SYSTEMS BIOLOGY OF AMINO ACID USE BY MAMMARY GLAND: MILK PROTEIN SYNTHESIS AND BEYOND

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EAAP 69th Annual Meeting
Dubrovnik, Croatia
August 27 - 31, 2018

ILLINOIS CLEMSON UNIVERSITY

Milk protein synthesis: a costly process

- ⇑ 5-fold in energy and protein requirements during lactation in dairy cows
- ⇑ 4-7-fold in translation activity of lactating mammary gland
- "Low" efficiency of dietary N ⇒ milk protein (25-30%)

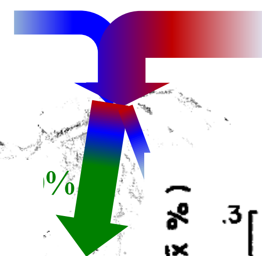
Nutritionally *and* Energetically costly process

Amino acids

Met

Lys

Energy (glucose)

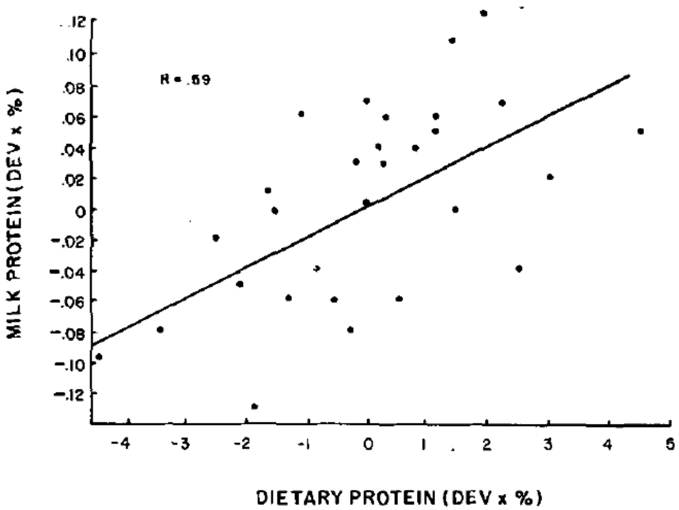


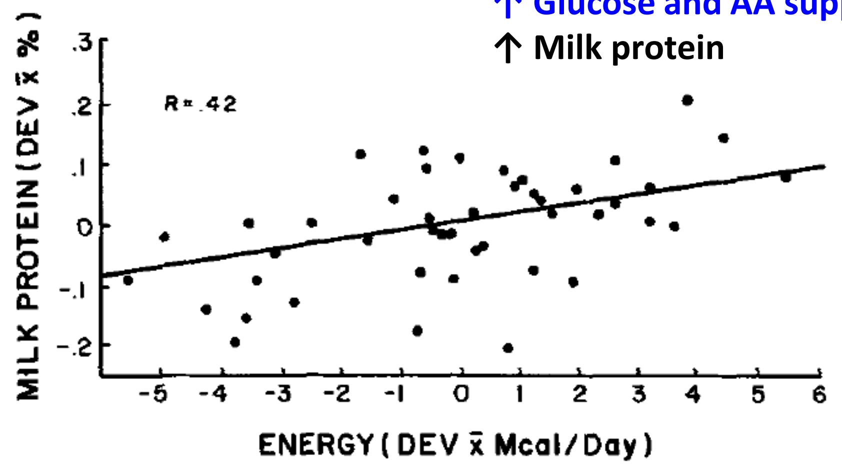
↑ Metabolizable E intake

↑ **Insulin**

↑ **Glucose and AA supply**

↑ **Milk protein**





Emery R. S., J Dairy Sci, 1978

Milk protein synthesis: a costly process

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Nutritionally *and* Energetically costly process

Amino acids **Energy (glucose)**

Met

Lys

Ile

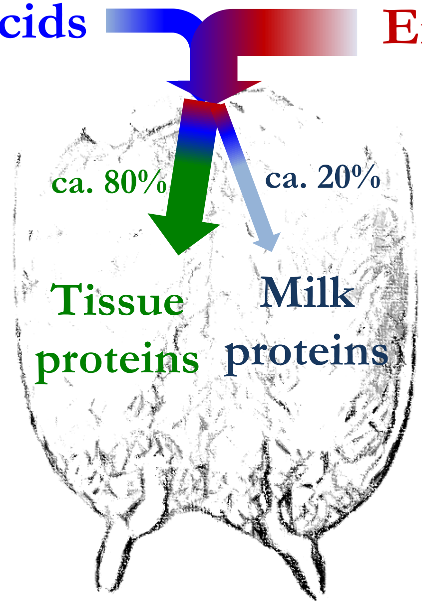
Leu

Thr

His

Arg

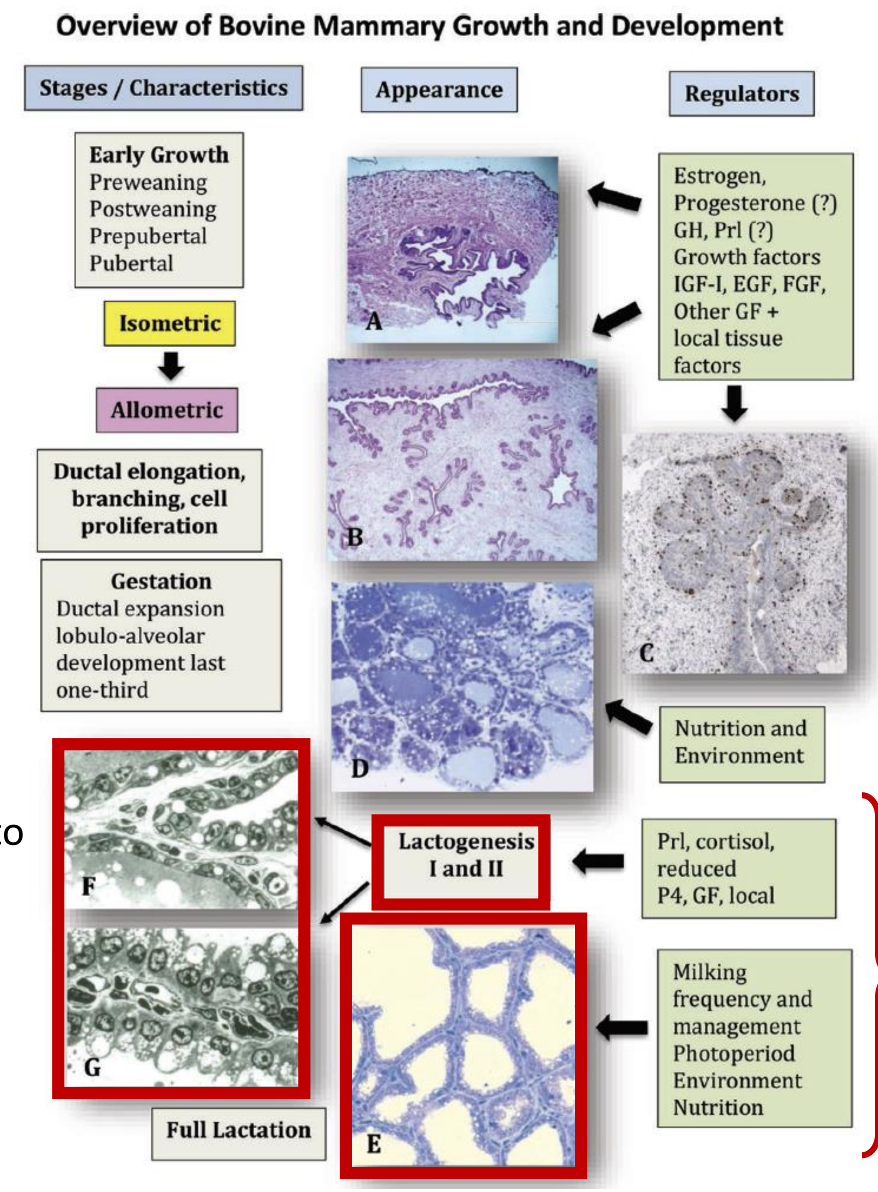
etc.



Single-limiting AA theory may not always apply

Saturable response in casein synthesis to EAA – efficiency of AA use <u>*not*</u> fixed

(Arriola-Apelo et al., 2014)



~2 week prior to calving

~2 week after calving

J. Dairy Sci. 100:10332–10352
https://doi.org/10.3168/jds.2017-12983
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A 100-Year Review: Mammary development and lactation[1]

R. Michael Akers[2]
Department of Dairy Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061

J. Dairy Sci. 89:1222–1234
© American Dairy Science Association, 2006.

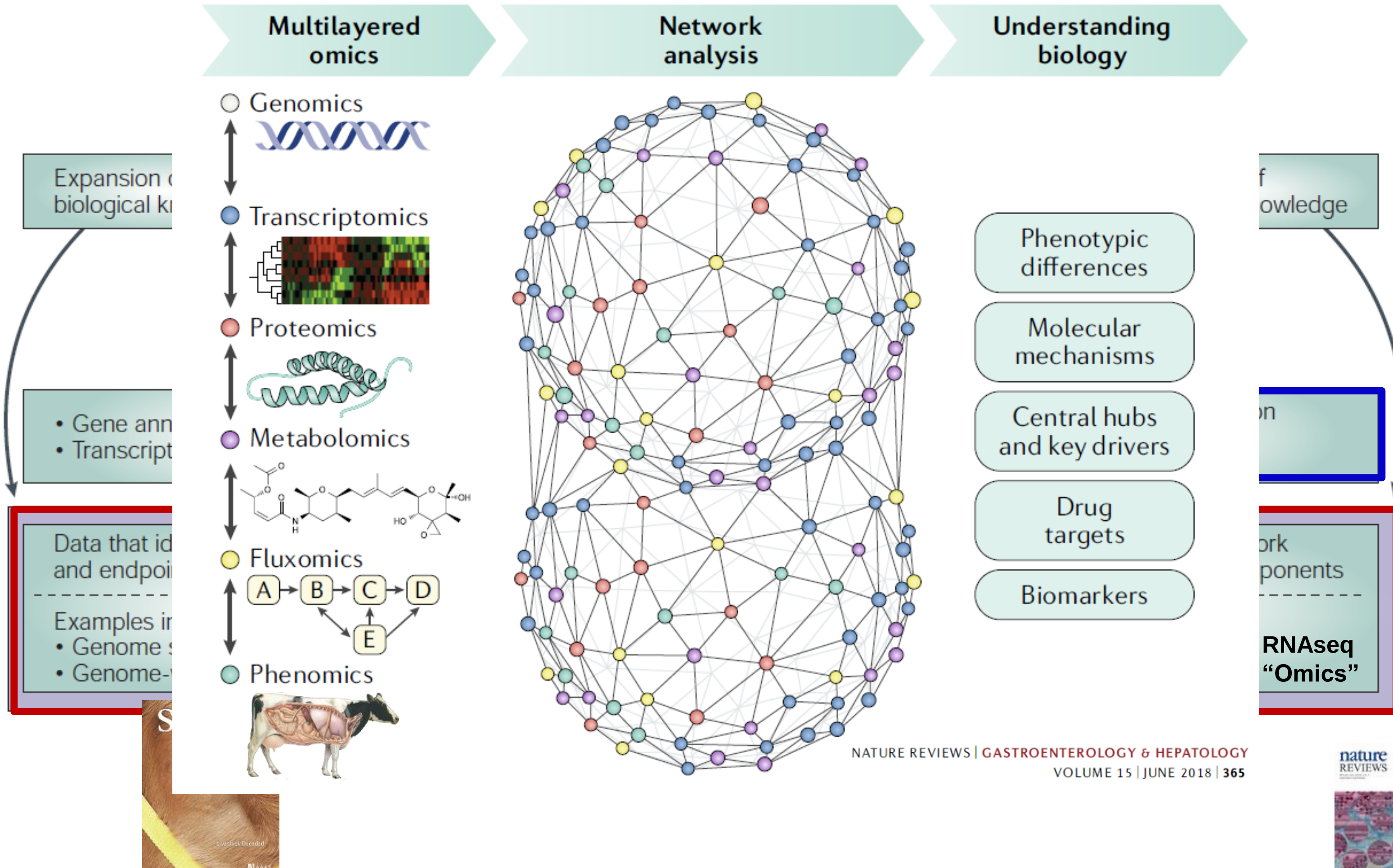
Major Advances Associated with Hormone and Growth Factor Regulation of Mammary Growth and Lactation in Dairy Cows

R. M. Akers
Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg 24061

Control of milk protein synthesis is complex!

- ✓ Prolactin ➔ JAK/STAT5
- ✓ **IGF-1, Insulin** ➔ IRS1, AKT1, mTOR
- ✓ Transcription binding sites for caseins: >100
- ✓ _FEW_ transcription factors experimentally verified

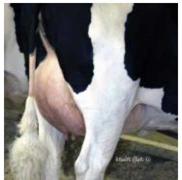
“Systems biology” approach: an integrative and iterative process ➔ reconstructing underlying biology



2009

(Papin et al., 2005)

Mammary gland reconstruction during lactation



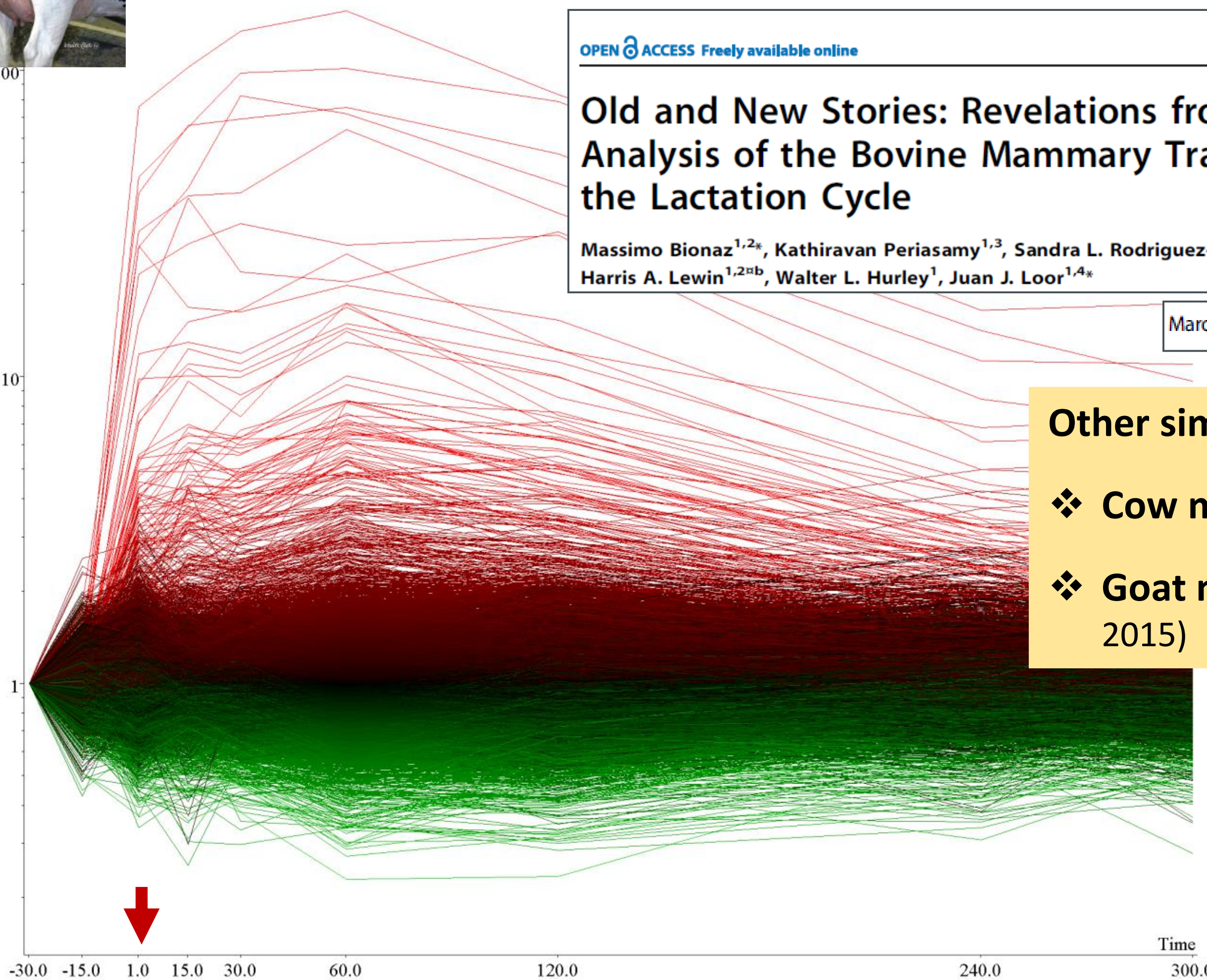
6,382 differentially expressed genes (FDR $P \leq 0.001$)

OPEN ACCESS Freely available online PLoS one

Old and New Stories: Revelations from Functional Analysis of the Bovine Mammary Transcriptome during the Lactation Cycle

Massimo Bionaz[1,2]*, Kathiravan Periasamy[1,3], Sandra L. Rodriguez-Zas[1,2], Robin E. Everts[1¤a], Harris A. Lewin[1,2¤b], Walter L. Hurley[1], Juan J. Loor[1,4]*

March 2012 | Volume 7 | Issue 3 | e33268

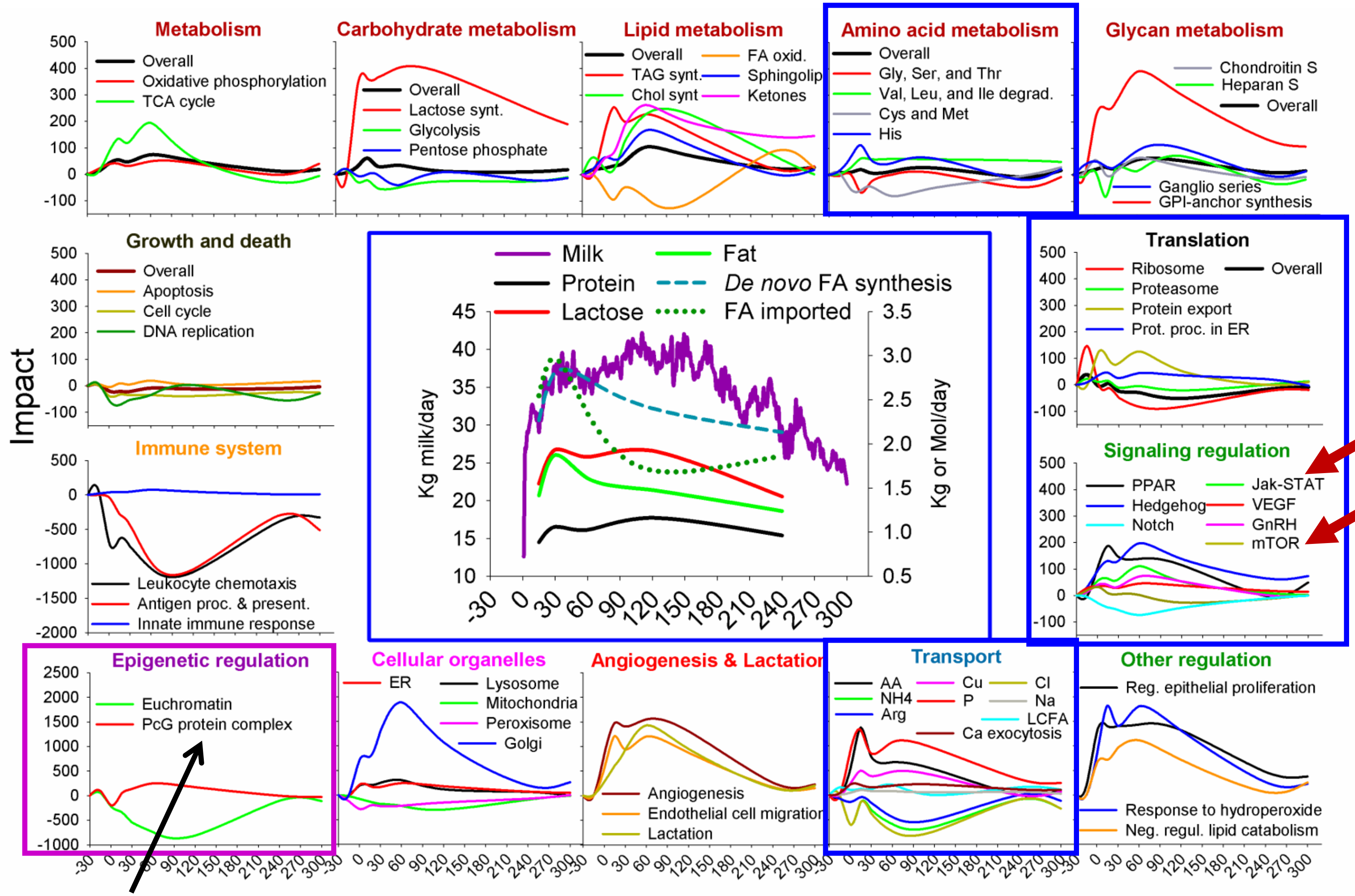


Parturition

Other similar research:

- **Cow milk** (Wickramasinghe et al., 2012)
- **Goat mammary gland** (Shi et al., 2015)

Most relevant impacted functions during lactation in cow mammary gland



Central in milk protein synth. regulation

Epigenetic "stabilizer"

(Bionaz et al., 2012ab)

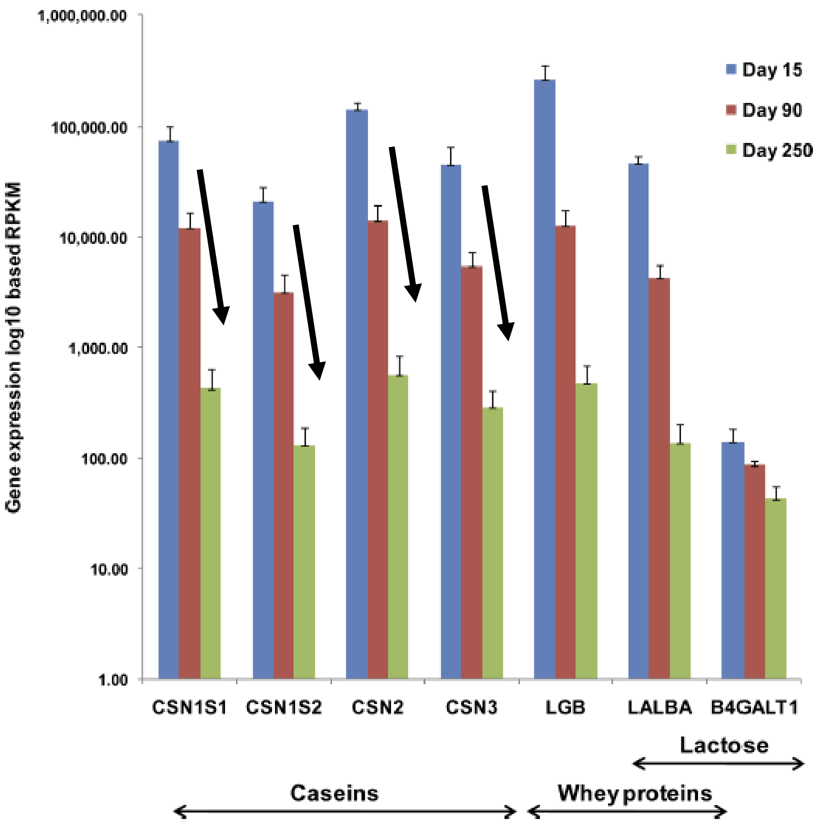
Wickramasinghe *et al. BMC Genomics* 2012, 13:45
http://www.biomedcentral.com/1471-2164/13/45

BMC Genomics

RESEARCH ARTICLE Open Access

Transcriptional profiling of bovine milk using RNA sequencing

Saumya Wickramasinghe, Gonzalo Rincon, Alma Islas-Trejo and Juan F Medrano*

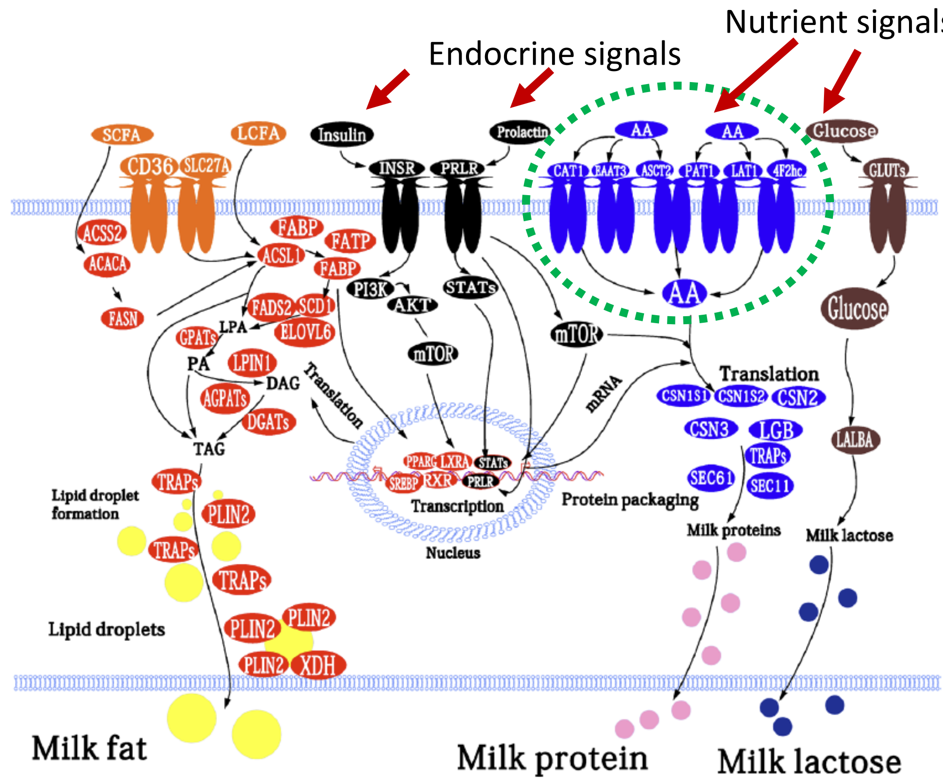


Funct Integr Genomics (2015) 15:309–321
DOI 10.1007/s10142-014-0420-1

ORIGINAL PAPER

Genes regulating lipid and protein metabolism are highly expressed in mammary gland of lactating dairy goats

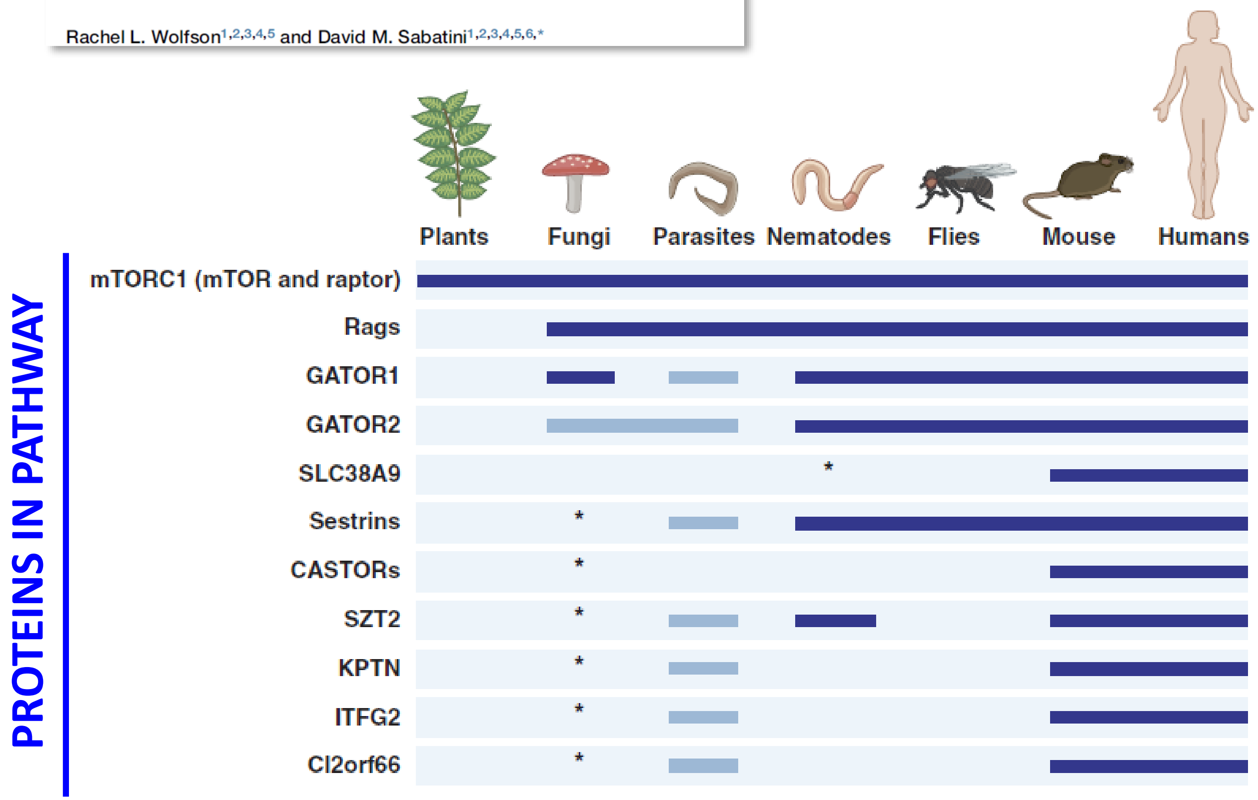
Hengbo Shi · Jiangjiang Zhu · Jun Luo · Wenting Cao ·
Huaiping Shi · Dawei Yao · Jun Li · Yuting Sun ·
Huifen Xu · Kang Yu · Juan J. Loor



Why the interest in "nutrient sensing pathways"??

The Dawn of the Age of Amino Acid Sensors for the mTORC1 Pathway

Rachel L. Wolfson[1,2,3,4,5] and David M. Sabatini[1,2,3,4,5,6,*]



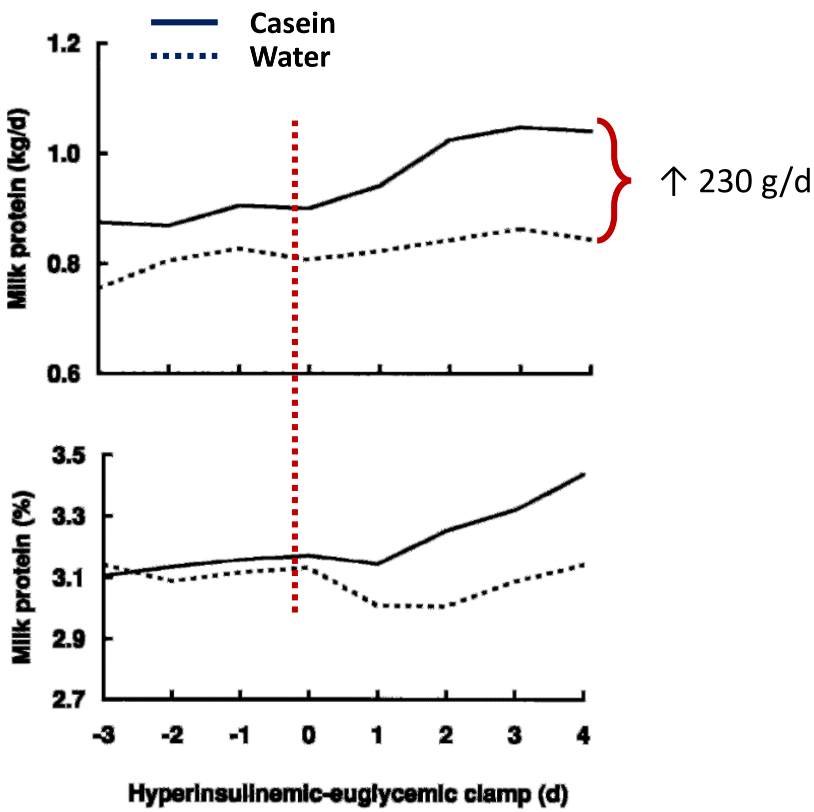
Cell Metabolism *26*, August 1, 2017

- Well-known that some components of nutrient sensing are conserved across evolution
- Allows for control of cellular growth and metabolism
- These processes can be ***fine-tuned:***
    - *AA profile?*
    - *"Optimal" concentrations?*
    - *How to deliver to mammary?*

The Role of Insulin in the Regulation of Milk Protein Synthesis in Dairy Cows[1,2]

J. M. GRIINARI,[3,4] M. A. MCGUIRE,[3,5] D. A. DWYER,[3]
D. E. BAUMAN,[3,6] D. M. BARBANO,[7] and W. A. HOUSE[8]
Cornell University, Ithaca, NY 14853

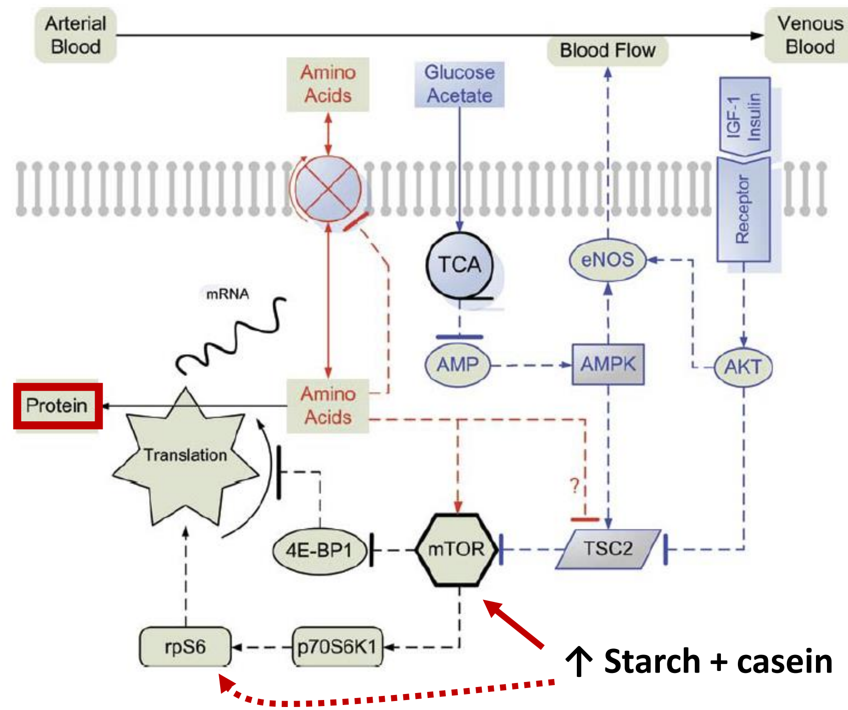
1997 J Dairy Sci 80:2361–2371



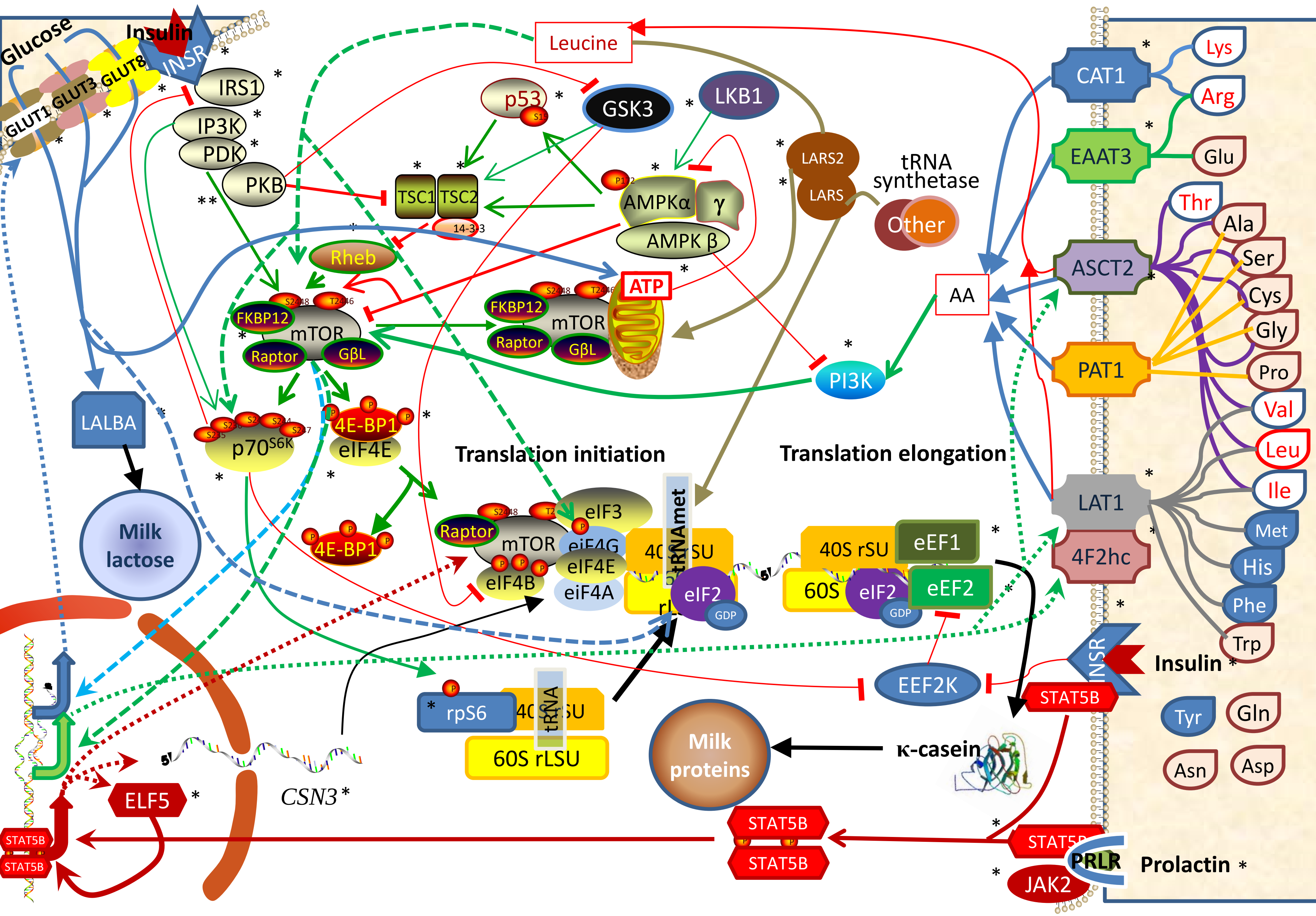
J. Dairy Sci. 93:3114–3127
doi:10.3168/jds.2009-2743
© American Dairy Science Association®, 2010.

Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids

A. G. Rius,*[1] J. A. D. R. N. Appuhamy,* J. Cyriac,* D. Kirovski,† O. Becvar,‡ J. Escobar,§ M. L. McGilliard,* B. J. Bequette,# R. M. Akers,* and M. D. Hanigan*[2]

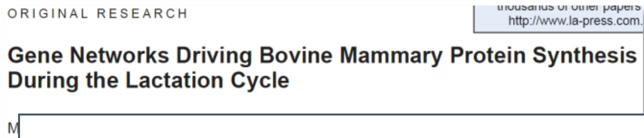


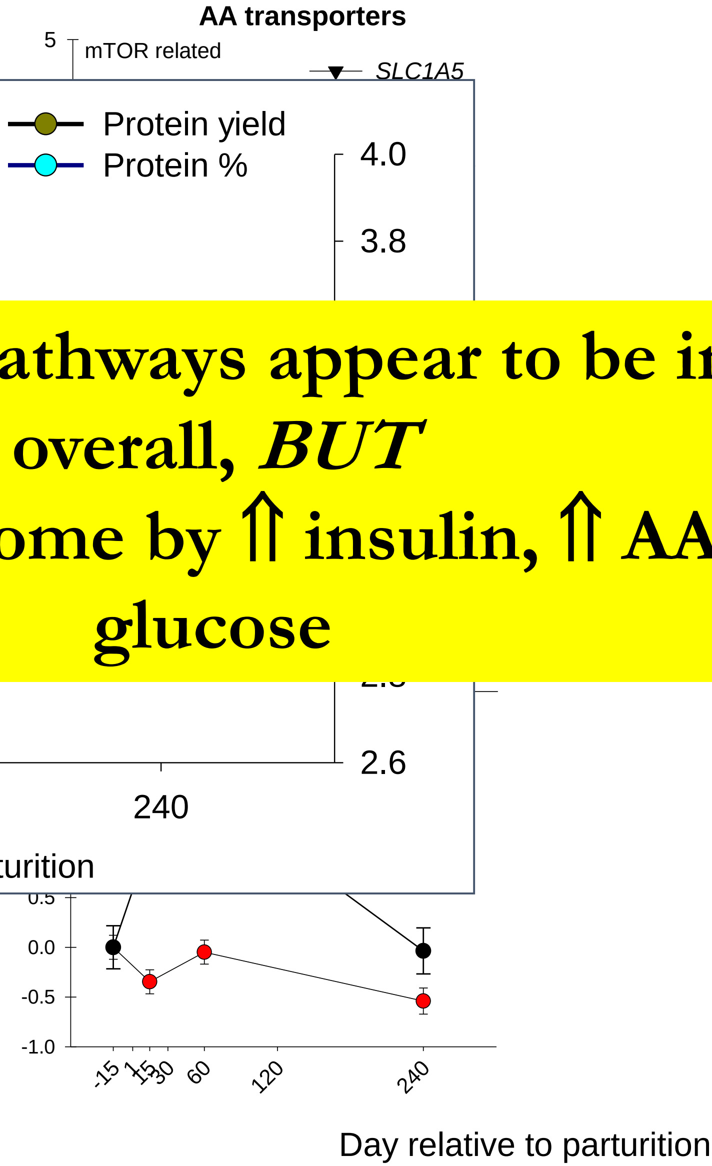
- Role of insulin first proposed in 1966 (Schmidt, JDS 49:381-385)
- One mechanism likely involves greater AA uptake by mammary tissue (Park et al., 1979)
- Feed restriction plus infusions of starch, casein, or both for 36 hours
- Mixed responses to starch with or without casein
- ↑ protein yield with starch + casein

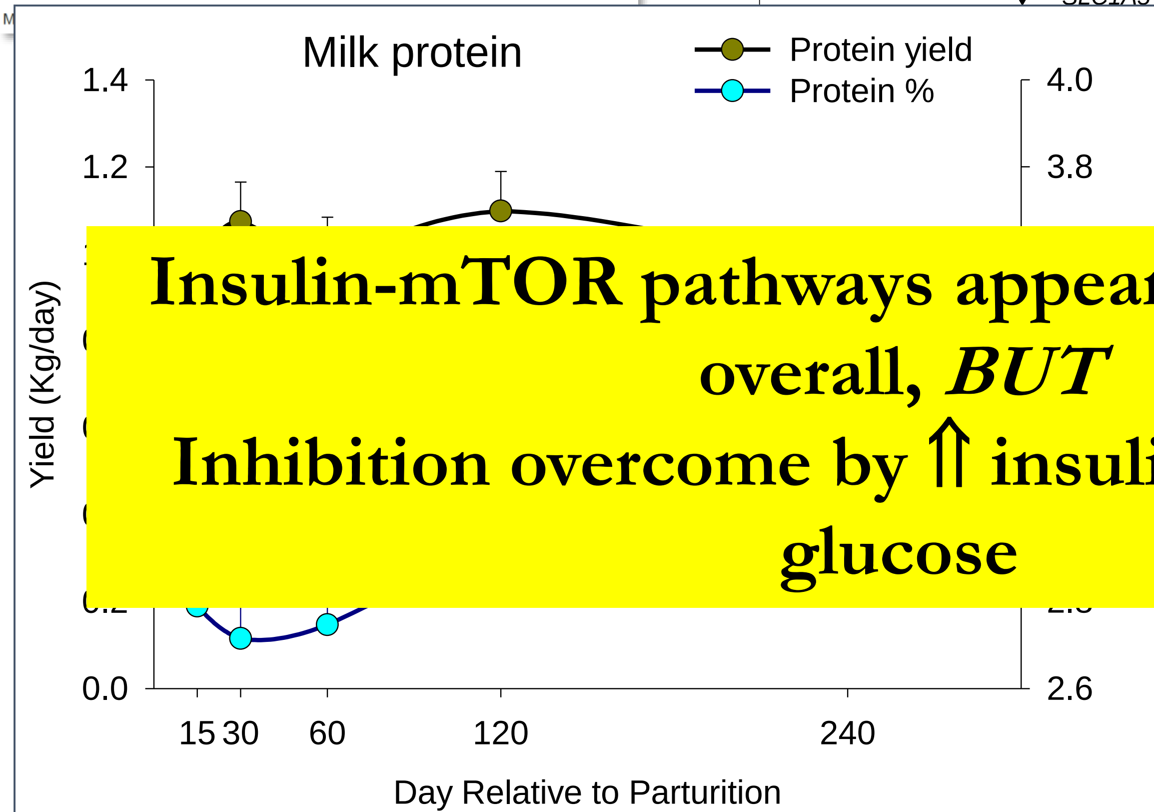


Bionaz and Loor, Bioinformatics and Biology Insights, 2011

Reconstruction of milk protein network during lactation







Insulin-mTOR pathways appear to be inhibited overall, *BUT*

Inhibition overcome by ⇑ insulin, ⇑ AA, and ⇑ glucose

mTOR signaling controls milk protein mainly via translation

Milk Protein Synthesis in the Lactating Mammary Gland: Insights from Transcriptomics Analyses

Massimo Bionaz, Walter Hurley and Juan Loor

Chapter 11 © 2012 Bionaz et al., licensee InTech.

Insulin, IGF-1 and regulation of milk protein synthesis networks:

"Missing link with nutrition"

- Clear role in transcription *and* translation
- Menzies et al. (2009):

  ↑ transcription, translation

  role for ELF5 (transc. factor)
- *Translation regulation via mTOR*
- *Various research groups over last 10 years*

Molecular branch studied	Tissue or cell type	Main objective*	Main conclusions*	Take home messages
Protein phosphorylation	Mammary tissue biopsy	Role of dietary AA and starch fed to lactating cows on MPS	mTOR and RPSK6 phosphorylation is enhanced by starch and AA	Glucose and AA are important
Protein phosphorylation	MacT cells	Role of the level of essential AA availability and insulin on phosphorylation of several mTOR pathways proteins and MPS	Essential AA enhance MPS rate by enhancing phosphorylation of 4EBP1 and eEF2	Essential AA are key
Protein phosphorylation	MacT cells	Role of specific essential AA on phosphorylation and MPS	Phosphorylation of mTOR and RPS6K decreases in the absence of leucine and isoleucine, and leads to lower protein synthesis rate	Leucine and Isoleucine are required
Protein phosphorylation and mRNA expression	Mammary tissue	Role of mRNA translation on MPS regulation	Lactating mammary tissue is associated with greater expression of RPS6, RPS6K, and eIF isoforms, thus, they play a key role in MPS	Translation machinery turned-on postpartum
Protein Phosphorylation	Mammary tissue biopsy	Role of mTOR signaling in nutritional regulation of MPS	Intravenous essential AA and glucose infusion enhance MPS via increased phosphorylation of mTOR	
Protein phosphorylation	Primary mammary epithelial cells	Role of mTOR signaling in nutritional and hormonal regulation of MPS	Nutrients and hormones are capable of regulating MPS through phosphorylation of the mTOR signaling pathway	mTOR pathway is sensitive to nutrients/hormones
Protein phosphorylation	MacT cells	Role of IGF-1 on mTOR phosphorylation and regulation of MPS	Exogenous IGF-1 increased RPS6K and mTOR phosphorylation and stimulated global protein synthesis	IGF-1 plays role in mTOR signalling

Ongoing efforts to identify "ideal" supply of amino acids and link with mTOR signaling

J. Dairy Sci. 97:4000–4017
http://dx.doi.org/10.3168/jds.2013-7392
© American Dairy Science Association®, 2014.

Invited review: **Current representation and future trends of predicting amino acid utilization in the lactating dairy cow**

S. I. Arriola Apelo,* J. R. Knapp,† and M. D. Hanigan*[1]

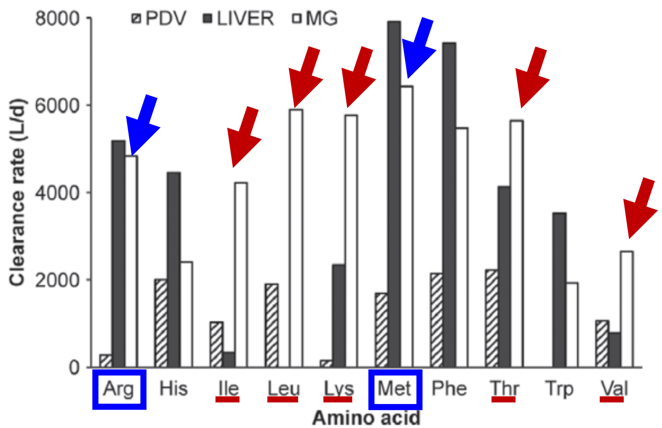


Figure 3. Portal-drained viscera (PDV), liver, and mammary glands (MG) clearance rate constants (L/d) as derived by Hanigan et al. (2001, 2004a,b). Leucine liver constant and Trp PDV constant are not reported.

- Some tissue "preference" for EAA seems to exist
- Mammary utilization of EAA changes with arterial concentration, physiological state, and hormonal status
- Transporter affinity differs, blood flow could be a factor, epithelial cell number, etc, etc

J. Dairy Sci. 101:5502–5514
https://doi.org/10.3168/jds.2017-13707
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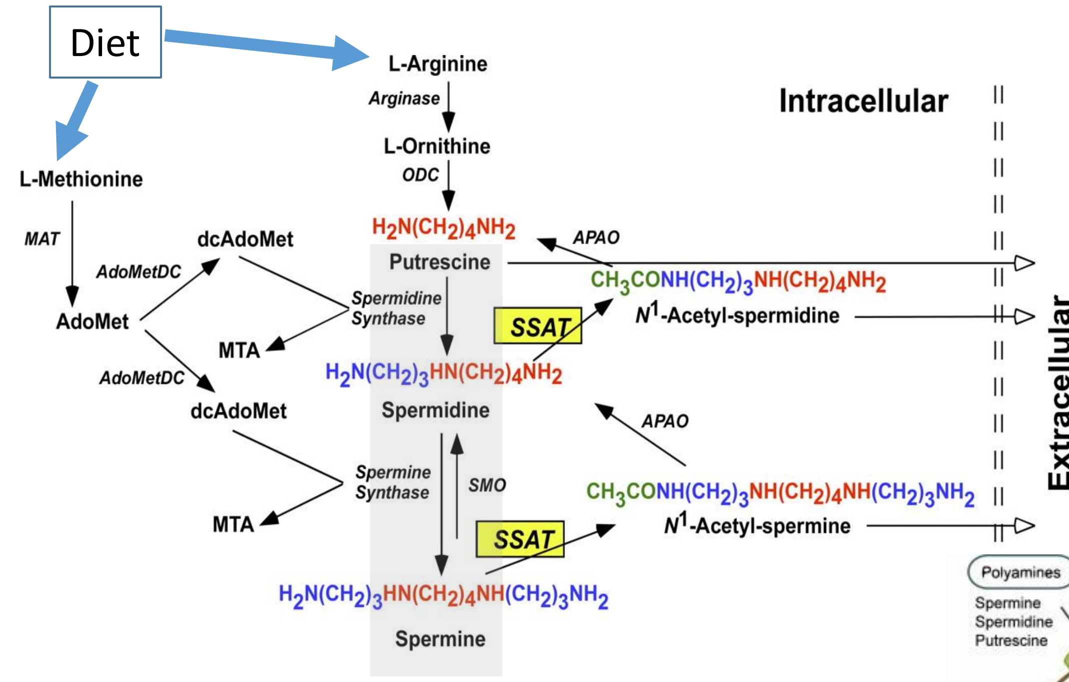
Increasing the availability of threonine, isoleucine, valine, and leucine relative to lysine while maintaining an ideal ratio of lysine:methionine alters mammary cellular metabolites, mammalian target of rapamycin signaling, and gene transcription

X. Dong,*†‡ Z. Zhou,†§ L. Wang,*# B. Saremi,|| A. Helmbrecht,|| Z. Wang,‡ and J. J. Loor†[1]

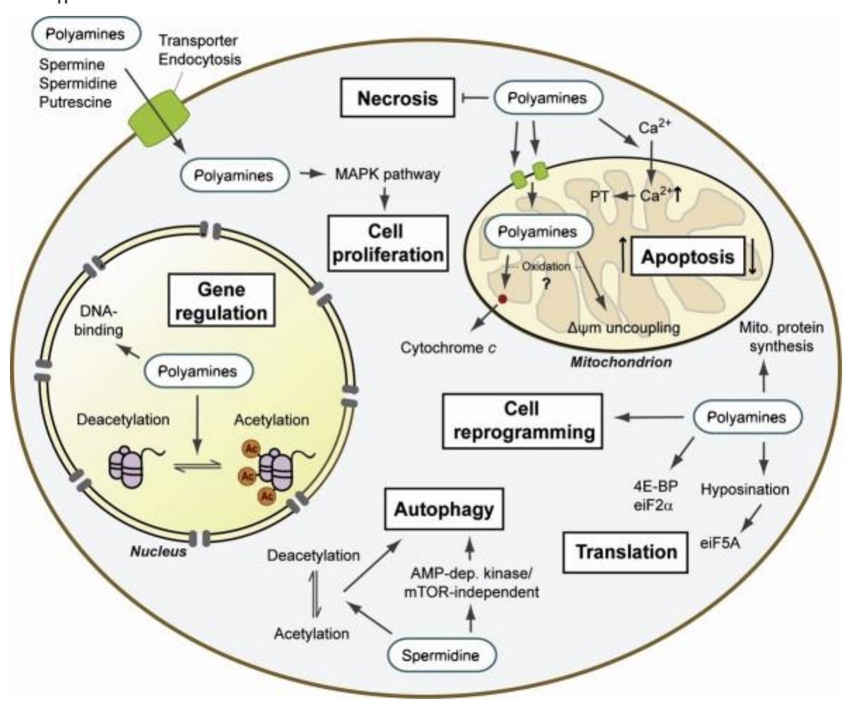
- Increased supply of Threonine, Isoleucine, and Valine particularly effective in ↑↑ p-mTOR, p-RPS6, and casein mRNA
- Positive effects in spite of ↓ mRNA for various AA transporters
- Post-transcriptional regulation appears very important…

There is a biologic link between Arginine and Methionine



- ❖ Polyamine synthesis may be responsive to supply of Arginine and Methionine
  - ❖ Importance??

Transcription
Cell proliferation
mTOR signaling
Oxidative stress



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Effects of Arginine Concentration on the *In Vitro* Expression of Casein and mTOR Pathway Related Genes in Mammary Epithelial Cells from Dairy Cattle

Mengzhi Wang[1,2,3], Bolin Xu[1], Hongrong Wang[1*¤], Dengpan Bu[2], Jiaqi Wang[2*¤], Juan-Jose Loor[3]

J. Dairy Sci. 100:4128–4133
https://doi.org/10.3168/jds.2016-11823
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Short communication: **Arginase inhibition reduces the synthesis of casein in bovine mammary epithelial cells**

M. Z. Wang,*[1,2] L. Y. Ding,*[1] C. Wang,† L. M. Chen,* J. J. Loor,‡ and H. R. Wang*[2]

L-Arginine

Arginase ↓

L-Ornithine

J. Dairy Sci. 101:3514–3523
https://doi.org/10.3168/jds.2017-13178
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Inhibition of arginase via jugular infusion of N^{ω}-hydroxy-nor-L-arginine inhibits casein synthesis in lactating dairy cows

L. Y. Ding,* L. M. Chen,* M. Z. Wang,*[1] J. Zhang,†[1] J. J. Loor,‡ G. Zhou,* X. Zhang,* and H. R. Wang*

J. Dairy Sci. 101:340–364
https://doi.org/10.3168/jds.2016-12493
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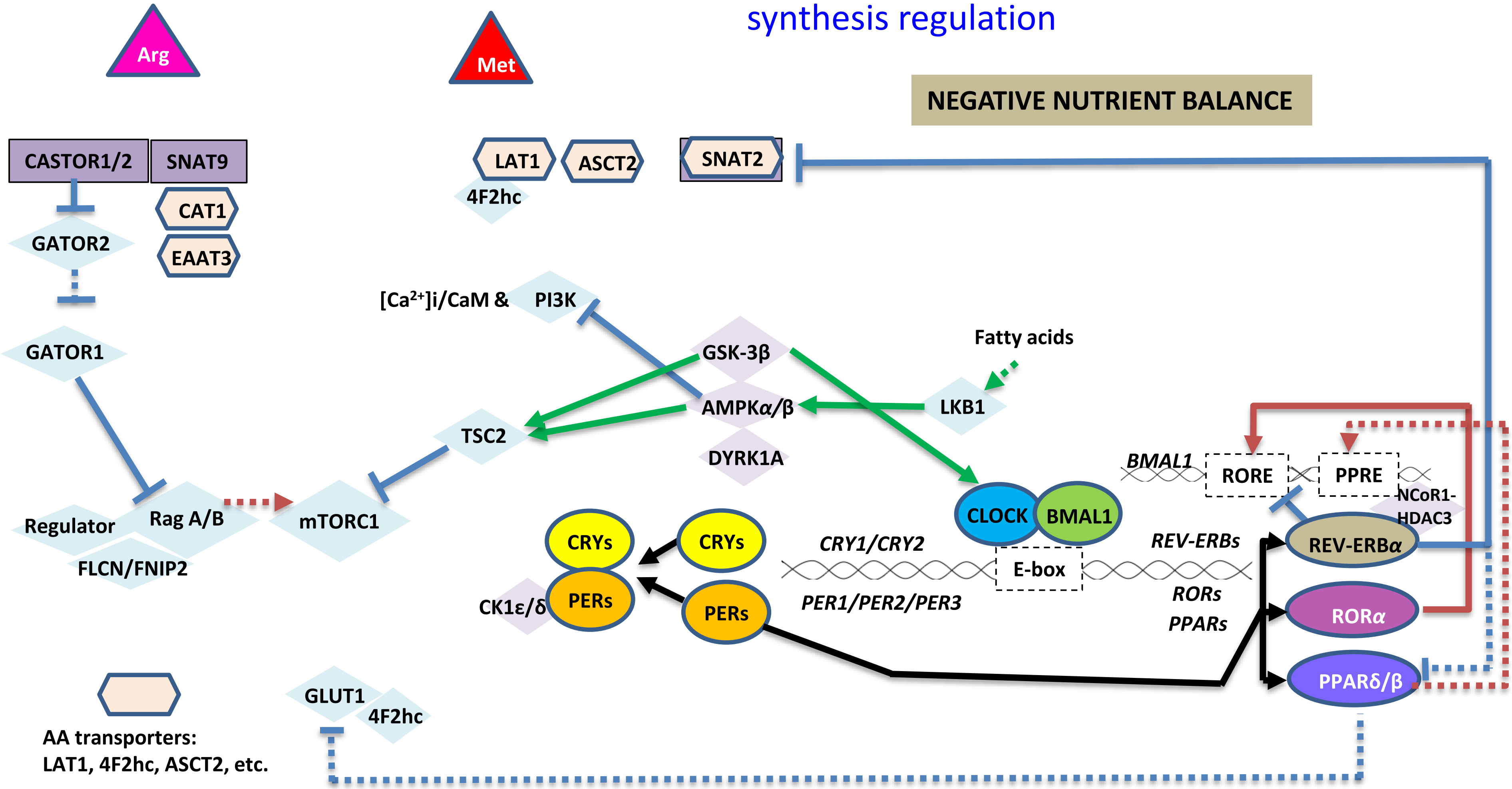
Meta-analysis to predict the effects of metabolizable amino acids on dairy cattle performance

I. J. Lean,* M. B. de Ondarza,† C. J. Sniffen,‡ J. E. P. Santos,§ and K. E. Griswold#[1]

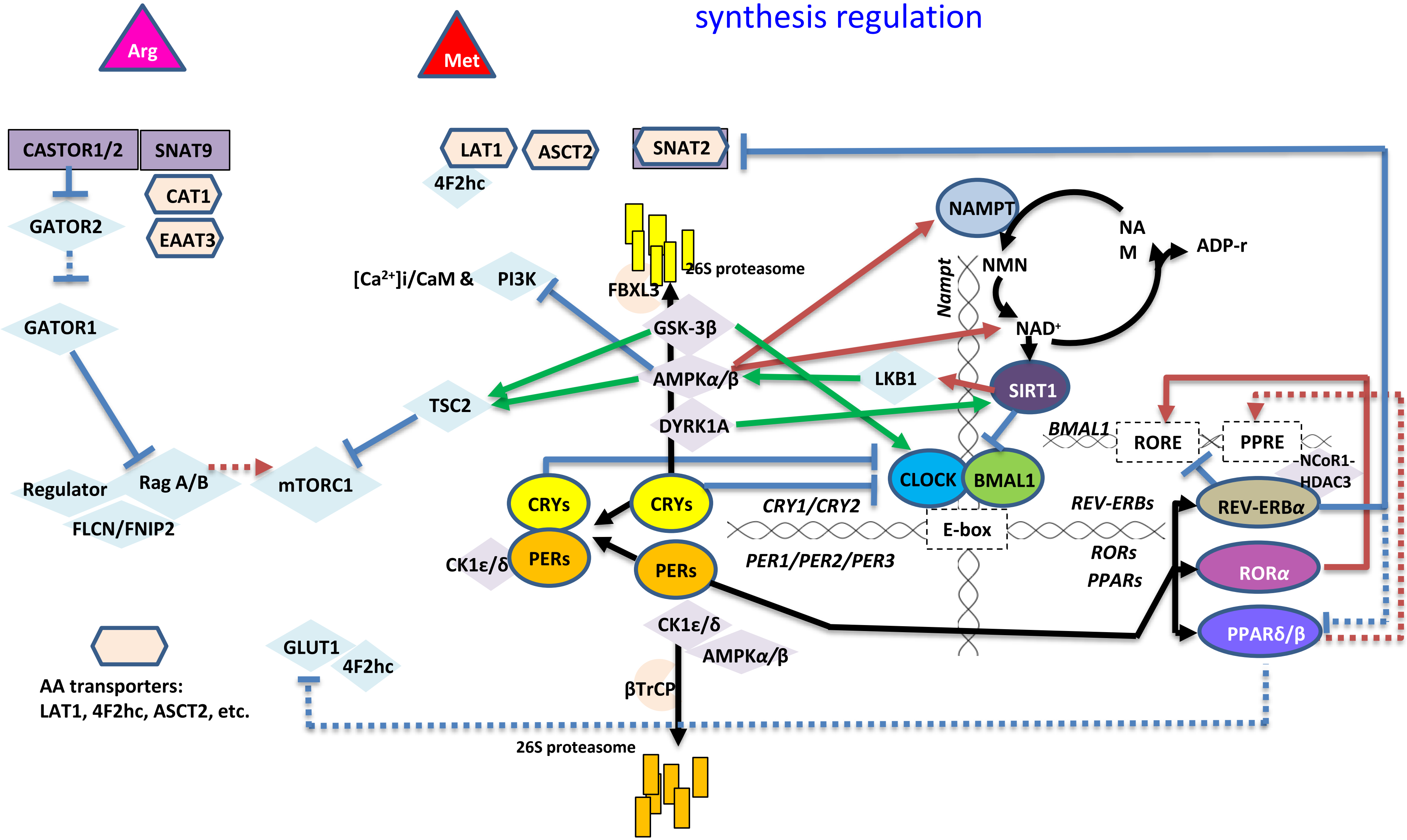
- Metabolizable <u>Methionine supply clearly</u> associated with milk protein % and yield
- Is there an **additive effect of Methionine and Arginine??**
- His, Trp, and Thr also may be limiting

Amino acid sensors, energy metabolism, CLOCK, and mTOR – novel mechanisms of protein synthesis regulation



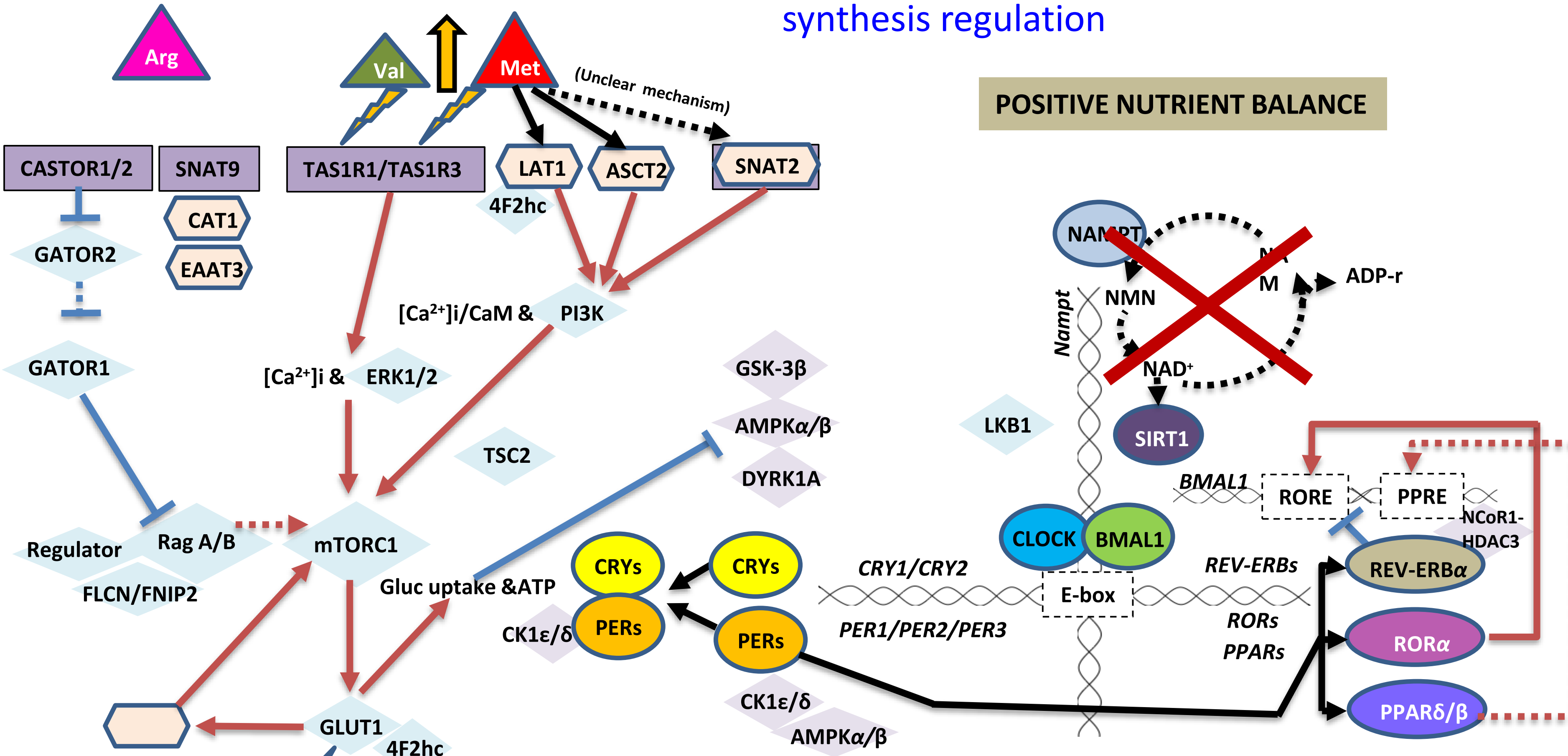
(Hu and Loor - unpublished)

Amino acid sensors, energy metabolism, CLOCK, and mTOR – novel mechanisms of protein synthesis regulation



(Hu and Loor - unpublished)

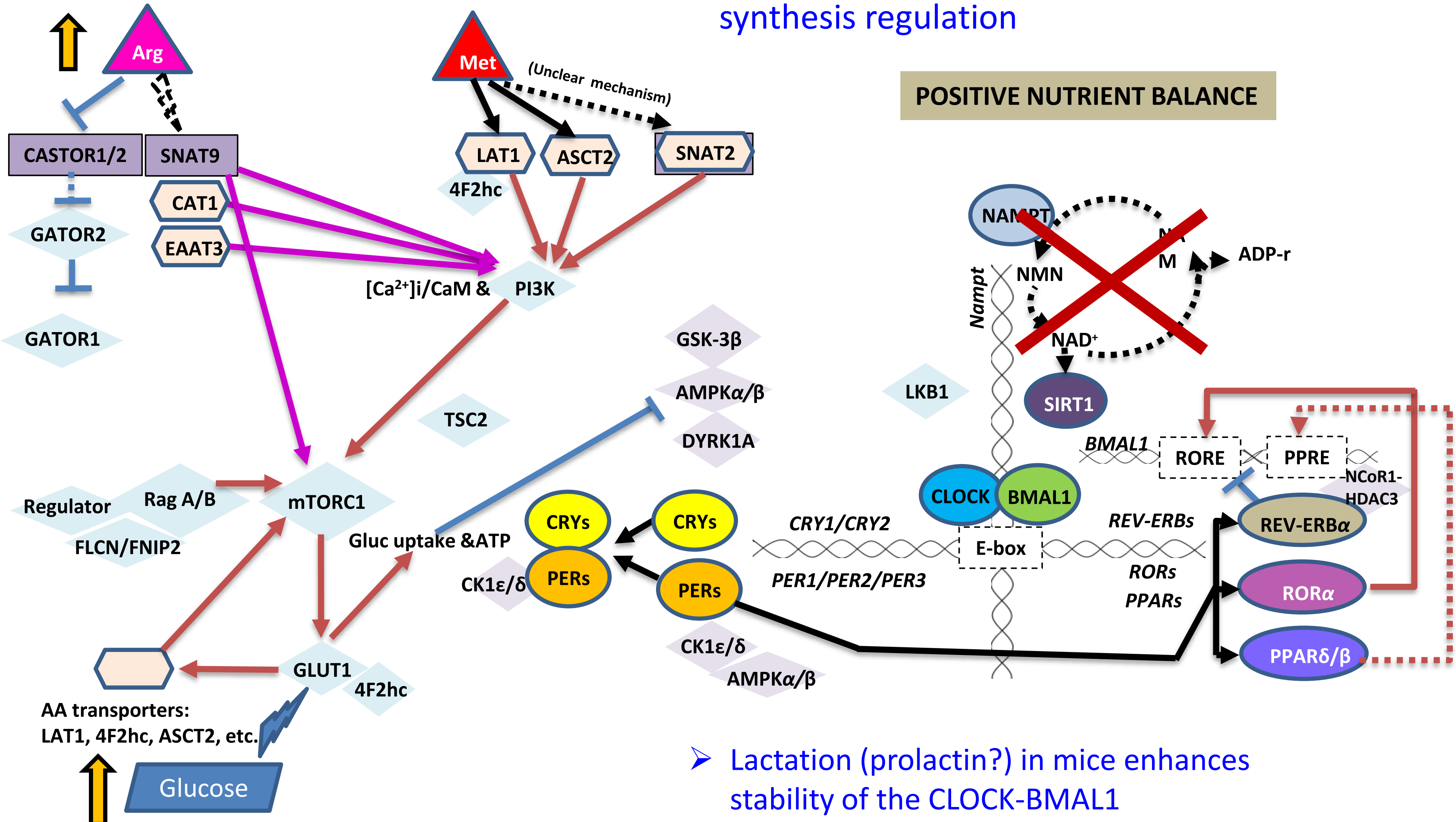
Amino acid sensors, energy metabolism, CLOCK, and mTOR – novel mechanisms of protein synthesis regulation



- Lactation (prolactin?) in mice enhances stability of the CLOCK-BMAL1 heterodimer ➔ ↑ metabolism??

(Hu and Loor - unpublished)

Amino acid sensors, energy metabolism, CLOCK, and mTOR – novel mechanisms of protein synthesis regulation



- Lactation (prolactin?) in mice enhances stability of the CLOCK-BMAL1 heterodimer ➔ ↑ metabolism??

(Hu and Loor - unpublished)

Beyond milk protein….

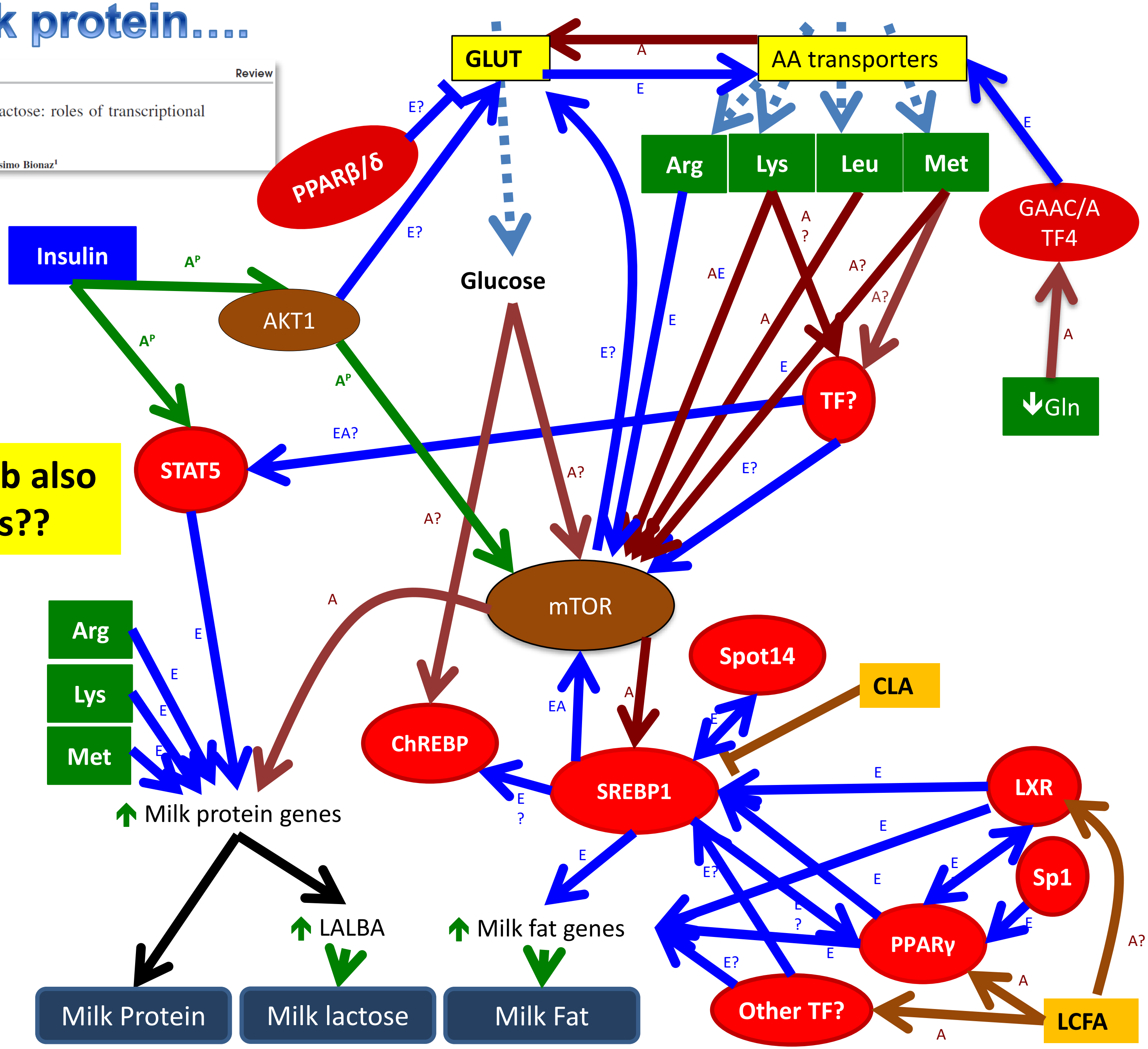
Physiol Genomics 48: 231–256, 2016.
First published January 26, 2016; doi:10.1152/physiolgenomics.00016.2015.

Review

Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation

Johan S. Osorio,[1] Jayant Lohakare,[1,2] and Massimo Bionaz[1]

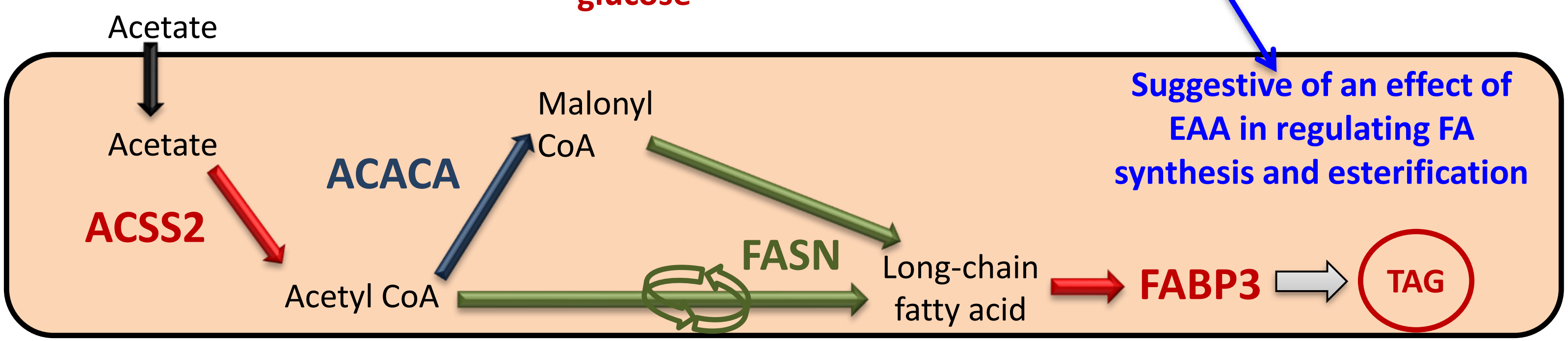
mTOR central hub also for lipid synthesis??



Lipogenic response of mammary gland to EAA

	Saline	Acetate	Casein	Glucose	Glu + Cas	Ace + Cas	SEM	*P*-value
Milk fat	1.41	1.58	1.42	1.55	1.51	1.65	-	-
ACSS2	0.48 **c**	0.80 **b**	0.90 **b**	1.17 **ab**	0.95 **b**	1.51 **a**	0.17	0.02
ACACA	0.51 **c**	0.75 **b**	0.85 **b**	0.79 **b**	0.87 **b**	1.34 **a**	0.16	0.05
FASN	0.45 **c**	0.79 **b**	0.91 **b**	0.95 **b**	0.72 **bc**	1.68 **a**	0.19	0.02
FABP3	0.01 **c**	0.47 **b**	0.01 **c**	0.06 **c**	0.01 **c**	1.80 **a**	0.62	0.01

Acetate response not greater than casein and glucose



- **Feed restriction to 85% of *ad libitum* intake (last 6 d of 14 d periods)**
- **Feeding 6 times/d**
- **Abomasal infusion of treatments**
- **Mammary biopsy on d 14**

(Danes, Batistel, Wattiaux, Broderick, and Loor - unpublished)

Summary and Perspectives

- Single-limiting amino acid theory may not always apply
  - Optimal"/"Ideal" intracellular concentrations? How to deliver to mammary cells?
  - Link with lipid synthesis?
  - Integrate transcription, translation, post-translational regulation: Systems approach
- Better description of transcription factor function
  - In silico analysis can provide viable candidates
  - In vitro culture (primary or immortalized cells) to determine function/s
- Epigenetic mechanisms
  - Methylation status of TF binding sites ➔ link with AA nutrition?
  - Programming effects of mammary gland ➔ in utero and/or prior to weaning?