

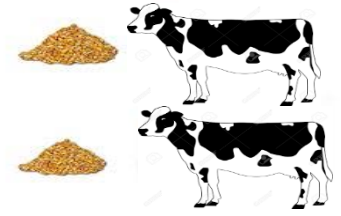


Identification of Genetic Markers Associated with Feed Efficiency in Holstein Calves

Miri Cohen-Zinder, Aviv Asher, Ehud Lipkin, Rotem Agmon, Arie Brosh, and Ariel Shabtay

Agricultural Research
Organization

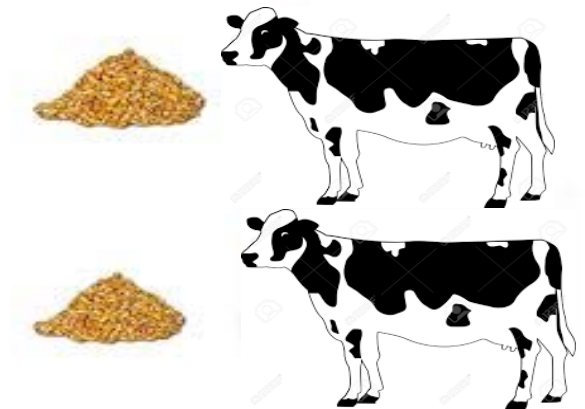
Feed Efficiency (FE): Importance



- A major component determining the **profit** of livestock production.
- Beef cattle production: **55-75% - feed costs** (NRC 2000)
 - Feed costs = ~65% in calf feeding systems
 - Feed costs = ~75% in yearling finishing systems
- Difficulties in FE measurements due to different:
 - Classes (growing, finishing, breeding)
 - Cattle Breeds
 - Biological systems (nutrition, reproduction, lactation, basal metabolism)

Feed Efficiency: why to improve?

- USA: annual cost savings of \$US 1 billion by increasing FE by 10% (equiv. to reduction of individual daily intake by 0.9 Kg).
- Feed-efficient cattle produce between 15-25% less enteric methane (CH₄) compared with non-efficient animals.
- **Economic and ecological concerns drive the need to improve feed efficiency.**



Residual Feed Intake (RFI) - phenotyping

- Efficient (Low score RFI) animals consume less feed than expected.
- Significant economic advantage over inefficient animals (High score RFI).
- Phenotyping is complex, expensive and requires special equipment. Hence, can not be achieved at the farm level.
- **Challenge: to develop phenotypic RFI-based programs for future genetic selection.**



Individual feeding system
(Newe - Ya'ar)

Genetics of RFI

- RFI is:
 - A complex trait
 - Controlled by various biological mechanisms
 - Affected by many genes
- **RFI has moderate heritability**
- The current use of genetic markers in selection programs is limited
- Genetic & phenotypic inconsistencies results from variations in:
 - **Breed**
 - **Age**
 - **Diet** (most studies report one-period diet)
- **Development of universal genetic markers which would reflect Genetic & phenotypic consistency across breeds, ages and diets**



Targeted sequence capture approach

Experimental design and Data generation



26 Holstein calves
#growing phase

3 Nutritional periods
#100d each

RFI 1:
TMR diet ME of 2.8 Mcal/kgDM
Ave. age: 7.26 MO

RFI 2:
TMR diet ME of 2.1 Mcal/kgDM
Ave. age: 11.5 MO

RFI 3:
TMR diet ME of 2.8 Mcal/kgDM
Ave. age: 18 MO

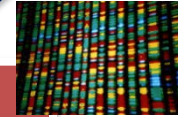
Measuring growth &
production parameters (mid-
#BW, ADG, DMI, RFI)

Selection of Calves with phenotypic
consistency: efficient (L) or inefficient (H)
(@ 3 RFIs)

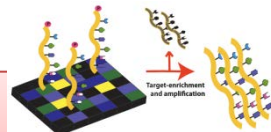
Low (L) RFI group
#N=7

High (H) RFI group
#N=8

#Genomic Analysis

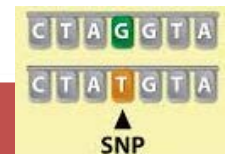


Targeted sequence capture
<260 genes of FE and RFI



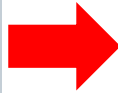
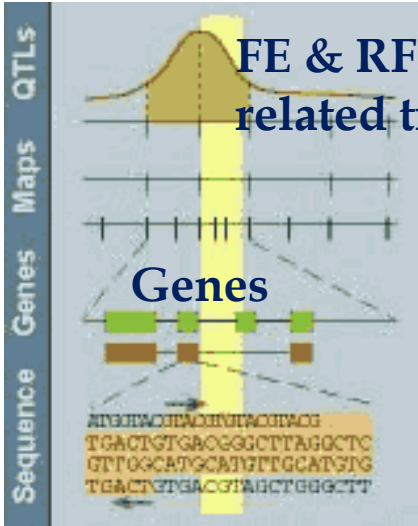
Data generation &
#Bioinformatic analysis

Detection of SNPs
#associated with RFI



#Targeted sequence capture approach:

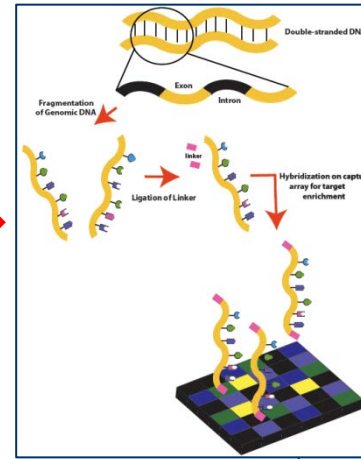
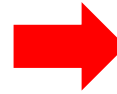
>260 genes



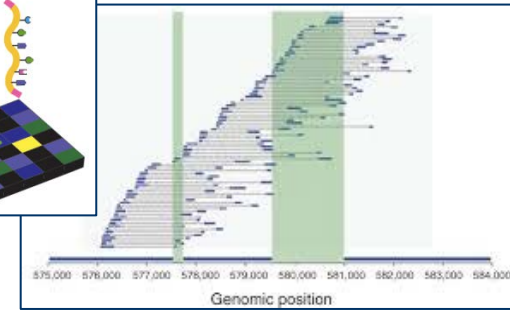
High RFI
N=8



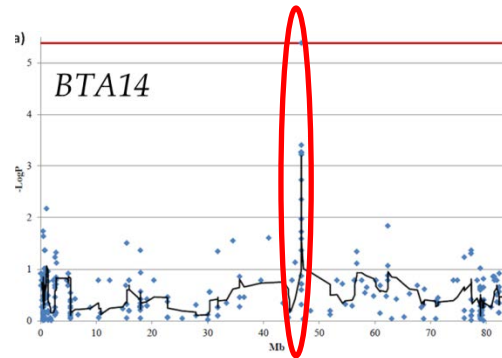
Low RFI
N=7



Targeted
sequence
capture

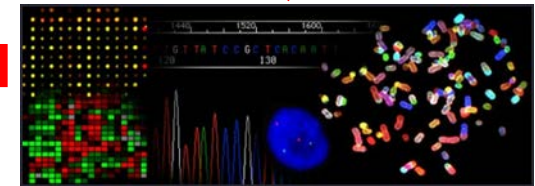


- Metabolism
- Muscle activity
- Thermoregulation
- Immunity
- Mitochondrial respiration

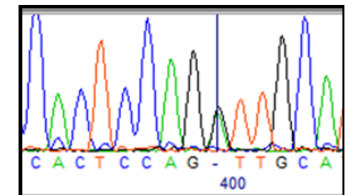


Detection of SNP
markers in
candidate genes

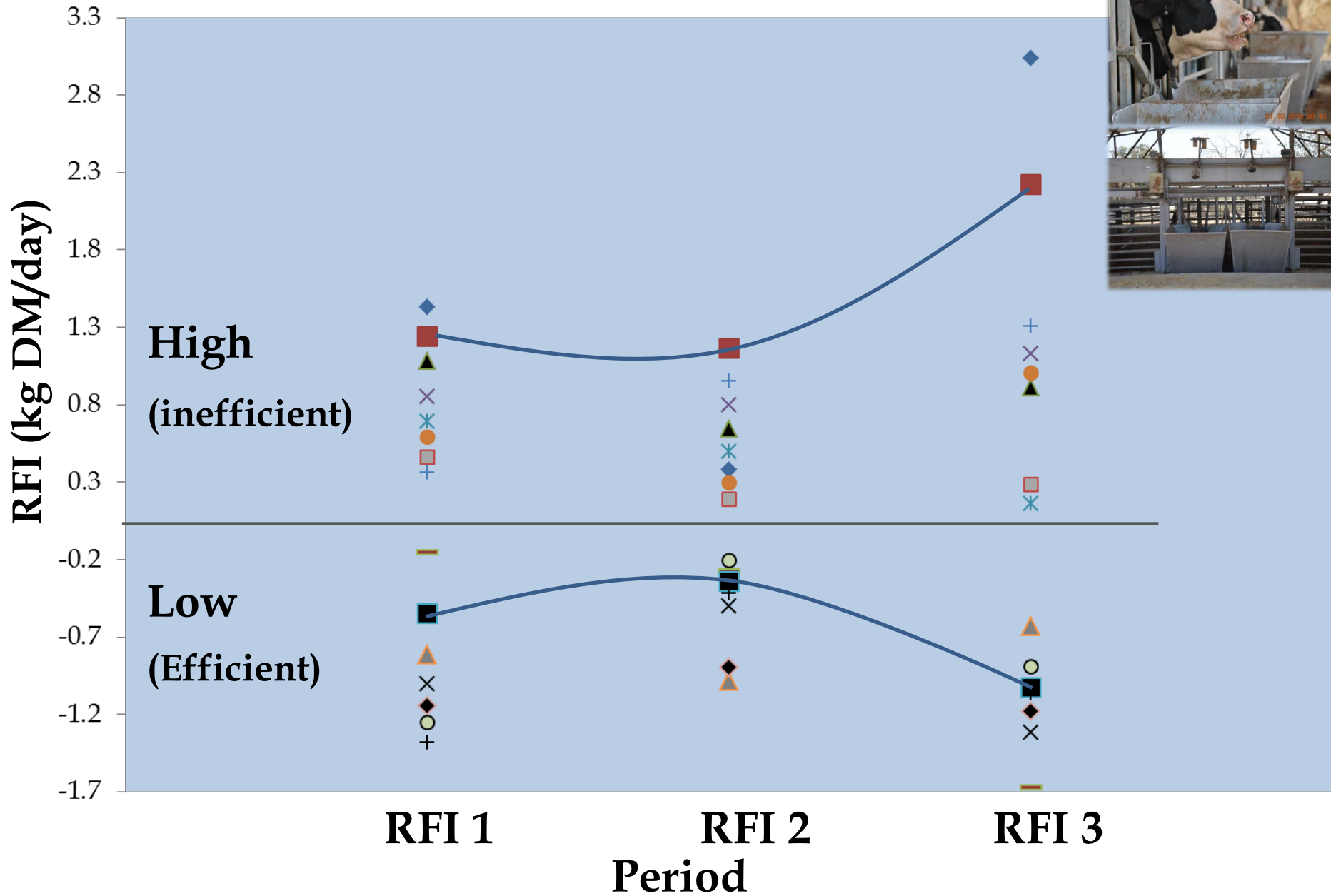
NGS



Genomic
analysis



RFI Performances & consistency



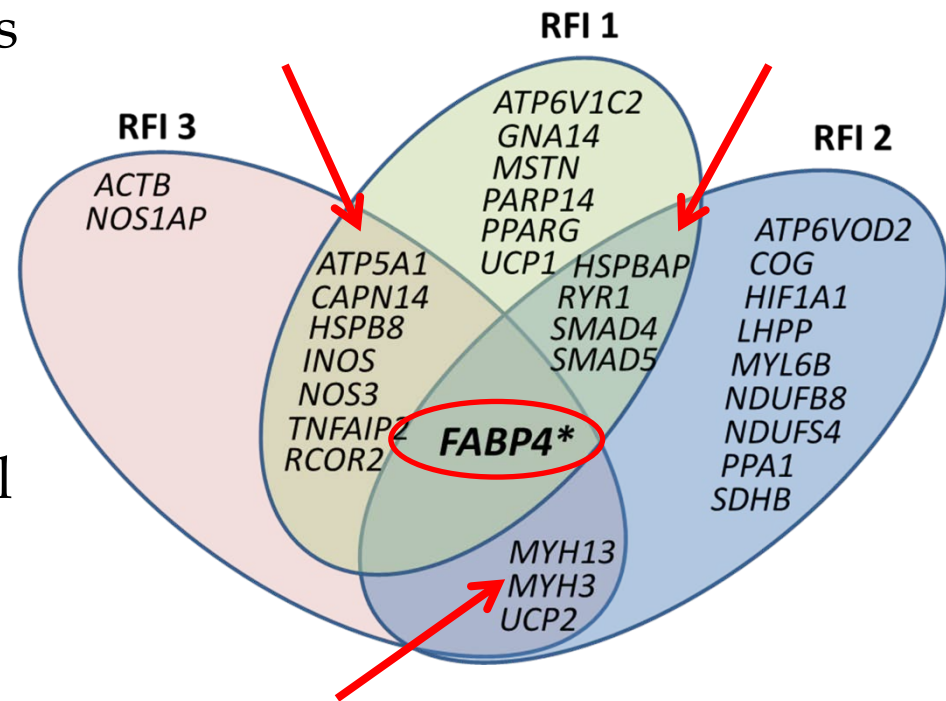
Targeted sequencing analysis - results

Region	SNPs	%	RFI 1	RFI 2	RFI 3
<u>Promoter</u>	1,451	10	5	0	7
<u>Exon</u>	756	5.2	8	6	8
Intron	4,305	29.7	2	6	7
<u>3'UTR</u>	214	1.5	8	1	1
Downstream	1,017	7.0	6	7	5
Intergenic	6,761	6.6	1	7	9
Total	4,504	100.0	00	00	00

Classification of SNPs (n=100) showing most significant P-values ($P \leq 0.001$) under RFI 1-3.

Polymorphisms present in RFI associated #genes

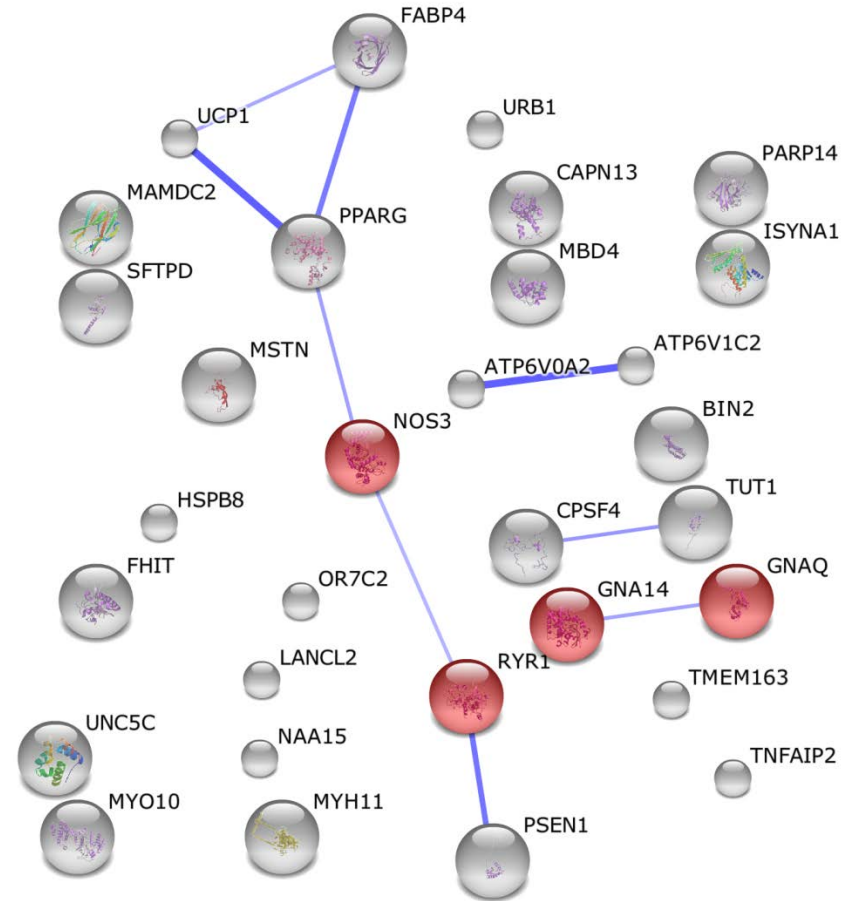
- Forty-eight SNPs from 32 genes were significant in at least one RFI period ($P \leq 0.05$).
- FABP4* was the only gene with a polymorphism (*FABP4_5*) that was significant between all three RFIs.



Overlap of genes harboring significant SNPs ($P \leq 0.05$) in three RFI periods.

Oxidative phosphorylation signaling Pathway (RFI 1 & RFI 3)

- Overlapping genes between RFI 1 and RFI 3
- Similar dietary conditions were shared between RFI 1 and RFI 3

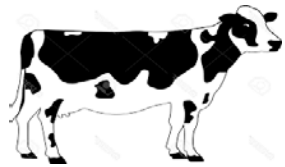


#1.3X10-6

STRING interaction network



RFI 2 – Low quality diet – (maintenance energy)



2.1 Mcal/kgDM

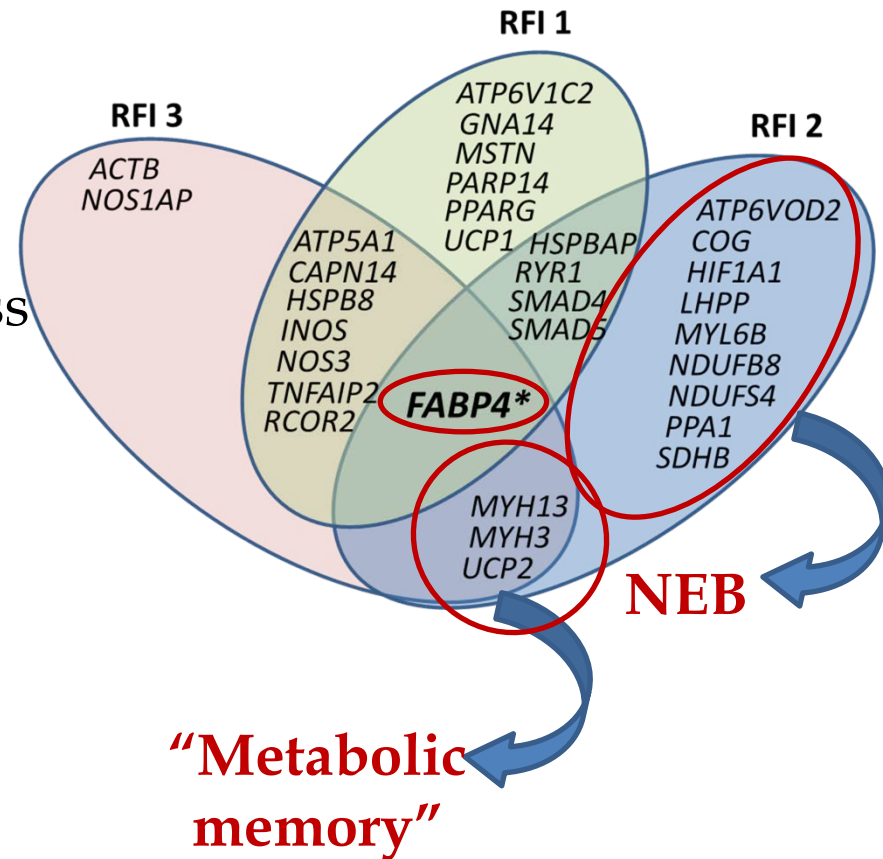


NEB during RFI2 --- metabolic stress

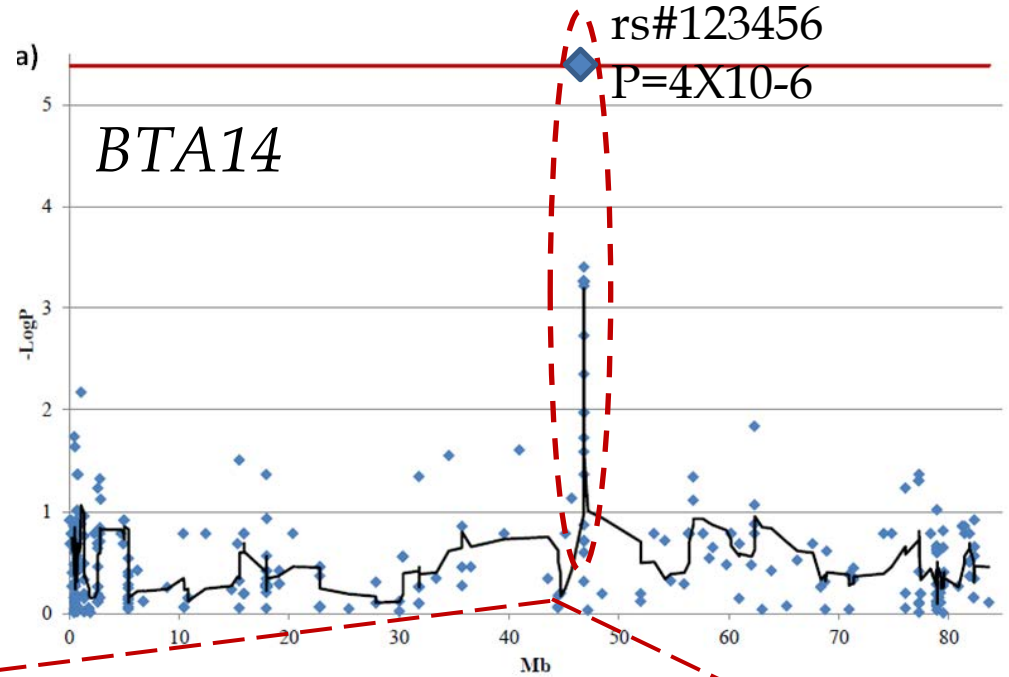
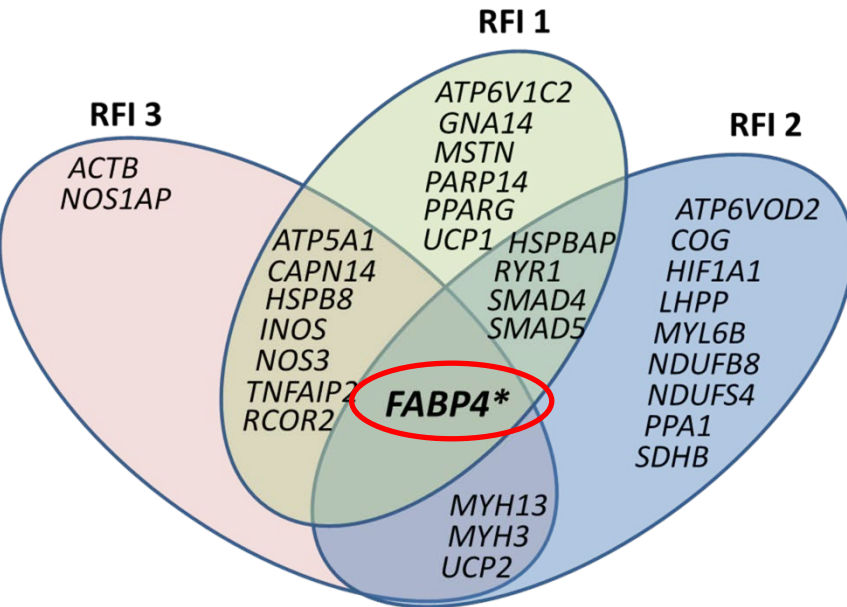
Adipose tissue degradation & a shift in protein turnover

NEB accompanied by imbalance between muscle protein synthesis & degradation

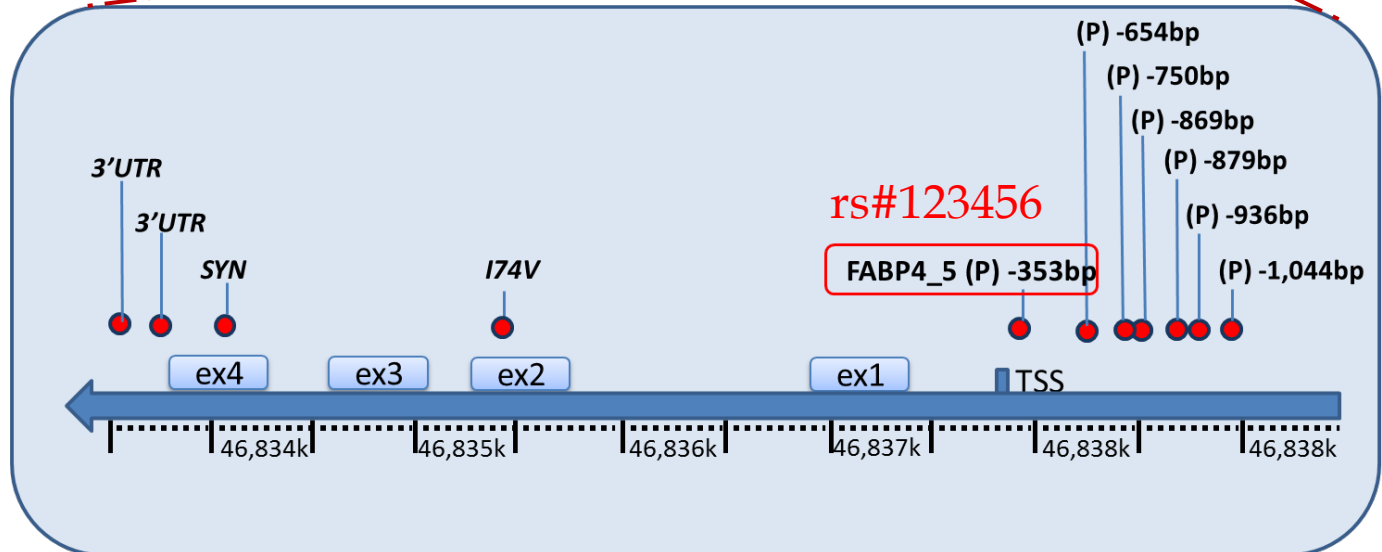
Common SNPs in MYH3, MYH13 muscle genes, shared between RFI 2 & RFI 3



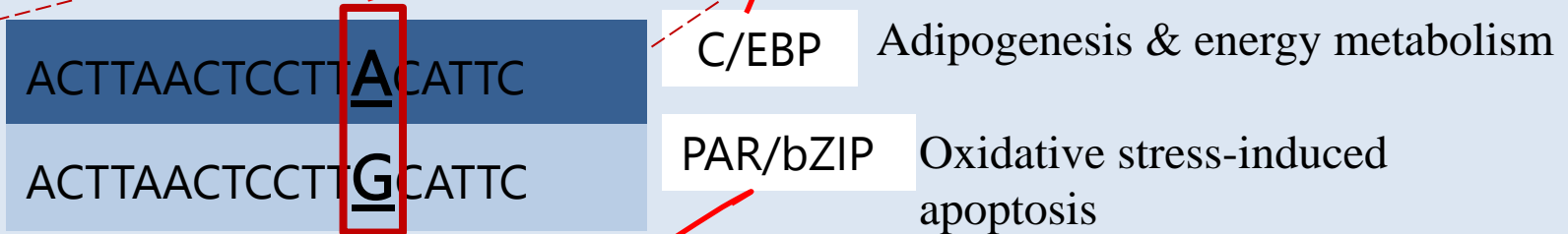
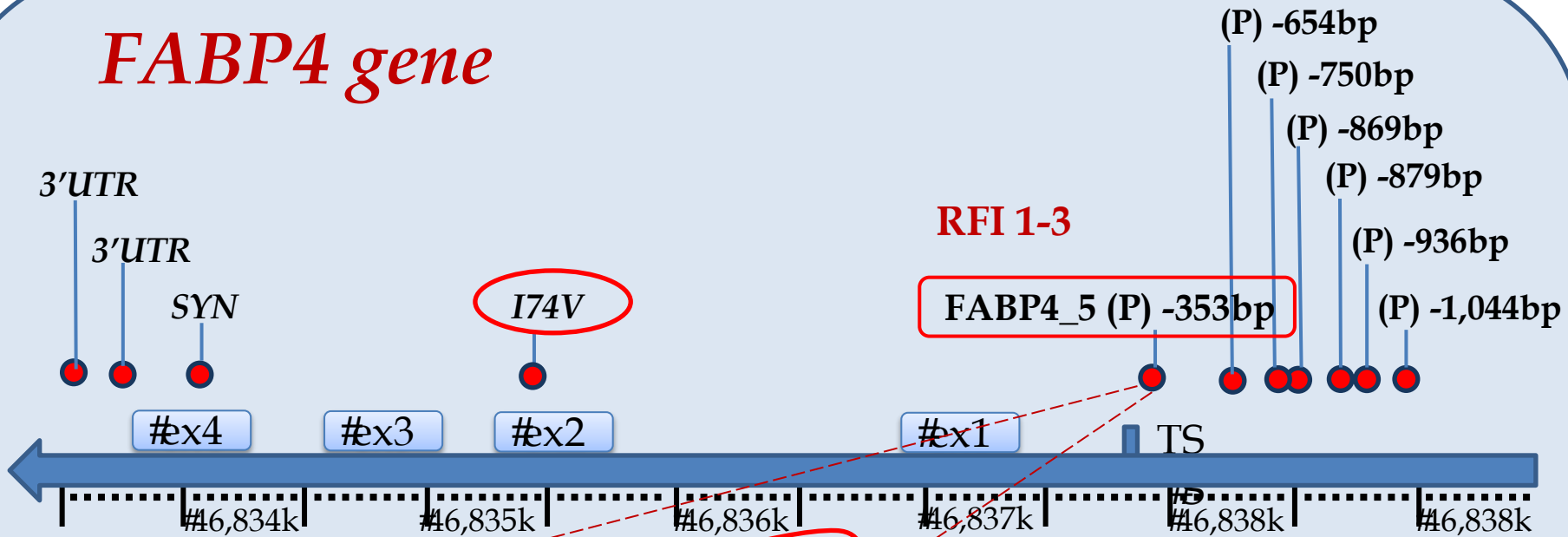
Association studies



FABP4
gene



FABP4 gene



Efficient-low RFI animals : A/G

Inefficient high-RFI animals : A/A

#Summary

- Using targeted sequencing, significant SNPs associated with each RFI (1-3) period were identified.
- The relatively high number of polymorphisms shared by RFI 1 & RFI 3 imply there is an effect of the diet on the genetics of FE.
- *FABP4* polymorphisms, across different diets and ages might reveal possible candidate markers for RFI and FE in the Holstein breed.
- The most significant SNP, FABP4_5 (4×10^{-6} in RFI 1), located in the promoter region of the gene – **a universal marker?**
- Following a larger scale validation, these currently identified genetic variants might be tested in Holstein cows and other cattle breeds.



#Acknowledgments

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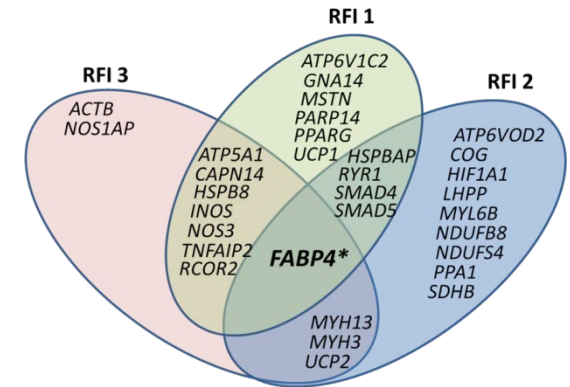
The Hebrew University, Jerusalem

Ehud Lipkin

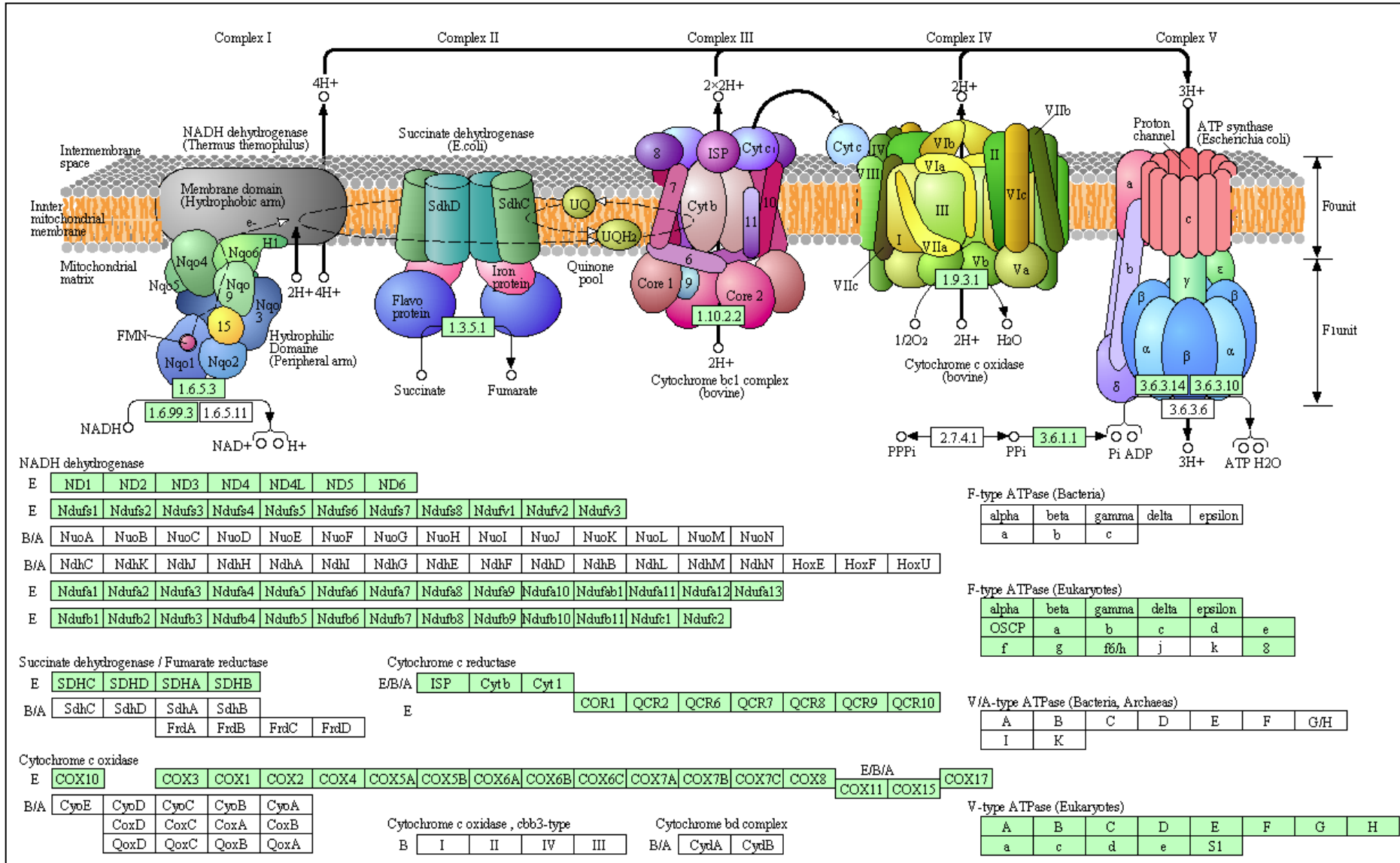


**Bar-Ilan University, Faculty
of Medicine in the Galilee**

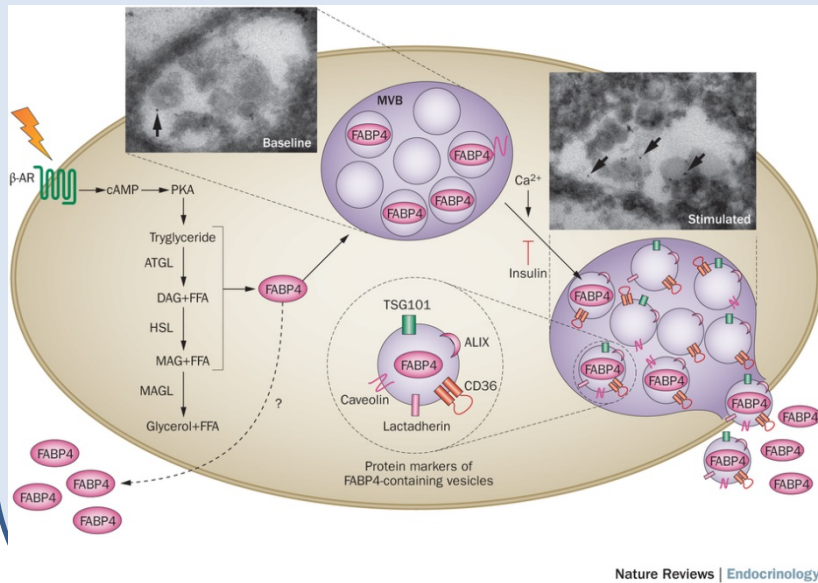
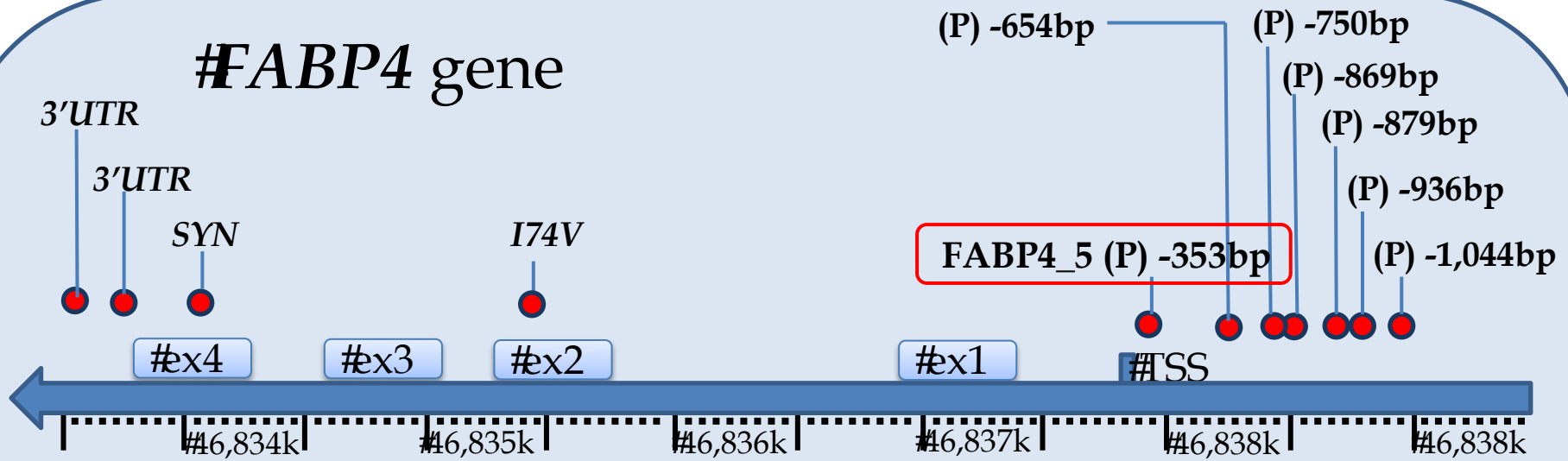
David Karasik
Roi Feingersch



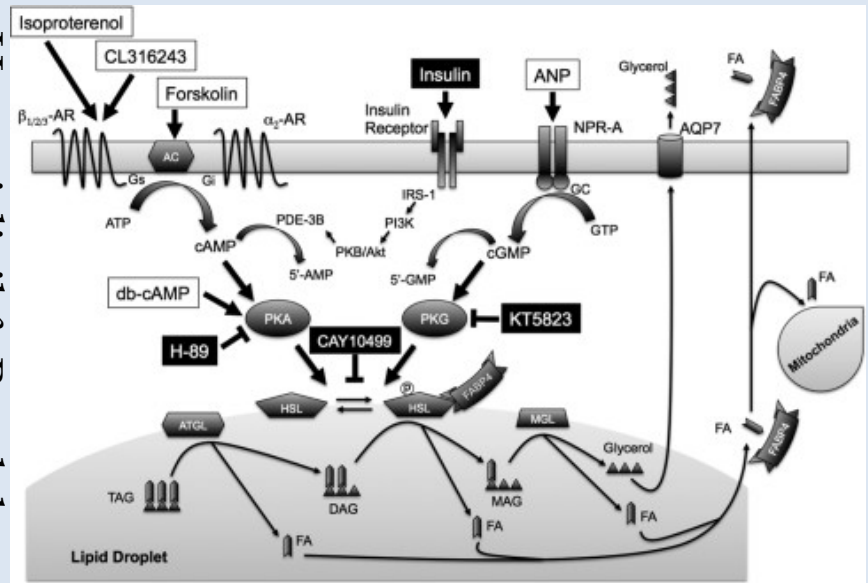
Oxidative phosphorylation signaling Pathway



#FABP4 gene

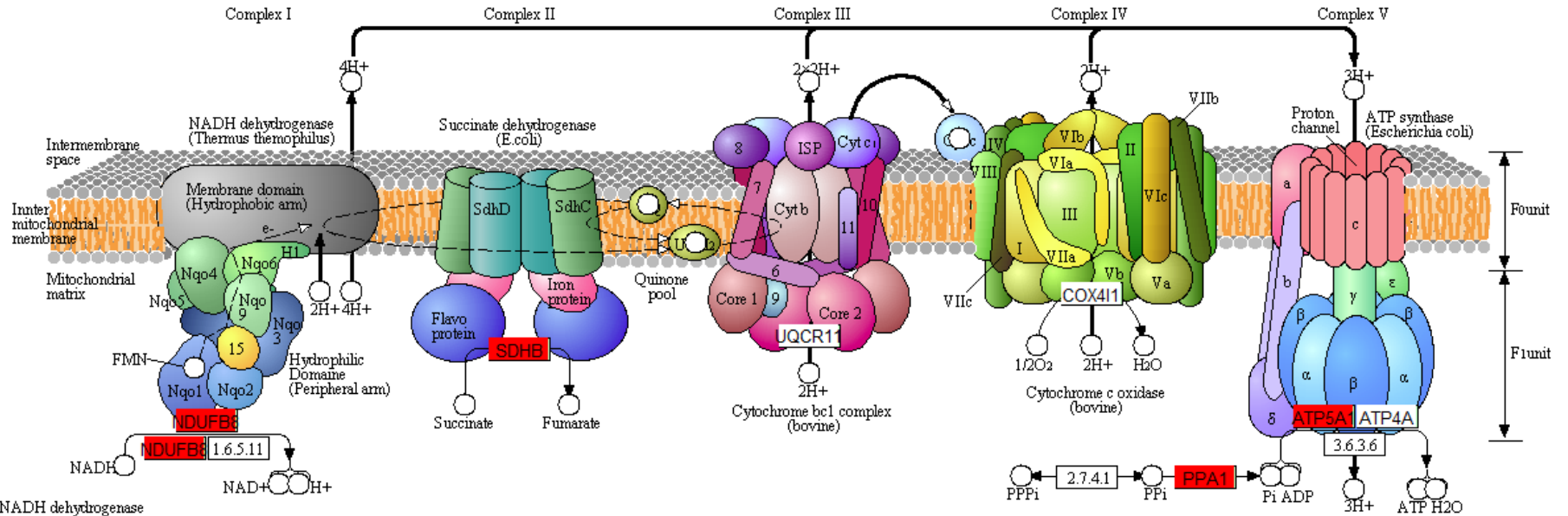


Hotamisligil & Bernlohr
2015



Mita et al. 2014

Oxidative phosphorylation signaling Pathway



NADH dehydrogenase

E ND1 ND2 ND3 ND4 ND4L ND5 ND6

E NDUFS1 NDUFS2 NDUFS3 NDUFS4 NDUFS5 NDUFS6 NDUFS7 NDUFS8 NDUFS9 NDUFV1 NDUFV2 NDUFV3

B/A NuoA NuoB NuoC NuoD NuoE NuoF NuoG NuoH NuoI NuoJ NuoK NuoL NuoM NuoN

B/A NdhC NdhK NdhJ NdhH NdhA NdhI NdhG NdhE NdhF NdhD NdhB NdhL NdhM NdhN HoxE HoxF HoxU

E NDUFA1 NDUFA2 NDUFA3 NDUFA4 NDUFA5 NDUFA6 NDUFA8 NDUFA9 NDUFA10 NDUFA11 NDUFA12 NDUFA13 NDUFA14 NDUFA15

E NDUFB1 NDUFB2 NDUFB3 NDUFB4 NDUFB5 NDUFB6 NDUFB7 NDUFB8 NDUFB9 NDUFB10 NDUFB11 NDUFC1 NDUFC2

Succinate dehydrogenase / Fumarate reductase

E SDHC SDHD SDHA SDHB

B/A SdhC SdhD SdhA SdhB
FrdA FrdB FrdC FrdD

Cytochrome c reductase

E/B/A JQCRF5 CYTB CYC1

E UQRCR1 UQRCR2 UQCRH1 UQCRB1 UQCRQ1 UQCR10 UQCR11

Cytochrome c oxidase

E COX10 COX3 COX1 COX2 COX411 COX5A COX5B COX6A COX6B COX1487 COX7A COX7B COX7C COX8A COX11 COX15 COX17

B/A CyoE CyoD CyoC CyoB CyoA
CoxD CoxC CoxA CoxB
QoxD QoxC QoxB QoxA

Cytochrome c oxidase, cbb3-type

B I II IV III

Cytochrome bd complex

B/A CyoA CyoB

F-type ATPase (Bacteria)

alpha	beta	gamma	delta	epsilon
a	b	c		

F-type ATPase (Eukaryotes)

ATP6A ATP5B ATP5C1 ATP5D ATP5E
ATP5O ATP6 ATP5F1 ATP5G2 ATP5H ATP5I
ATP5J2 ATP5L ATP5J j k ATP8

V/A-type ATPase (Bacteria, Archaeas)

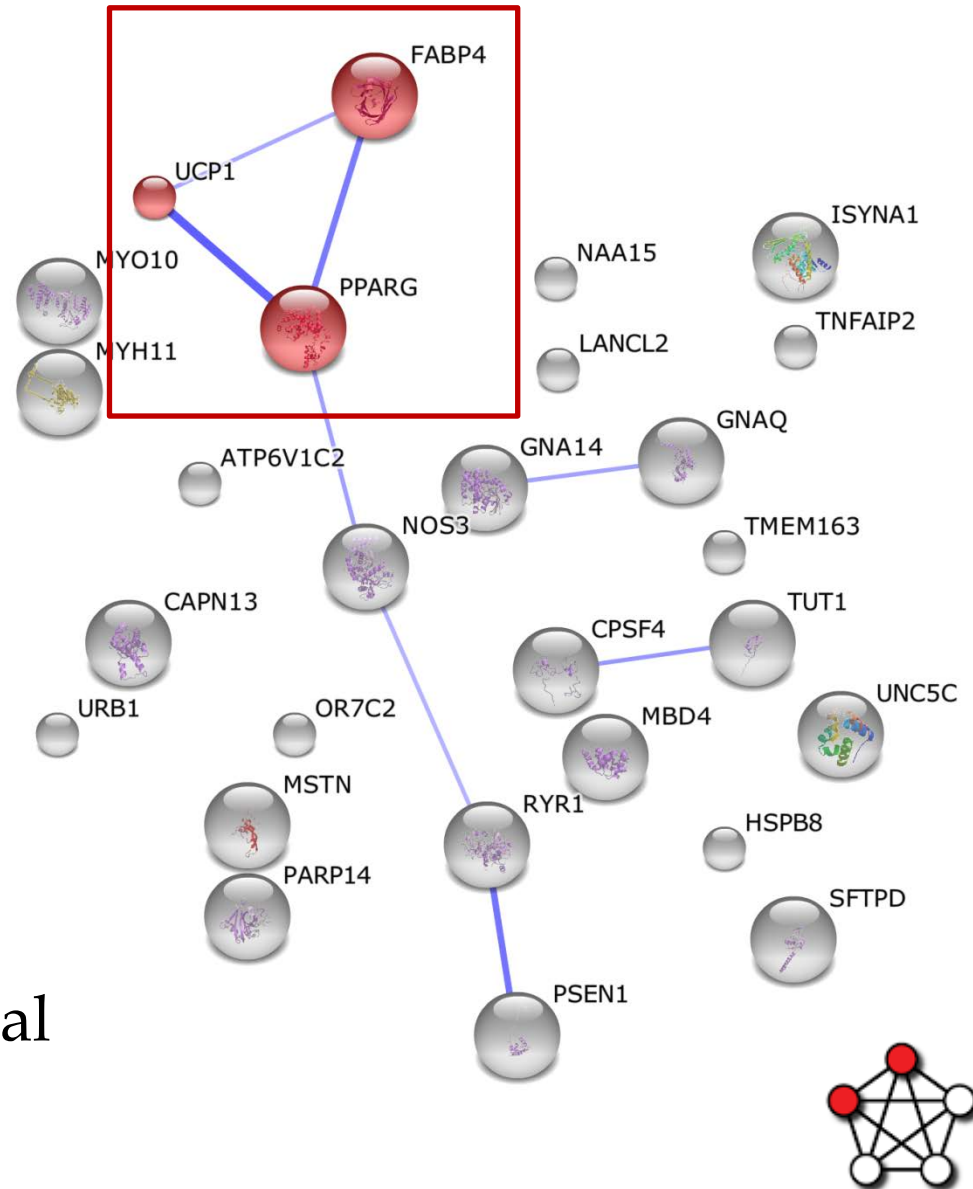
A	B	C	D	E	F	G/H
I	K					

V-type ATPase (Eukaryotes)

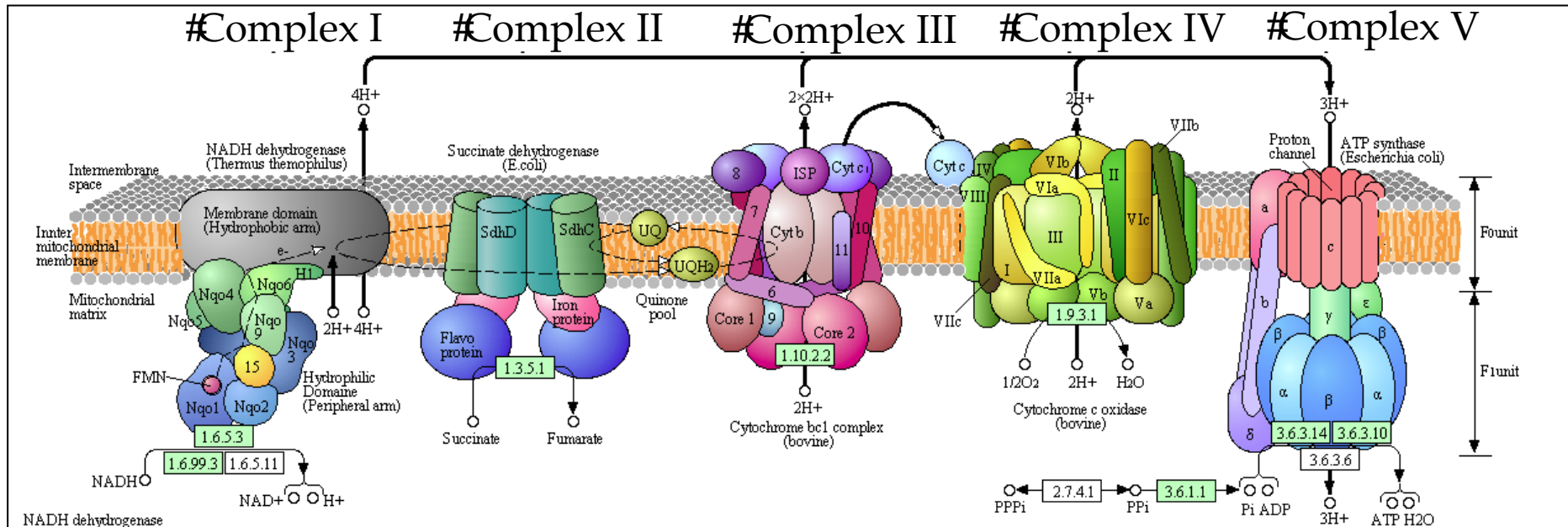
ATP6V1 ATP6V1A ATP6V1B ATP6V1C ATP6V1D ATP6V1E ATP6V1F ATP6V1G ATP6V1H
ATP6V0 ATP6V0A ATP6V0B ATP6V0C ATP6V0D ATP6V0E ATP6V0F

PPAR-gamma signaling Pathway (RFI 1 & RFI 3)

- PPARG: promotes adipocyte differentiation to enhance blood glucose uptake.
- Similar dietary conditions between RFI 1 and RFI 3
- FABP4: Lipid transport protein in adipocytes.
- UCP1 - mitochondrial, proton carrier creates proton leaks across the inner mitochondrial membrane



Complexes of the OXPHOS metabolic Pathway (inner membrane of the cell's mitochondria)



NDUFS4
NDUFB8

SDHB

PPA1

ATP5A1

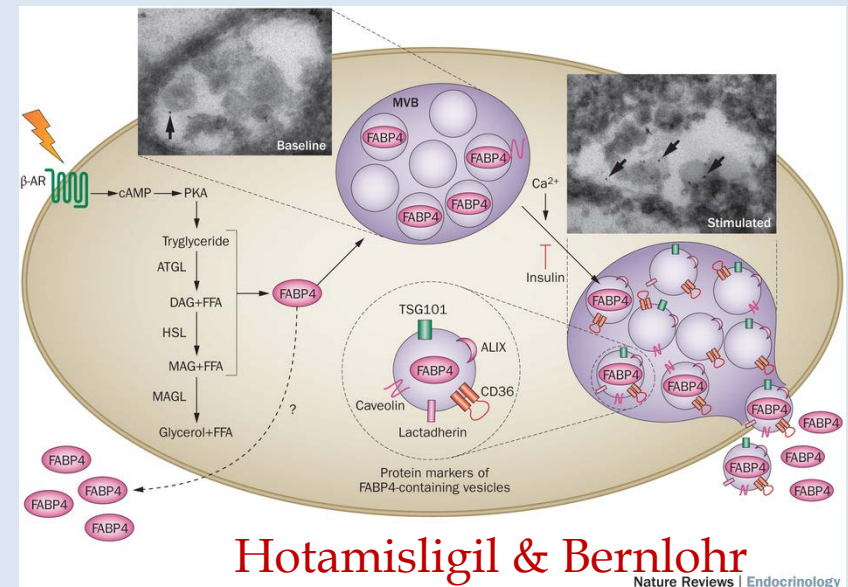
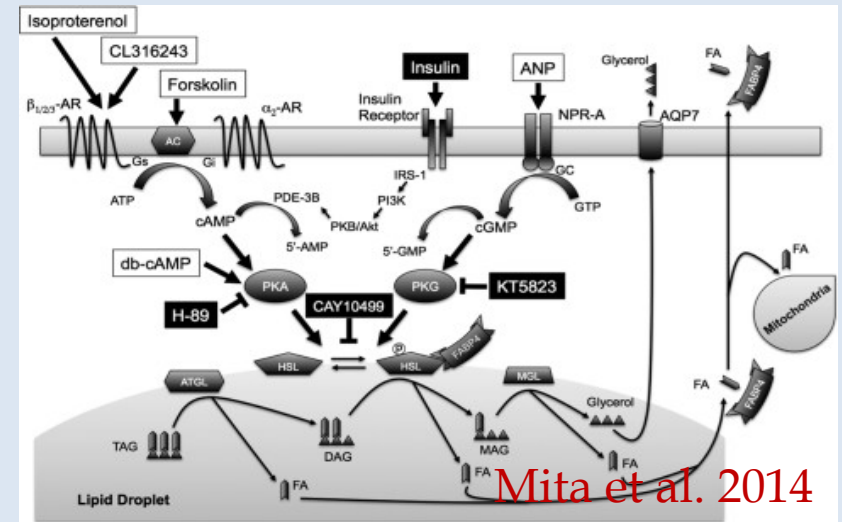
ATP6V0A2

ATP6V1C2

KEGG pathway diagram

#FABP4 gene

- Cytosolic proteins
- Bind hydrophobic ligands
- Saturated & unsaturated long chain FA
- Transports lipids to specific compartments in the cell.
- FABP4 is expressed in adipocytes and macrophages.
- FABP4 secretion from adipocytes is in association with lipolysis



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2015

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