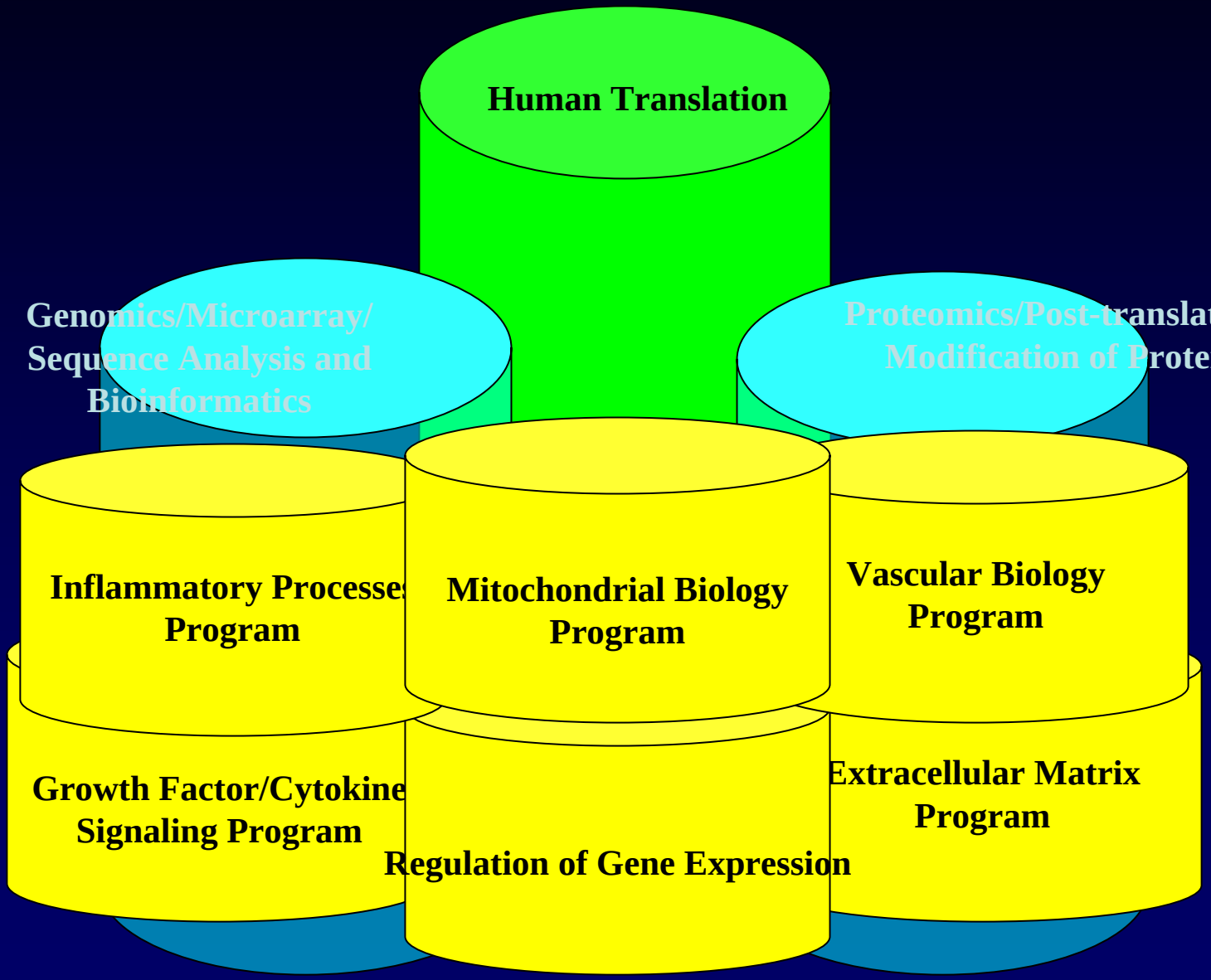
Center for Metabolic Biology

Lawrence J. Mandarino, Ph.D.
ZhengPing Yi, Ph.D.
School of Life Sciences
Department of Kinesiology
Department of Chemistry & Biochemistry

A Multidisciplinary Approach to the Metabolic Syndrome



Current Research Projects

- Insulin Signaling
  - Role of IRS-1 serine/threonine phosphorylation in insulin resistance
- Mitochondrial dysfunction
  - Expression of nuclear encoded mitochondrial genes
- Extracellular matrix
  - Relationship between insulin sensitivity and extracellular matrix remodeling

Projects Using Mass Spectrometry Techniques

- IRS-1 S/T phosphorylation
- Mitochondrial proteome

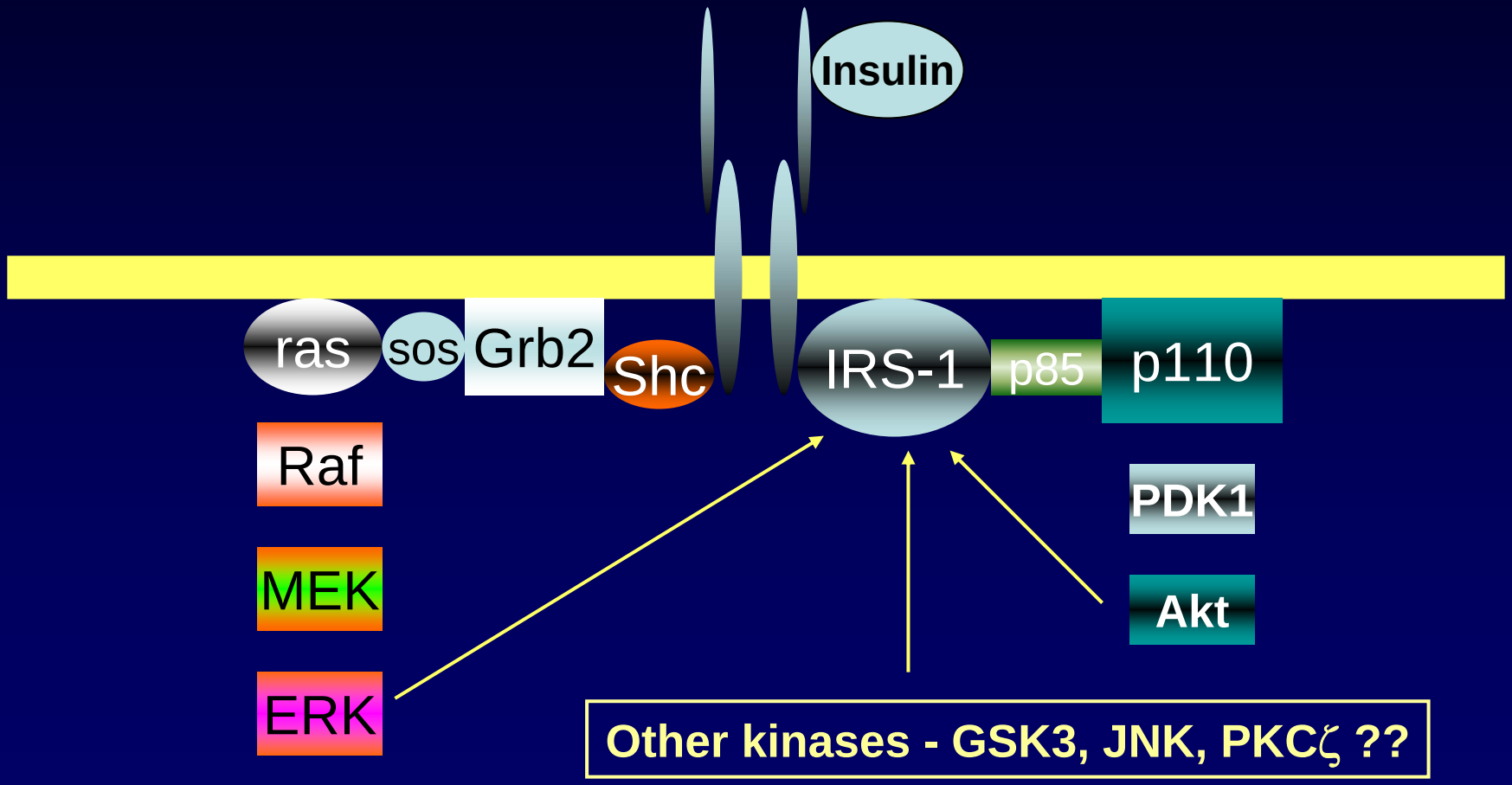
Mass Spectrometry and Related Instrumentation

- Thermo HPLC-nanospray-LTQ/FT-ICR
  - High resolution – mass accuracy
  - High sensitivity – 0.5 attomole (500 zeptomoles = 301,000 molecules)
- Thermo vMALDI-LTQ
- 2D gel electrophoresis, imaging and quantification

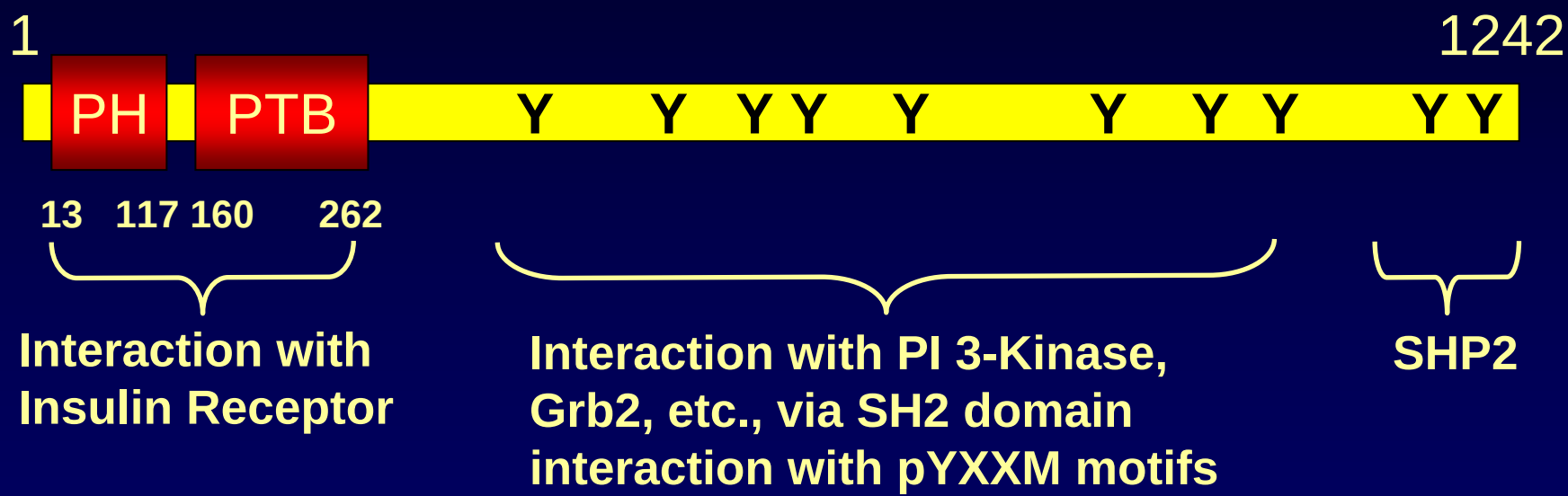
IRS-1 S/T phosphorylation

- Human IRS-1
  - 1242 amino acids
  - ~15% serine/threonine
  - Tyrosine phosphorylation transmits insulin signal
  - S/T phosphorylation positively and negatively influences IRS-1 tyrosine phosphorylation and may be involved in insulin resistance

Insulin Receptor Signaling and IRS-1 Serine Phosphorylation



Human IRS-1 Domain Structure



A “Few” Serine/Threonine Phosphorylation Sites

Sequence	Motif
339PASVDGSPVpSPSTNR353	PXpSP
525THSAGpTpSPTITHQK538	XpTpSP
889EPKpSPGEYVNUEFGSD904	PXpSP
1075VNLpSPNRNQSAK1086	XXpSP
1097RRHpSpSETFSSTPSATR1112	RRHpSpS*
1140RHSSApSFENVWLRP1153	RHSSApS*
1220RRpSpSEDLSAYASISFQK1236	RRpSpS*

*potential PKA, Akt, RSK2, or other AGC kinase site

The ultimate goal is discovery and “global” quantification of S/T phosphorylation sites in IRS-1 from small biopsies of human muscle from insulin resistant patients and insulin sensitive control subjects.

Mitochondrial Proteomics

- Global gene expression analysis (microarray analysis of mRNA) shows a pattern of decreased expression of nuclear encoded mitochondrial genes.
- How is this reflected, under conditions of insulin resistance, in changes in protein abundance, and, ultimately, function?