Signal Transduction and Cancer
Generation of High-Quality Data

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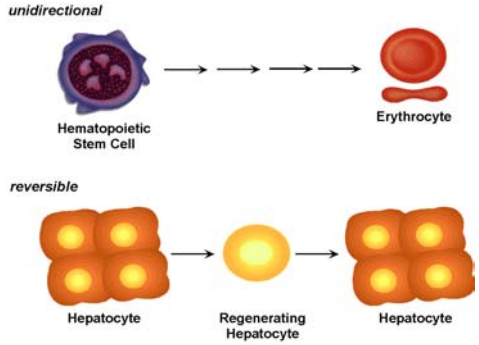
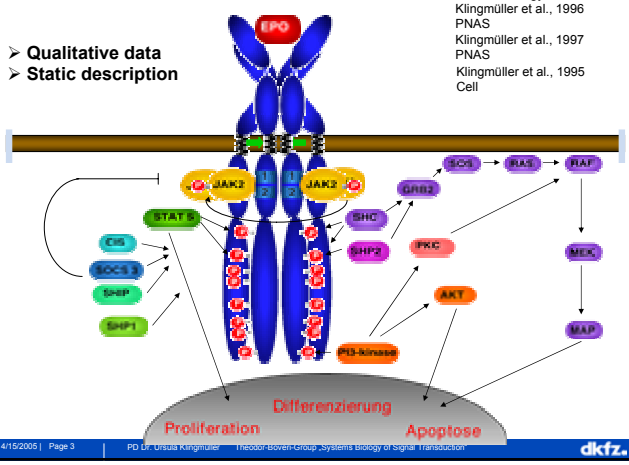
Dynamic Growth and Differentiation Processes



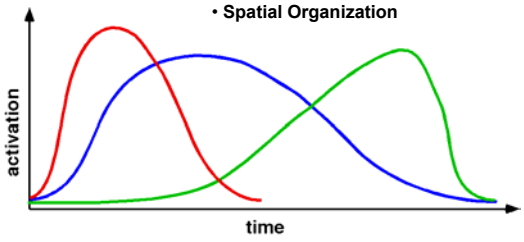
- **Qualitative data**
- **Static description**

Kubatzky et al, 2001 Current Biology
Klingmüller et al., 1996 PNAS
Klingmüller et al., 1997 PNAS
Klingmüller et al., 1995 Cell

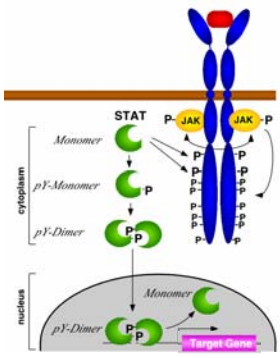


Dynamic Behavior - Decision Making Process

- **Timing**
- **Amplitude**
- **Duration**
- **Spatial Organization**



JAK-STAT Signaling Cascade



JAK (*Janus-Kinase*)
cytoplasmic,
receptor associated tyrosine kinase

STAT
(*Signal Transducer and Activator of Transcription*)
latent transcription factor

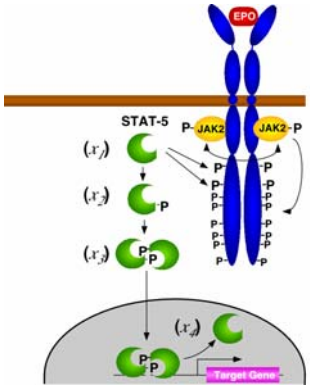
Role for Signaling through:

hematopoietic cytokine receptors
receptor tyrosine kinases
hepta-Helix receptors

⇩

constitutively activated in tumors

Mathematical Model (1): JAK-STAT Feed-Forward Cascade



(1) Unphosphorylated STAT-5

$$\dot{x}_1(t) = -k_1 x_1 EpoR_A$$

(2) Tyrosine Phosphorylated STAT-5 Monomer

$$\dot{x}_2(t) = -k_2 x_2^2 + k_1 x_1 EpoR_A$$

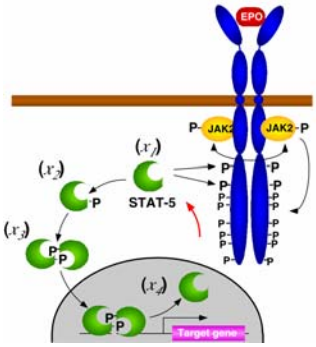
(3) Tyrosine Phosphorylated STAT-5 Dimer

$$\dot{x}_3(t) = -k_3 x_3 + \frac{1}{2} k_2 x_2^2$$

(4) Tyrosine Phosphorylated STAT-5 Dimer in the nucleus

$$\dot{x}_4(t) = \quad + k_3 x_3$$

Mathematical Model (2): Cycling STAT-Signaling Pathway



(1) Unphosphorylated STAT-5

$$\dot{x}_1(t) = -k_1 x_1 EpoR_A^2 k_4 x_5^*$$

(2) Tyrosine Phosphorylated STAT-5 Monomer

$$\dot{x}_2(t) = -k_2 x_2^2 + k_1 x_1 EpoR_A$$

(3) Tyrosine Phosphorylated STAT-5 Dimer

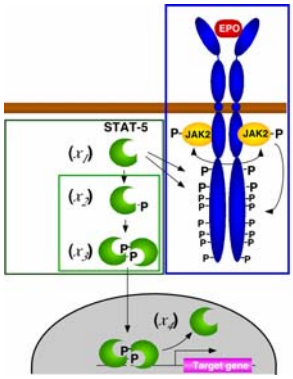
$$\dot{x}_3(t) = -k_3 x_3 + \frac{1}{2} k_2 x_2^2$$

(4) Tyrosine Phosphorylated STAT-5 Dimer in the Nucleus

$$\dot{x}_4(t) = -k_4 x_4^* + k_3 x_3$$

JAK2-STAT5 Pathway

Estimating the dynamical parameters (*k*)



Quantified Components:

Input-Function:

Epo-induced Tyrosine Phosphorylation of the Erythropoietin-Receptor ($EpoR_A$)

Cytoplasm:

Tyrosine Phosphorylated STAT-5

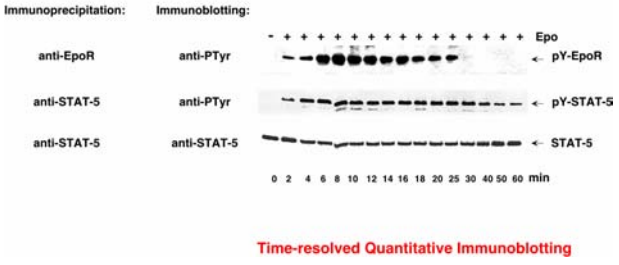
$$y_1 = k_5(x_2 + x_3)$$

STAT-5

$$y_2 = k_6(x_1 + x_2 + x_3)$$

JAK2-STAT5 Pathway

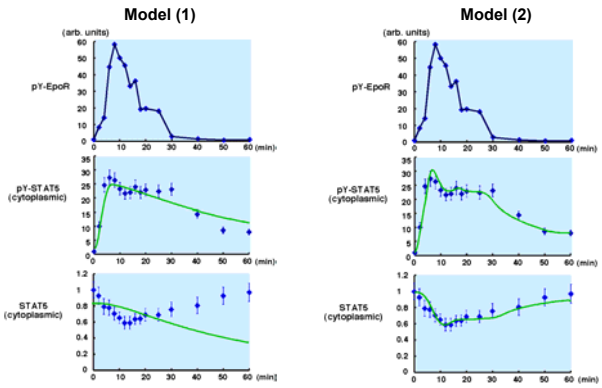
Quantitative, time-resolved measurements



Time-resolved Quantitative Immunoblotting

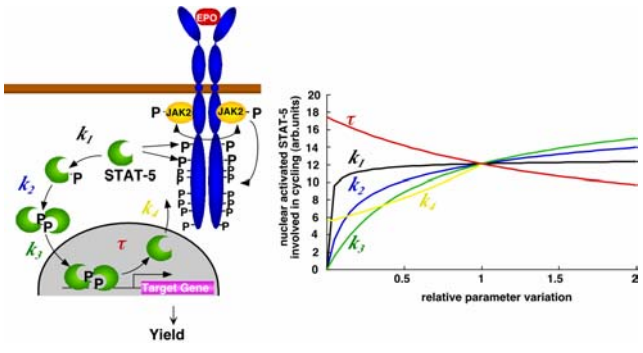
JAK2-STAT5 Pathway

Fit of Experimental Data: Feed-Forward Cascade (1) versus Cycling (2)



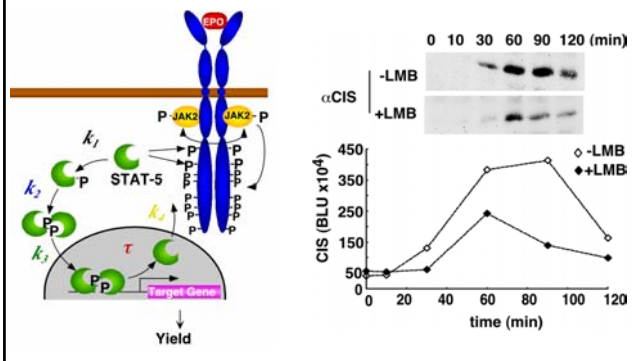
JAK2-STAT5 Pathway

Predicting Steps Most Sensitive for Perturbation

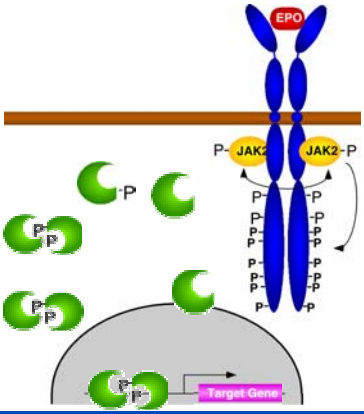


JAK2-STAT5 Pathway

Experimental validation of prediction



JAK2-STAT5 Pathway
Remote Sensor

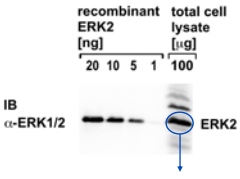


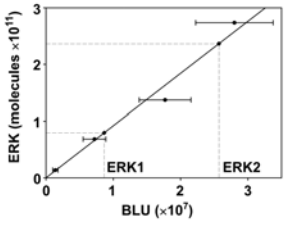
Bottle neck of systems biology approaches:
Generation of high quality quantitative data

Development/ Improvements of Quantitative Immunoblotting

- CCD camera based analysis
- Conversion of arbitrary units to molecules per cell
- Determining steps prone to errors
- Normalizers
- Calibrators

Determination of molecules per cell





2.57x10^7 BLU

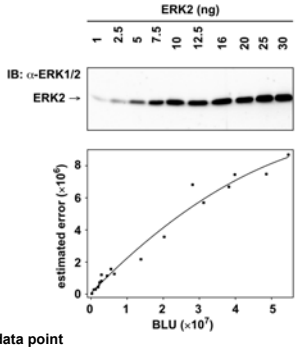
2.37x10^{11} molecules

7.5x10^5 cells

3.16x10^5 molecules per cell

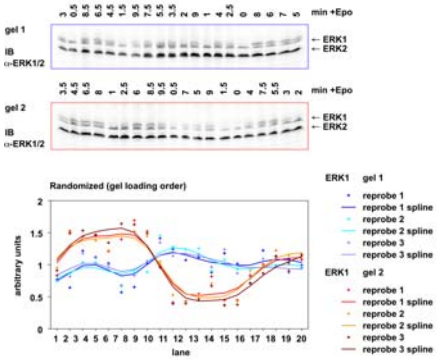
- linear detection
- generation of absolute numbers

Determination of the Error of Immunoblotting Measurements



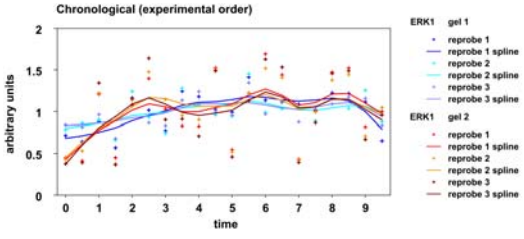
- error < 20% for each data point
- uncorrelated error (pipetting, antibody detection)
- correlated error (gel/transfer inhomogeneities)

Determining Steps Prone to Error



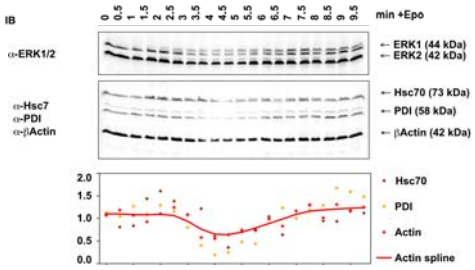
- uncorrelated error (pipetting, antibody detection) is small
- strong impact of correlated error (gel/transfer inhomogeneities)

Determining Steps Prone to Error



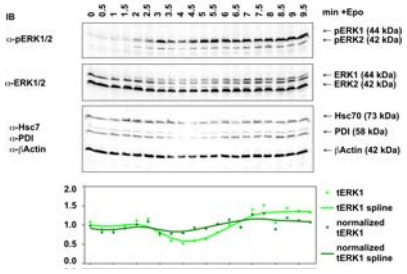
- randomized gel loading destroys correlated error (gel/transfer inhomogeneities)

Use of Normalizers



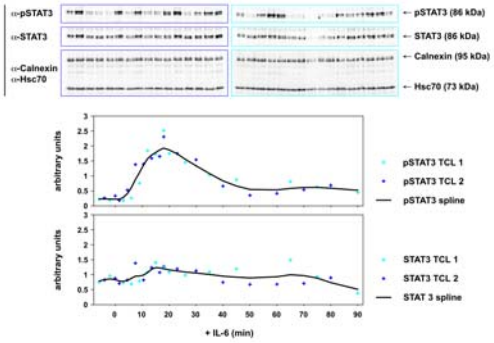
➡ Use of housekeeping protein with related molecular weight

Use of Normalizers



➡ as expceted total ERK1 is constant over time

Time-course of STAT3 Activation in Primary Hepatocytes
Total Cell Lysate



➢ calnexin as normalizer and randomized gel loading facilitates the generation of quantitative data for primary adherent cells

Calibrators

- Prepurification of proteins by immunoprecipitation
- Quantitative analysis of rare proteins from cell lysate by immunoprecipitation
- Error prone multi-step process

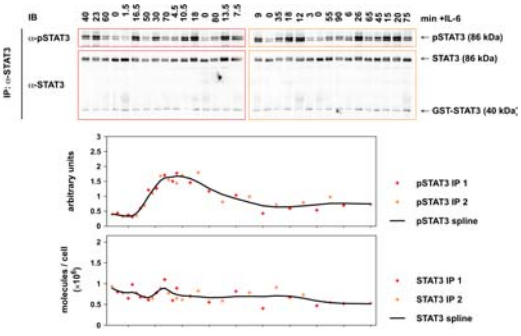
Calibrator

(recombinant protein that harbors the epitope recognized by the antibody used for immunoprecipitation)

➢ added to cell lysates

➢ used for normalization

Time-course of STAT3 Activation in Primary Hepatocytes
Immunoprecipitation



➢ GST-STAT3 as calibrator and randomized gel loading facilitates the generation of quantitative data for 40 time points

➢ discovery of true dynamic behavior

Generation of High Quality Quantitative Data
Identification of error prone steps - Improvements

Major source for errors:

- SDS-PAGE and transfer

Less important:

- Incubation with antibody and luminol

Improvements:

- Randomized gel loading
- Prepurification by immunoprecipitation
- Use of appropriate normalizers and calibrators

Summary

- **New biological knowledge can be generated by data-based mathematical modeling**
- **High quality data can be generated by quantitative immunoblotting applying randomized gel loading and appropriate normalizers and calibrators**

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