Why bother with Proteomics?

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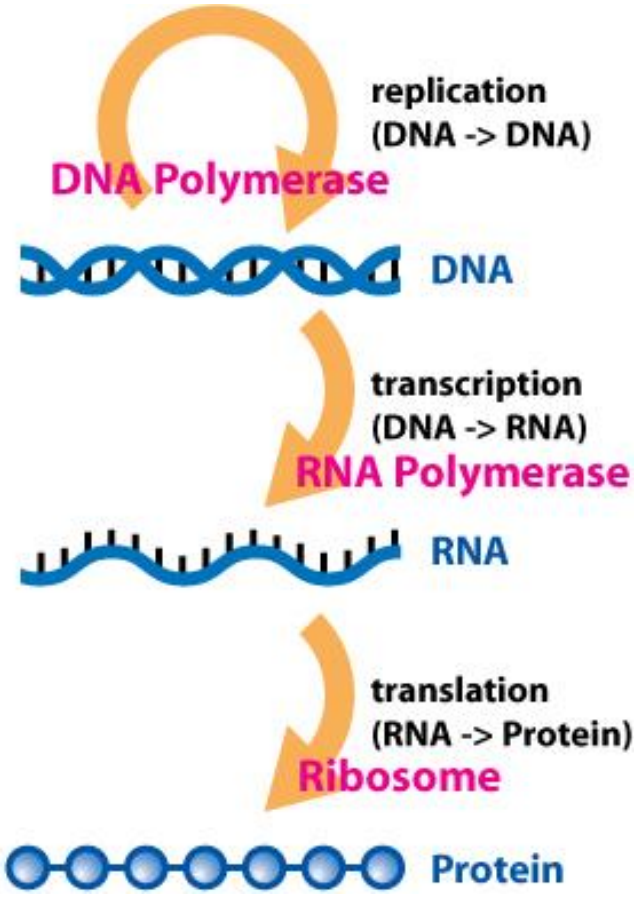
MOLECULAR BIOLOGY AND HUMAN GENETICS

Overview

- You want to measure gene expression as close to the "coalface" as possible.
- The biological process depends heavily on post-translational modification.
- A molecular biology kit is not available for the species you want to study.
- You want to translate your biomarker findings to an ELISA test.

What you measure determines what you can learn



- DNA Sequencing: does this genome contain unusual variants that alter function?
- RNA Sequencing: how do the genes being transcribed differentiate this tissue?
- Protein Identification: what proteins differentiate this tissue?

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Discovery: shotgun proteomics

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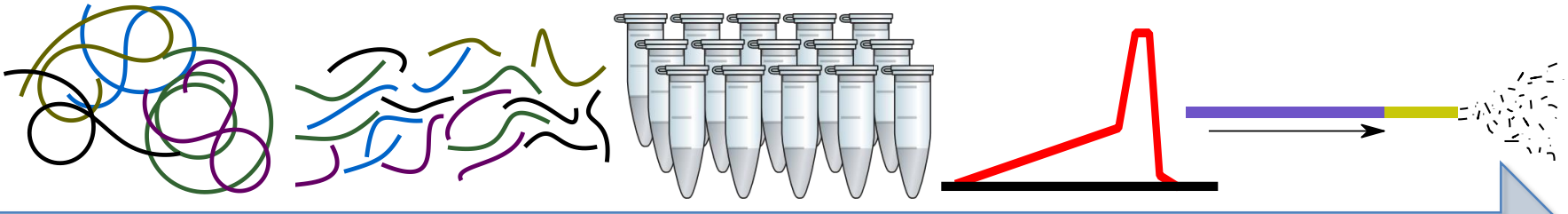
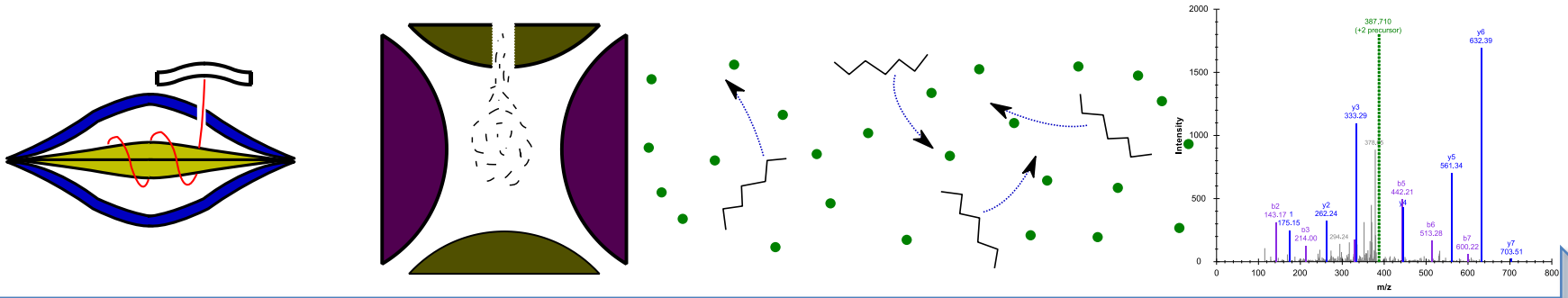


Protein Mixture → Peptide Mixture → Peptide Fractionation → Liquid Chromatography → Electrospray Ionization



High-Resolution Mass Spectrometry → Isolate Ions of Peptide → Collide Ions to Dissociate → Collect Fragments in Tandem MS

M. Bantscheff et al. *Anal. Bioanal. Chem.* (2007) 389: 1017

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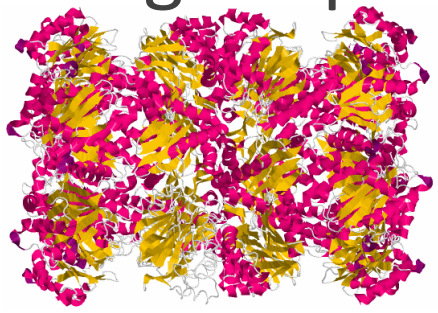
Proteins frequently differ from transcripts in abundance

SYNTHETIC REGULATION

- Unfolding mRNA
- Biasing tRNA pool
- Folding polypeptide
- Cleaving sequence
- Transporting protein
- Modifying for activity

CATABOLIC REGULATION

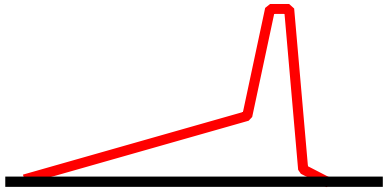
- Oxidizing with age
- Denaturing structure
- Marking via ubiquitin
- Cleaving via protease

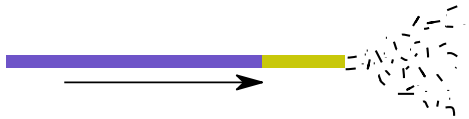


1PMA
RCSB.ORG

"Half-life" may differ substantially among proteins and across tissues.

SRM: Targeted Proteomics

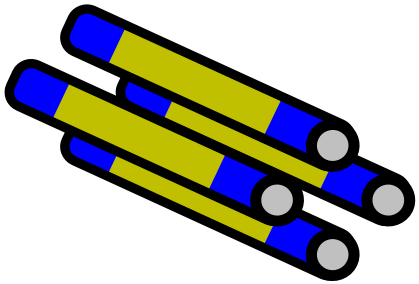


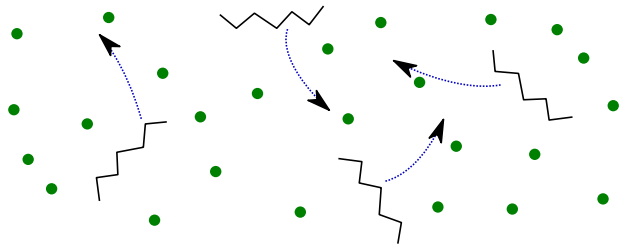


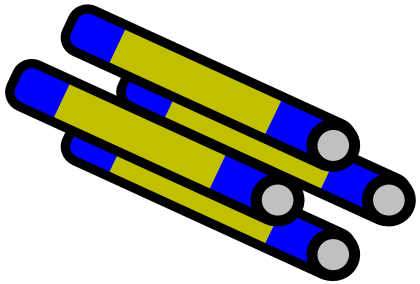
Peptide Mixture

Liquid Chromatography

Electrospray Ionization



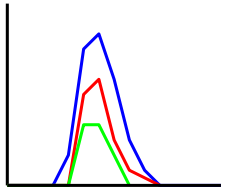


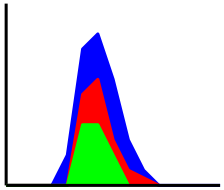


Screen out all but Target Mass

Collide Ions to Dissociate

Screen out all but Fragment Mass



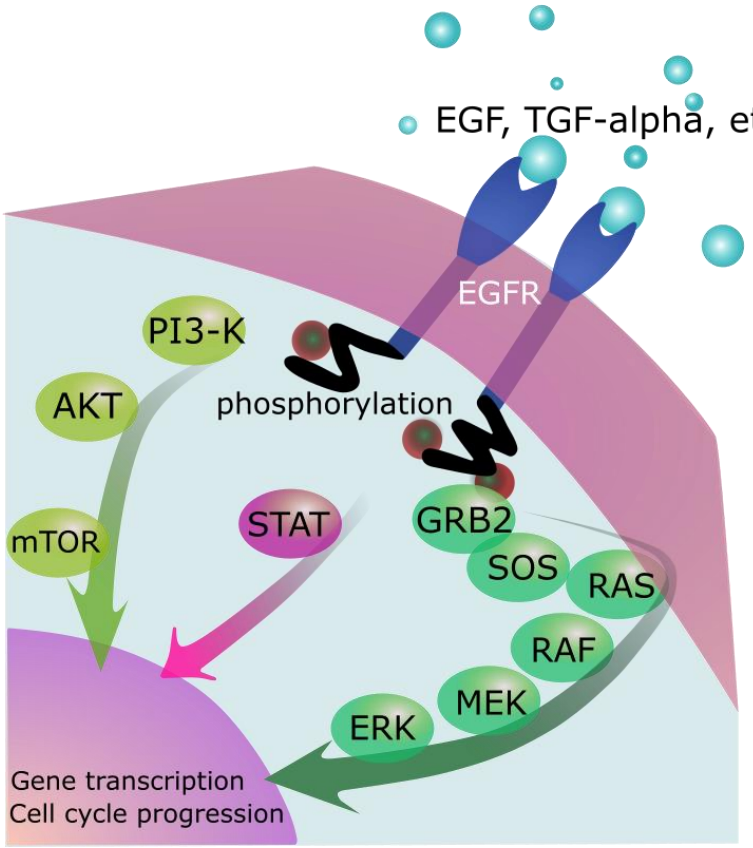


Find Peaks from Related Traces

Integrate Peak Areas

Compare Areas to Reference Areas

Post-translational modification is essential to function



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- A protein may be present but inactive.
- Phosphorylation is one of hundreds of potential PTMs.
- Rapid processes cannot wait for transcript and translation.

Khoury et al. *Scientific Reports* (2011) 1: 90

PTMs alter intact mass, fragment masses, and retention time

D I G S E S T E K
D I G S*E S T E K
D I G S E S*T E K
D I G S E S T*E K
D I G S*E S*T E K
D I G S*E S T*E K
D I G S E S*T*E K
D I G S*E S*T*E K

- Each PO_4 adds 80 Da to mass of peptide.
- Fragments that contain PO_4 will increase by 80 Da.
- PO_4 content generally makes peptides more hydrophilic.

Steen et al. *Mol. Cell. Proteomics* (2006) 5: 172-181

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Case study: black rhino cytokines

- Study of black rhino immune response uses QIAGEN qPCR kit for horse!

- Selected Reaction Monitoring assays can be designed with peptide sequences known only from draft genome assembly.

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Case study: biomarkers for colon cancer

- "Shotgun" proteomics finds a dozen proteins with expression changes in early cancer.

- After SRM confirmation, low-cost ELISA assays can be developed for each protein in panel.

Takeaways

- If you want direct evidence of catalysis, measure the enzyme or its reactants.
- Proteomic inventories get all the attention, but directly quantitative methods are ready.
- Post-translational modifications are invisible to genetic sequencers!
- Proteomics methods are adaptable to your species, if the genome is assembled.