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Modelling Methods: High Throughput Time-Course Data

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Overview

- Introduction to p53
- Estimating p53 Activity
 - Identifiability
- (Not) Verifying Predicted Activity
 - New Biological Hypothesis
- Predicting New Targets
- Screening for New Transcription Factors





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Example: p53

p53 Network Controls Response to DNA Damage



Repair or Die?

p53 Mutations Implicated in Most Cancers



Role of p53

Key Transcription Factor



p53: More Realistic View



Kohn, Biochem. Biophys. Res. Comm, 2005



Whole Cell Cycle Network

Intuitive understanding of overall behaviour is impossible



Kohn, Mol. Bio. Cell, 1999



Understanding p53 Function





p53: Two Sub-Systems

Effector



Understanding p53 Function

- Protein Side
 - Difficult to Get Data
 - Well Modelled
 - Bar-Or et al, PNAS 00
 - Monk, Curr. Bio. 03
 - Ma *et al*, *PNAS* 05
 - Ciliberto, Novak and Tyson, Cell Cycle 05
- Effector Side
 - Transcription Factor
 - Controls Many Downstream Genes
 - Can Measure These Using Microarrays



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Example: DNA Damage Time-Course

Irradiate Cells and Take Microarray Time Series





Estimate p53 Activity

Use 5 Known Targets to Fit This Equation







Parameter Scaling

- We Can Scale and Shift Origin of f By Changing B_j and S_j Without Changing the Solution x_j
- We Remove This Ambiguity by Fixing

$$S_j = 1$$

for One Gene (p21) and Assuming

$$f(0) = 0$$



Estimate p53 Activity

Estimate Unknown p53 Profile







Identifiability Problem

• Predicted p53 Profile Has Huge Variance





Data Anchoring

• Measure Degradation of p21 Using RT-Q-PCR





Predicted p53 Activity





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Experimental Verification of p53 Activity







Experimental Verification of p53 Activity





Possible Explanation

Model Predicts: Translocation of p53 Out of Nucleus After 6h



Prediction: Translocation Out of Nucleus





Moral

- The Most Useful Models Are Often Those That
 Do Not Fit the Data
- Aside: Every Positive Significance Test Is an Example of This





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Use Estimate to Predict p53 Targets

Good Fit: Likely to Be p53 Target



Use Estimate to Predict p53 Sensitivity

Poor Fit But Sensitive: Co-regulated?



Experimental Verification of p53 Targets

Inactivate p53 Using siRNA





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Screening For New Transcription Factors

Unpublished Work



Extensions

- Co-regulation
 - Rel1 and Rel2 in Mosquito Response to Malaria
 - Macrophage and DC Response to LPS
- Repression
 - Bonneau et al, Genome Biology, 7 (2006), R36
 - Khanin et al, PNAS, 103 (2006), 18592-18596
- Targets of Targets
 - Horrendous Identifiability Problems





Morals

- Dynamics Must Be Incorporated in Analysis of Time-Course Data
- We Can Build Useful Models Without Measuring All The Key Variables
- Models Must Be Identifiable
- The Most Useful Models Are Often Those That
 Do Not Fit the Data

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