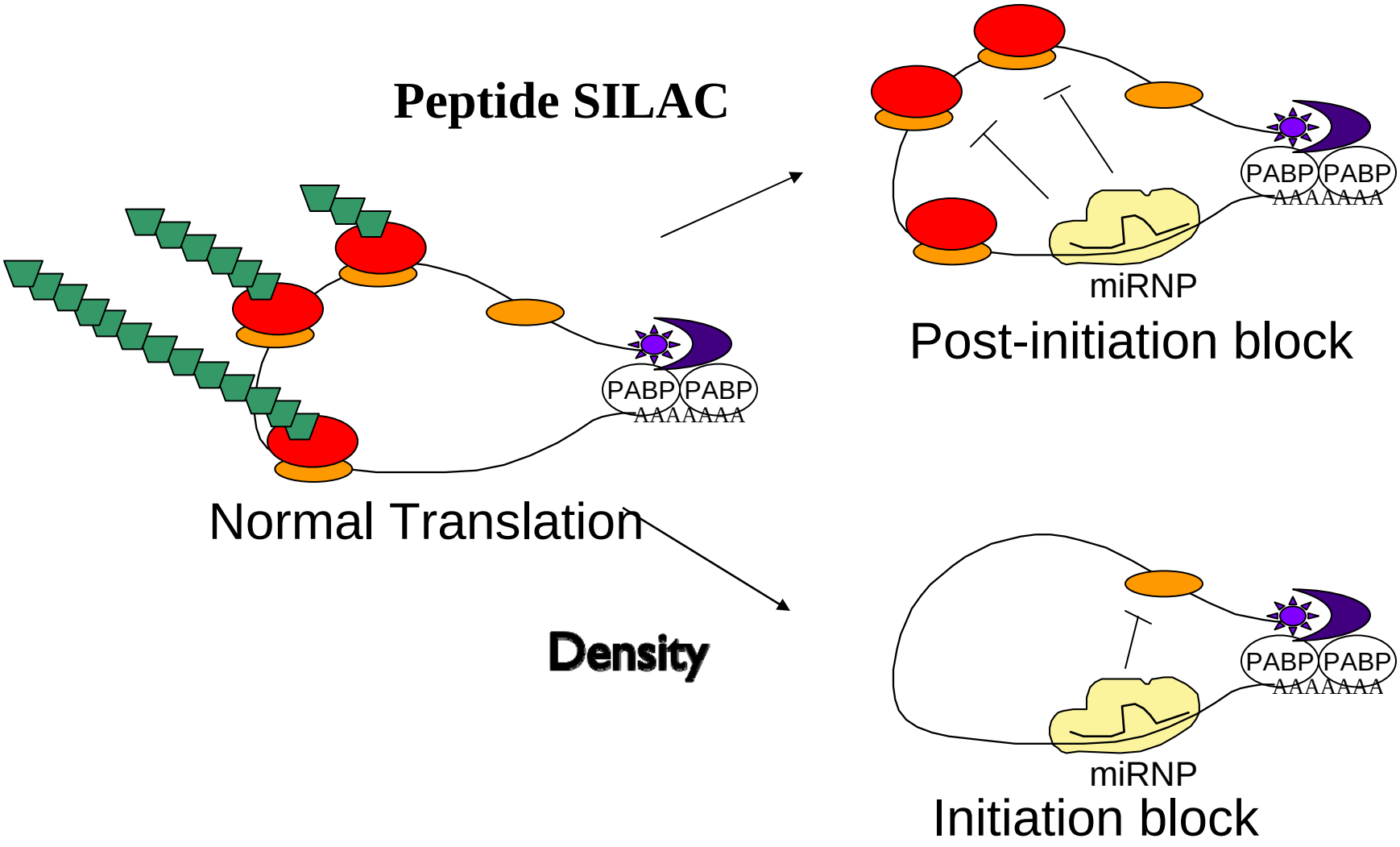
Less protein production

A Correlation Between the Change in mRNA and Protein Abundance



Niklaus Rajewsky, (2008) Nature

Modes of miRNA-Induced Translational Repression



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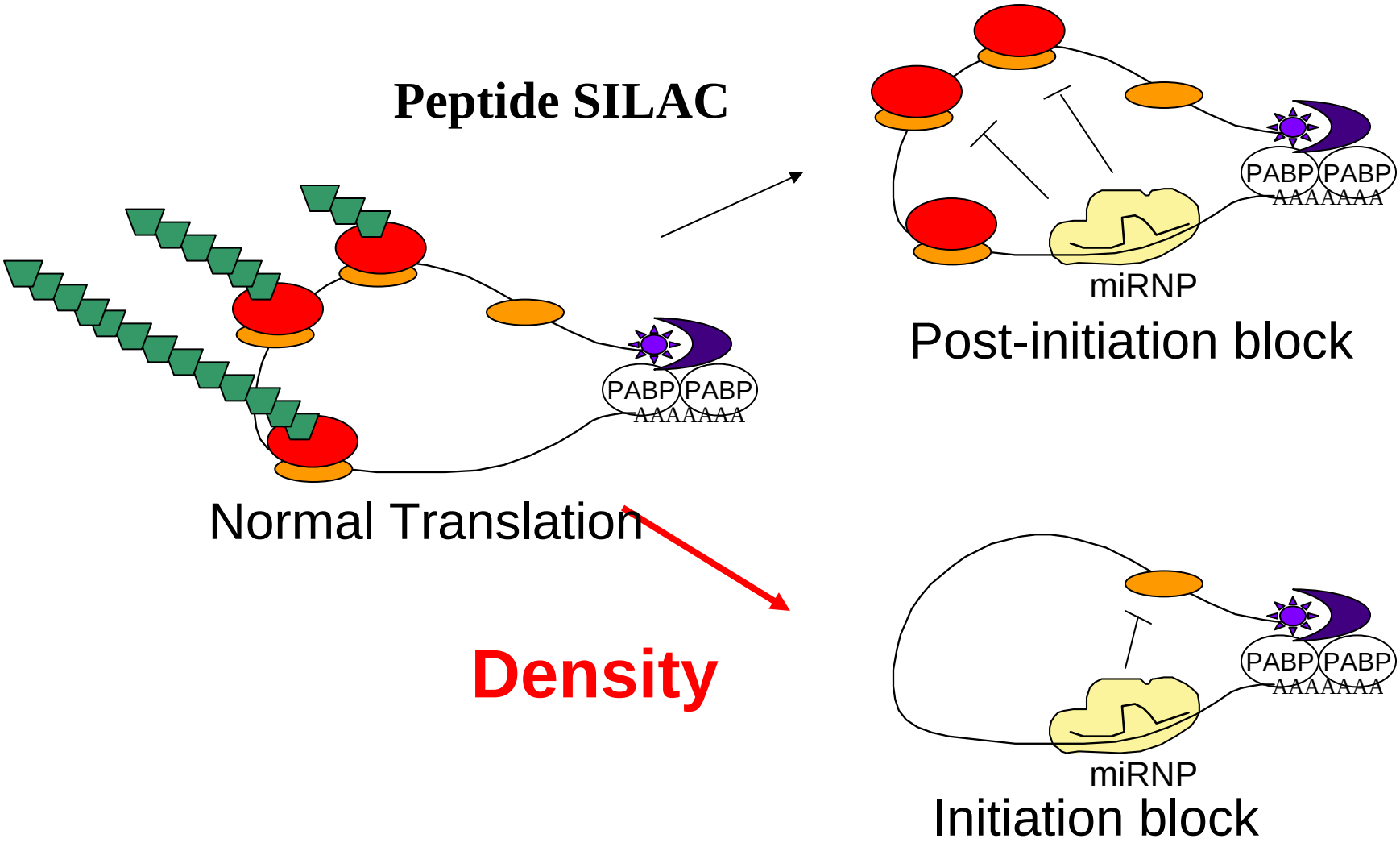
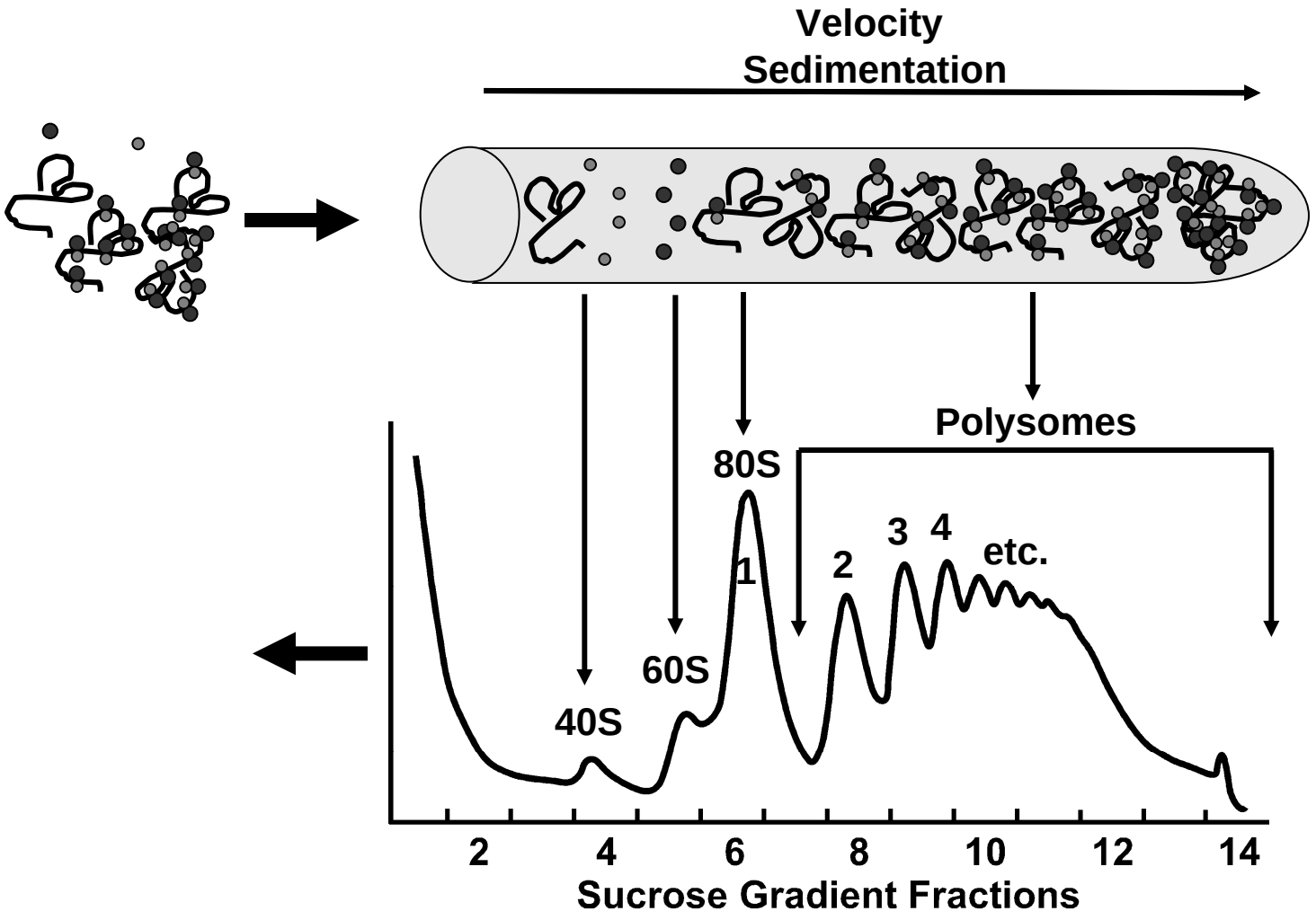


Polysome Profiling: Measuring Ribosome Density and Occupancy



JWM, 2007

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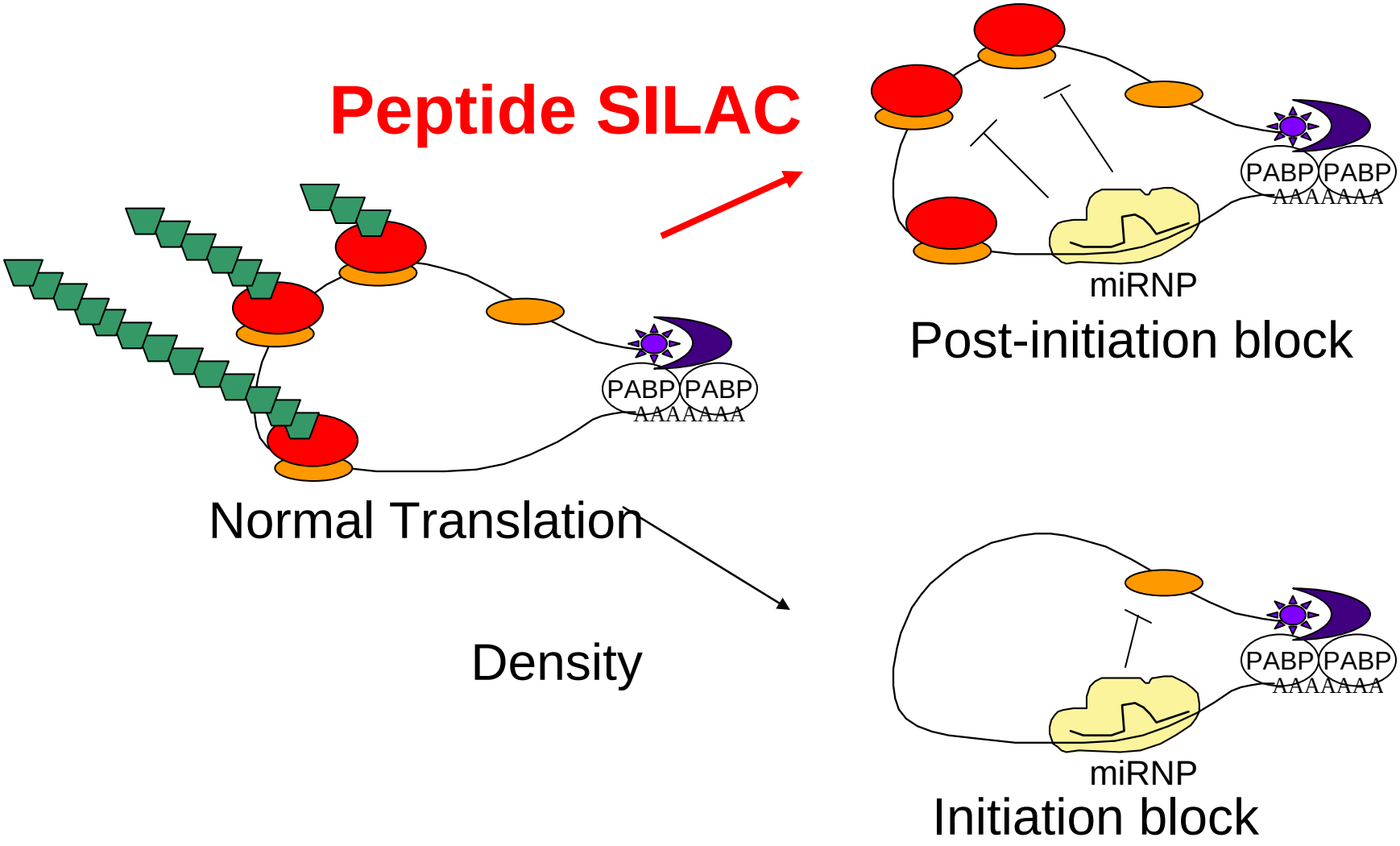

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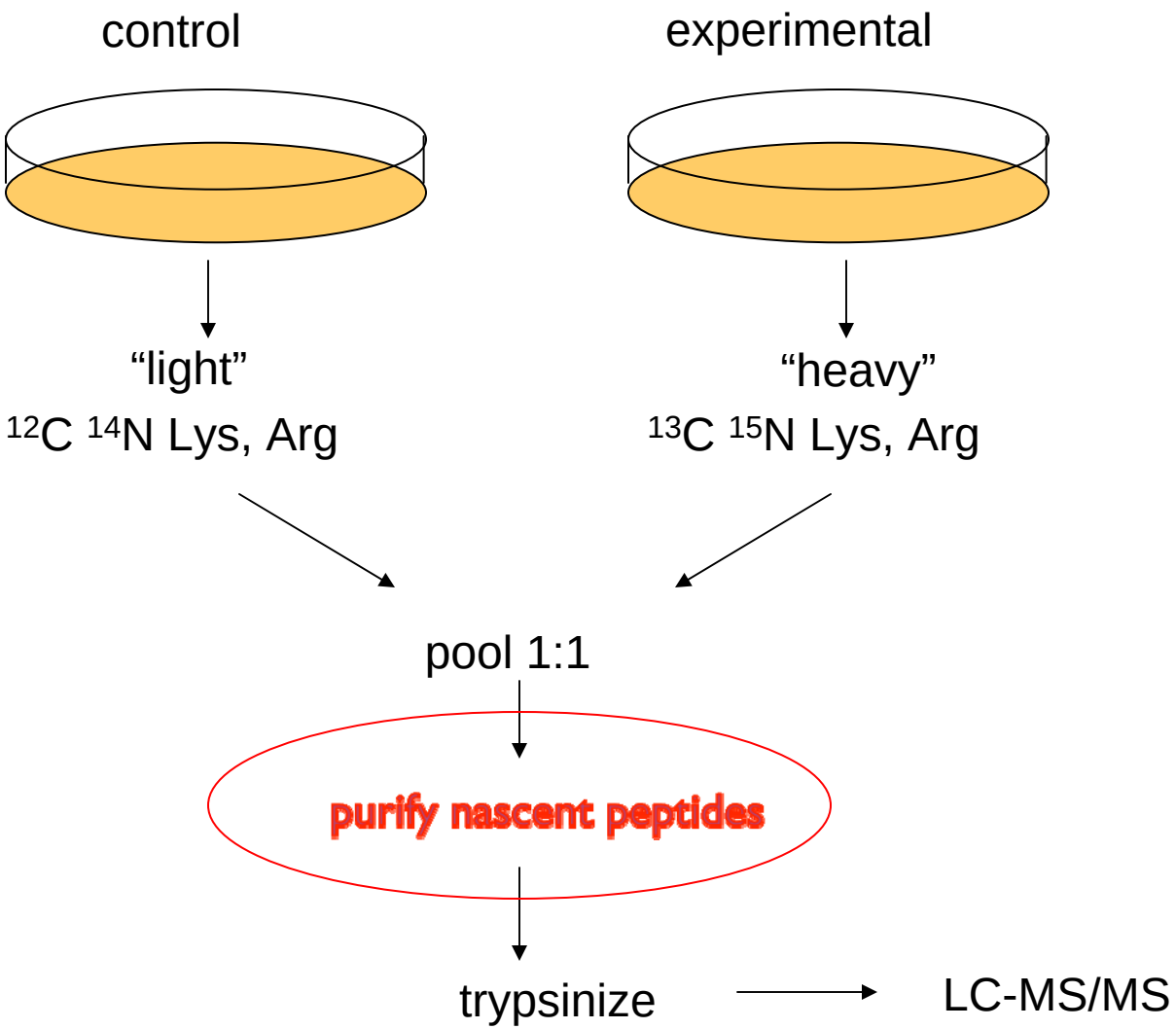
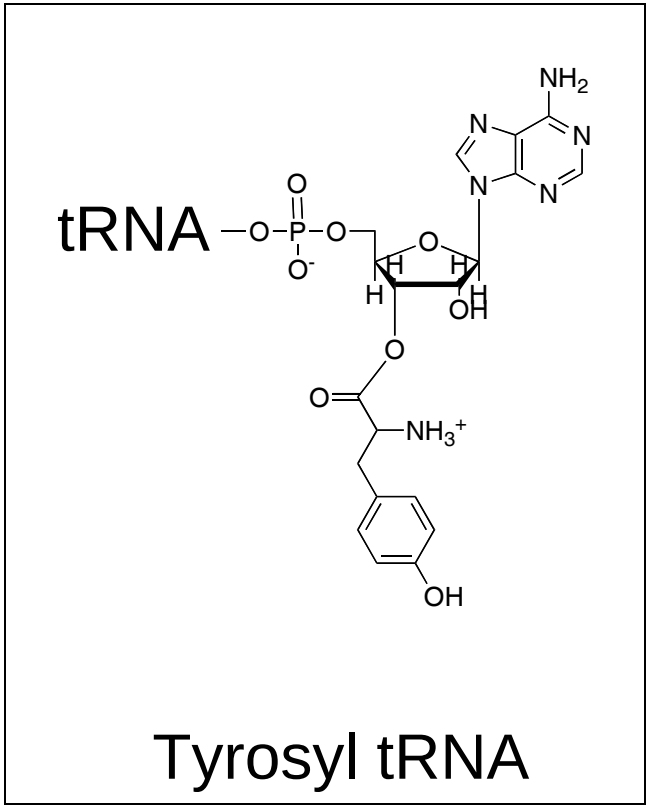
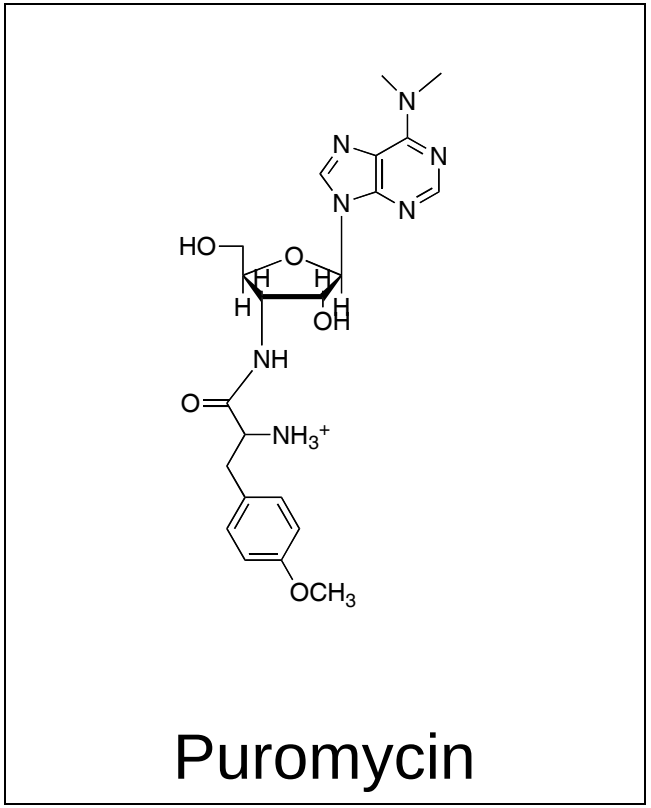


Use Puromycin to Pull Out Nascent Peptides

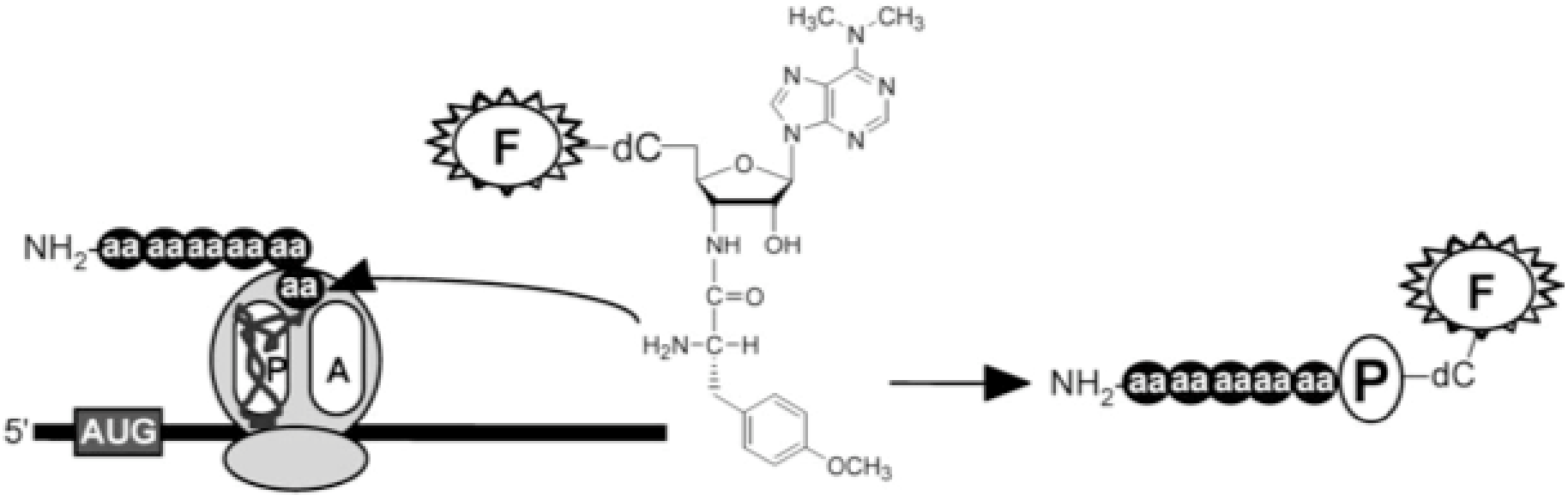


Tyrosyl tRNA



Puromycin

Conjugation of Tagged Puromycin to Peptides

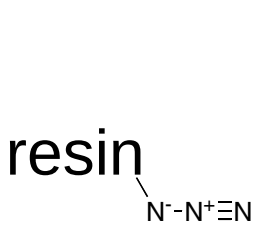


Richard Roberts, (2004) Chemistry and Biology

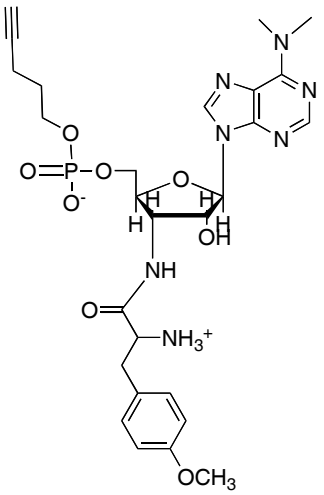
Using Click Chemistry to Purify Alkyne-Tagged Puromycin + Peptide

Click Chemistry

Azide + alkyne



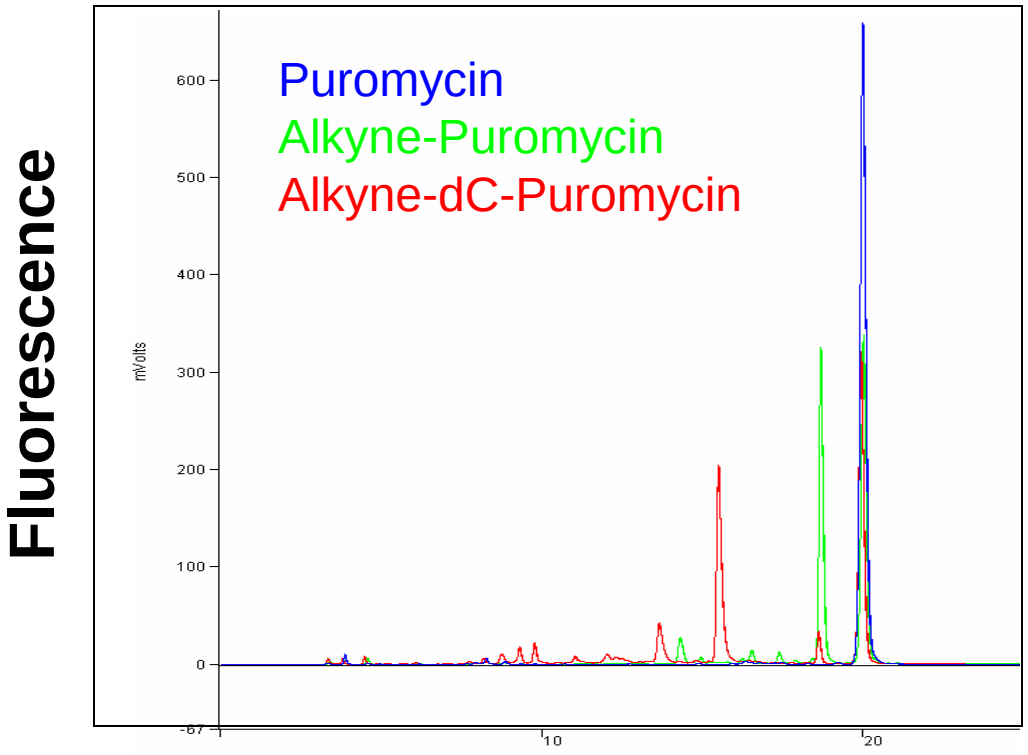
+



Azide resin **Alkyne-puromycin**

Alkyne-Puromycin Derivatives are “Clickable”

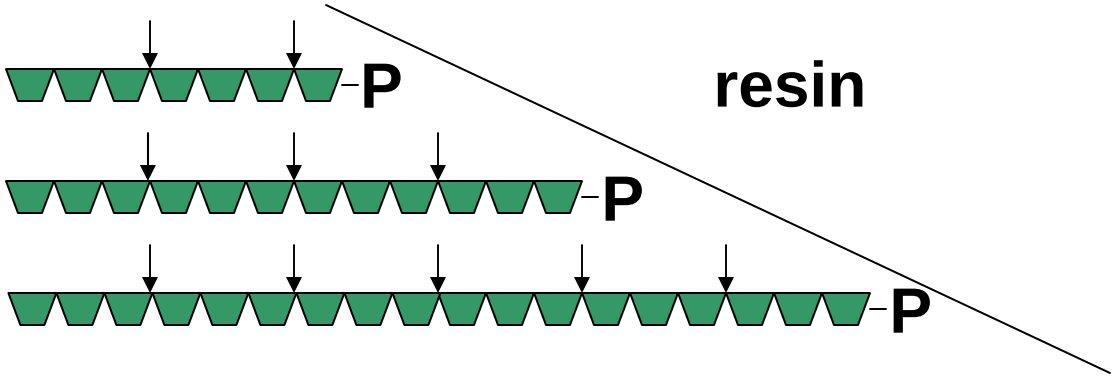
Alkyne Puromycin reacts with Fluorescent Azide



Currently optimizing incorporation into peptides

Mapping Peptides

Elute with Trypsin digestion



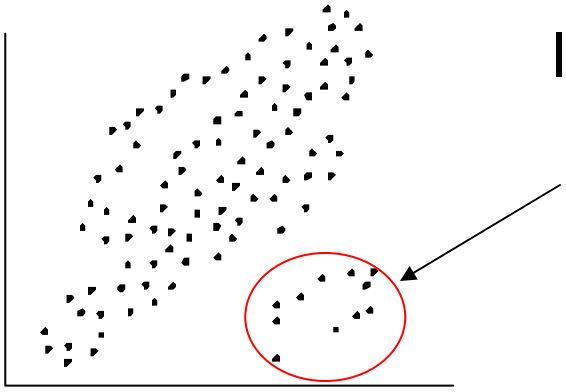
Analysis of N-terminal tryptic peptides should allow quantitation of peptide production

One Possible Scenario

We can compare nascent peptide production to ribosome density (and mRNA expression)

*

Peptide abundance

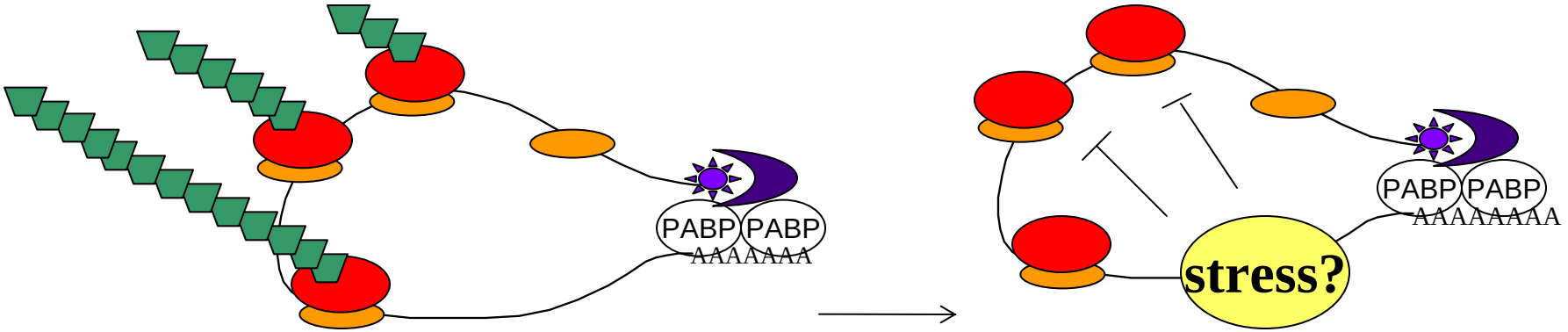


Interesting candidates for post-initiation translational regulation?

Ribosome Density

*Example of possible dataset- not real data

Getting a Handle on Translation

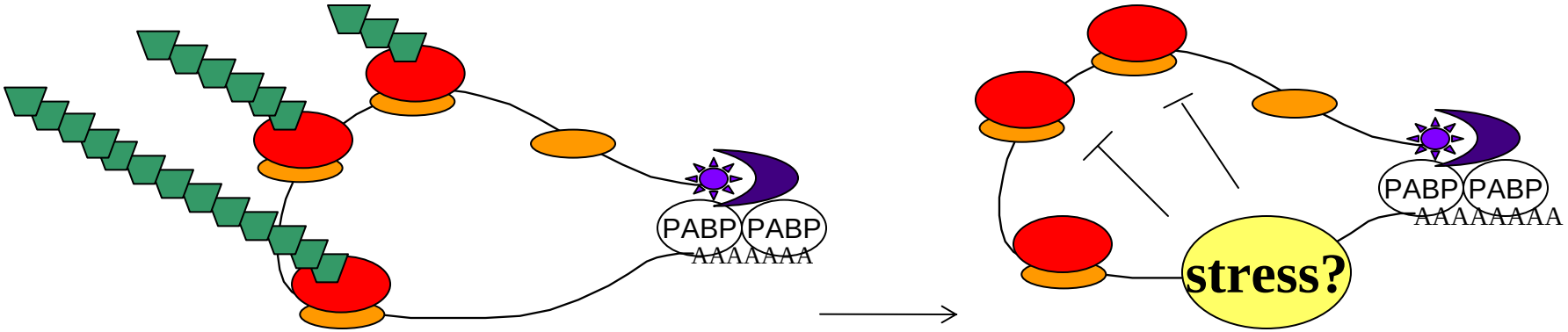


mRNA expression
(microarray)

Ribosome occupancy
(density gradient)

New protein synthesis
(peptide SILAC)

Getting a Handle on Translation



- **Short timescale**
- **Sequence of events (mRNA turnover vs translation inhibition)**
- **Localization of translation**

Acknowledgements

Patrick Brown

Brown lab members
Jason Meyers
Heather McCullough

Dan Herschlag

Pehr Harbury

W. Lance Martin
Rebecca Weisinger