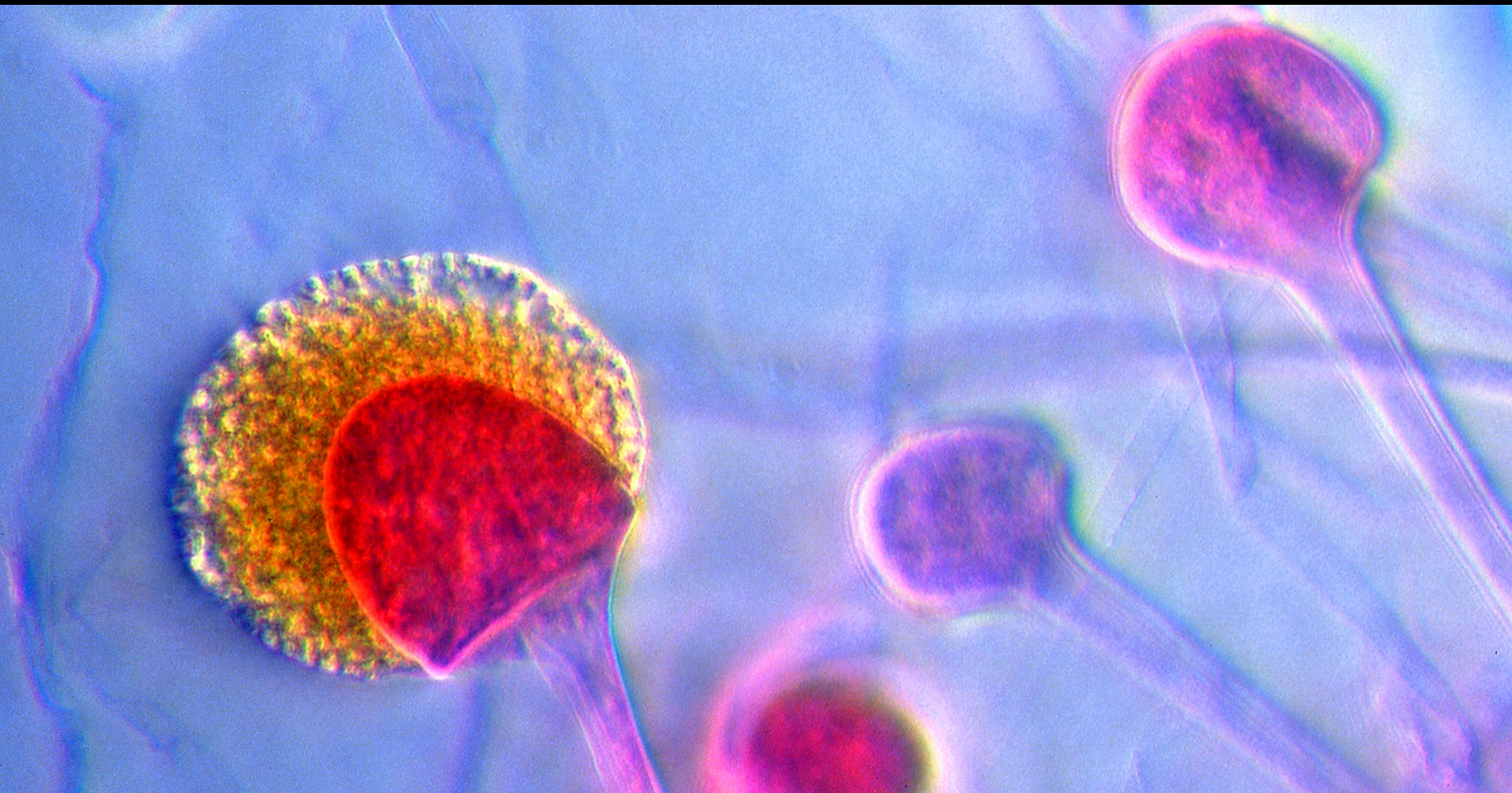
Molecular Medicine Meets CIRS

James Ryan, PhD
Progene DX LLC
jryan@progenedx.com



Disclosures

James Ryan is a principal in Progene DX, a company that sells the transcriptomic test called GENIE

Gene ExpressioN: Inflammation Explained

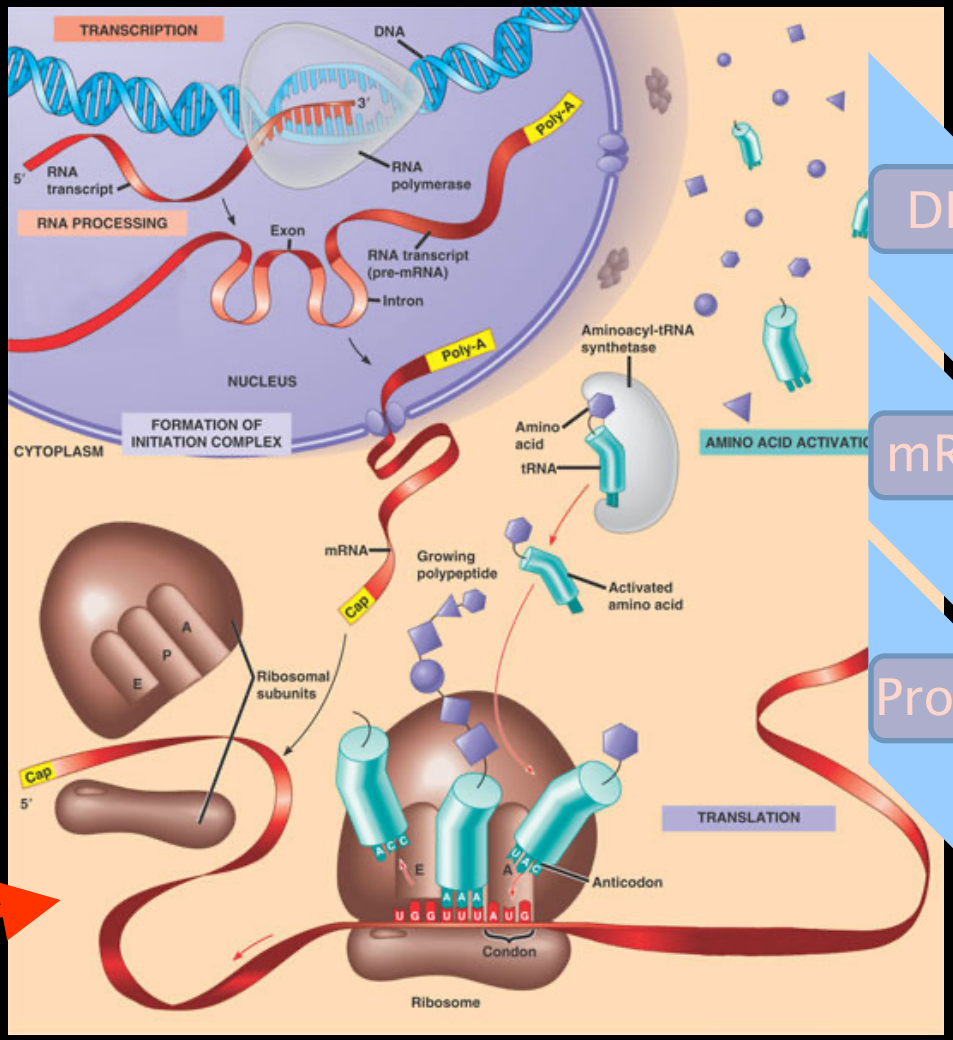
Basics – Why are cells different

1. Our bodies are made of tissues, cells organized for a common function
2. Cells derive their function by differential gene expression
3. Although some cells are highly specialized, e.g., neuron vs epithelial cell, they all use the same basic machinery, e.g., ribosomes, mitochondria.

Specialized function

1. Cells derive their purpose by differential gene expression – HOWEVER – it's the translation of genes into proteins that give each cell its character
2. Transcription and translation have several regulatory mechanisms to control the fate of a cell.

Central Dogma of Molecular Biology



DNA
- Highly stable (yrs)

mRNA
- Highly unstable (hrs)

Protein
- Moderately stable (days)

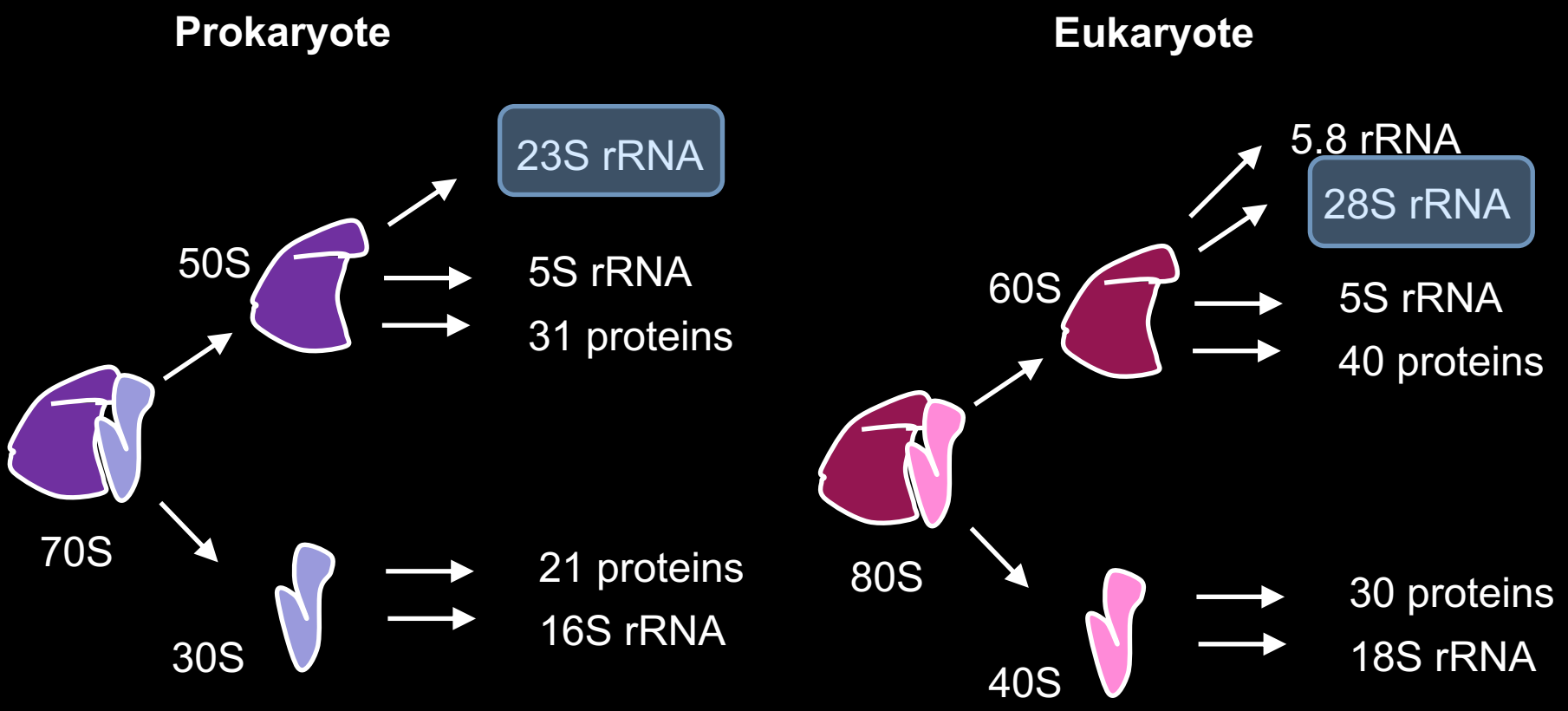
Post Transcriptional Control

(Transcript degradation)

Eukaryotic Ribosomes

- Protein biosynthesis
- Made of 2 subunits: Eukaryotes 40S and 60S
- Functional unit referred to 80S ribosome in 5M ribosomes/cell, 25% of cell's dry mass
- Mass of ribosomes is roughly $^{1}/_{2}$ RNA

Conserved Evolution



Sarcin-Ricin Loop (SRL)

1. All living organisms need to make protein

2. Regardless of species, ribosomes must read the messenger RNA codons, bind proper charged transfer RNAs, join amino acids and then move to the next codon

3. These highly specific requirements lead to what is one of the most highly conserved sequences in all living organisms – Sarcin-Ricin loop of ribosomal RNA

Translation Inhibition

- Dunbar et al, C. elegans Detects Pathogen-Induced Translational Inhibition to Activate Immune Signaling. (Cell Host & Microbe *11*, 2012)

- McEwan et al, Host Translational Inhibition by Pseudomonas aeruginosa Exotoxin A Triggers an Immune Response in Caenorhabditis elegans (Cell Host & Microbe *11*, 364–374, 2012)

"Here we show that *P. aeruginosa* infection inhibits mRNA translation in the intestine via the endocytosed translation inhibitor Exotoxin A…

Thus, translational inhibition, a common pathogenic strategy, can trigger activation of an immune surveillance pathway to provide host defense"

Ribosomal Control of Cell Fate

- Ribosomes were long thought to be homogeneous nanomachines
- Recently, ribosomes were found to have different subunit compositions based on tissue in animal models
- Ribosomes were also found to have subunit heterogeneity according to developmental stages – embryogenesis

Ribosomal Control of Cell Fate

- Now we understand ribosome subunit heterogeneity can preferentially translate certain transcripts.
- Additionally, there are hundreds of proteins that interact with the ribosome, potentially tuning preferences even further.
- Do CIRS patients show ribosome heterogeneity?

Post Transcriptional Control

(Ribosomal selection – IRES)

Ribosomal Control of Cell Fate

Do CIRS patients show ribosome heterogeneity?

MitoRibo Large Subunit - CV

Subunit	CV
MRPL	0.14
MRPL	0.15
MRPL	0.15
MRPL	0.20
MRPL	0.15
MRPL	0.16
MRPL	0.21
MRPL	0.16

MitoRibo Small Subunit - CV

Subunit	CV
MRPS	0.14
MRPS	0.15
MRPS	0.16
MRPS	0.20
MRPS	0.20
MRPS	0.17
MRPS	0.16

Ribosome Large Subunit - CV

Subunit	CV
RPL	0.40
RPL	0.35
RPL	0.34
RPL	0.33
RPL	0.31
RPL	0.29
RPL	0.29
RPL	0.27
RPL	0.26

Ribosome Small Subunit - CV

Subunit	CV
RPS	0.41
RPS	0.39
RPS	0.38
RPS	0.34
RPS	0.33
RPS	0.27
RPS	0.25
RPS	0.23

Correlations to Ribosome Decline

- What else happens when ribosomal gene expression declines in CIRS?
- Other parts of the metabolic panel also fall
- After mitochondrial panels, T cell markers are the most impacted

Ribosomes Sorted t-test - B lymphocytes

Gene	CD79a	CD79b	CD81
Delta Z	1.23	1.34	1.12
P value	4.9E-01	5.1E-02	2.3E+00

Ribosomes Sorted t-test - T lymphocytes

Gene	CD3D	CD127	CD25	CD4
Delta Z	1.95	1.32	1.23	-0.19
P value	4.8E-08	8.2E-04	4.4E-02	5.7E-01

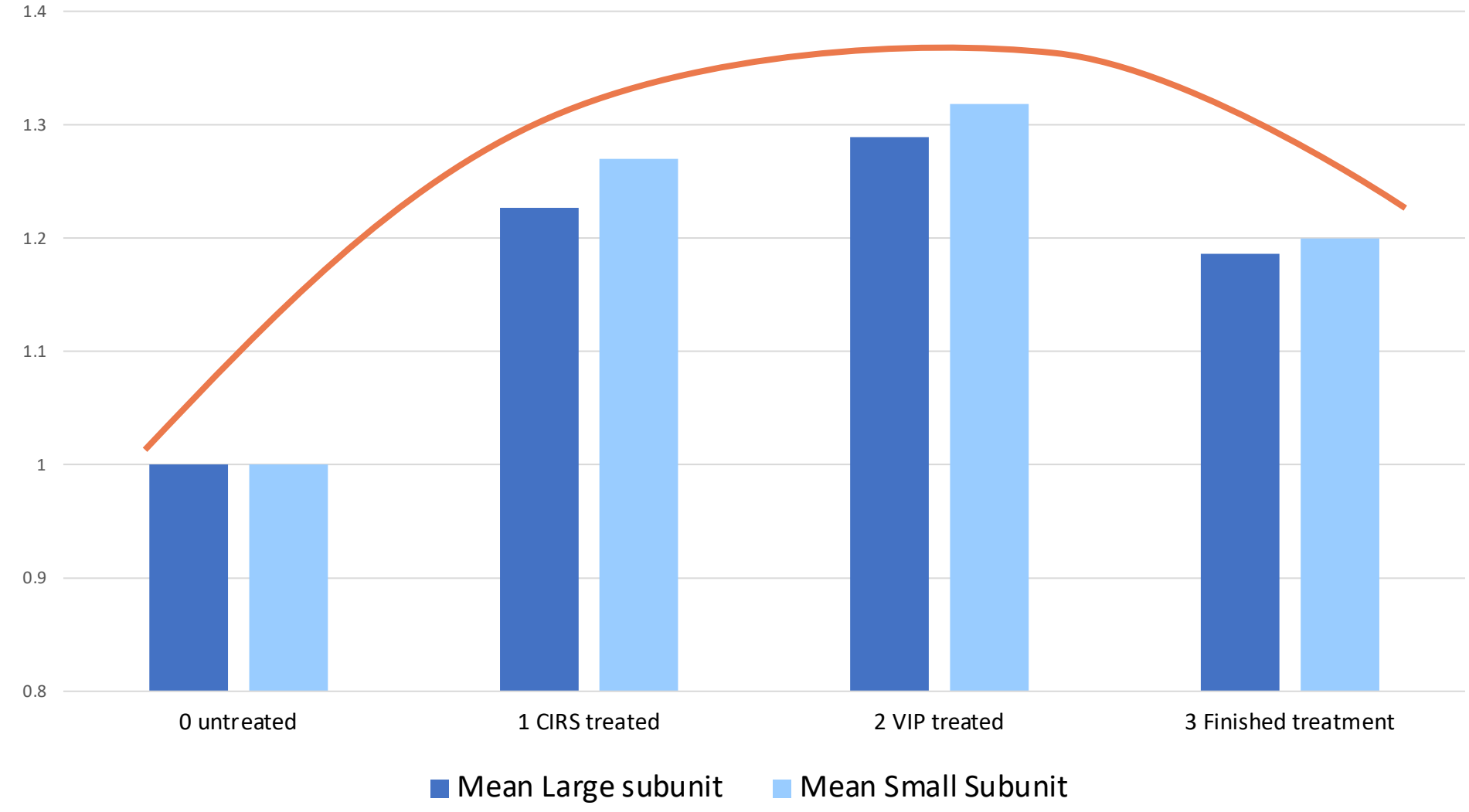
Ribosomes Sorted t-test - Other

Gene	CD14	CD40LG	CD48	CD52
Delta Z	-1.31	1.13	2.17	1.96
P value	2.6E-03	1.2E-01	5.0E-06	1.4E-08

Cellular Decline

- Molecular hypometabolism – decreased transcriptional output (IF on GENIE).
- A plethora of triggers can act at different points on the path of a transcript becoming a protein.
- Quiescence
- Senescence

Ribosomes Over Rx

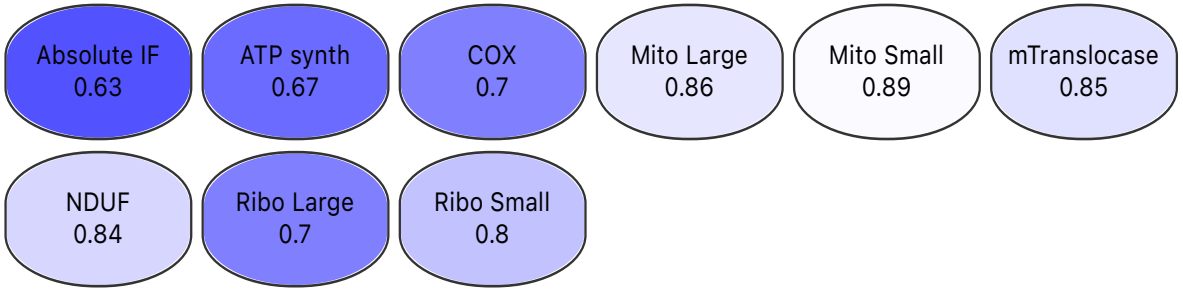


Altitude vs Trajectory

GENIE REPORT - *Gene Expression:Inflammation Explained*

1) Metabolism

Ratio for metabolic gene families compared to normal controls. 1 equals control value.



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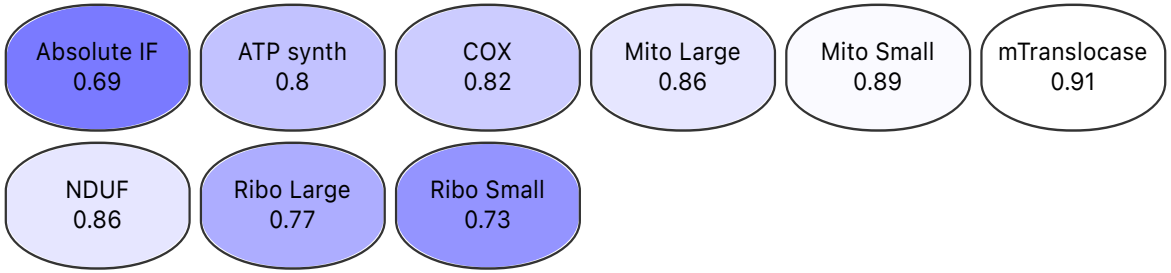

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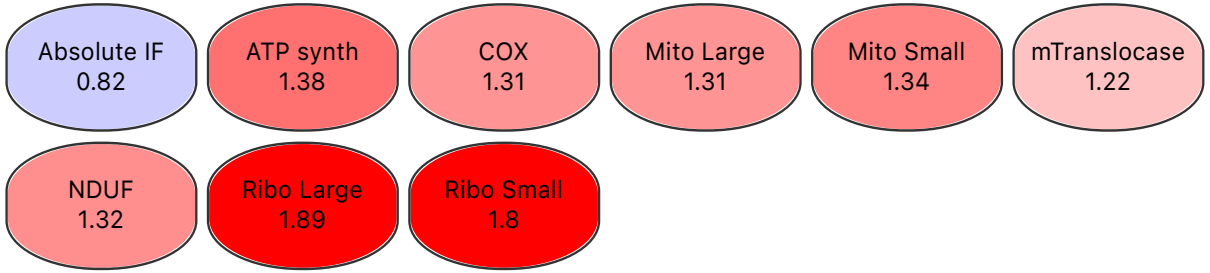

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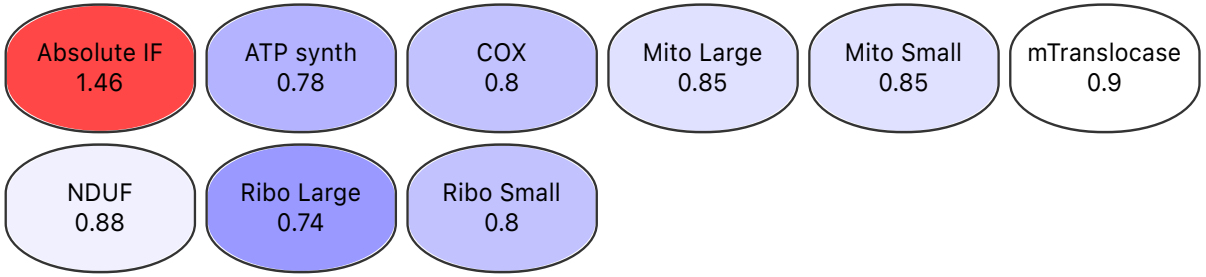

The Molecular Arc of CIRS

- Microbial toxin exposure leads to ribosomal damage by ribotoxins and ribosomal inhibitory proteins, among others.

- Additionally, microbial genotoxic products can damage transcription efficiency, also reducing transcriptional output.

- Removal of toxins corrects the above deficiencies, but now the patient has to play catch up on output that was marginalized.

Conclusions

- Untreated CIRS patients showed a marked down regulation of ribosomal genes, which control basic metabolic activities.
- While molecular hypometabolism can lead to innate immune activation, the time to ribosomal rebound will be different for different cases.
- Heterogeneity in ribosomal subunits for CIRS patients may be leading to preferential translation of stress specific mRNA transcripts.