Network and systems biology of cancer: implications for molecular targeted therapy

Yosef Yarden, PhD
Department of Biological Regulation
The Weizmann Institute of Science
Rehovot, Israel

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Antitumor Activity of Rapamycin in a Phase I Trial for Patients with Recurrent PTEN-Deficient Glioblastoma

Tim F. Cloughesy[1], Koji Yoshimoto[2¤], Phioanh Nghiemphu[1], Kevin Brown[3], Julie Dang[2], Shaojun Zhu[2], Teli Hsueh[4], Yinan Chen[4], Wei Wang[5], David Youngkin[3], Linda Liau[6], Neil Martin[6], Don Becker[6], Marvin Bergsneider[6], Albert Lai[1], Richard Green[7], Tom Oglesby[5], Michael Koleto[5], Jeff Trent[3], Steve Horvath[8], Paul S. Mischel[2,4], Ingo K. Mellinghoff[4], Charles L. Sawyers[9]*

ABSTRACT

Background

There is much discussion in the cancer drug development community about how to incorporate molecular tools into early-stage clinical trials to assess target modulation, measure anti-tumor activity, and enrich the clinical trial population for patients who are more likely to benefit. Small, molecularly focused clinical studies offer the promise of the early definition of optimal biologic dose and patient population.

Methods and Findings

Based on preclinical evidence that phosphatase and tensin homolog deleted on Chromosome 10 (PTEN) loss sensitizes tumors to the inhibition of mammalian target of rapamycin (mTOR), we conducted a proof-of-concept Phase I neoadjuvant trial of rapamycin in patients with recurrent glioblastoma, whose tumors lacked expression of the tumor suppressor PTEN. We aimed to assess the safety profile of daily rapamycin in patients with glioma, define the dose of rapamycin required for mTOR inhibition in tumor tissue, and evaluate the antiproliferative activity of rapamycin in PTEN-deficient glioblastoma. Although intratumoral rapamycin concentrations that were sufficient to inhibit mTOR in vitro were achieved in all patients, the magnitude of mTOR inhibition in tumor cells (measured by reduced ribosomal S6 protein phosphorylation) varied substantially. Tumor cell proliferation (measured by Ki-67 staining) was dramatically reduced in seven of 14 patients after 1 wk of rapamycin treatment and was associated with the magnitude of mTOR inhibition ($p = 0.0047$, Fisher exact test) but not the intratumoral rapamycin concentration. Tumor cells harvested from the Ki-67 nonresponders retained sensitivity to rapamycin ex vivo, indicating that clinical resistance to biochemical mTOR inhibition was not cell-intrinsic. Rapamycin treatment led to Akt activation in seven patients, presumably due to loss of negative feedback, and this activation was associated with shorter time-to-progression during post-surgical maintenance rapamycin therapy ($p < 0.05$, Logrank test).

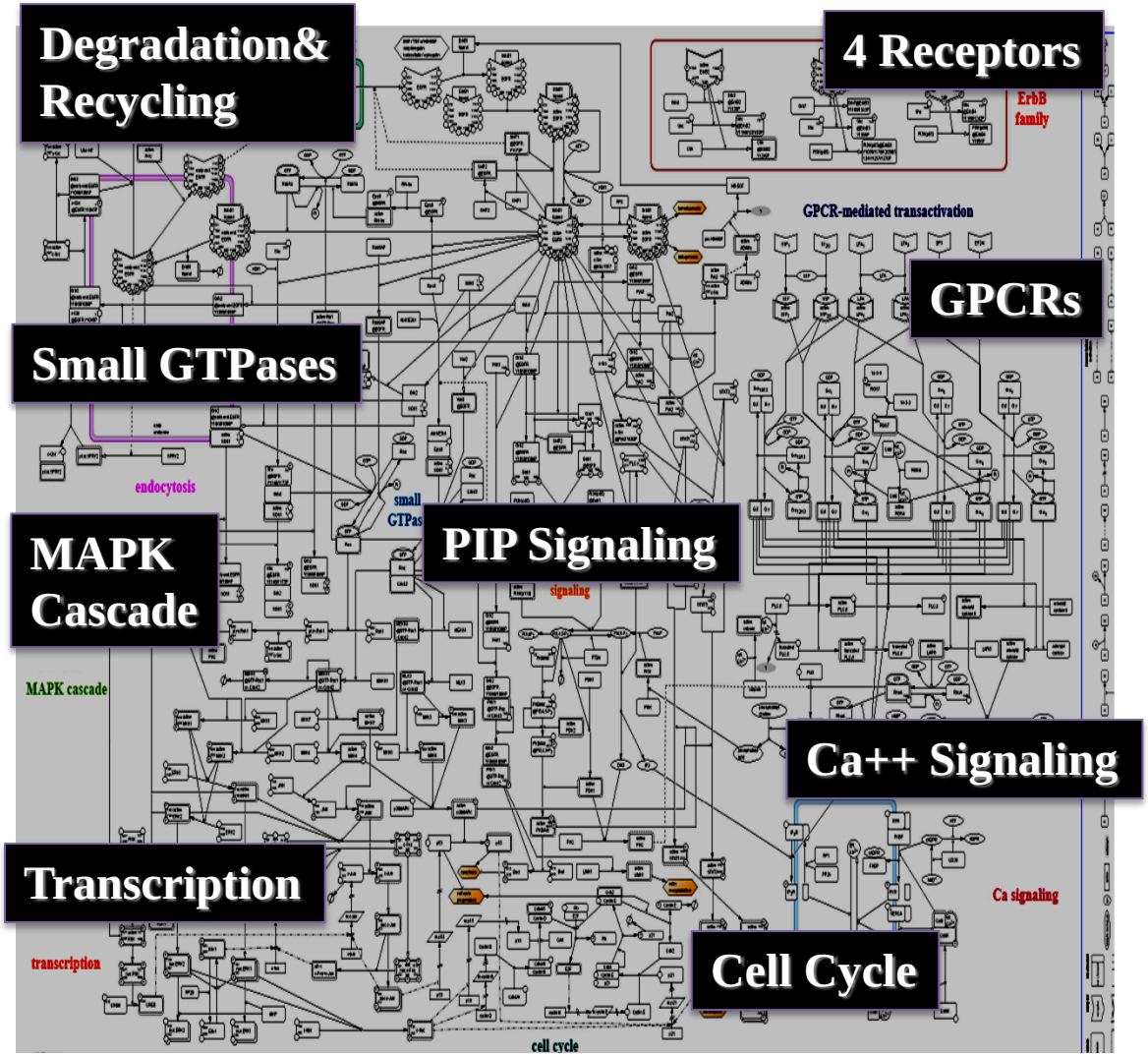
Conclusions

Rapamycin has anticancer activity in PTEN-deficient glioblastoma and warrants further clinical study alone or in combination with PI3K pathway inhibitors. The short-term treatment endpoints used in this neoadjuvant trial design identified the importance of monitoring target inhibition and negative feedback to guide future clinical development.

Trial registration: http://www.ClinicalTrials.gov (#NCT00047073).

The Editors' Summary of this article follows the references.

Network Complexity and Drugs Targeting HER2 and EGFR



- EGFR and HER2 effective targets for many drugs
  - *-Examples: Gefitinib, Erlotinib, Lapatinib, Cetuximab, Panitumumab and Trastuzumab*
- EGFR and HER2 are effective targets in several clinical indications
  - *-Examples: Lung cancer, breast cancer, colorectal tumors, head and neck cancer and pancreatic cancer*

K. Oda et al., Mol. Syst. Biol. 1:8-24 (2005)

Mechanisms that ensure robustness of engineered (and biological) systems

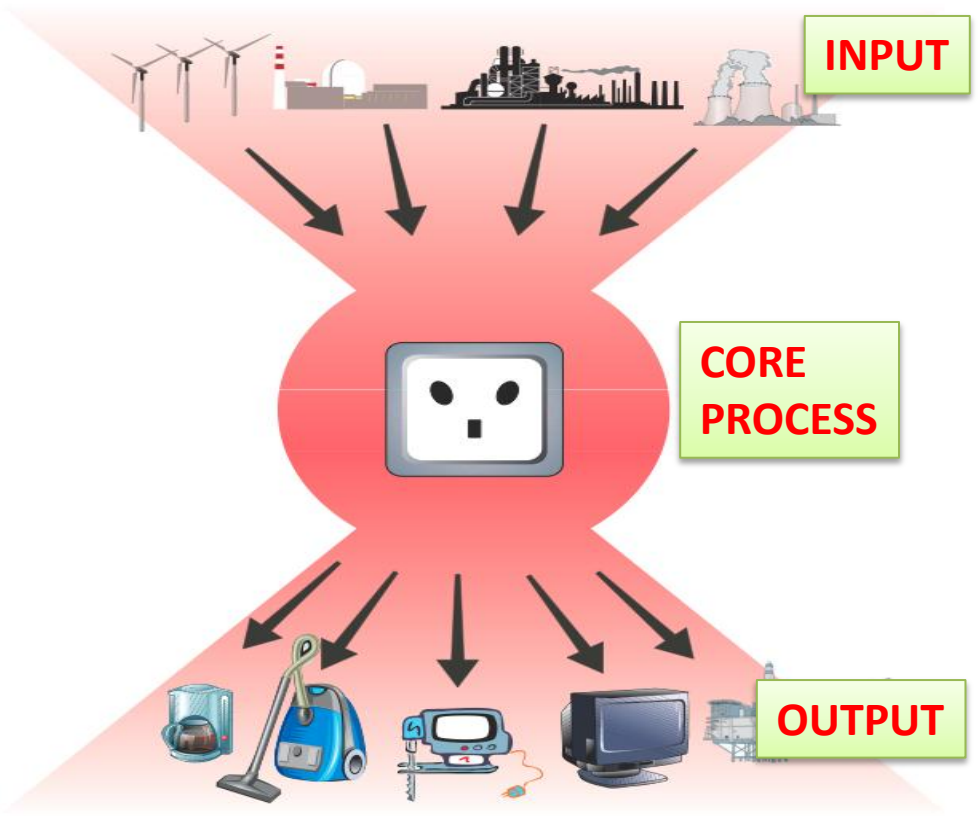
Modularity: ***Organization in units that enable damage containment***

Redundancy and diversity: ***input and output diversity and multiple pathways to achieve a specific function***

System controls:

Positive control leading to amplification and negative feedback control

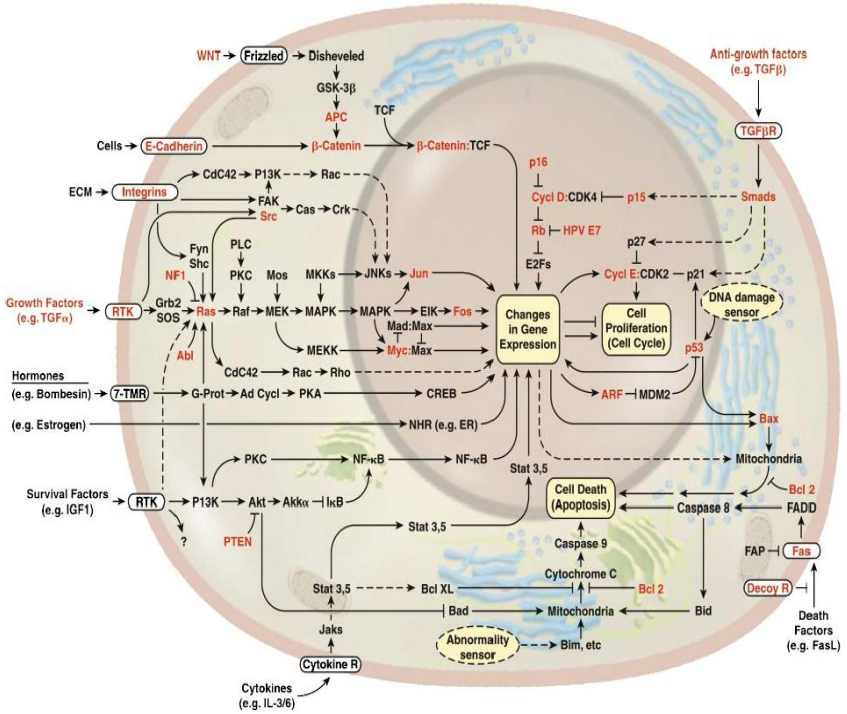
System adaptability ***(training)***



Citri and Yarden (2006) Nature Rev. Mol. Cell Biol. 7: 505

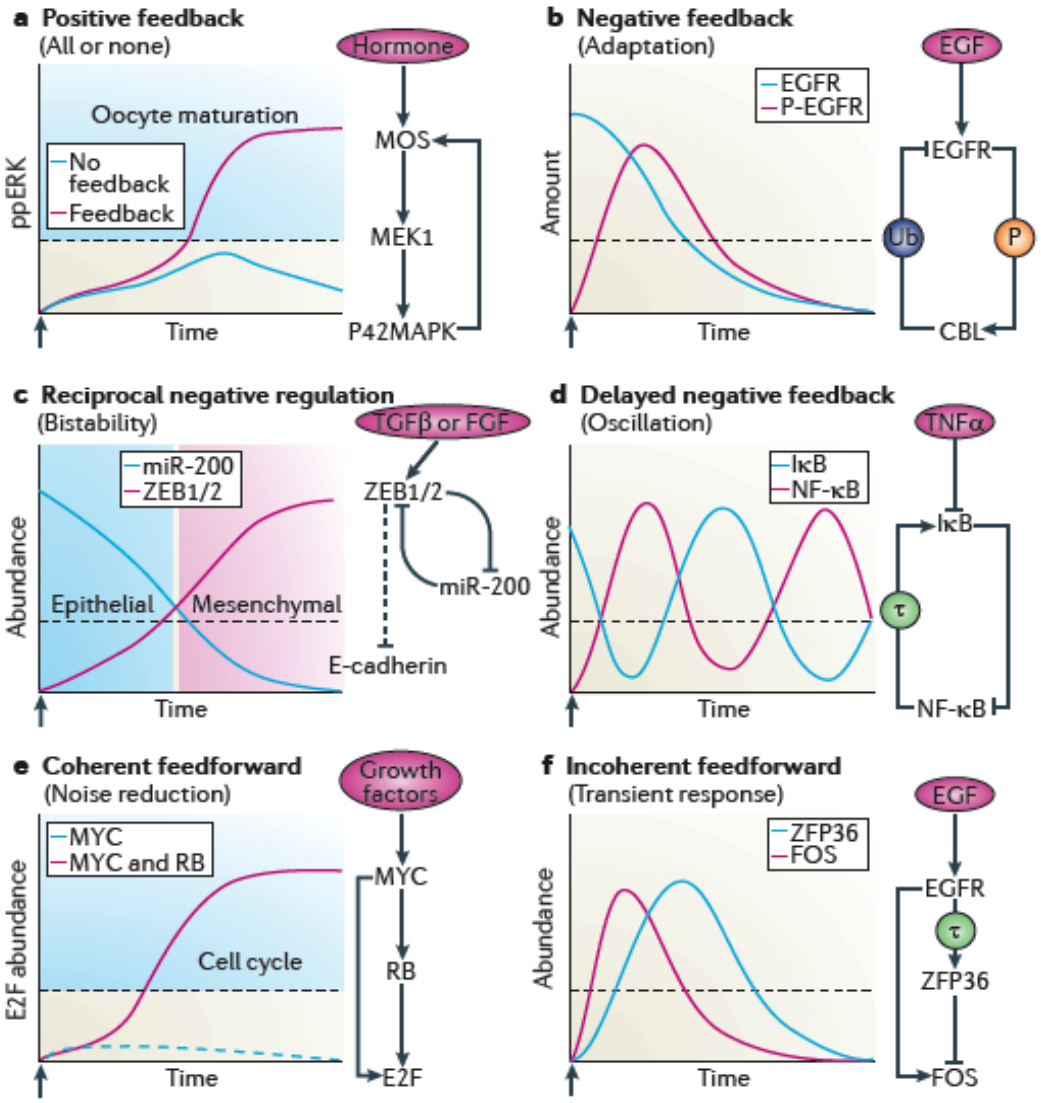
Biological and engineered systems share structural and functional features

- Component multiplicity
- Rich connectivity
- Fail-safe functioning



However, the single supervisory component of engineered systems is often replaced in biological systems by multiple control loops

Feedback Loops Carve Network's Output



Avraham & Yarden (2011) Nature Rev. Mol. Cell Biol.

Computational tasks of feedback regulatory loops

- **Fold change detection:** The output of a network depends on the relative change in input signal, rather than on the absolute levels.
- Reference: The incoherent feedforward loop can provide fold-change detection in gene regulation. Goentoro L, Shoval O, Kirschner MW, Alon U. Mol Cell. (2009)

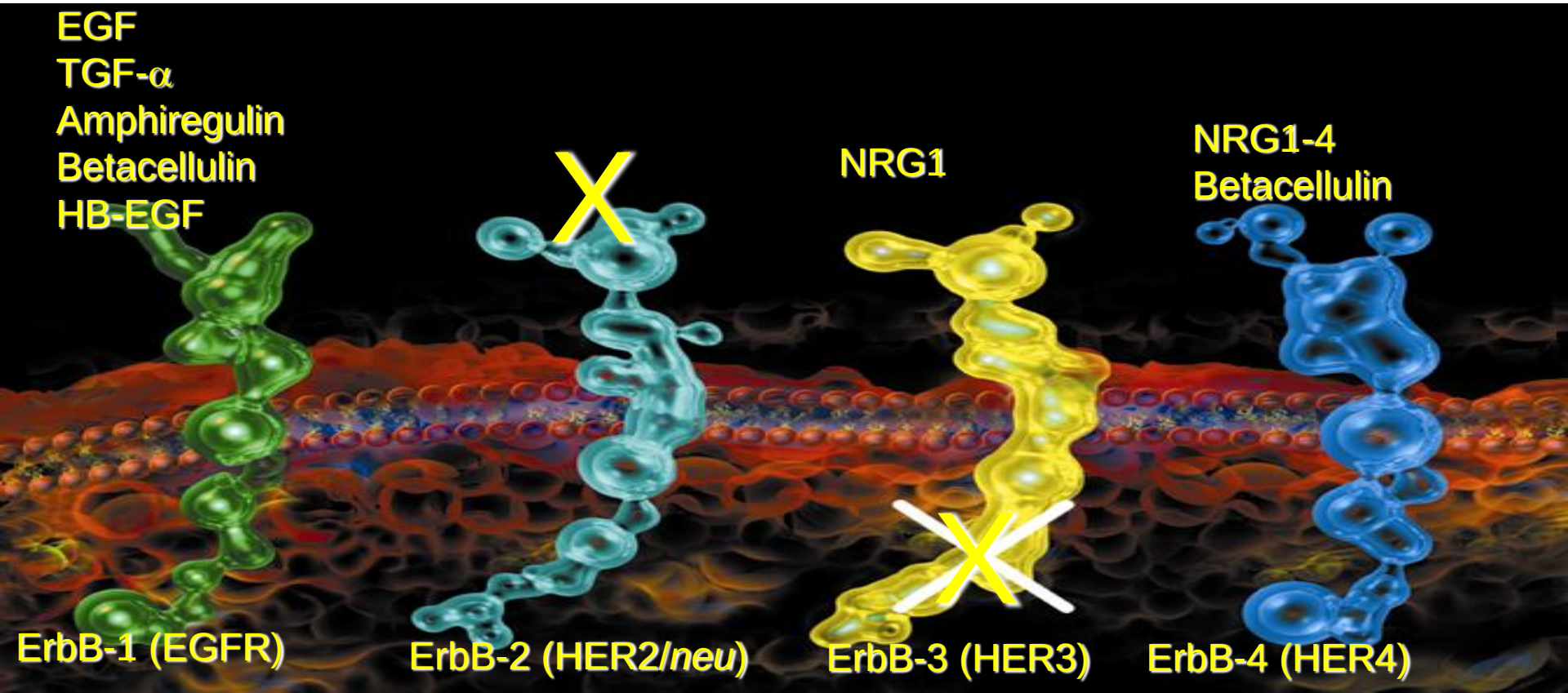
Overall, feedback loops are the guardians of the cell's steady state. Hence, pharmacological interventions would eventually be restrained.

- Reference: Growth-factor-dependent mitogenesis requires two distinct phases of signalling. Jones SM and Kazlauskas A. *Nat Cell Biol* (2001)
- **Decoding ligand specificity:** Although different signals are funneled into the same pathway, specificity is maintained by feedback regulation.
- Reference: Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. Santos SD, Verveer PJ, and Bastiaens PI. *Nat Cell Biol* (2007)

The EGFR/HER2 Family and the Double Enigma

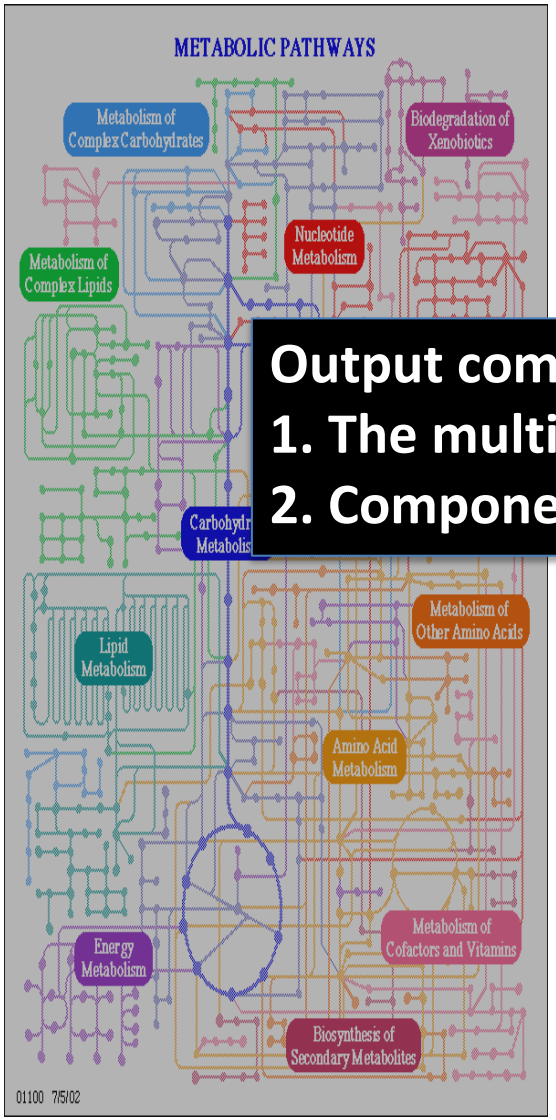
HER2 is highly related to EGFR, but it binds no known ligand

ErbB-3 ***binds several ligands, but its kinase is inactive***

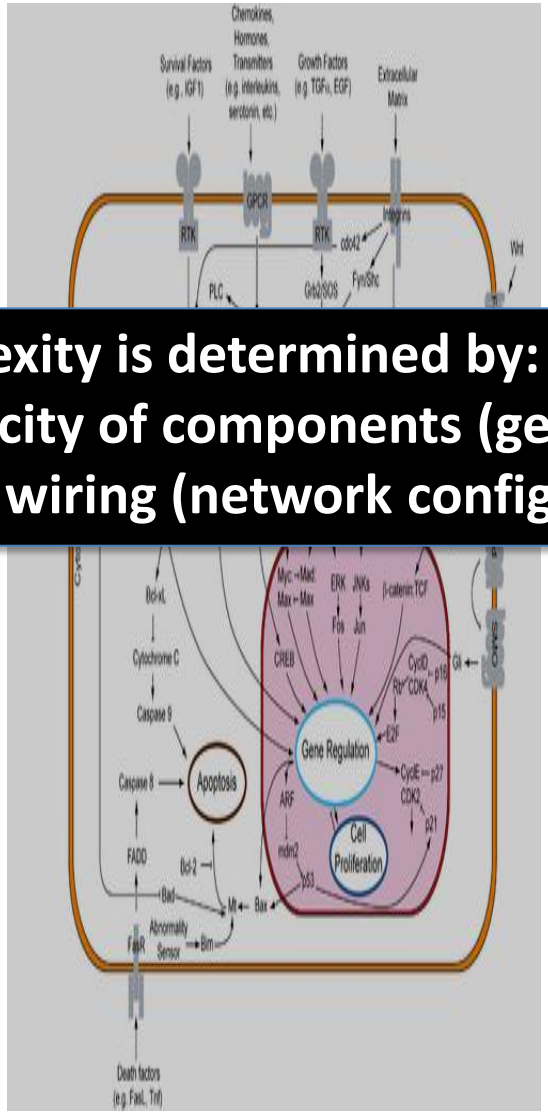


Systems biology of signal transduction: Integration of networks

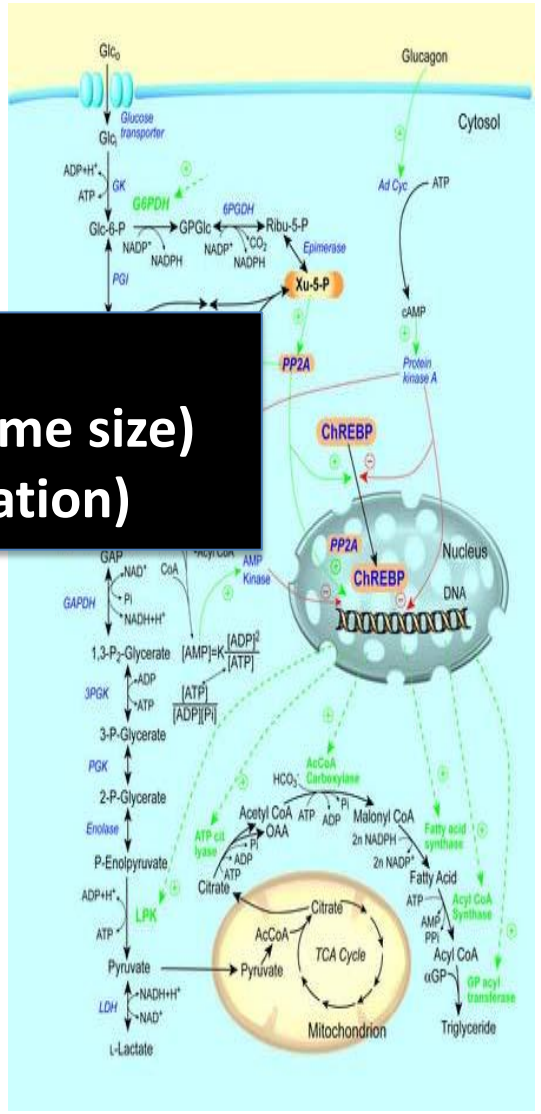
Metabolism



Information/signaling



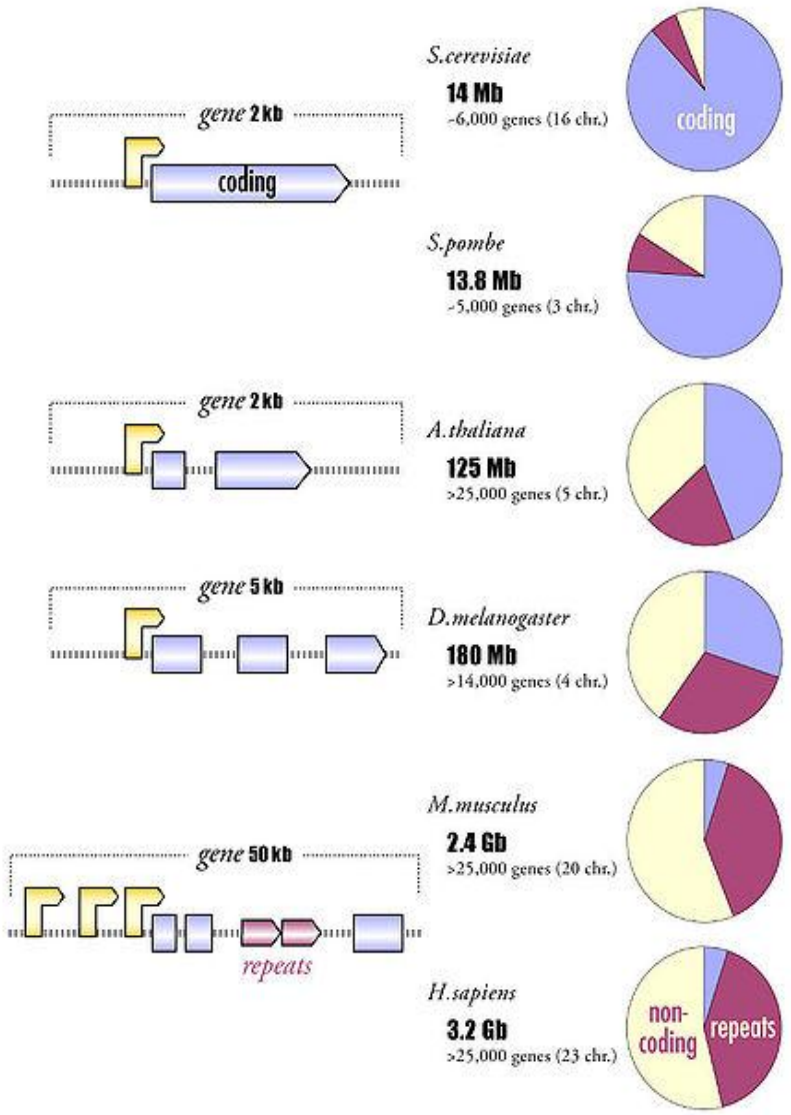
Energy



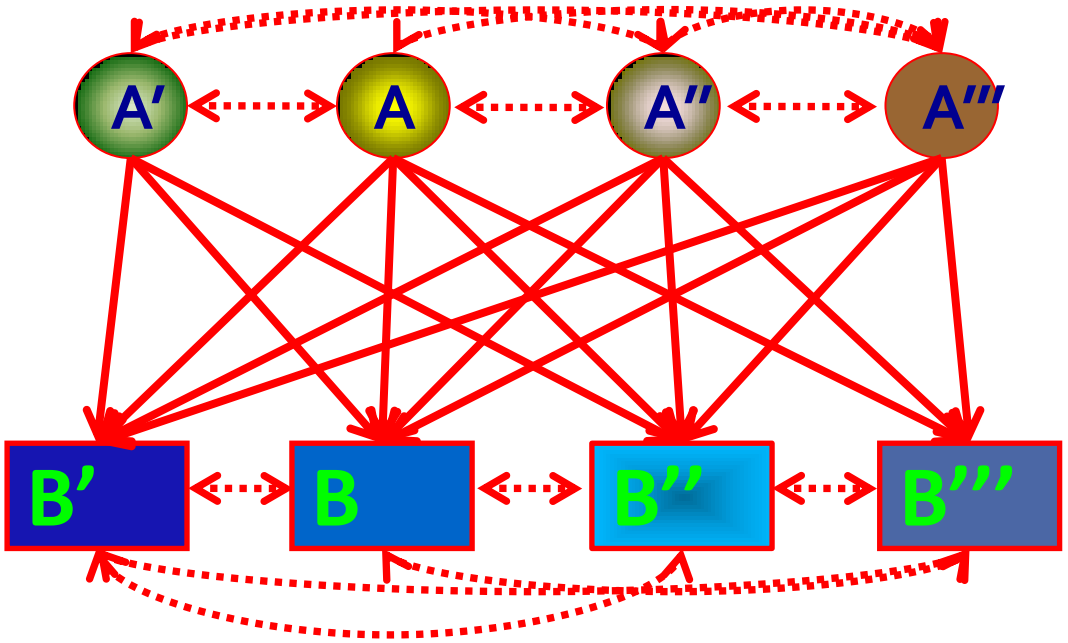
Output complexity is determined by:
1. The multiplicity of components (genome size)
2. Component wiring (network configuration)

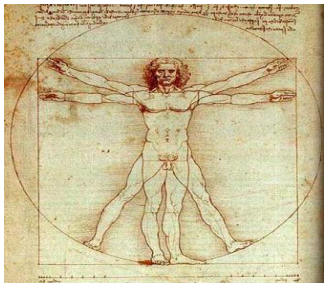
Networks evolved to compensate for the limited size of genomes

- Genomes expand by duplications
- Trade-offs of Mega-genomes:
  - Logistics of Replication
  - Challenges for DNA repair
  - Excessive regulatory sequences
- The alternatives:
  - Simple proteins-->multi-domain
  - Splice variants and PTMs
  - Pathways-->networks

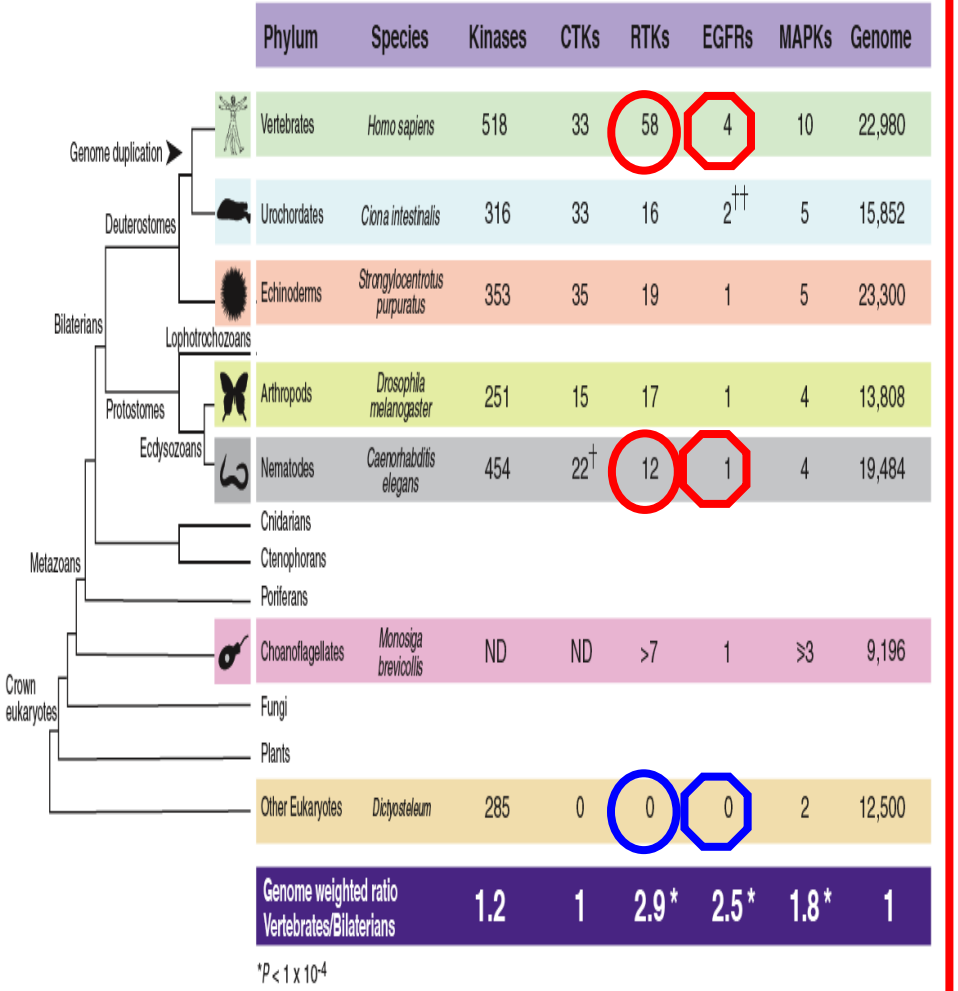


The Origin of Biological Complexity: Whole Genome and Chromosome Duplications





The Evolution of RTKs: Roles for Sub-Functionalization



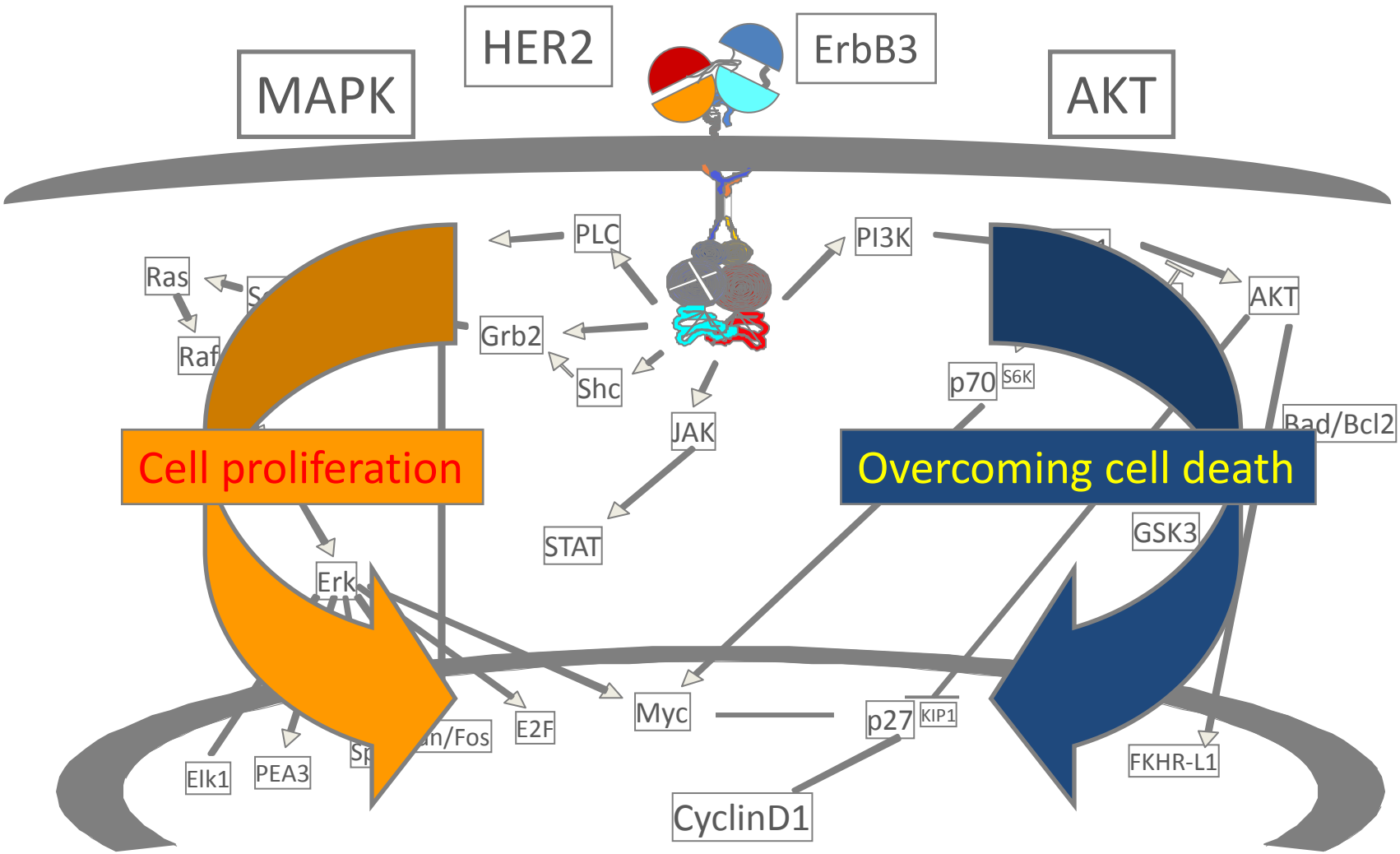
Phylum	Species	Kinases	CTKs	RTKs	EGFRs	MAPKs	Genome
Vertebrates	*Homo sapiens*	518	33	58	4	10	22,980
Urochordates	*Ciona intestinalis*	316	33	16	2††	5	15,852
Echinoderms	*Strongylocentrotus purpuratus*	353	35	19	1	5	23,300
Arthropods	*Drosophila melanogaster*	251	15	17	1	4	13,808
Nematodes	*Caenorhabditis elegans*	454	22†	12	1	4	19,484
Choanoflagellates	*Monosiga brevicollis*	ND	ND	>7	1	⩾3	9,196
Other Eukaryotes	*Dictyosteleum*	285	0	0	0	2	12,500
Genome weighted ratio Vertebrates/Bilaterians		1.2	1	2.9*	2.5*	1.8*	1

*$P < 1 \times 10^{-4}$

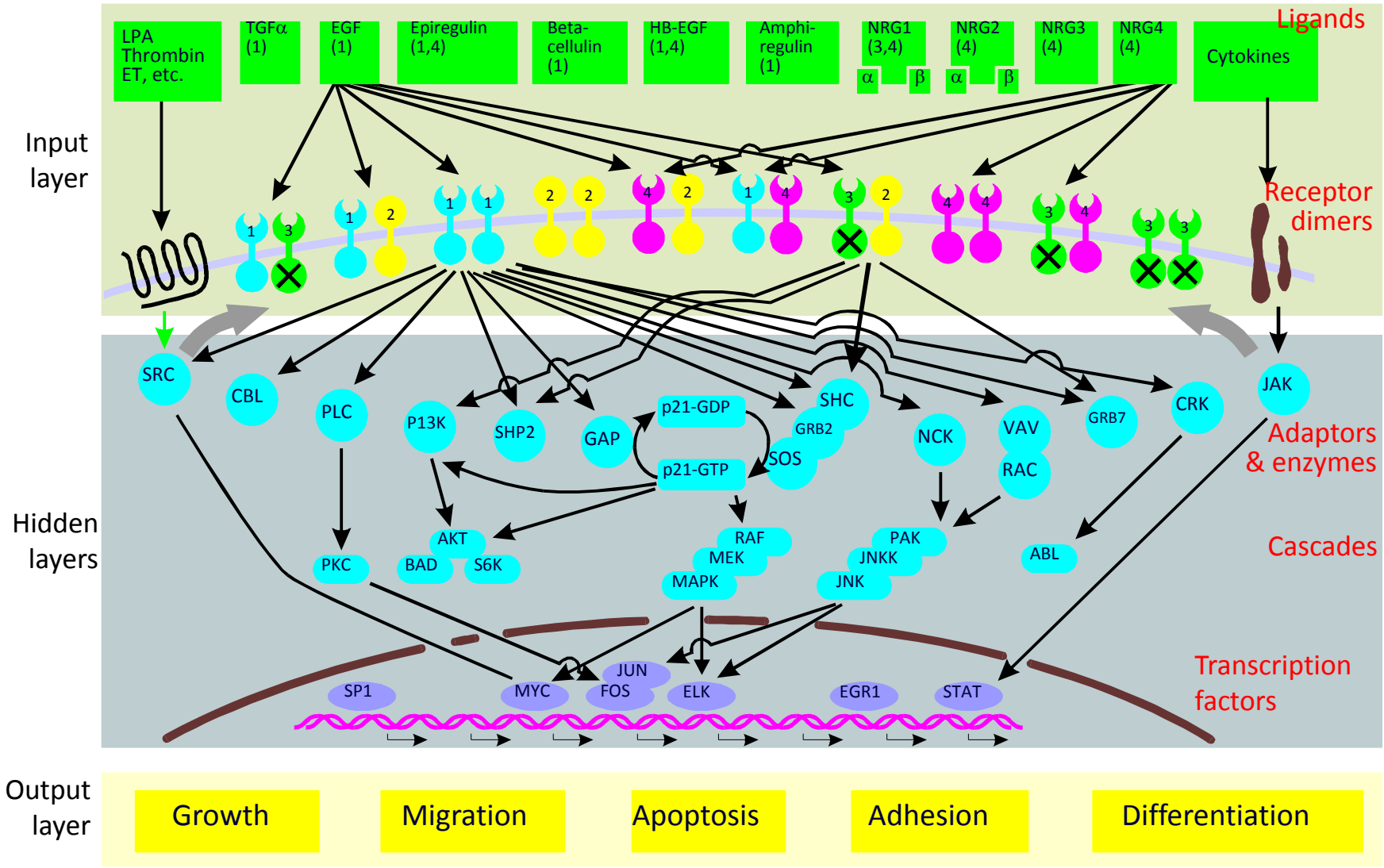
- There have been two genome-wide duplications and numerous smaller scale events
- Most duplicated genes are lost; sub-functionalization retains duplicated genes by enabling complementary functions

Amit, Wides &Yarden (2007) Molecular Systems Biology 3:151-163

Sub-functionalization: Heterodimers comprising ErbB3 (kinase-dead) and HER2 (ligand-less) are Highly Mitogenic

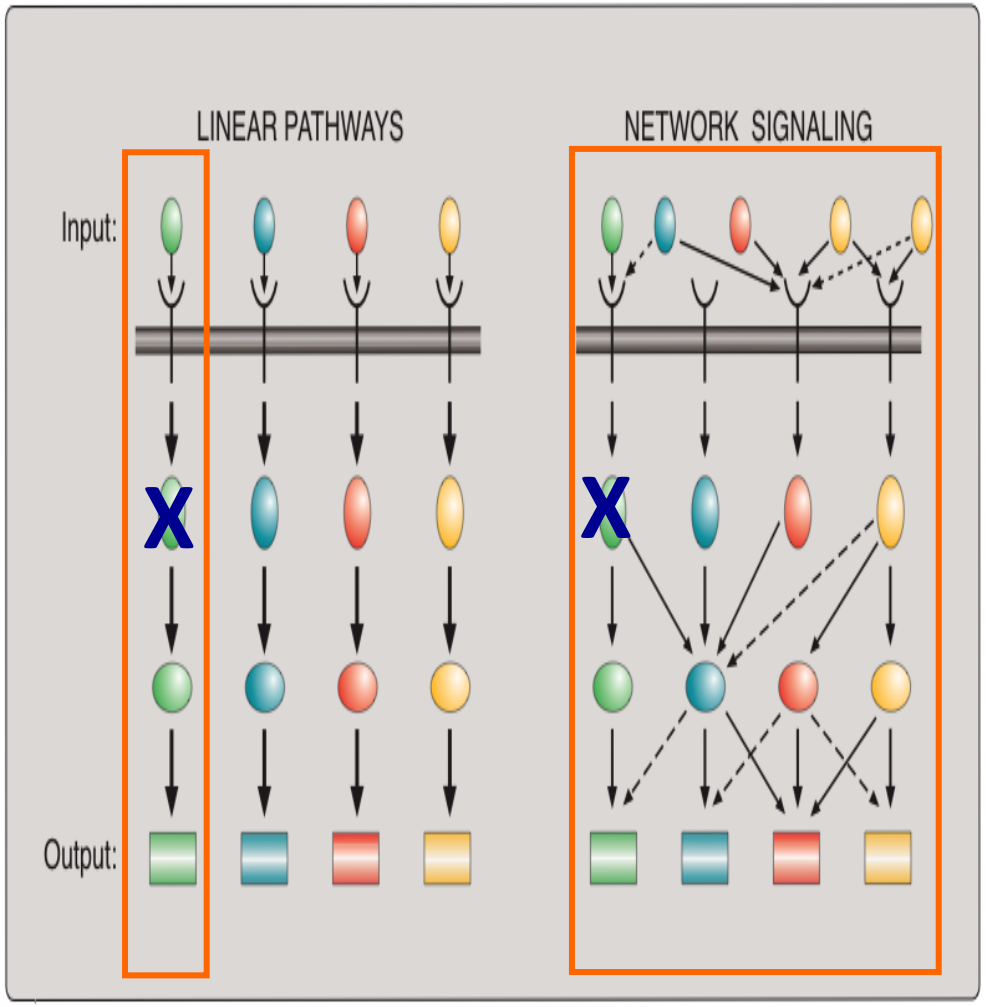


The EGFR/HER2 Signaling Network



Yarden and Sliwkowski (2001) Nature Rev. Mol. Cell Biol, 2:127-137.

Evolution Transformed a Pathway Into a Layered Signaling Network and Trained it to Resist Common Perturbations



<u>Robustness</u> is a property that enables a system to function despite external

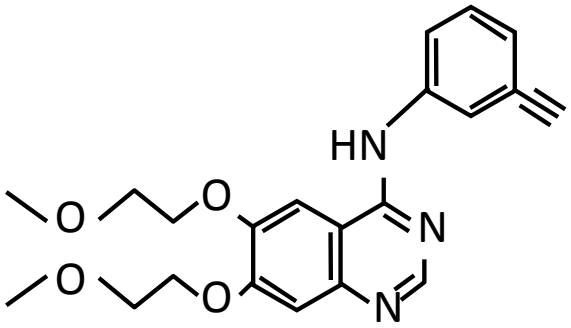
(environmental) and internal (genetic)

perturbations.

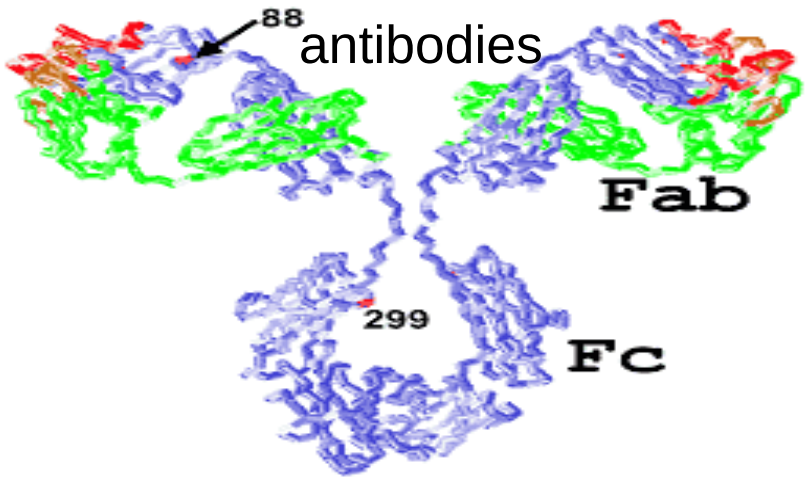
<u>Evolvability</u> is the capacity of a system to generate stable variance.

Two major therapeutic strategies in cancer

Kinase inhibitors



Monoclonal antibodies



Kinase inhibitors

Synthetic

- Low molecular weight
- Intracellular action
- Medium-low cost
- Narrow/broad target specificity
- MOA: well understood
- Rapid onset of patient resistance

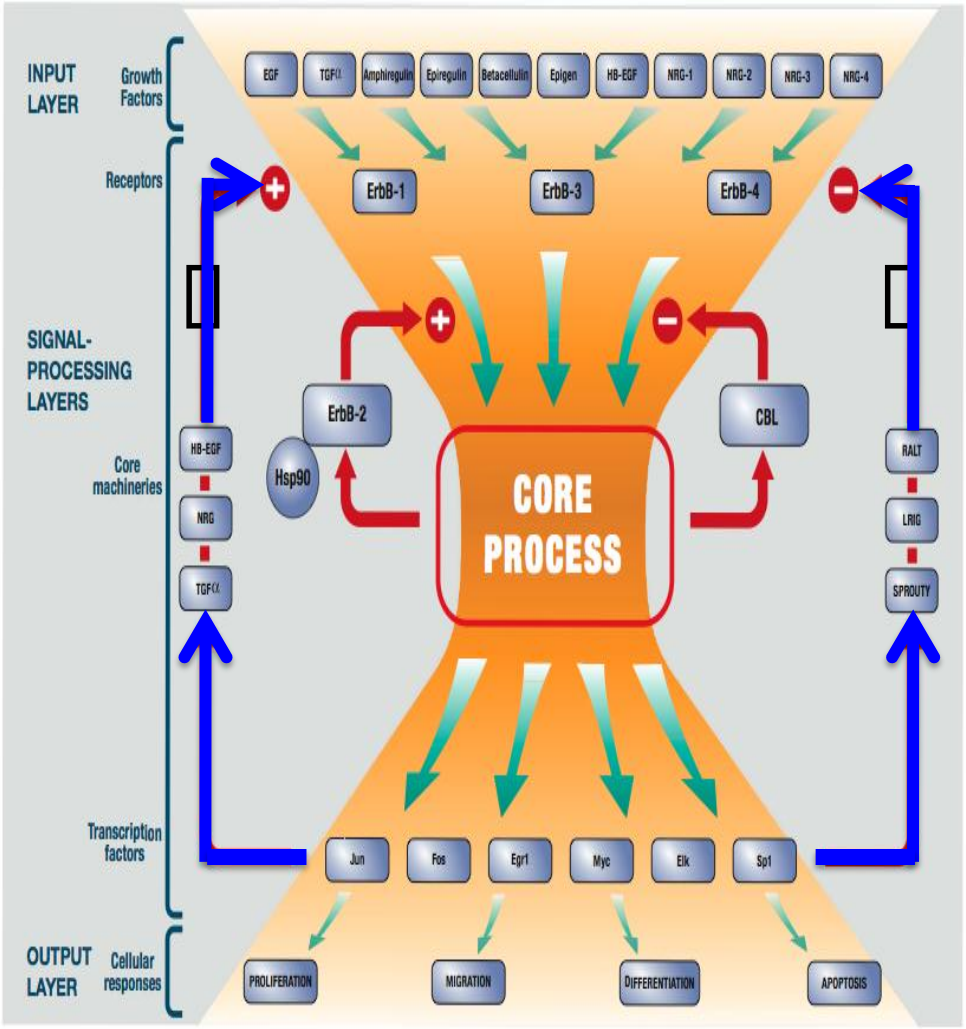
Monoclonal antibodies

Recombinant

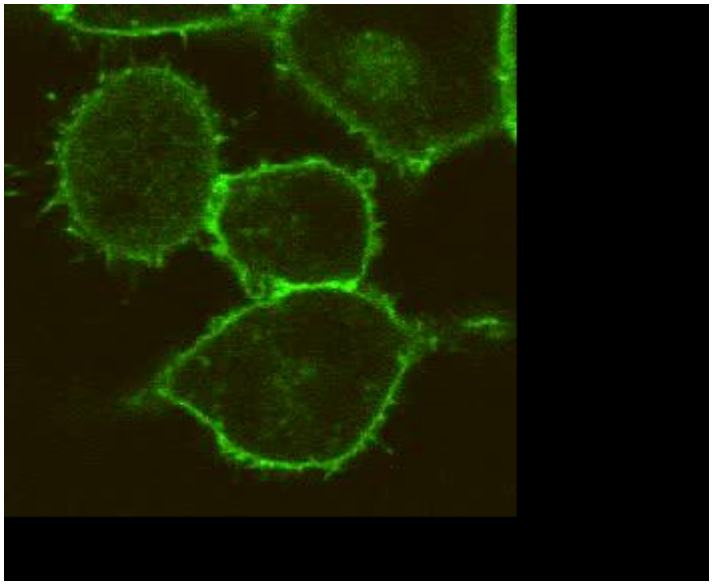
- High molecular weight
- Extracellular action
- High cost
- Absolute target specificity
- MOA: Incompletely understood
- Slow onset of patient resistance

Control loops ensure robustness

- *Modularity: Organization in units that enable damage containment*
- *Redundancy and diversity: input and output diversity and multiple pathways to achieve a specific function*
- *Positive and negative feedback control loops*
- *Plasticity (short-term) and adaptability (long-term)*

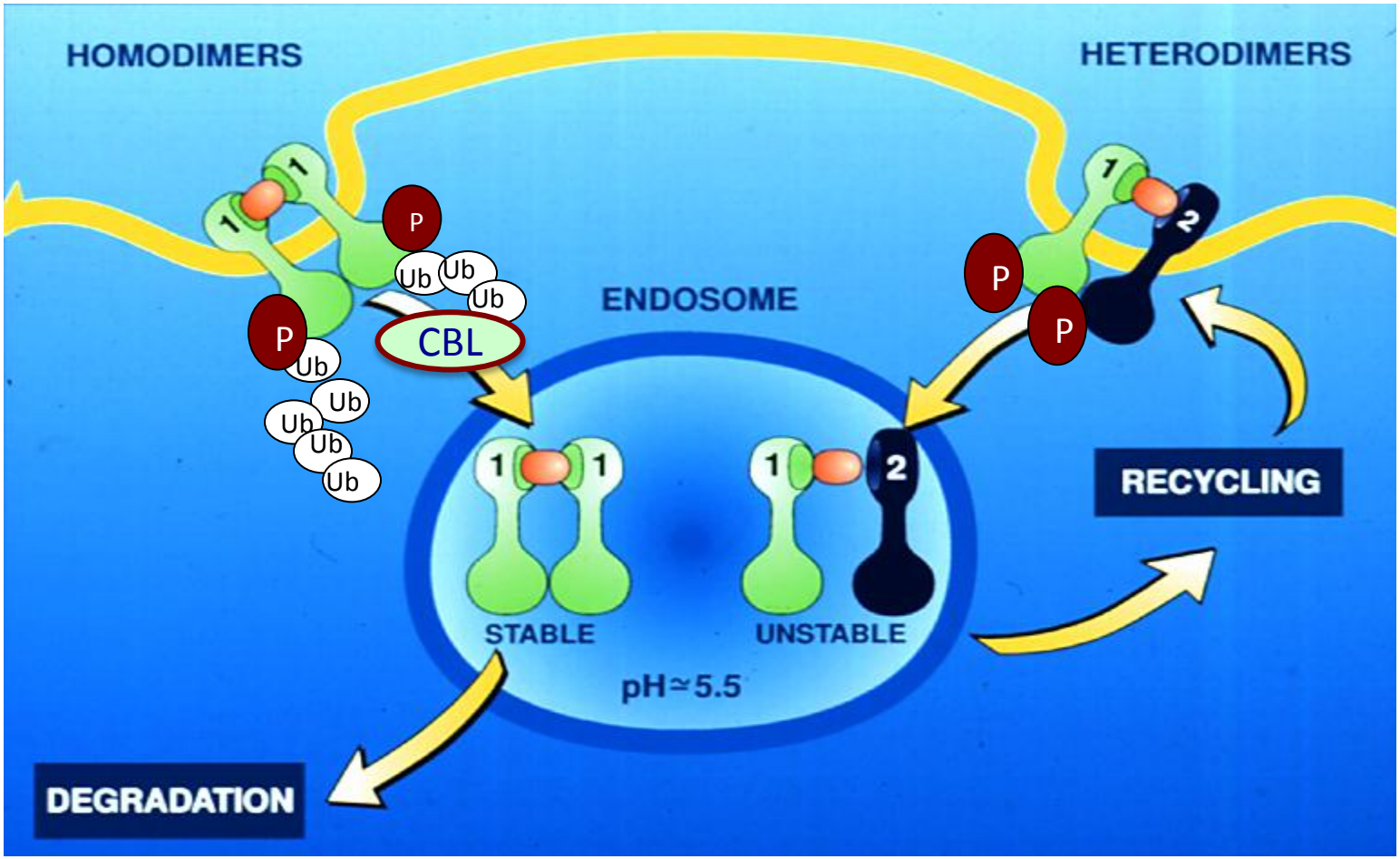


Citri and Yarden (2006) Nature Rev. Mol. Cell Biol. 7: 505



Courtesy of Tom and Donna Jovin (MPI).

HER2 and Cezanne-1 Recycle EGFR



Mosesson, Mills & Yarden (2008) Derailed endocytosis: an emerging feature of cancer. Nature Rev. Cancer 8;835-50

System's Control: Newly Synthesized Proteins and Transcription Regulation

Wave 1 (IEGs)

Peak: Sharp, 10-30 min

Examples: Fos, Jun

Function: Transcription factors

Wave 2 (DEGs)

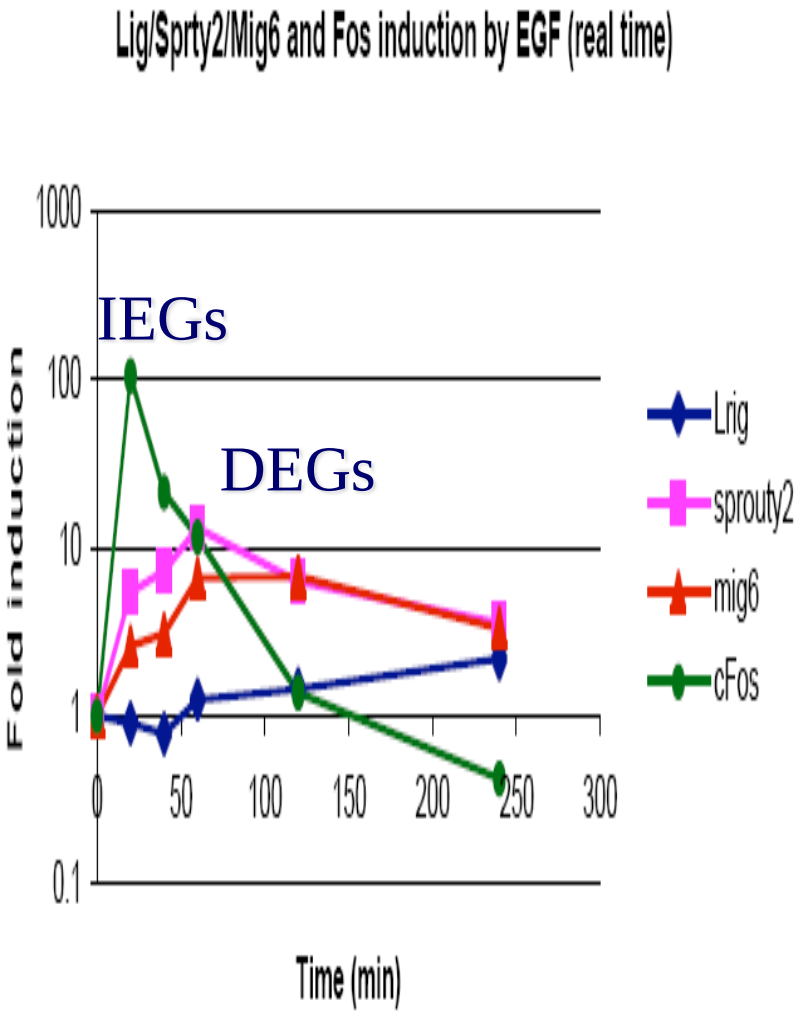
Peak: Broad, 40-480 min

Examples:

MAPK phosphatases

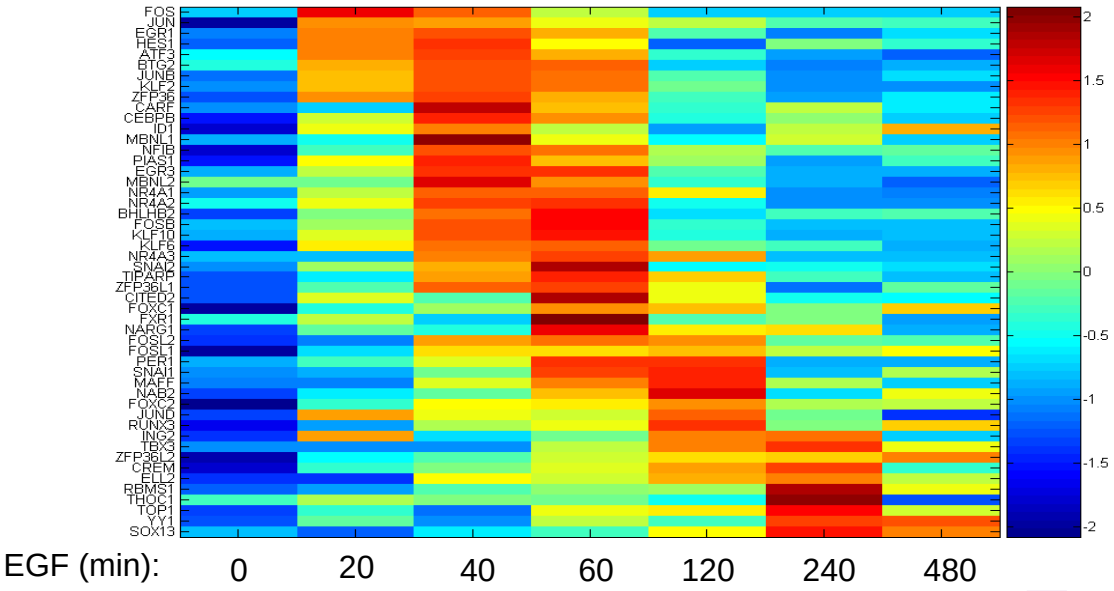
Transcription repressors Growth factors & cytokines

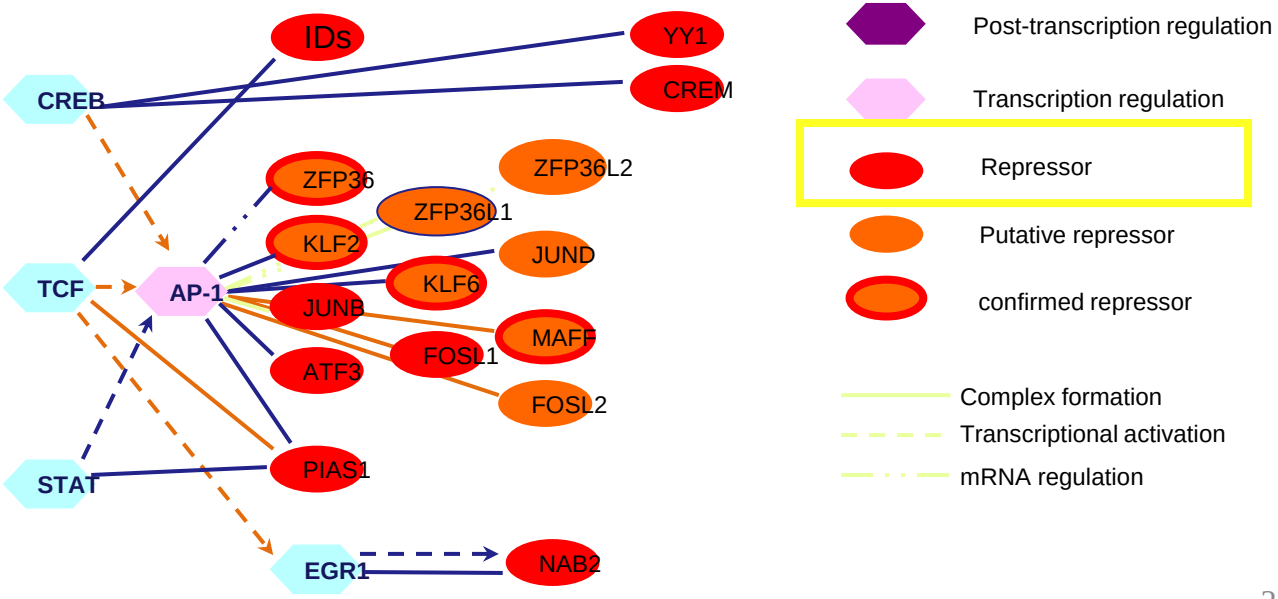
RNA-binding proteins



I. Amit and A. Citri

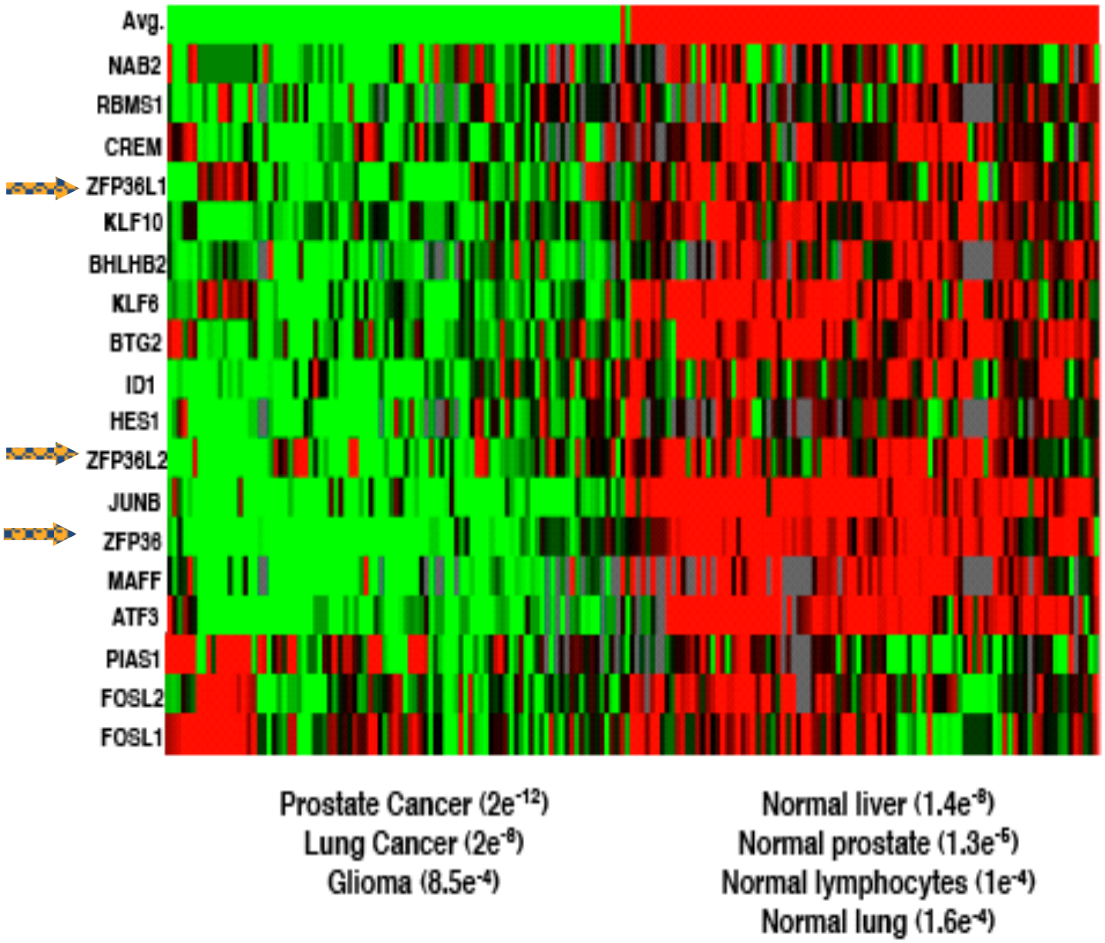
Delayed Early Genes: Transcription repressors





Delayed Early Genes are Down-regulated in Carcinomas

Tissue: Malignant Normal



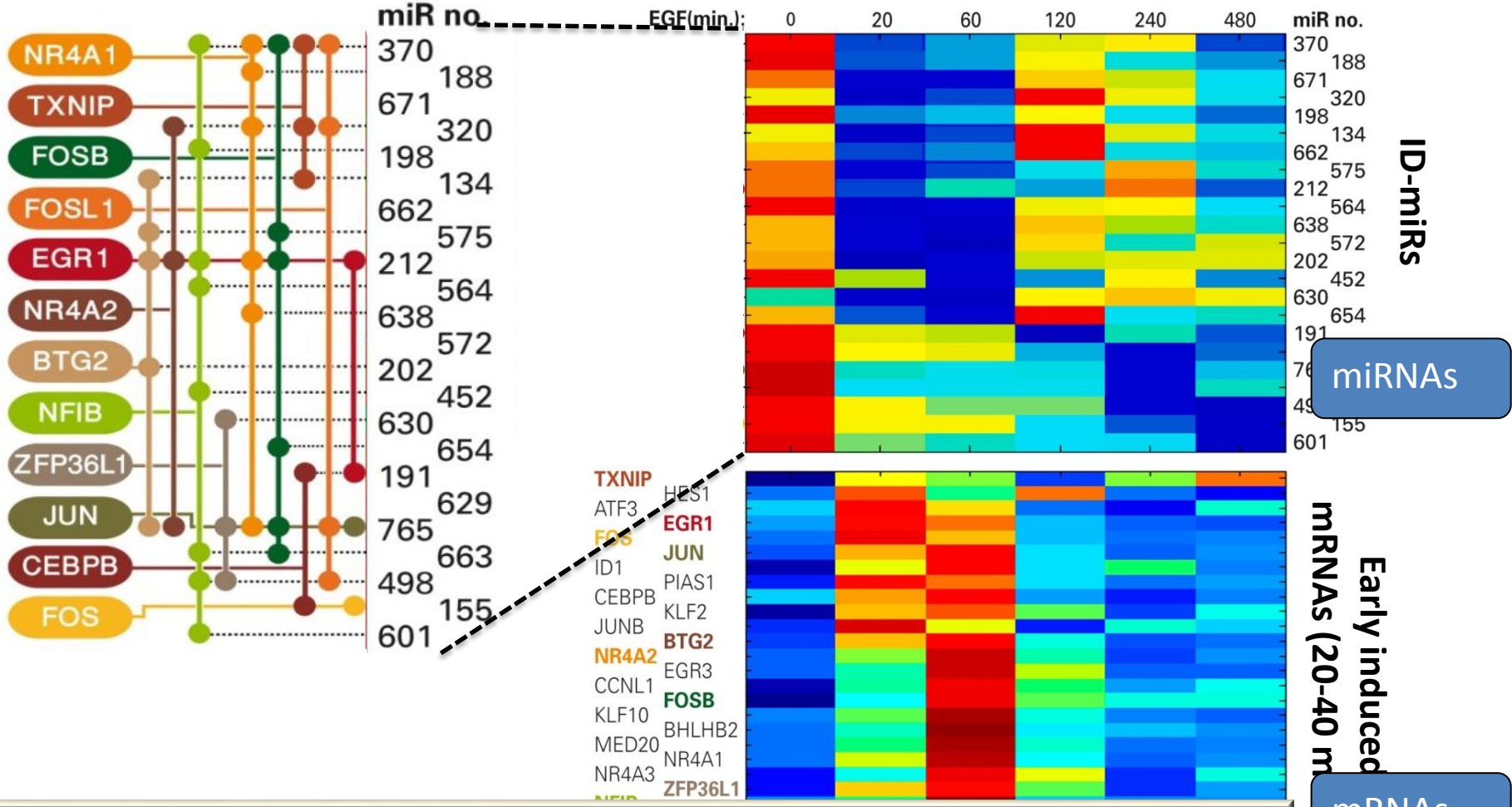
- Analysis: A human cancer compendium (1975 published micro-arrays; 22 tumor types)
- Observation: A large proportion of DEGs (18 of 25) are coordinately down-regulated in carcinomas.

I. Amit et al., Nature Genetics (2007)

The connectivity map of ID-miRs and mRNA targets based on expression and predictions

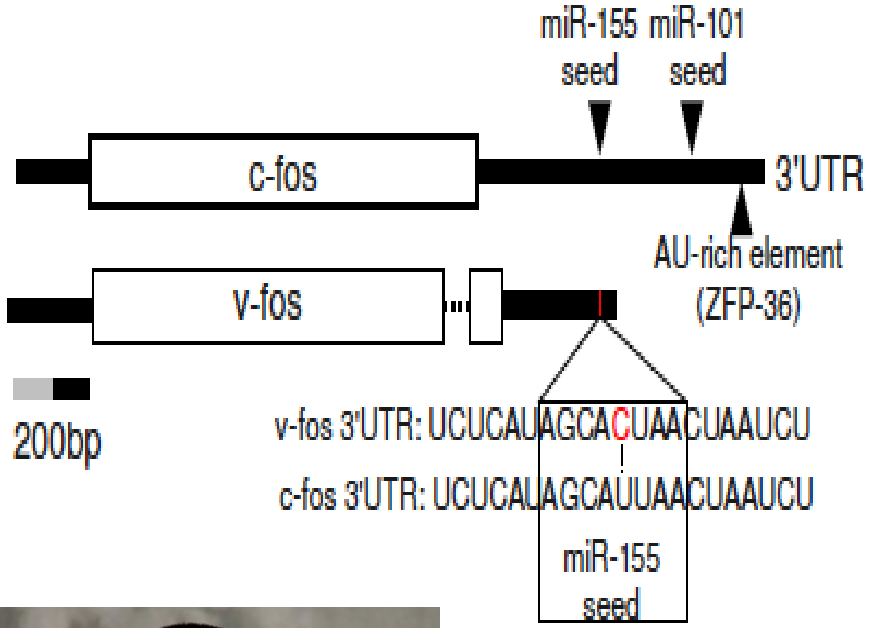
Early induced mRNAs (20-40 min) which are predicted targets of ID-miRs

Inversed correlation between ID-miRs and early induced mRNAs

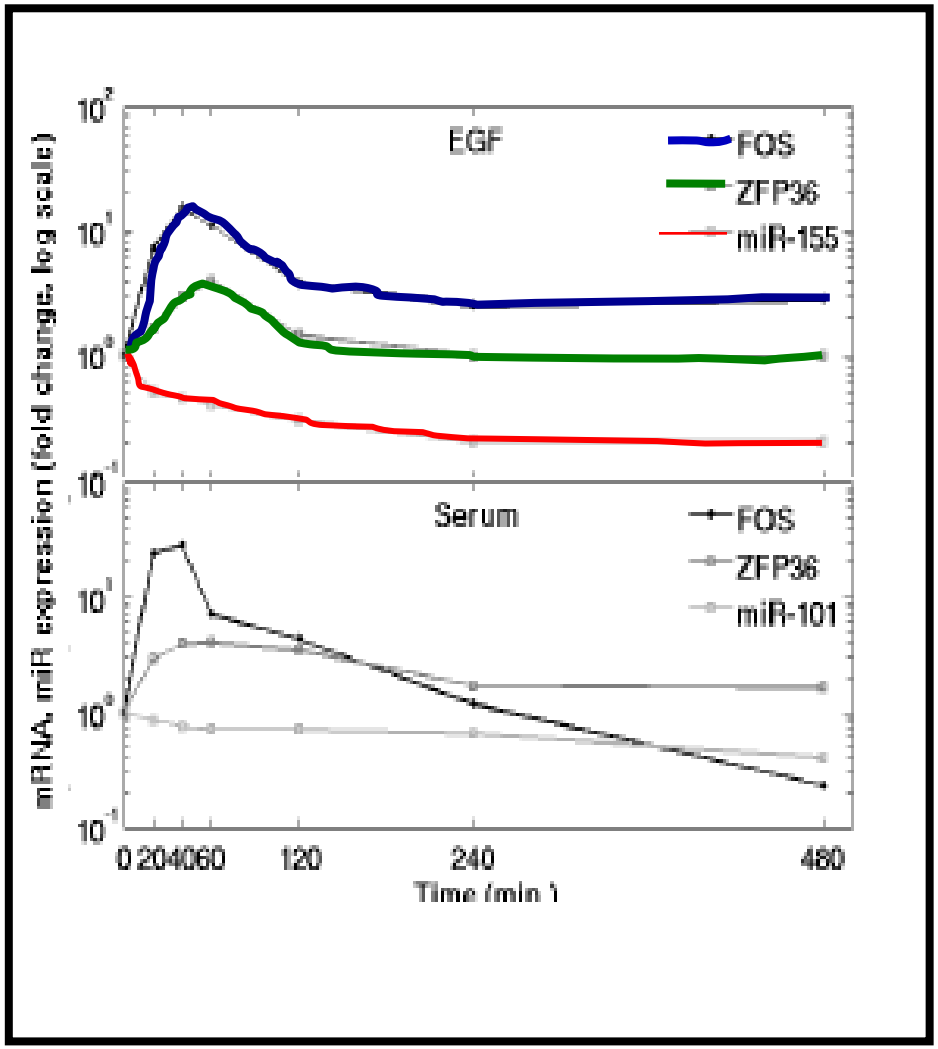


The targets of ID-miRs are a group of oncongenic transcription factors that are immediately induced in response to EGF (IEGs)

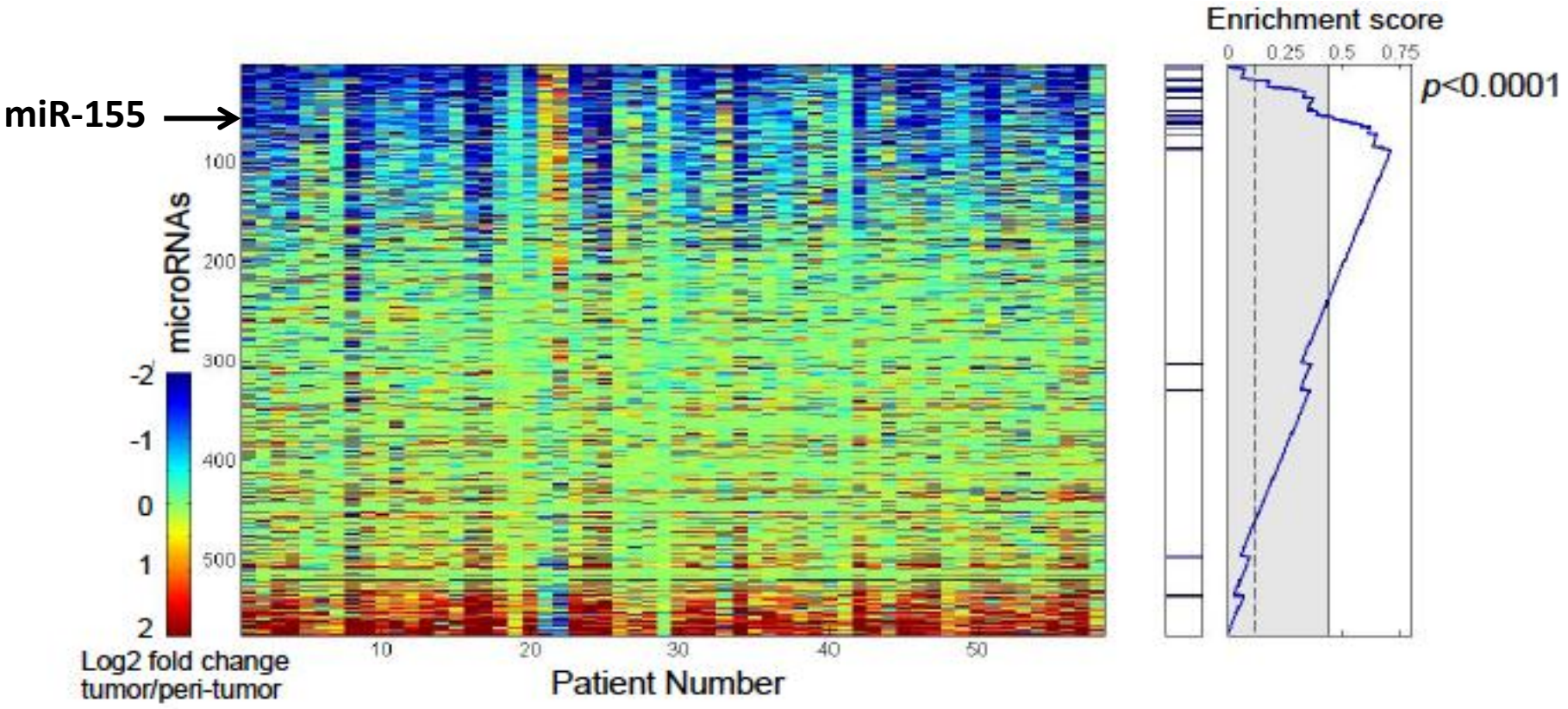
Coordinate regulation of c-FOS by miRNAs and the DEG called ZFP36



Roi Avraham

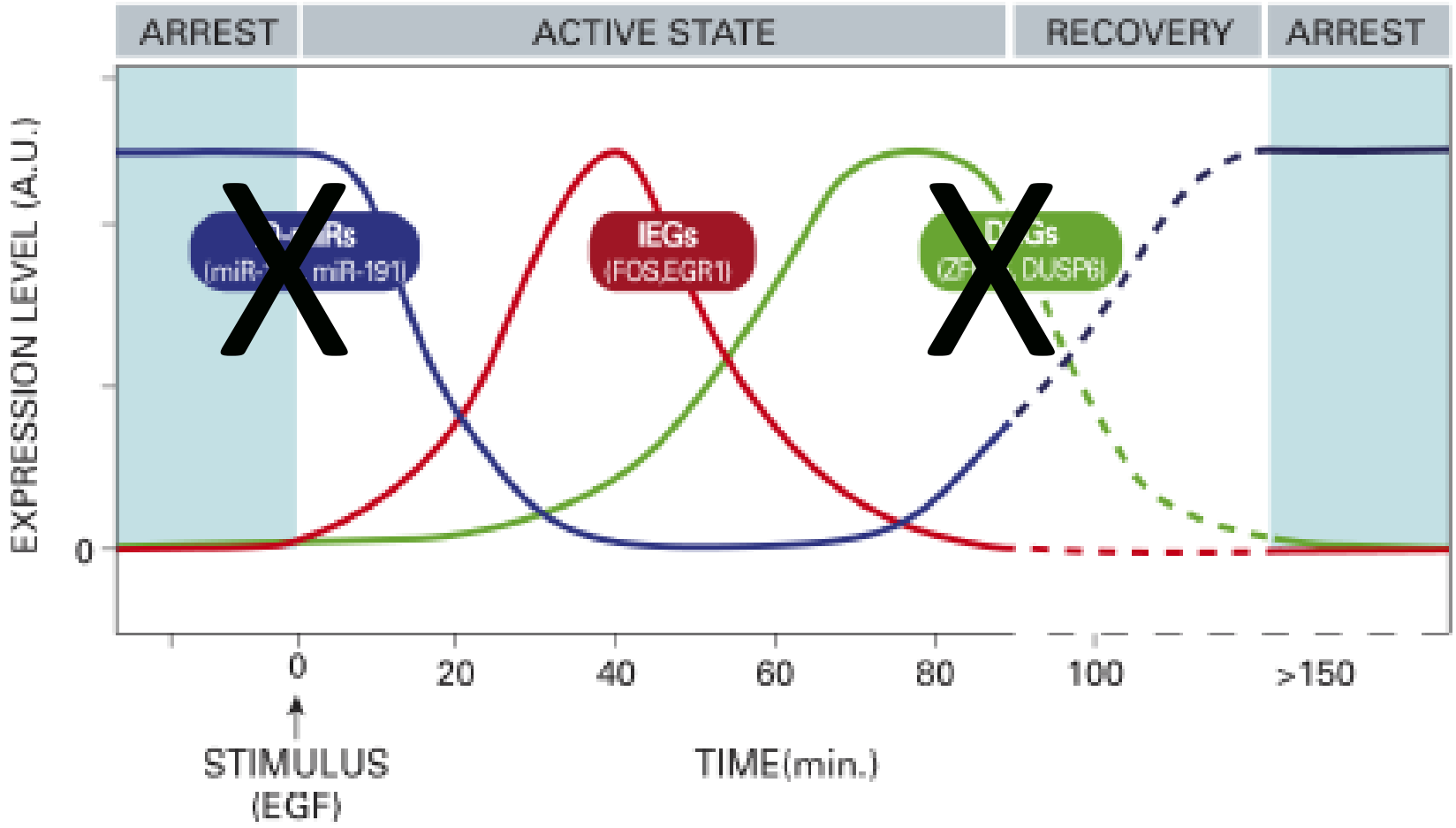


ID-miRs are commonly downregulated in mammary tumors vs peri-tumors

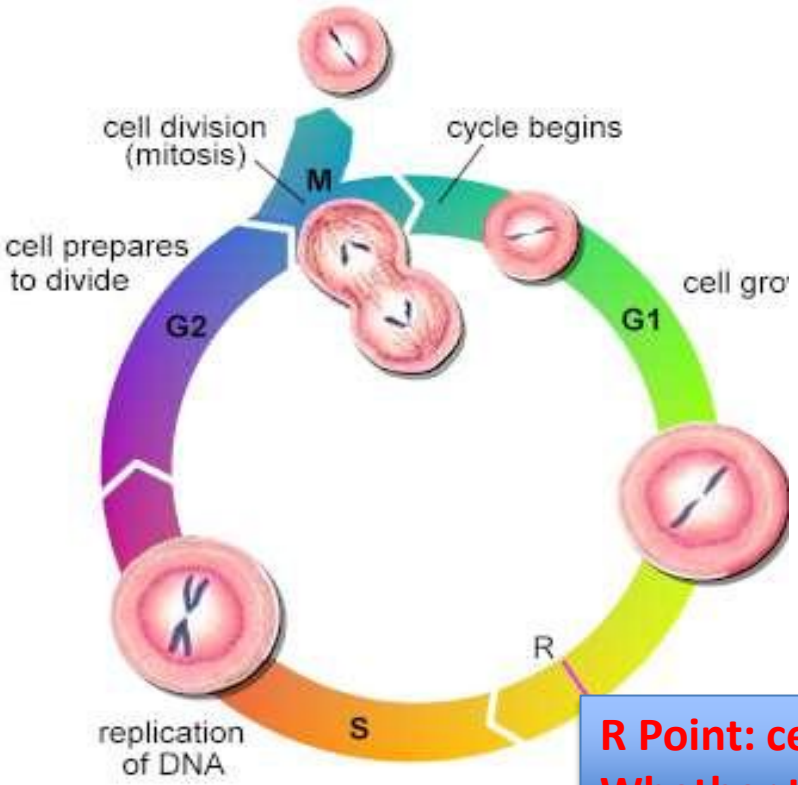


Noa Bossel and Amit Zeisel & Giovanni Blandino

Micro-RNAs and DEGs Define a Window of IEG expression



EGF-induced proliferation of mammary cells



R Point: cell decides Whether to continue

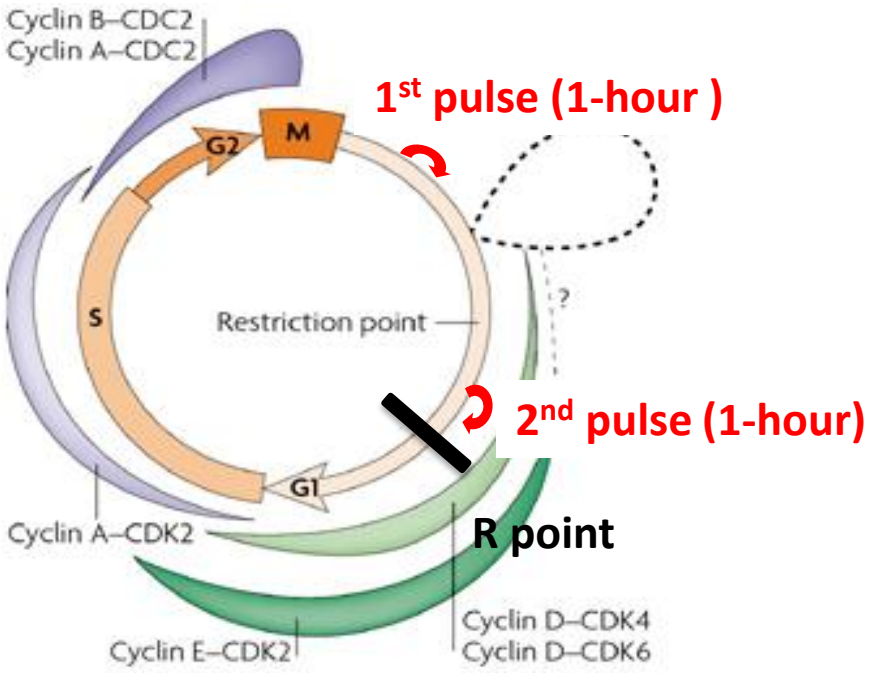
Yaara Zwang

R crossing requires continuous (>6 hours) presence of growth factors

Growth-factor-dependent mitogenesis requires two distinct phases of signalling

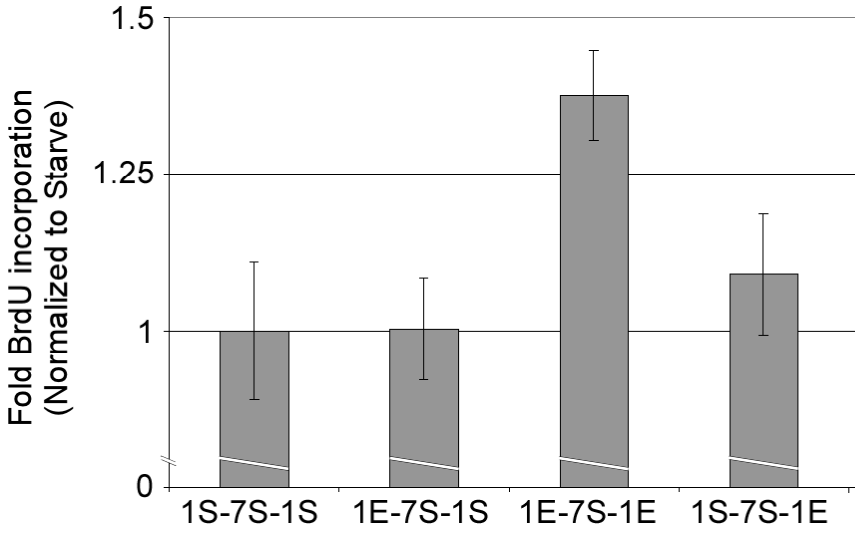
NATURE CELL BIOLOGY | VOL 3 | FEBRUARY 2001

Steven M. Jones*† and Andrius Kazlauskas*†‡



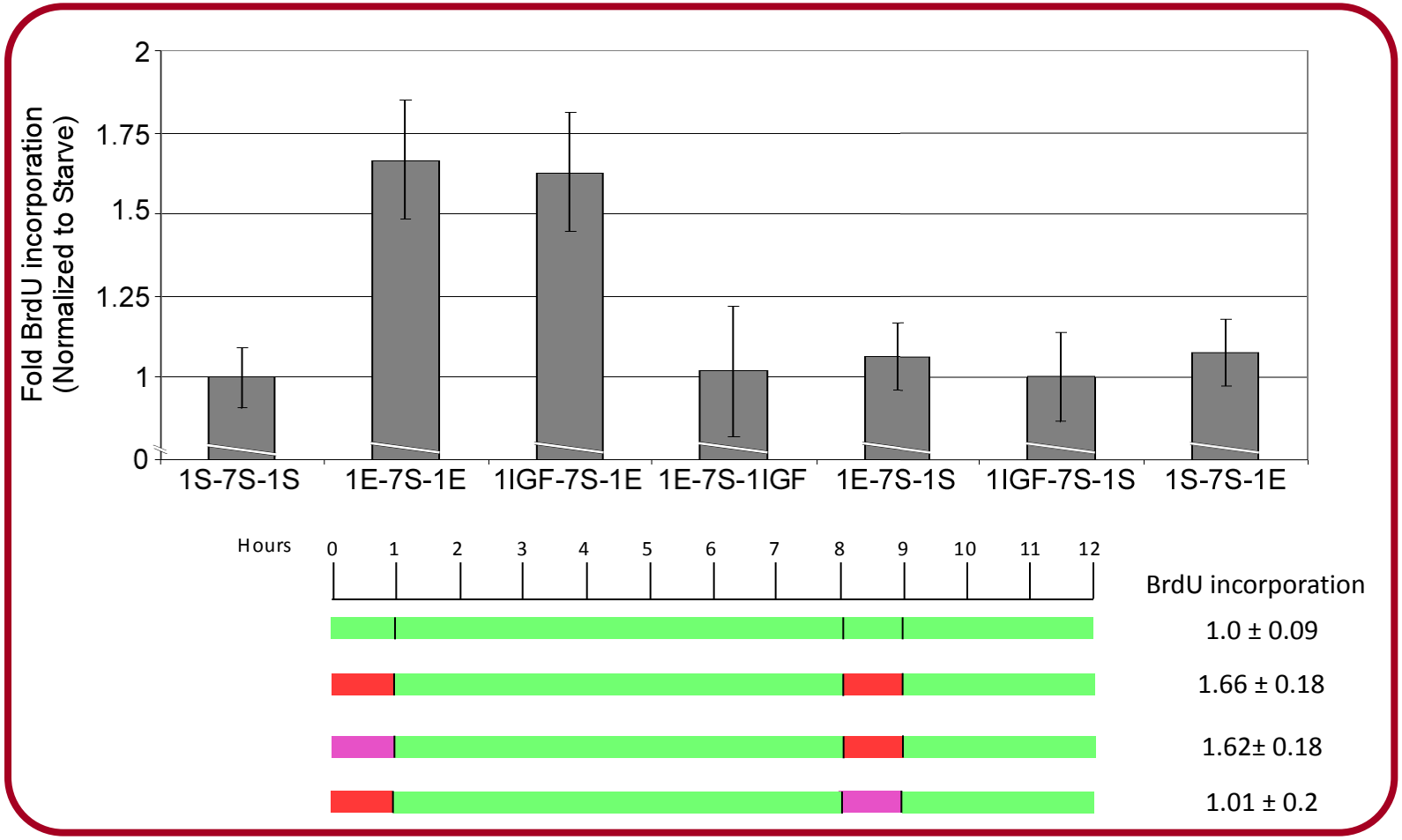
R-crossing is enabled by two short pulses of growth factors

Proliferation induced by two-pulses of EGF



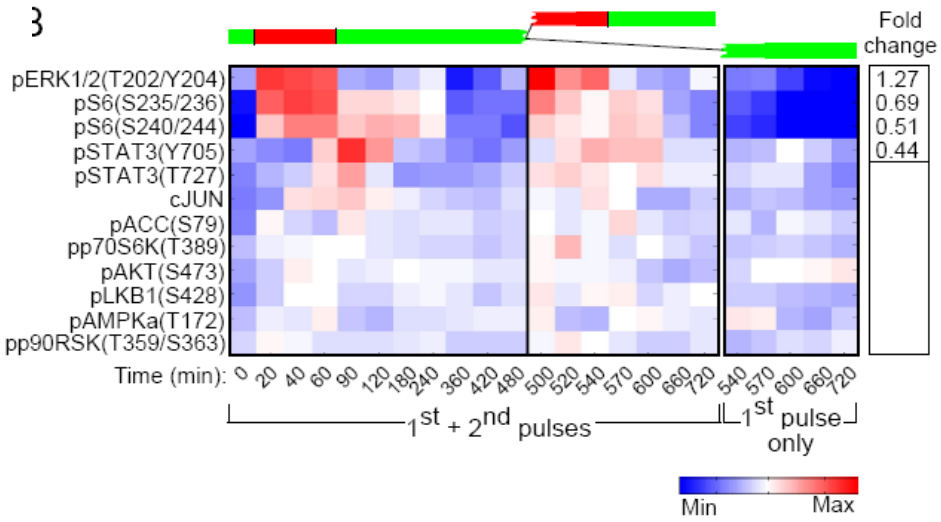
	BrdU incorporation
1S-7S-1S	1.0 ± 0.1
1E-7S-1S	1.0 ± 0.08
1E-7S-1E	1.37 ± 0.07
1S-7S-1E	1.09 ± 0.09

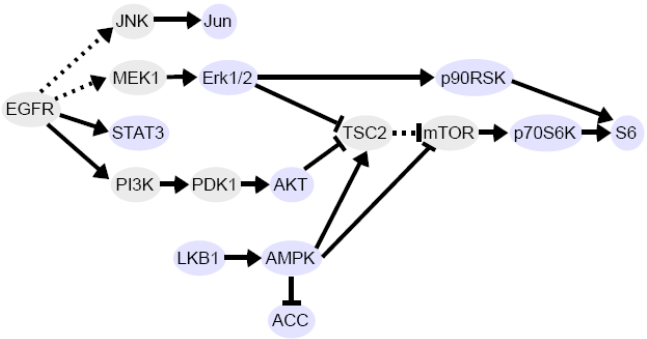
IGF1 may replace EGF in the 1st, not the 2nd pulse

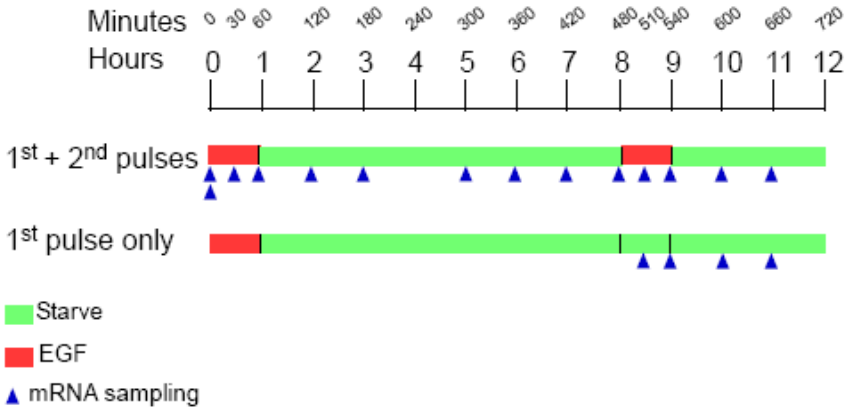


IGF-1 can substitute 1st pulse, but not 2nd pulse EGF.

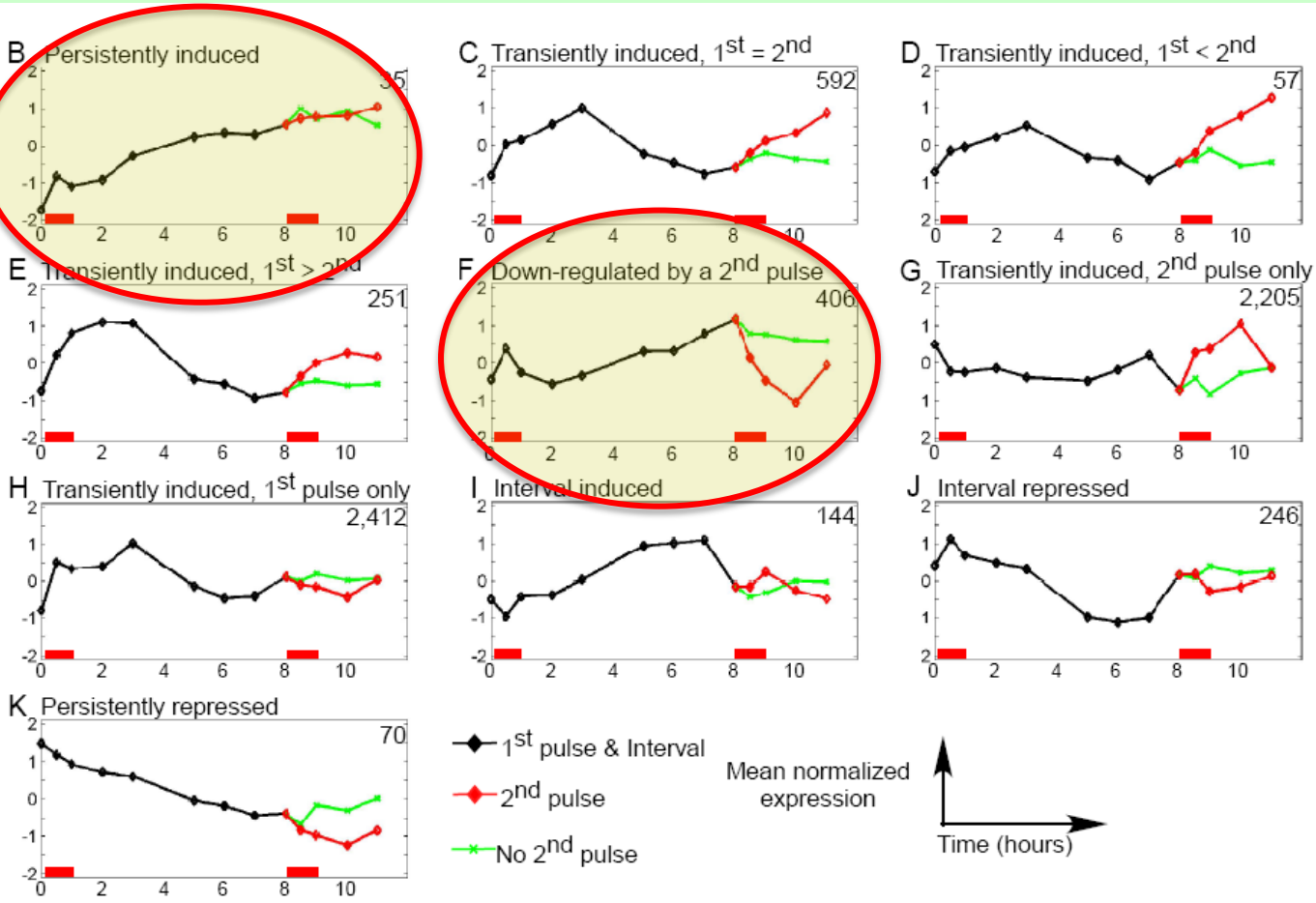
RPPA and Transcriptomic Analyses of the Two-Pulses



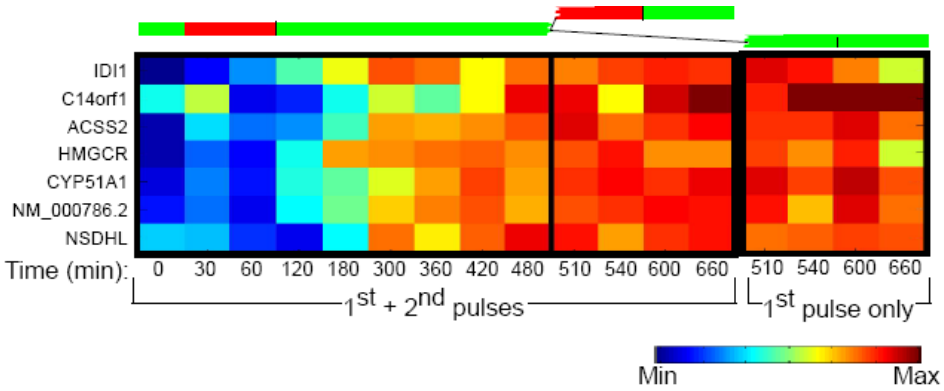


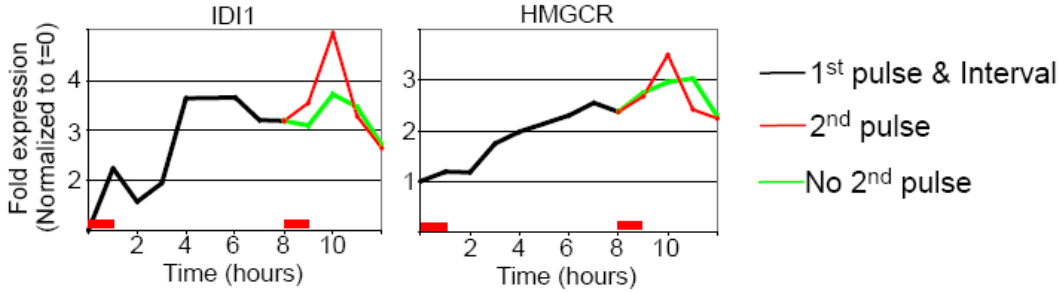


10 expression profiles are induced by EGF (two pulses)

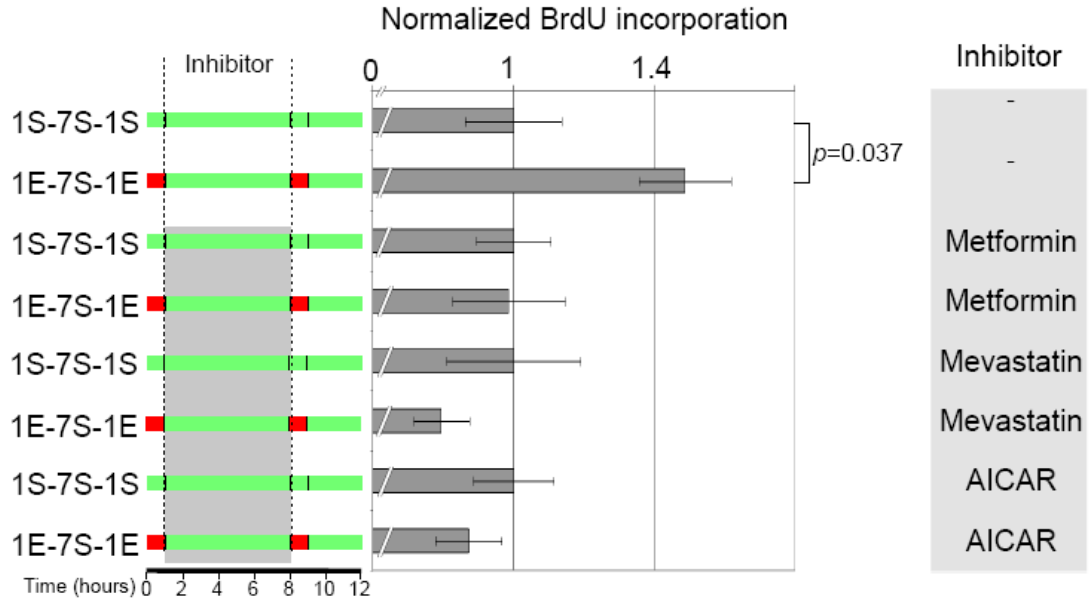


The *Persistently Induced* module is enriched for metabolic genes





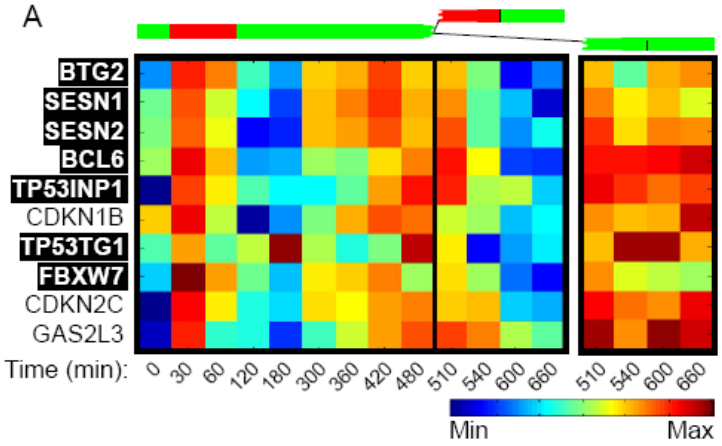
Induction of metabolic processes is essential for R-crossing



Lipid metabolism and membrane biogenesis initiate at the 1st pulse and might be essential for R crossing

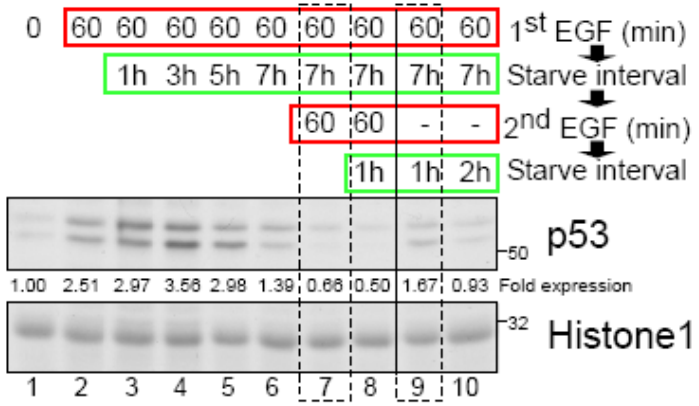
The module *"Down-regulated by 2nd Pulse"* comprises several p53 regulated genes

The module includes well-established p53 target genes

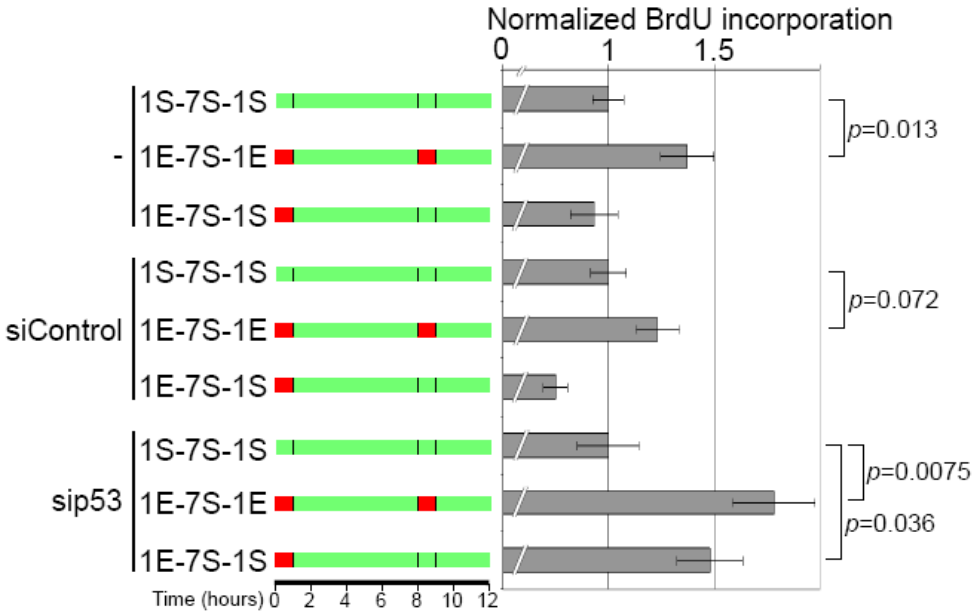


And

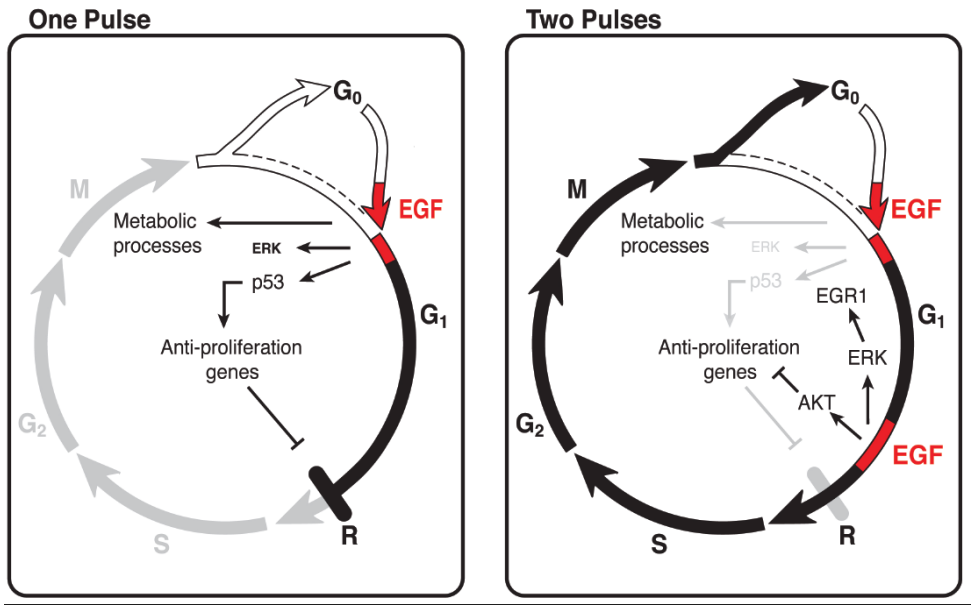
p53 associates with chromatin upon the 1st pulse, remains active during the interval and dissociates on the 2nd pulse



Knockdown of p53 enables R-crossing in the absence of a second pulse



The Paradigm of “Consistency Test”



- The 2-pulse mode of commitment might filter the "noise" of growth factor bursts, which are often short and inconsistent

- In the absence of p53 (e.g., cancer cells), this filtering mechanism is defective

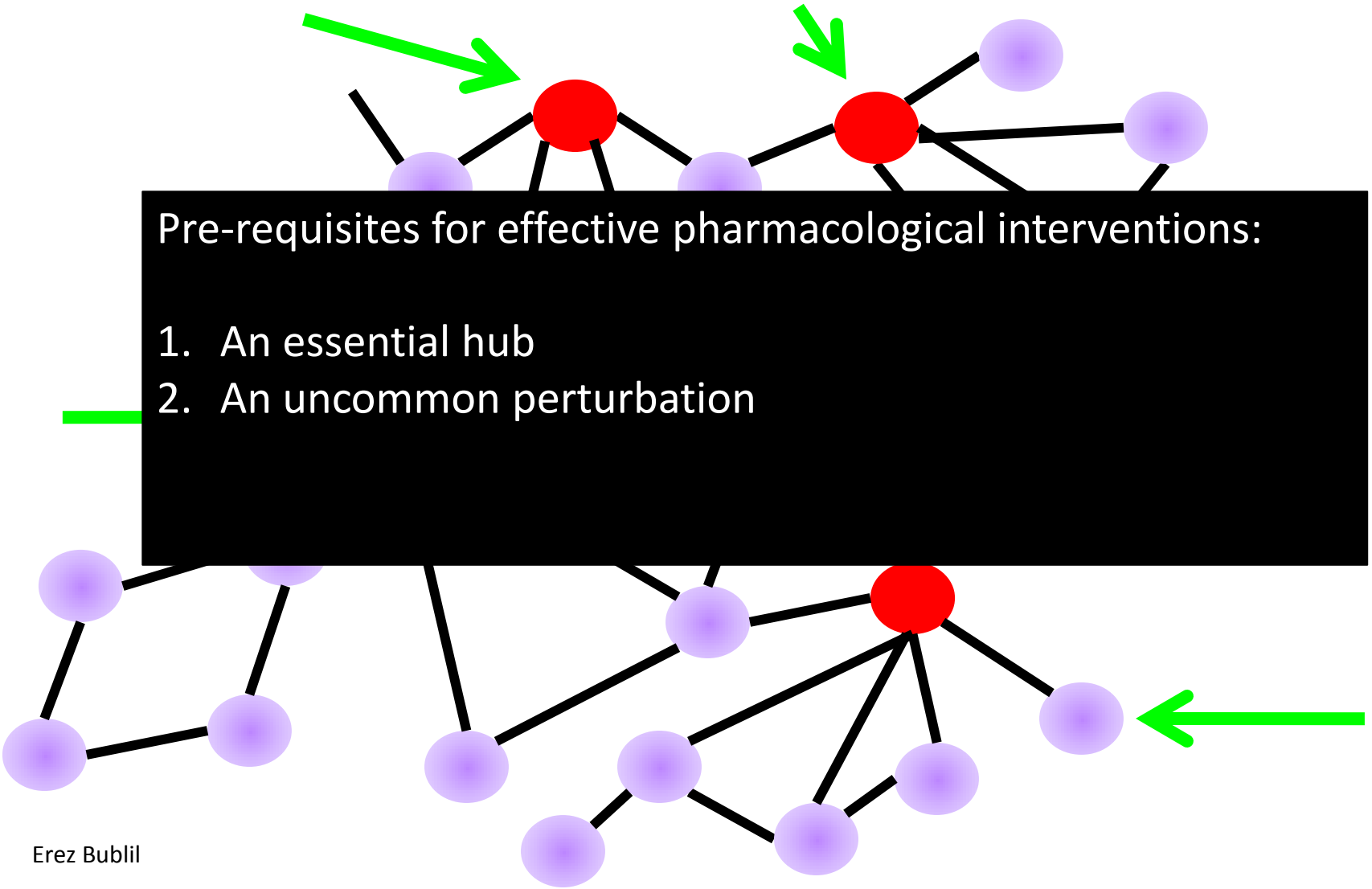
Y. Zwang et al., Molecular Cell (2011)

Back to Complexity: Lessons from Graph (Network) Theory

- While networks expand, rich nodes become richer[1]
- Networks are trained to resist common perturbations; they show extreme fragility to uncommon attacks (or double attacks)[2]
- Robust networks are hub-addicted, uncommon interceptors (drugs) targeting major hubs may collapse a network[3]
- Hub centrality breeds lethality[4]

[1.] A. Wagner, 2001; [2.] Carlson & Doyle, 2000; [3.] I.B. Weinstein, 2002; [4.] Barbasi & Oltavi; 2001

Centrality-Lethality Principle

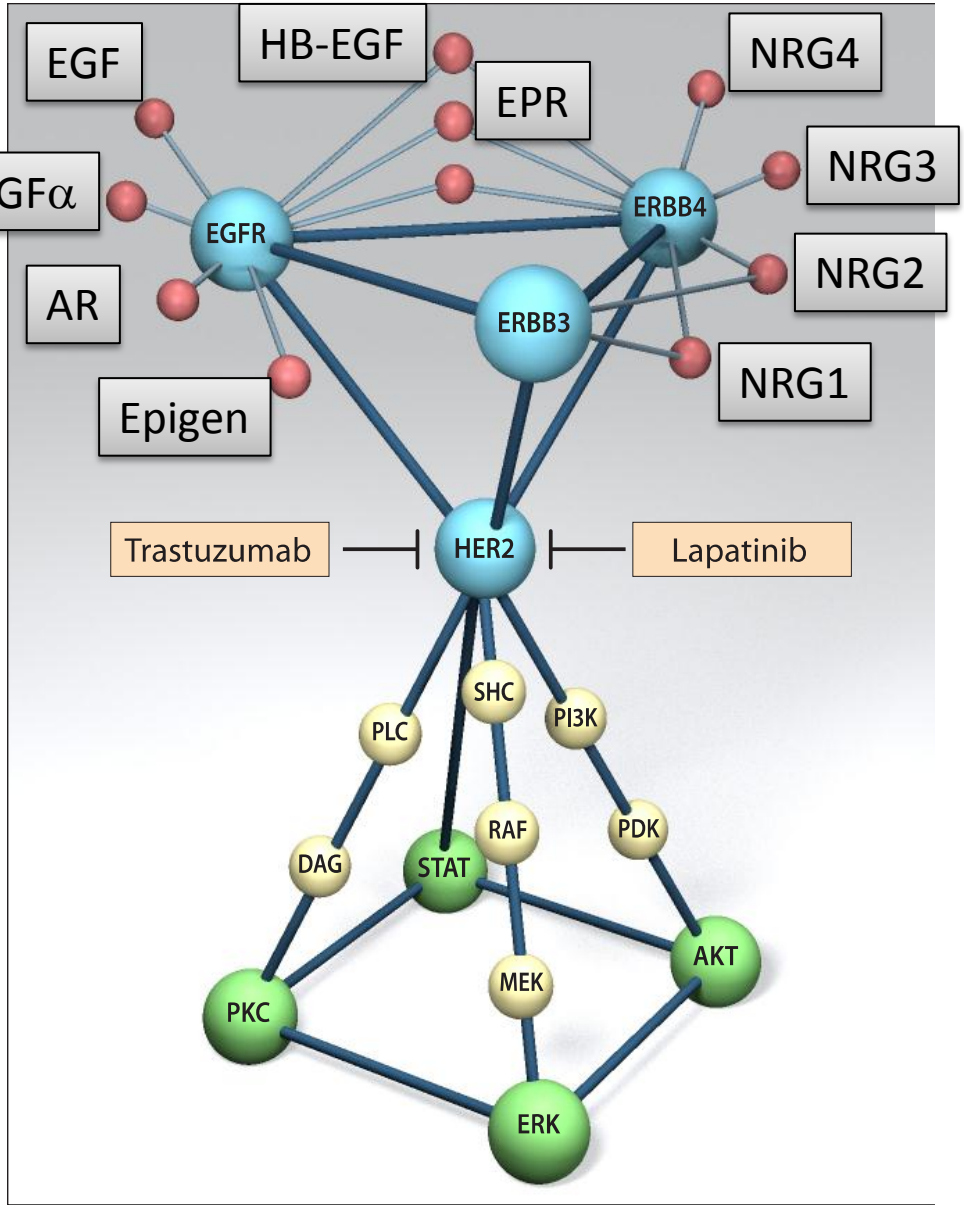


Pre-requisites for effective pharmacological interventions:

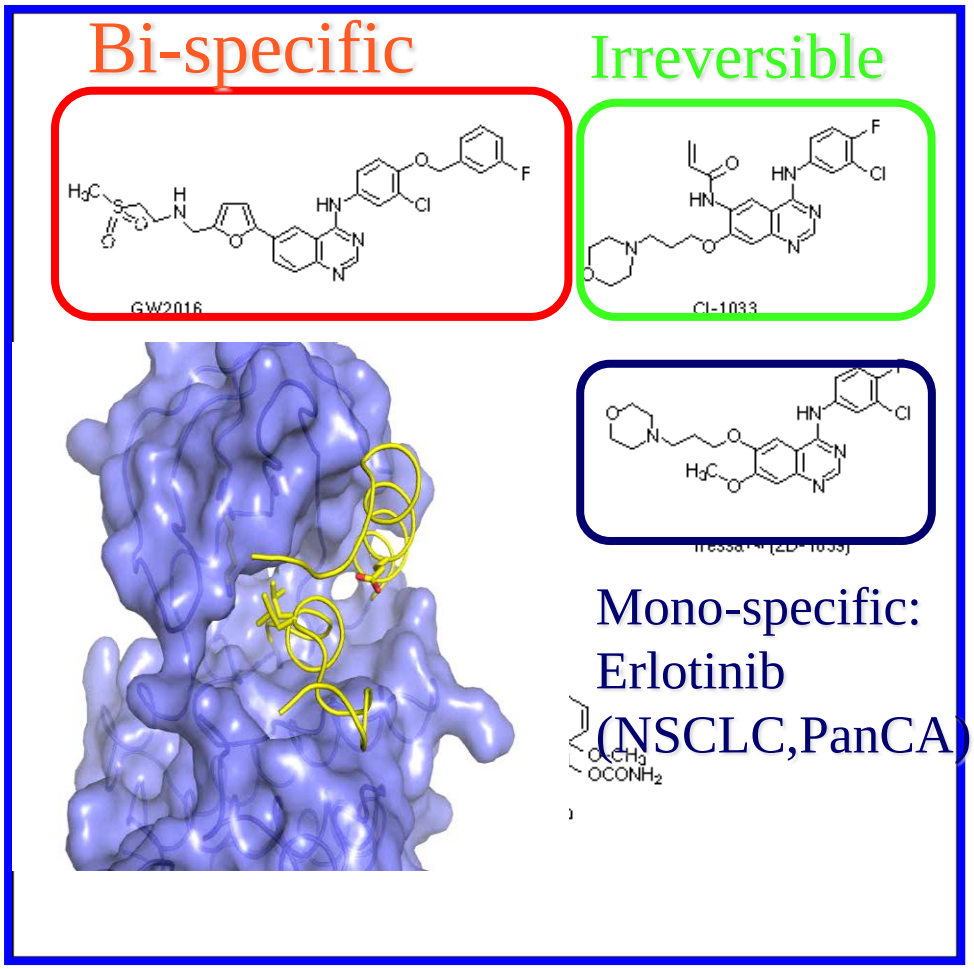
1. An essential hub
2. An uncommon perturbation

HER2+ Breast Tumors: excessive reliance (addiction) on heterodimers

I. Bernard Weinstein



Uncommon Perturbation #1: Double-hit drugs (e.g., Lapatinib)

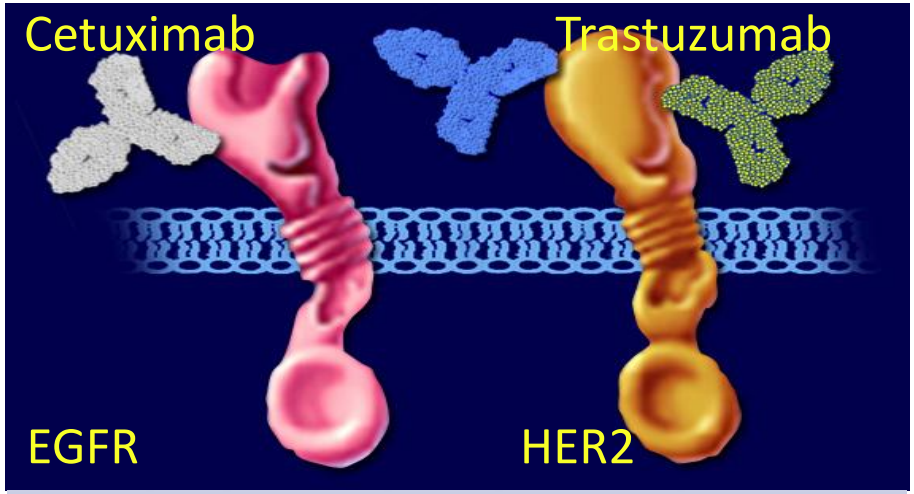


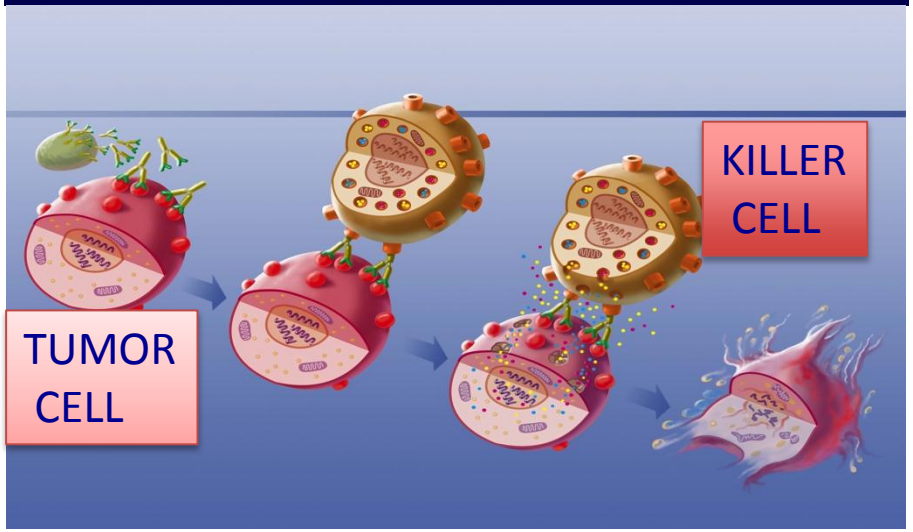
Burris HA, 3rd et al. J Clin Oncol 2005;23:5305

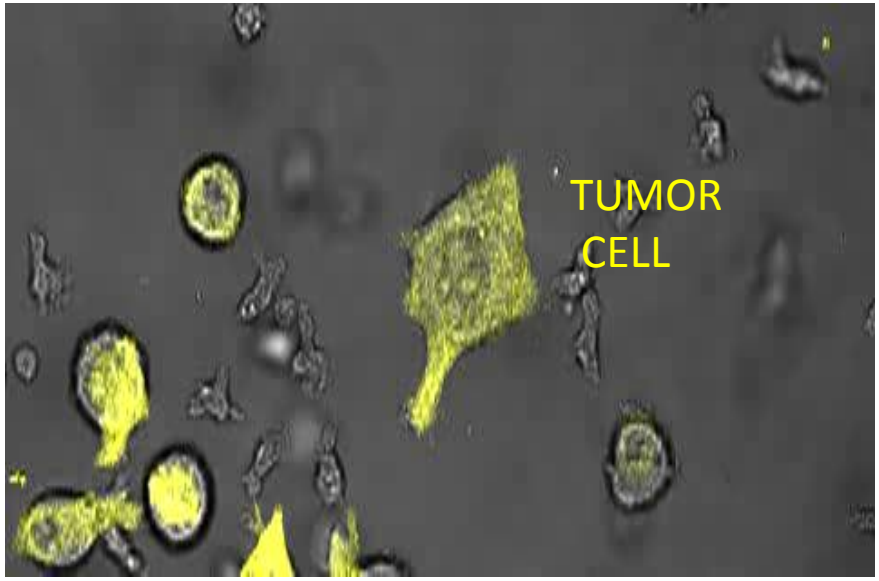
Title: Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas

Abstract: …. Heavily pretreated patients with ErbB1-expressing and/or ErbB2-overexpressing metastatic cancers were randomly assigned to one of five dose cohorts of lapatinib administered once daily……Four patients with trastuzumab-resistant metastatic breast cancer – two of whom were classified as having inflammatory breast cancer – had partial responses (PRs).

Uncommon Perturbation #2: Recruitment of the immune system by monoclonal antibodies







Courtesy of Dr. Chris Bleackley (Univ. of Alberta)

Messages: Therapeutic harnessing of biological complexity

- Networks evolved to compensate for the limited coding capacity of complex genomes

- While undergoing transformation from pathways to networks, biological systems gained robustness by means of training to withstand common, single perturbations (mono-therapies)

- Feedback loops are the guardians of the cell's steady state; perturbing the steady state would invoke resistance, unless feedback loops are restrained

- In conclusion: Blocking a cancer network translates to:

-Targeting a major (addicting or survival) hub
-Using multiple or uncommon perturbations
-Restraining the respective feedback loop

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Dr. Erez Bublil
Nir Ben-Chetrit
Hadas Cohen
Sivan Abramovitch
Jean Wakim

My Collaborators

Eytan Domany (WIS)
Michael Sela (WIS)
Giovanni Blandino (Rome)
Francesca Biagioni (Rome)
Marcella Mottolese (Rome)
Sabrina Strano (Rome)
Tzachi Pilpel (WIS)
Gideon Rechavi (Tel Hashomer)
Moshe Oren (WIS)
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Christos Sotiriou (Jules Bordet)
Noa Bossel (WIS)
Amit Zeisel (WIS)

Past fellows

Hadassa Waterman
Menachem Katz
Gil Levkowitz
Daniel Harari
Leah Klaper
Gal Gur-Shachar
Mina Marmor
Ariel Rinon
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Judith Gan
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