Gene expression, Protein abundance and Enzyme activity – How well do they correlate?

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1. Introduction

Gene expression is the translation of genetic information into functional macromolecules

1. Functional RNAs
2. Proteins

Examples to the two categories of gene products, (1.) hammerhead ribozyme and (2.) tertiary structure of a polypeptide. Adopted from Protein Data Bank ID 2GOZ and bioinformatik.uni-leipzig.de
Gene Regulation

**Gene expression:**

I. Epigenetic Regulation – by Histone modification, DNA methylation, ncRNAs, Prions

II. Transcriptional Regulation – by Specificity & General factors, Repressors, Activators, Enhancers, Silencers, DNA-binding proteins

III. Post-transcriptional Regulation – by Capping, Splicing, Poly-A tailing, RNA processing

IV. Translational Regulation – control of the polypeptide level →

DNA methylation as control mechanism of gene reexpression. Adopted from http://learn.genetics.utah.edu/content/epigenetics/
Regulation of Transcript stability & Translation

Major mechanisms of the transcript and translation regulation. Adopted from de Sousa A. et al., 2009
Protein Regulation

Protein abundance:

I. Translational Regulation – by Conformational masking, Ribosome control, Protein binding, 5' UTR like m7G capping & AUG-Codon

II. Polypeptide stability - by Exonucleases, AU-rich elements, Non-sense mediated decay (NMD) & Non-stop decay, siRNA, miRNA, piRNA

III. Polypeptide folding – by Cellular milieu, Chaperons

IV. Polypeptide targeting – Signal recognition particle & Receptor, ER & Golgi

V. Post-translational Regulation – control of the active protein →

Illustration of the process of protein folding. Adopted from wikipedia.org
Enzyme Regulation

**Enzyme activity:**

I. Compartmentalisation - by Organella

II. Post-translational modification - by Addition of functional groups & proteins/peptides, Chemical conversion, Structural changes

III. Regulation - by Inhibitors (competitive, uncompetitive, non-competitive, suicide, mixed), Activators (allosteric, co-factors like vitamins, non-vitamins & minerals), precursors

IV. Environmental – by Changing pH

Illustration of the substrate specificity of enzymes. Adopted from chem4kids.com
Correlation of mRNA transcript and protein levels

Ratio between mRNA and protein levels, as observed in proteome- and transcriptome-profiling experiments. Adopted from de Sousa A. et al., 2009
Diurnal Protein & Non-diurnal mRNA

Metabolic coordination depends upon temporal regulation of proteins:

- 20% of hepatic proteins are cycling
- Half of them lack the cycling transcript
- Revealing the extent of post-transcriptional regulation

Comparison of protein abundance and mRNA level under diurnal cycling in mouse liver. Adopted from Reddy, A. et al., 2006
Transcript levels indicate metabolic changes

Silicon (Si) starvation in *T. pseudonana* induces lipid formation and 3 clusters of RNA response:

- 44% of mRNA are dissociated
- 30% of mRNA are transient
- 26% of mRNA are associated

Comparison of mRNA levels in diatom *T. pseudonana* under silicon starvation. Adopted from M. Hildebrand lab.
2. *Haematococcus pluvialis*

- Green plant (Chlorophyta)
- Pigments chlorophyll a + b and carotenoide
- Carotenoide Astaxanthin protects the cell umbrella-like against excessive light
- Economically important as antioxidant in human nutrition
- Non-sequences genome
3. Glutamine synthetetase

each subunit binds glutamate + NH$_3$ + ATP and converts them to glutamine + ADP + P$_i$. Glutamine synthetase

Simplified illustration of ammonia assimilation by GS. Adopted from Goodsell, D., 2002

Central role of glutamine synthetase in the acquisition and in the C-N interlink. Adopted from Miflin & Habash, 2002
4. Regulation of GS in *H. pluvialis*

Regulation of the GS activity by compartmentalisation. Modified from Inokuchi & Okada, 2001

Regulation of the GS activity by Post-translational modification through structural changes. Adopted from Goodsell, D., 2002

Regulation of the GS activity by Post-translational modification through adenylation and uridylation. Adopted from wjgrimes@u.arizona.edu

Regulation of the GS activity through inhibitors and activators. Adopted from wjgrimes@u.arizona.edu
5. Results

Continuous Light Regime
75 µE/ m²s
$t_0 = 0.2 \cdot 10^5$ cells/ mL

Effect of continuous normal light on relative GS activity, GS isoform abundances and gene expression of chloroplastic ($gln1$) and cytosolic ($gln2$) GS.
Diurnal Light Cycling
75 µE/ m²s
L:D/ 16:8 hours
\( t_0 = 0.2 \cdot 10^5 \) cells/ mL

Effect of diurnal normal light on relative GS activity, GS isoform abundances and gene expression of chloroplastic \((gln1)\) and cytosolic \((gln2)\) GS.
### Summary

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<thead>
<tr>
<th>Enzyme activity</th>
<th>Protein abundance</th>
<th>Transcript level</th>
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<tr>
<td><strong>Diurnal cycling:</strong></td>
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<td>Continuous light:</td>
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<td>GS $\downarrow$ $\uparrow$</td>
<td>GS$_1$ $\times$</td>
<td>$gln1$ $\uparrow$</td>
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<td>associated mRNA</td>
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<td>GS$_2$ $\downarrow$ $\uparrow$</td>
<td>$gln2$ $\leftrightarrow$</td>
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<td>dissociated mRNA</td>
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<td>Non-rhythmic mRNA</td>
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6. Take-home message

1. Changes in mRNA levels just indicate metabolic changes.
2. Changes in mRNA levels moderately correlate with changes in protein abundance.
3. Disparity between mRNA levels and protein abundance make it difficult to predict the enzyme activity.

in *Haematococcus pluvialis*:

- GS transcript-, protein- and activity- levels are significantly affected by the light regime
- $GS_1$ protein and activity increase in the dark
- $GS_2$ protein and activity increase in the light
- $gln$ gene transcript levels are moderately associated to GS levels

Genetic networks & Regulatory mechanisms require more research.
Thank you for your attention.

“And some Haematococcus for anti-ageing.”