

# HER2 and EGFR: at last, cancer therapy meets systems biology

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**BioNetVisA**

**Strasbourg, September 7, 2014**

## Forthcoming Events

Conference Series | Heidelberg, Germany | 8 – 11 November 2014  
**From functional genomics to systems biology**

Practical Course | Heidelberg, Germany | 8 – 20 June 2015  
**Synthetic biology in action**

[events.embo.org](http://events.embo.org)

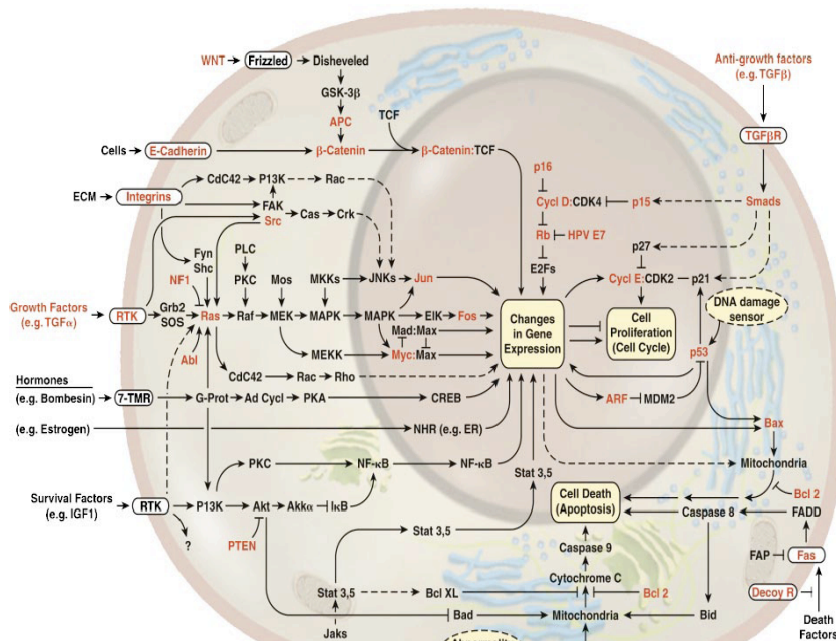
# EMBO Courses and Workshops: approximately 80 events annually

|   |  |   |   |
|---|--|---|---|
| <p>UK-Bristol   6–11 July 2014   P. Verkade<br/><b>Correlative light electron microscopy</b></p> <p>IT-Sesto Fiorentino   13–19 July 2014   P. Turano<br/><b>Solution and solid-state NMR of paramagnetic molecules</b></p> <p>FR-Paris   20–27 July 2014   M. Nilges<br/><b>Biomolecular simulation</b></p> <p>UK-Hinxton   28 July–3 August 2014   L. Emery<br/><b>Genotype to phenotype mapping of complex traits</b></p> <p>DE-Joachimsthal   10–15 August 2014   C. Griesinger<br/><b>Multidimensional NMR in structural biology</b></p> <p>DE-Dresden   18–29 August 2014   P. Tomancak<br/><b>Light sheet microscopy</b></p> <p>DE-Heidelberg   31 August–9 September 2014   J. Briggs<br/><b>Cryo-electron microscopy and 3D image processing</b></p> <p>IT-Alghero   6–13 September 2014   K. Hofmann<br/><b>Ubiquitin and related modifiers</b></p> <p>DE-Hamburg   8–16 September 2014   R. Meijers<br/><b>Protein expression, purification, and characterization (PEPC9)</b></p> <p>DE-Heidelberg   8–20 September 2014   F. Nédélec<br/><b>Microscopy, modelling and biophysical methods</b></p> <p>DE-Heidelberg   18–25 September 2014   J. Dreyer<br/><b>Single-cell gene expression analysis</b></p> <p>ES-Barcelona   28 September–3 October 2014   E. Sabidó<br/><b>Targeted proteomics: Experimental design and data analysis</b></p> <p>ZA-Cape Town   29 September–3 October 2014   N. Mulder<br/><b>Computational analysis of protein–protein interactions: From sequences to networks</b></p> <p>DE-Würzburg   12–18 October 2014   J. Vogel<br/><b>Non-coding RNA in infection</b></p> <p>UK-Hinxton   20–25 October 2014   G. Rustici<br/><b>Analysis of high-throughput sequencing data</b></p> <p>DE-Heidelberg   20–26 October 2014   R. Pepperkok<br/><b>High-throughput microscopy for systems biology</b></p> <p>DE-Hamburg   27 October–3 November 2014   D. Svergun<br/><b>Solution scattering from biological macromolecules</b></p> | <h2>Workshops</h2> <p>UK-Chelmsford   7 July 2014   S. Bustin<br/><b>Simultaneous profiling of RNA and protein using proximity ligation assay</b></p> <p>FR-Bischoffsheim   24–29 August 2014   M. Heinlein<br/><b>Intercellular communication in plant development and disease</b></p> <p>IL-Ma'ale Hachamisha   7–12 September 2014   E. Cohen<br/><b>The regulation of aging and proteostasis</b></p> <p>FR-Hyères   9–12 September 2014   K. Morelli<br/><b>Advances in protein–protein interaction analysis and modulation</b></p> <p>CL-Puerto Natales   9–14 September 2014   M. Marzolo<br/><b>Current advances in membrane trafficking: Implications for polarity and diseases</b></p> <p>DE-Heidelberg   25–26 September 2014   J. Dreyer<br/><b>Unravelling biological secrets by single-cell expression profiling</b></p> <p>TR-Istanbul   26–28 September 2014   A. Celik<br/><b>Decoding neural circuit structure and function</b></p> <p>ES-Sigües   1–4 October 2014   E. Martí<br/><b>Development and regeneration of the spinal cord</b></p> <p>IT-Catanzaro   3–6 October 2014   G. Morrone<br/><b>Cancer stem cells 20 years later: Achievements, controversies, emerging concepts and technologies</b></p> <p>TR-Istanbul   6–8 October 2014   A. Marcello<br/><b>Human RNA viruses</b></p> <p>AU-Broome   6–9 October 2014   E. Vincan<br/><b>Wnt signalling: Stem cells, development and disease</b></p> <p>ES-Bilbao   7–9 October 2014   A. Carracedo<br/><b>Translational advances in cancer cell signalling and metabolism</b></p> <p>FR-Cargèse (Corsica)   7–10 October 2014   A. Morillon<br/><b>Non-coding RNAs in genome expression, maintenance and stability</b></p> <p>SG-Singapore   12–15 October 2014   C. Stewart<br/><b>Cell plasticity and nuclear dynamics</b></p> <p>CN-Hong Kong   16–18 October 2014   E. So<br/><b>Cancer stem cells and epigenetics</b></p> | <p>Brain development and disorders</p> <p>ES-Sant Feliu de Guixòls   6–10 September 2014   E. Saló<br/><b>The molecular and cellular basis of regeneration and tissue repair</b></p> <p>UK-Cambridge   21–24 September 2014   O. Leyser<br/><b>Interdisciplinary plant development</b></p> <p>FR-Paris   29 September–3 October 2014   G. Eberl<br/><b>Innate lymphoid cells</b></p> <p>PT-Lisbon   30 September–3 October 2014   M. Bettencourt-Dias<br/><b>Centrosomes and spindle pole bodies</b></p> <p>DE-Heidelberg   9–12 October 2014   C. Nerlov<br/><b>Stem cells in cancer and regenerative medicine</b></p> <p>DE-Heidelberg   12–15 October 2014   L. Steinmetz<br/><b>Experimental approaches to evolution and ecology using yeast</b></p> <p>AR-Buenos Aires   19–24 October 2014   F. Pelisch<br/><b>Ubiquitin and ubiquitin-like proteins: At the crossroads from chromatin to protein</b></p> <p>ES-Girona   26–31 October 2014   M. Schuldiner<br/><b>The Endoplasmic Reticulum (ER) as a hub for organelle communication</b></p> <p>DE-Heidelberg   6–7 November 2014   H. Stefánsson<br/><b>15th EMBL   EMBO Science and Society Conference</b></p> <p><b>Foods are us! On eating and becoming</b></p> <p>DE-Heidelberg   8–11 November 2014   E. Furlong<br/><b>From functional genomics to systems biology</b></p> <p>HR-Dubrovnik   21–25 March 2015   P. Rehling<br/><b>Mechanisms and regulation of protein translocation</b></p> <p>DE-Heidelberg   6–10 May 2015   S.A. Teichmann<br/><b>Chromatin and epigenetics</b></p> <p>UK-Egham   27–31 July 2015   J.E. Diffey<br/><b>DNA replication, chromosome segregation and cell division</b></p> <h2>Symposia</h2> | <p>Frontiers in stem cells and cancer</p> <p>DE-Heidelberg   15–18 April 2015   L. Pelkmans<br/><b>Cellular heterogeneity: Role of variability and noise in biological decision-making</b></p> <p>DE-Heidelberg   14–17 June 2015   C. Haass<br/><b>Mechanisms of neurodegeneration</b></p> <p>DE-Heidelberg   21–23 June 2015   K.R. Patel<br/><b>Enabling technologies for eukaryotic synthetic biology</b></p> <h2>Lecture Courses</h2> <p>EMBO Global Exchange Lecture Courses</p> <p>TN-Tunis   15–25 September 2014   F. Guerfall<br/><b>High-throughput NGS applied to infectious diseases</b></p> <p>BR-Cuiabá/Poconé   27 October–7 November 2014   L. Cameron<br/><b>Biochemistry and molecular biology bench to bedside approaches</b></p> <h2>Funding Available</h2> <p>Apply now for 2016 funding</p> <p>Courses, workshops, conferences and symposia<br/>1 March and 1 August 2015</p> <p>Keynote lectures given by EMBO members at</p> |
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# Biological and engineered systems share structural and functional features

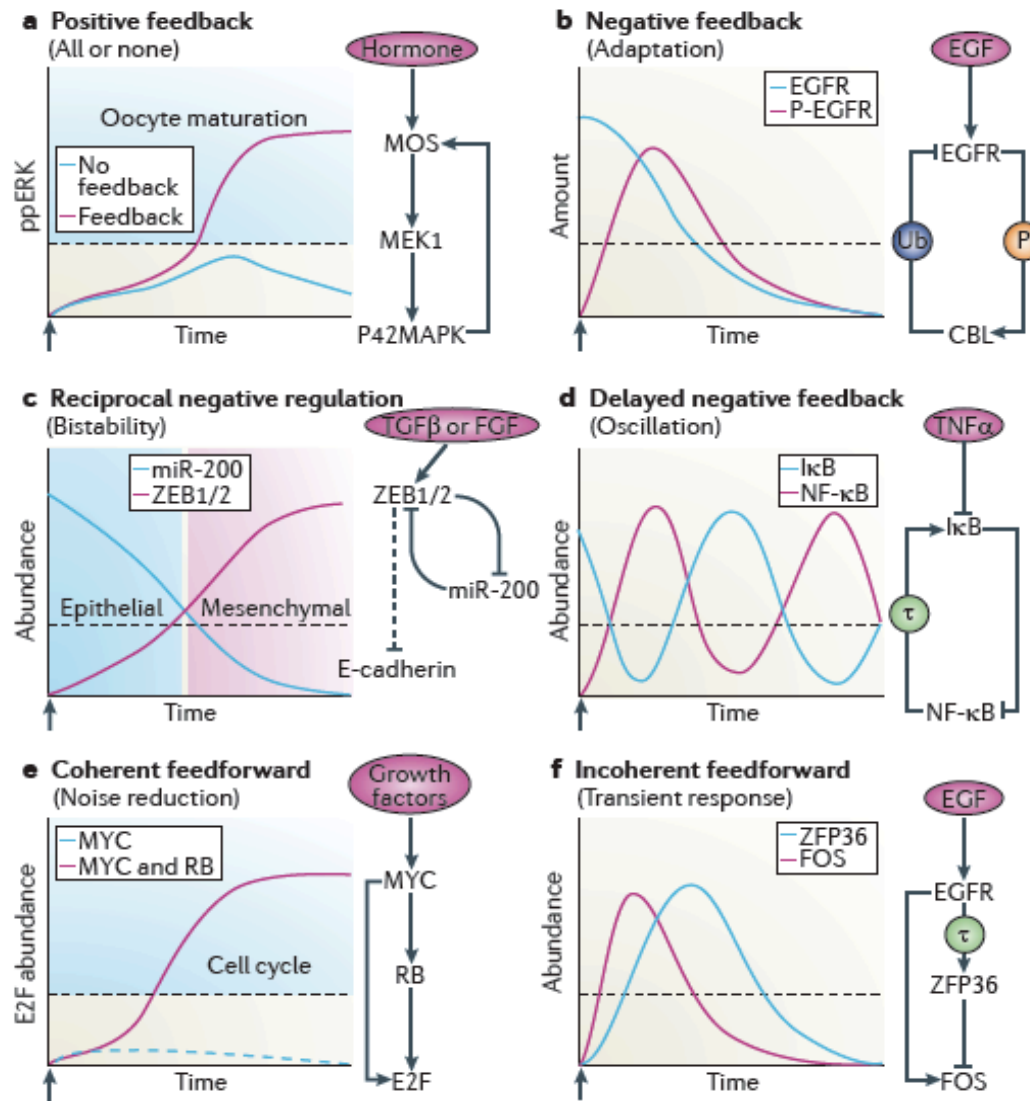
- Component multiplicity
- Rich connectivity
- Fail-safe functioning



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



# Feedback Loops Carve Network's



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

# Computational tasks of feedback regulatory loops

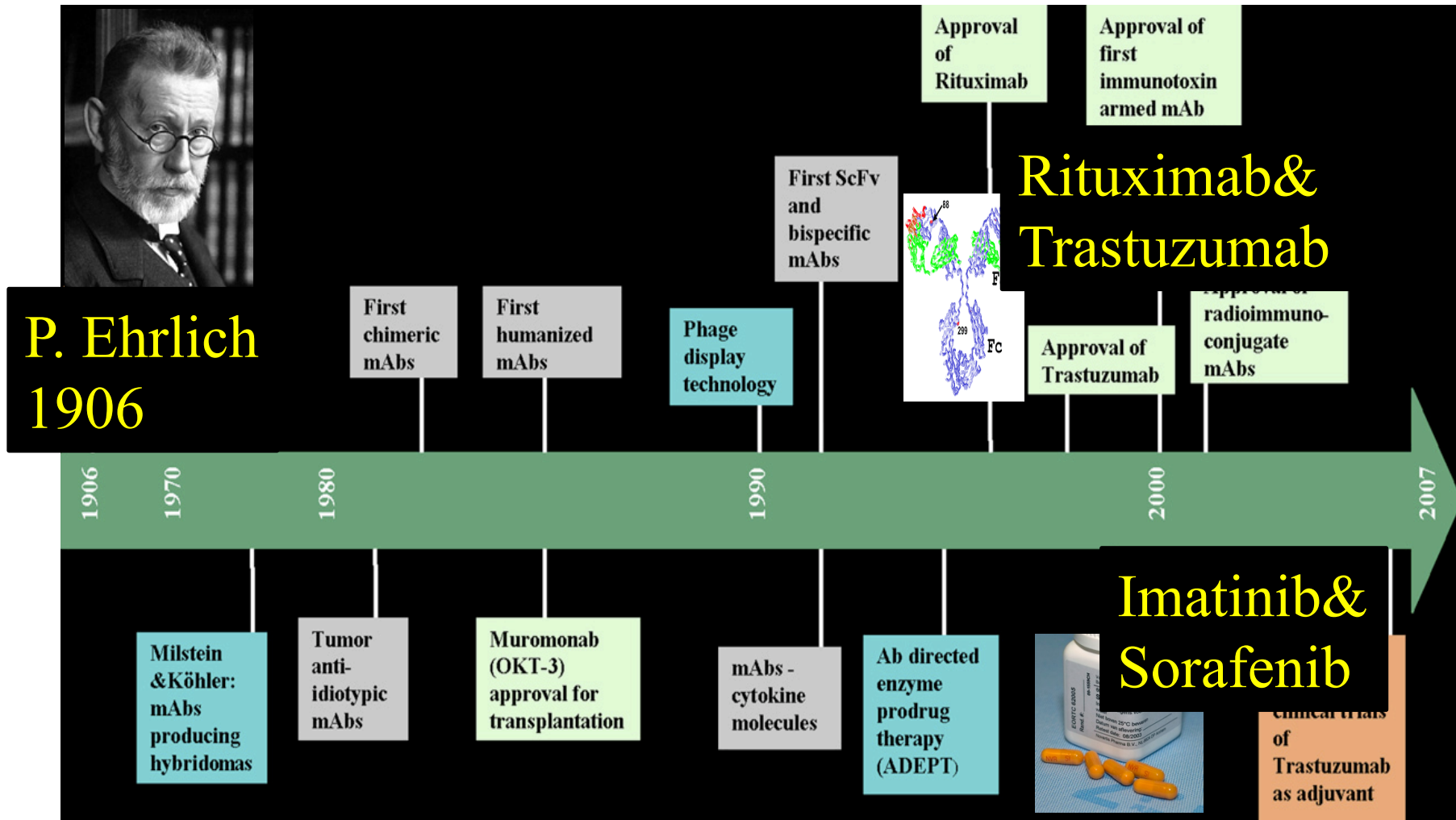
- **Fold change detection:** The output of a network depends on the relative change in input signal, rather than on the absolute levels.
- Reference: The incoherent feedforward loop can provide fold-change detection in gene regulation. Goentoro L, Shoval O

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

distinct phases of signalling. Jones SM and Kaziauskas 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 Era of Genome-Based Targeted Cancer Therapy



Ben-Kasus et al., (2007) Molecular Oncology



# Growth Factors Control All Phases of Tumor Progression

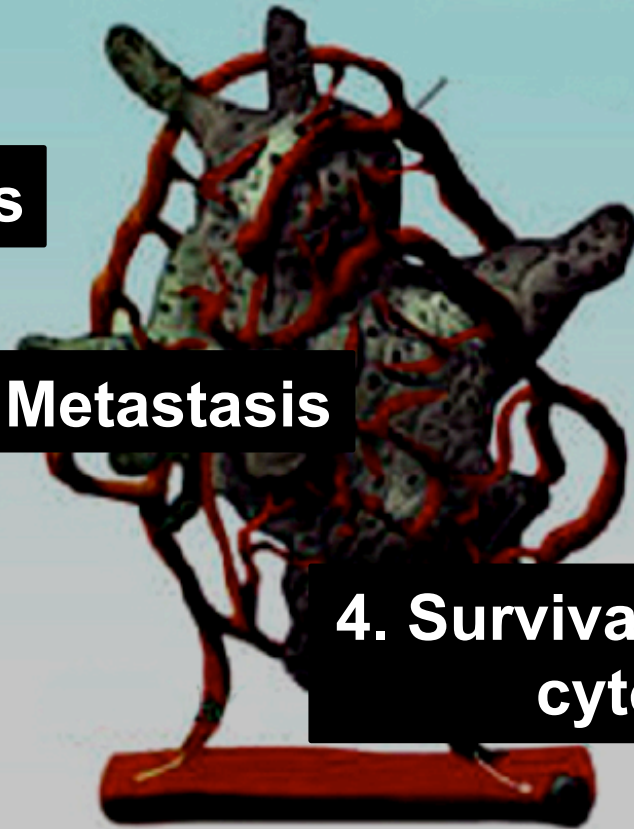
**1. Clonal expansion**



**2. Angiogenesis**



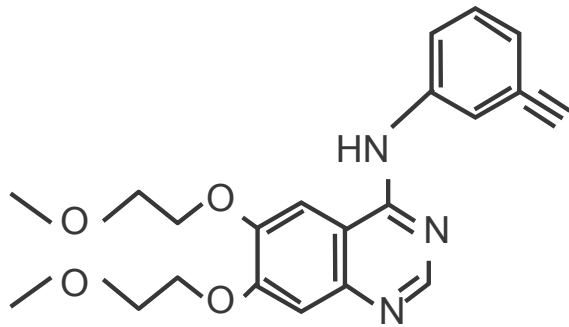
**3. Metastasis**



**4. Survival under cytotoxics**

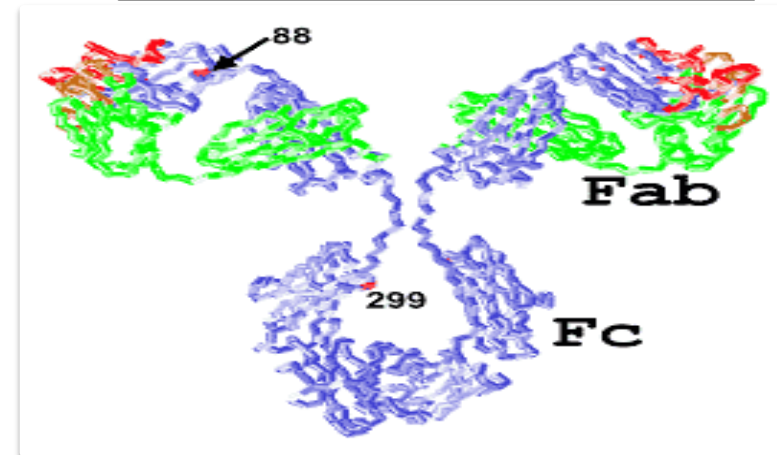
# Two Major Therapeutic Strategies Targeting EGFR/HER2 Signaling

## Kinase inhibitors



**Gefitinib (lung CA)**  
**Erlotinib (lung&pancreatic CA)**  
**Afatinib (lung CA)**  
**Lapatinib (breast CA)**

## Monoclonal antibodies

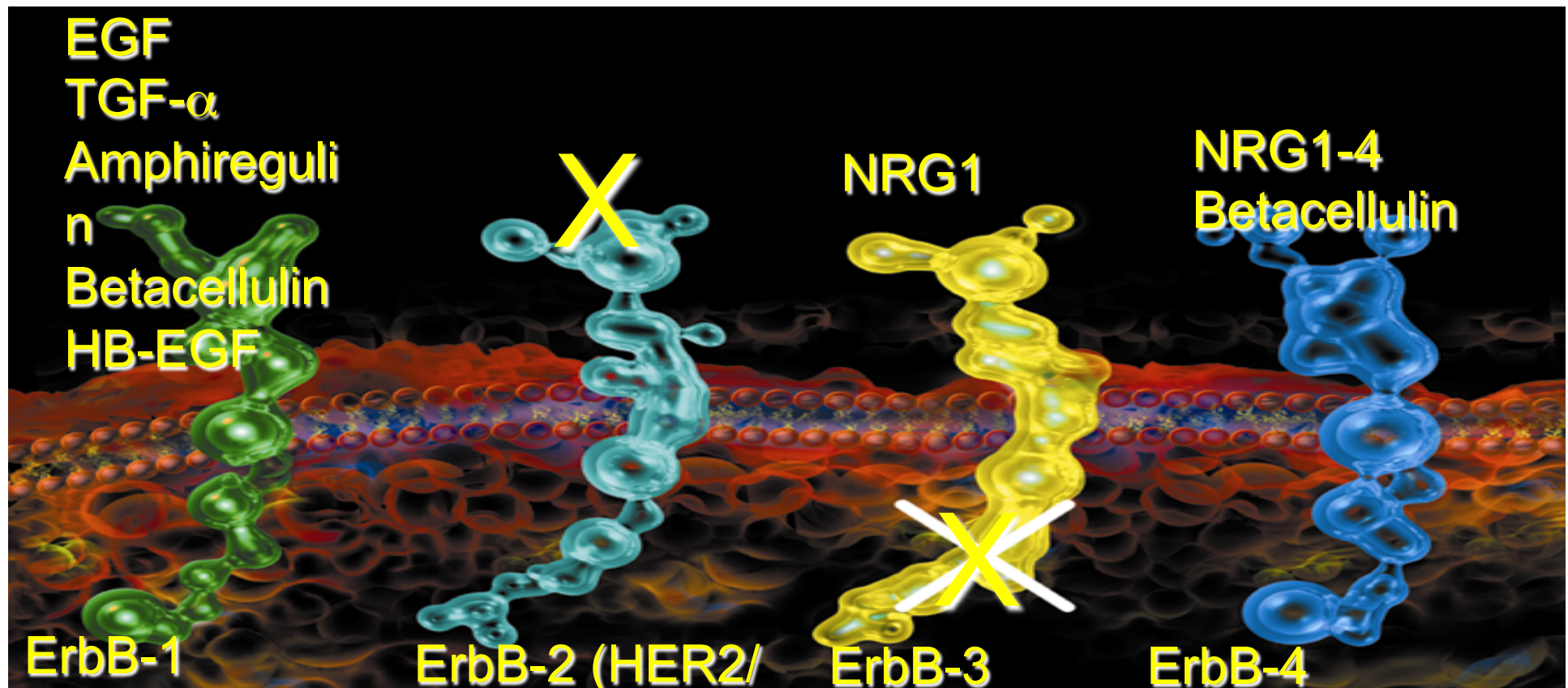


**Trastuzumab (breast&gastric CA)**  
**Cetuximab (colorectal&head CA)**  
**Panitumumab (colorectal CA)**  
**Pertuzumab (breast CA)**

# The EGFR/HER2 Family and the Double Enigma

***HER2, a strongly oncogenic kin of EGFR, binds no known ligand***

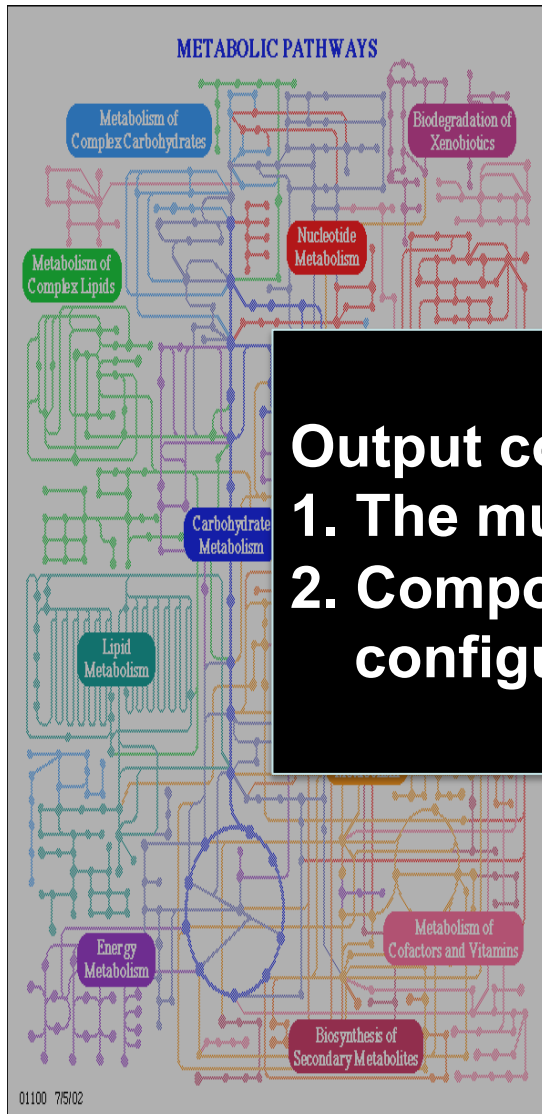
***ERBB3 binds several ligands, but its kinase is inactive***



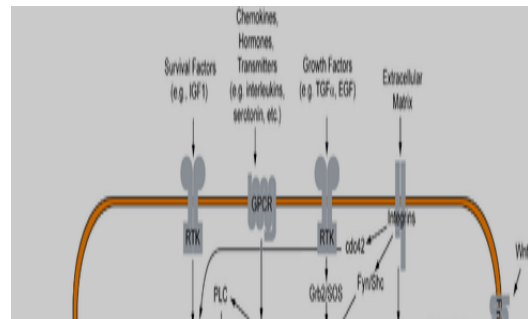


# Systems biology of signal transduction: Integration of networks

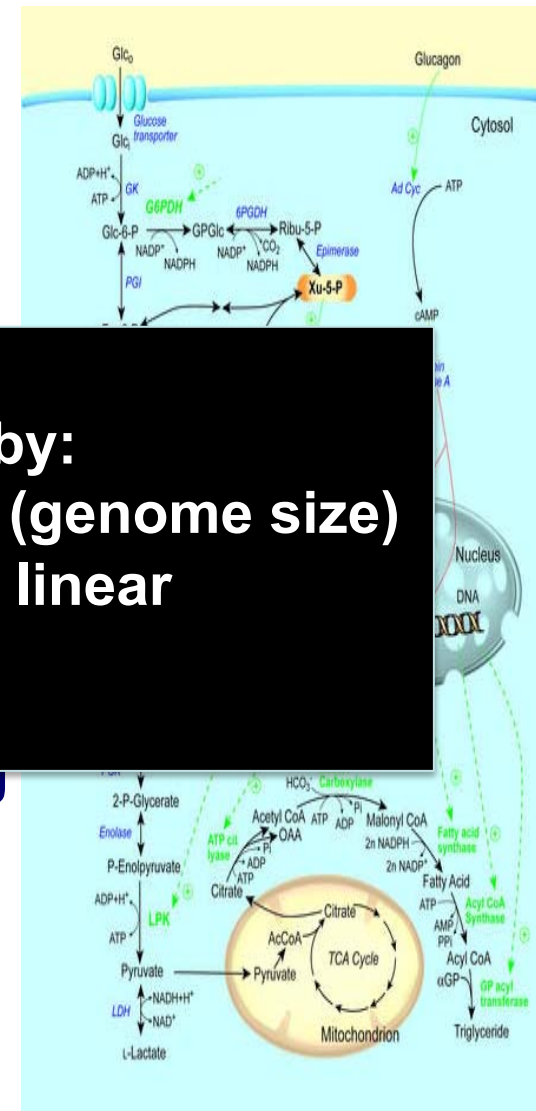
## Metabolism



## Information/signaling

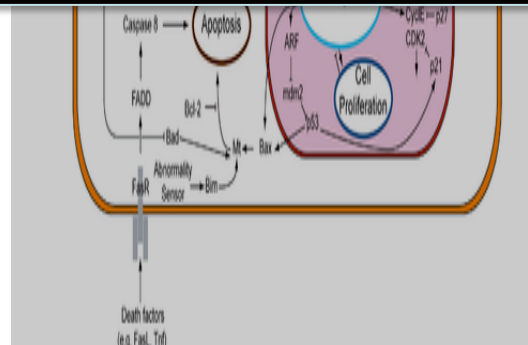


## Energy



**Output complexity is determined by:**

- 1. The multiplicity of components (genome size)**
- 2. Component wiring (network vs. linear configuration)**



# Networks evolved to compensate for the limited size of genomes

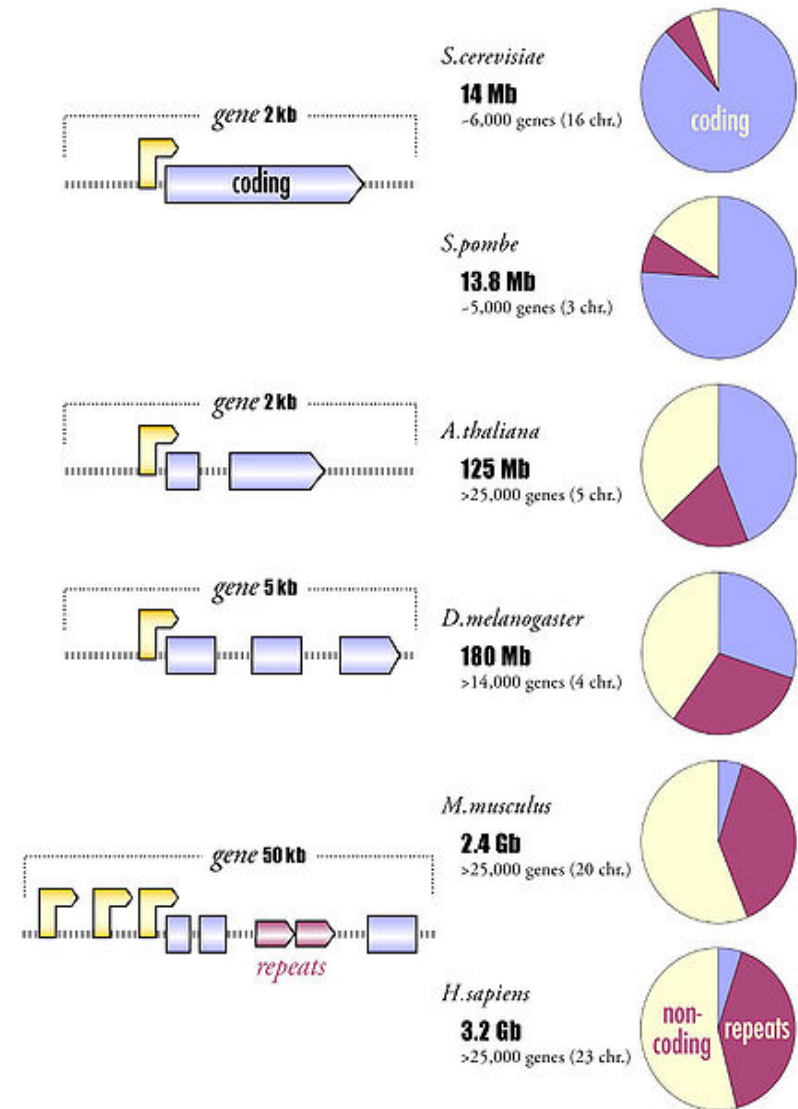
- Genomes expand by duplications

- Trade-offs of Mega-genomes:

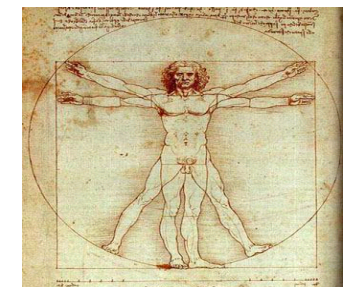
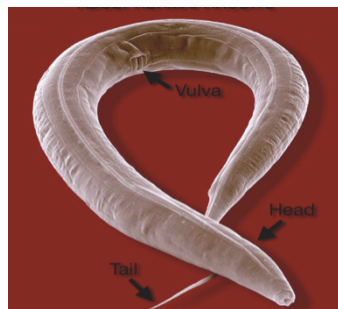
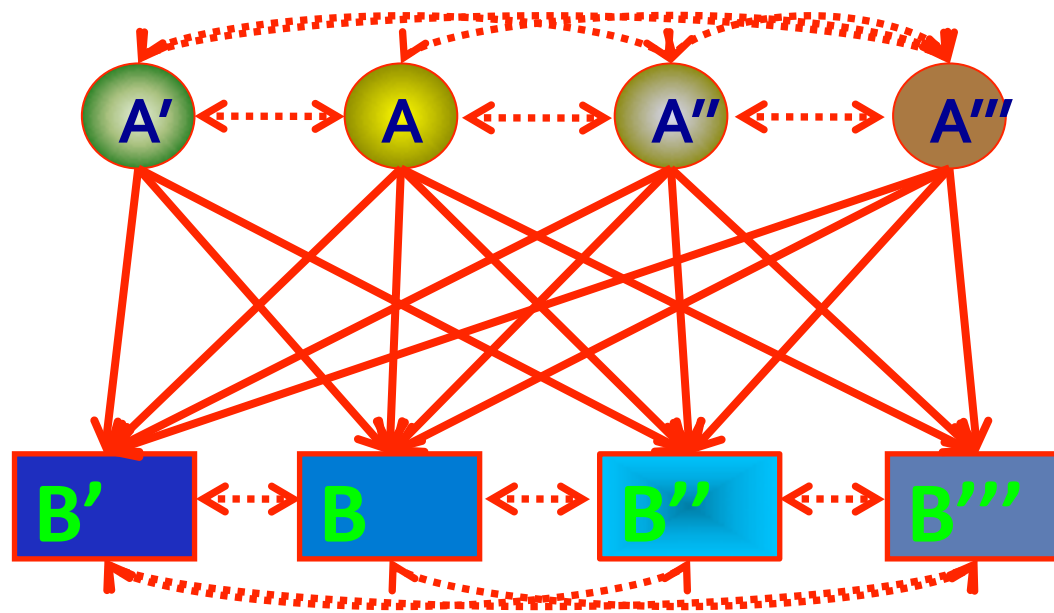
- Logistics of Replication
- Challenges for DNA repair
- Excessive regulatory sequences

- The alternatives:

- Simple proteins-->multi-domain
- Splice variants and PTMs
- Pathways-->networks

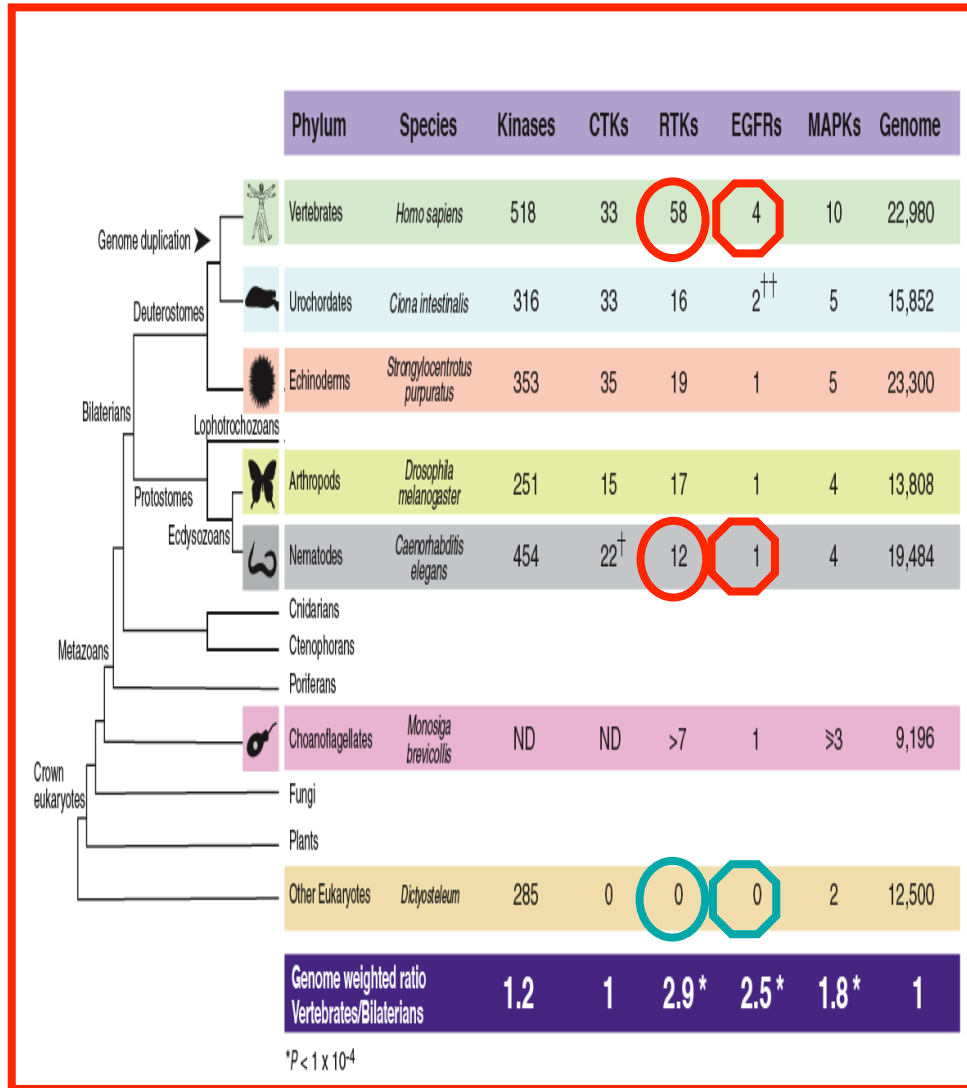


# The Origin of Biological Complexity: Whole Genome and Chromosome Duplications





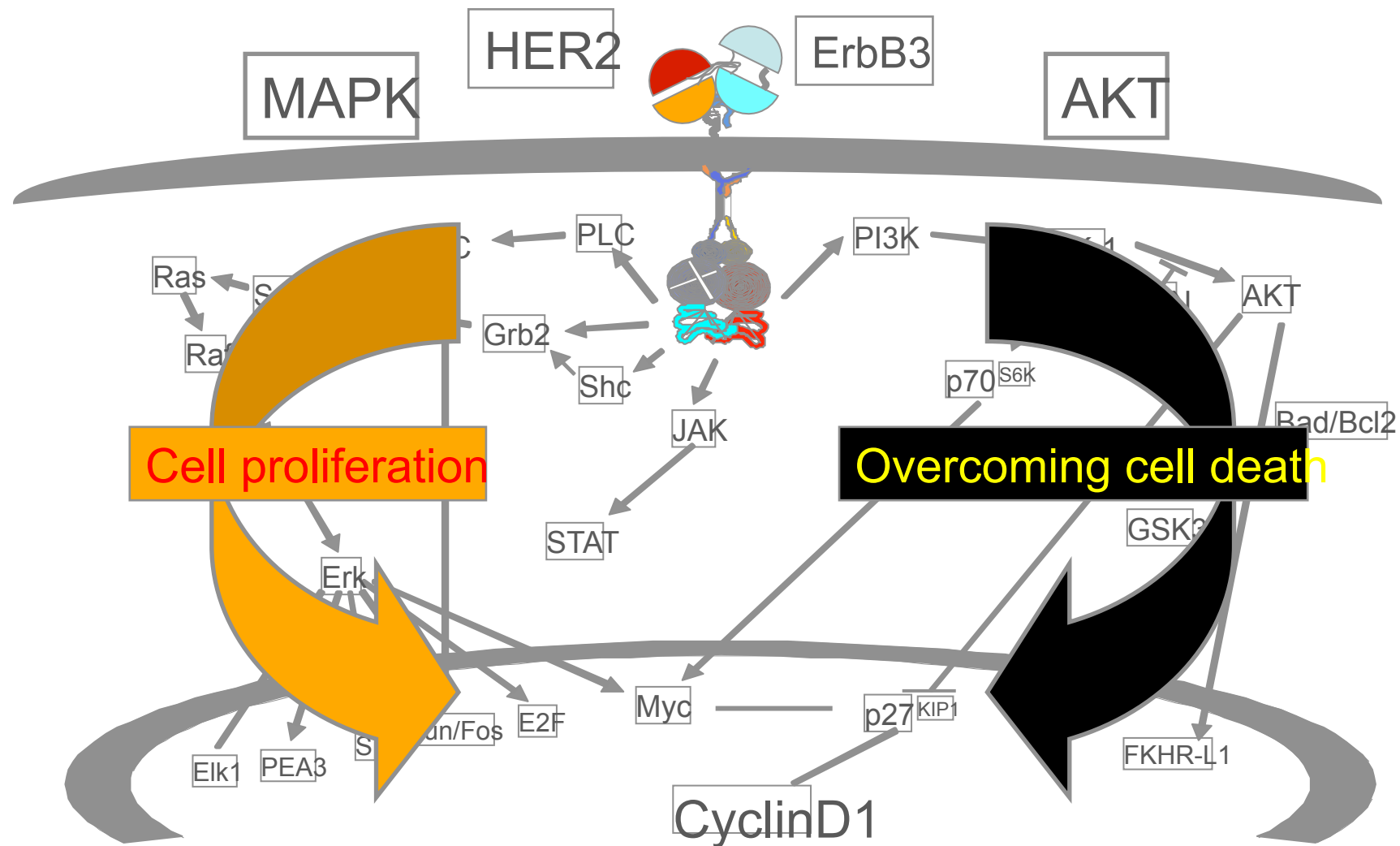
# The Evolution of RTKs: Roles for Sub-Functionalization



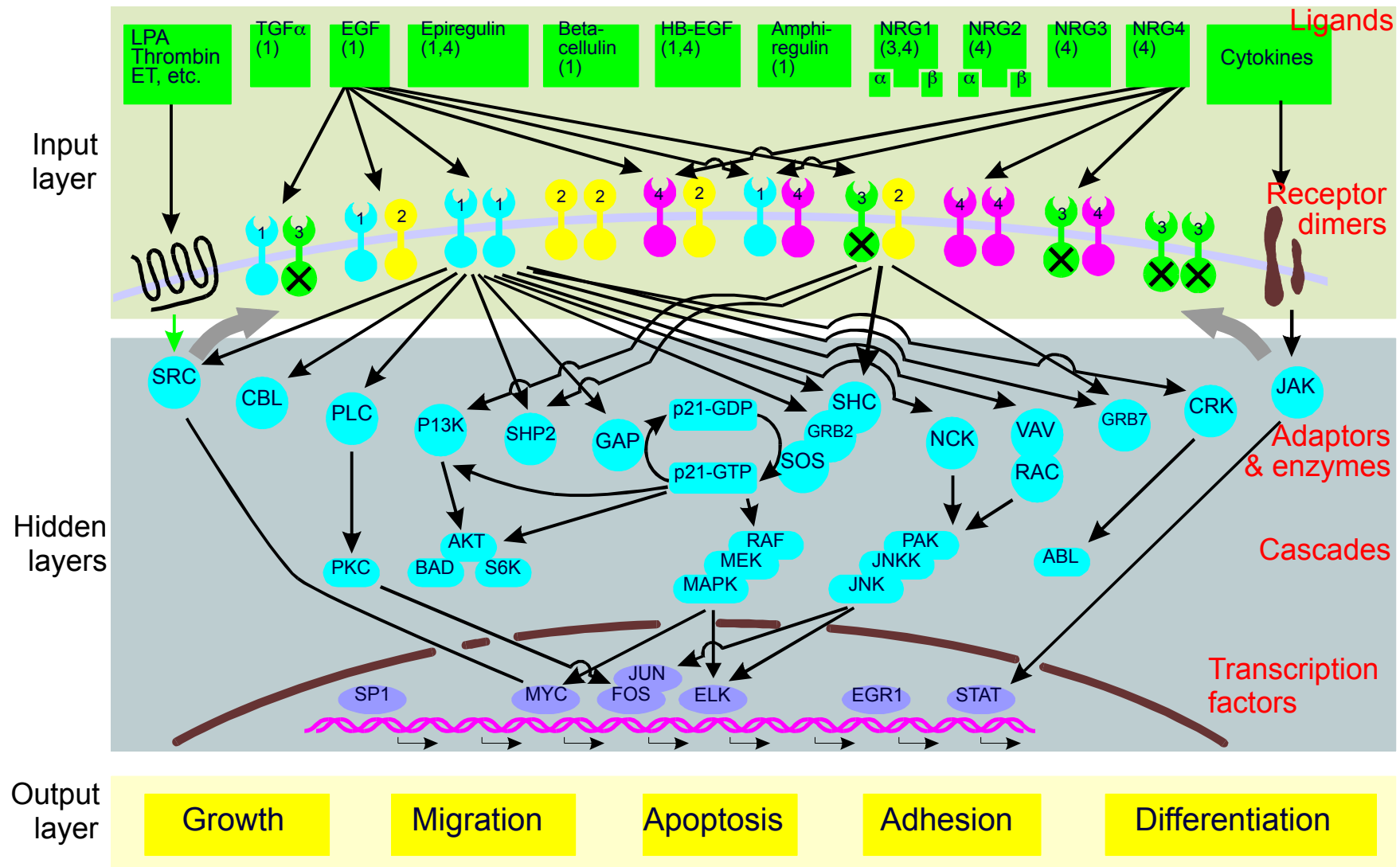
- There have been two genome-wide duplications and numerous smaller scale events

- Most duplicated genes are lost; sub-functionalization retains duplicated genes by enabling complementary functions

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

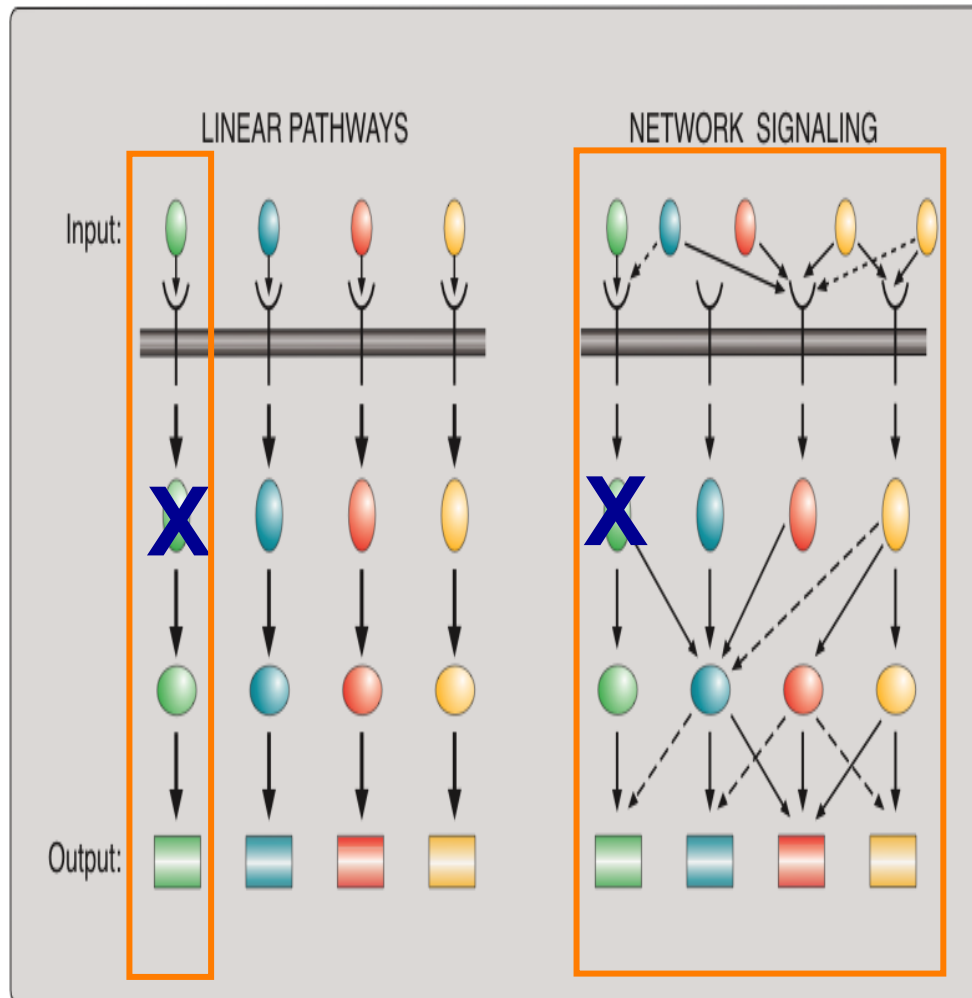


# The EGFR/HER2 Signaling Network



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



*Robustness is a property that enables a system to function despite external (environmental) and internal (genetic) perturbations.*

*Evolvability is the capacity of a system to generate stable variance.*



# Mechanisms that ensure robustness of engineered (and biological) systems

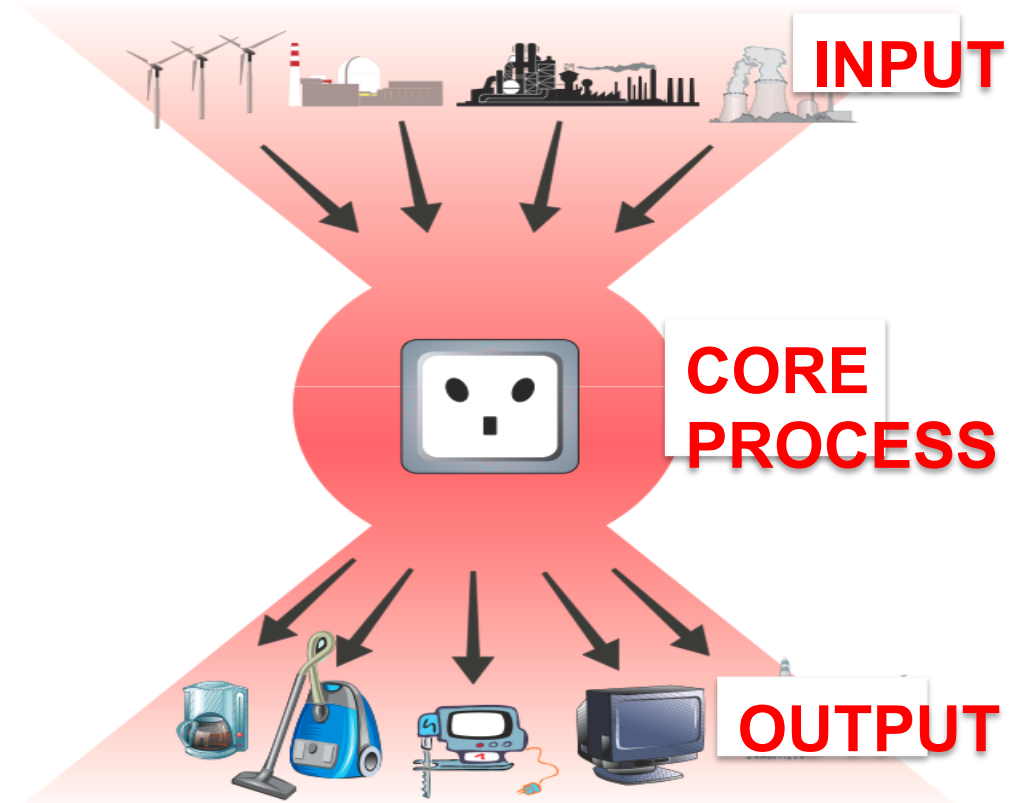
**Modularity:** Organization in units that enable damage containment

**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)**



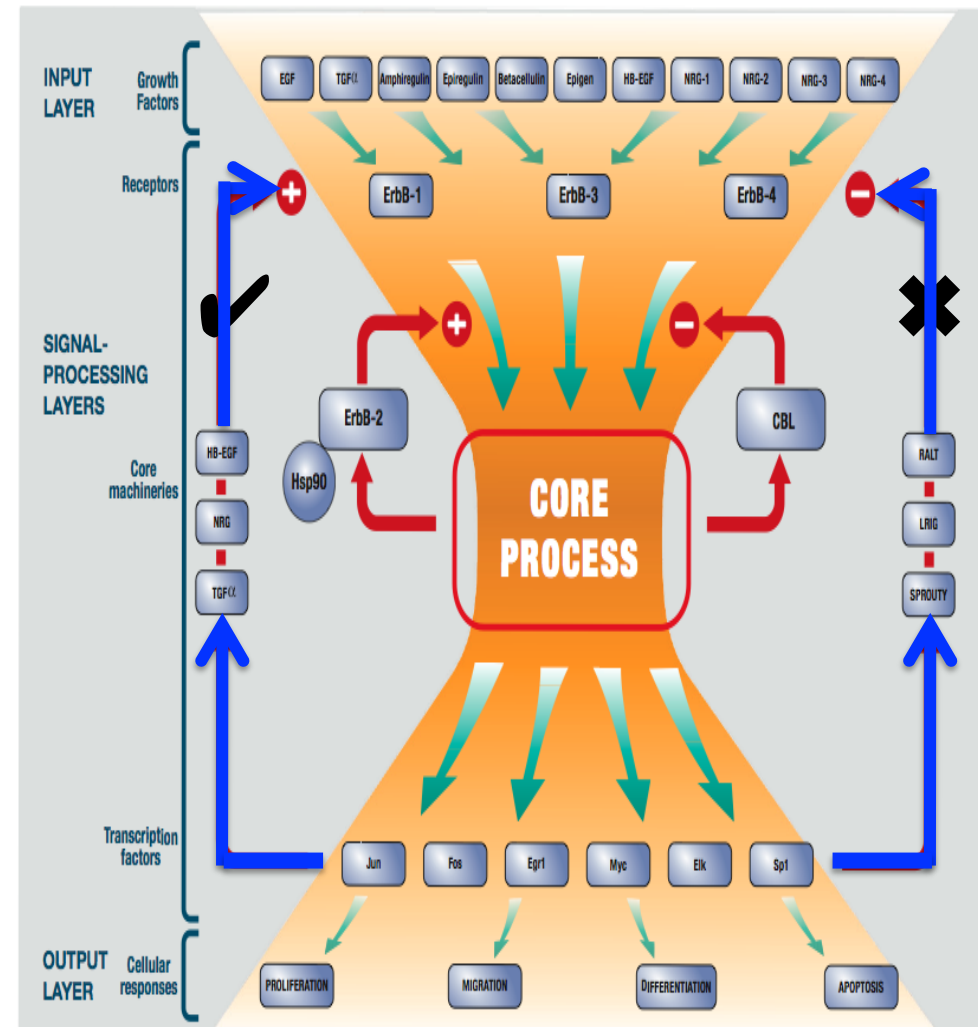
# Control loops ensure robustness

*Modularity: Organization in units that enable damage containment*

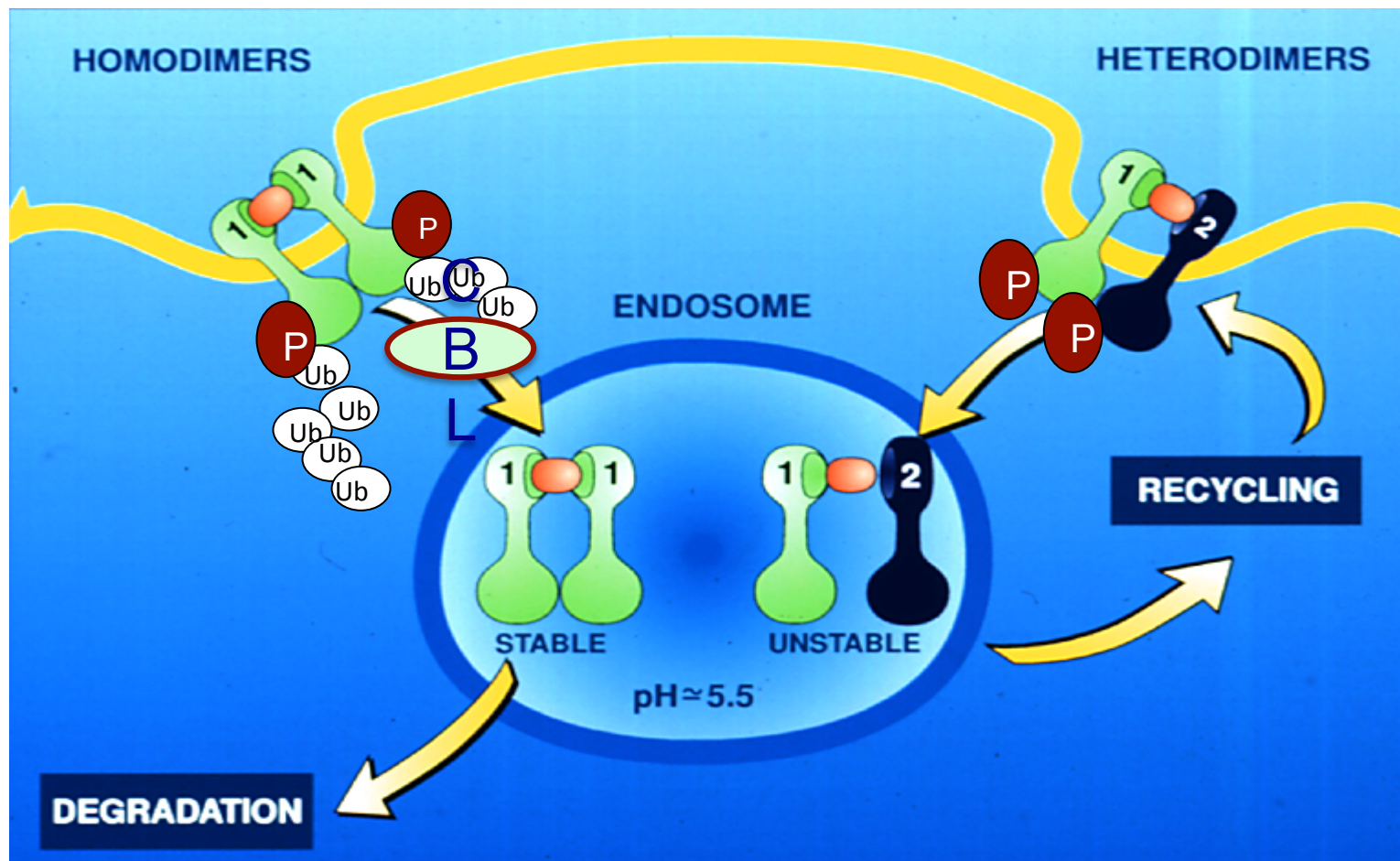
*Redundancy and diversity: input and output diversity and multiple pathways to achieve a specific function*

*Positive and negative feedback control loops*

*Plasticity (short-term) and adaptability (long-term)*

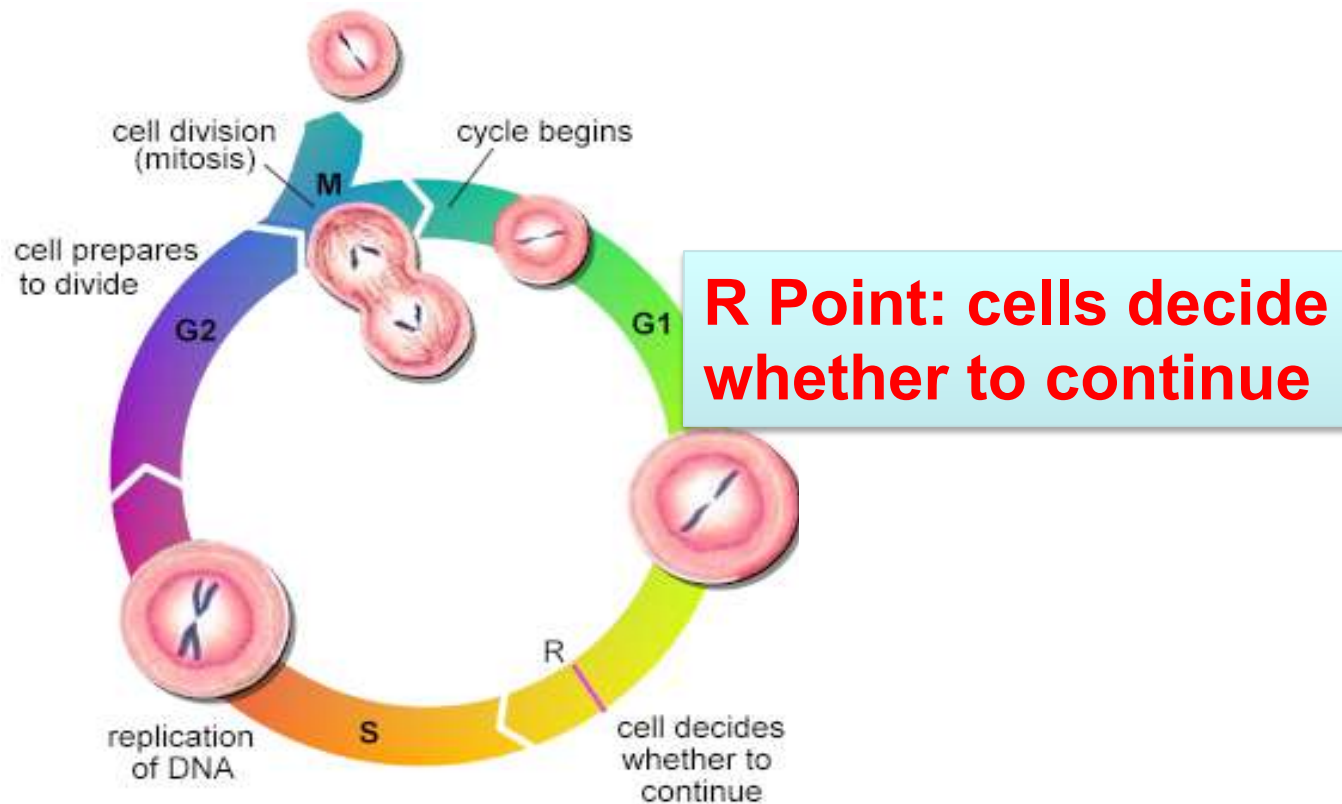


# HER2 Recycles EGFR



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

# EGF-induced proliferation of mammary cells (HMECs)



**Yaara Zwang**

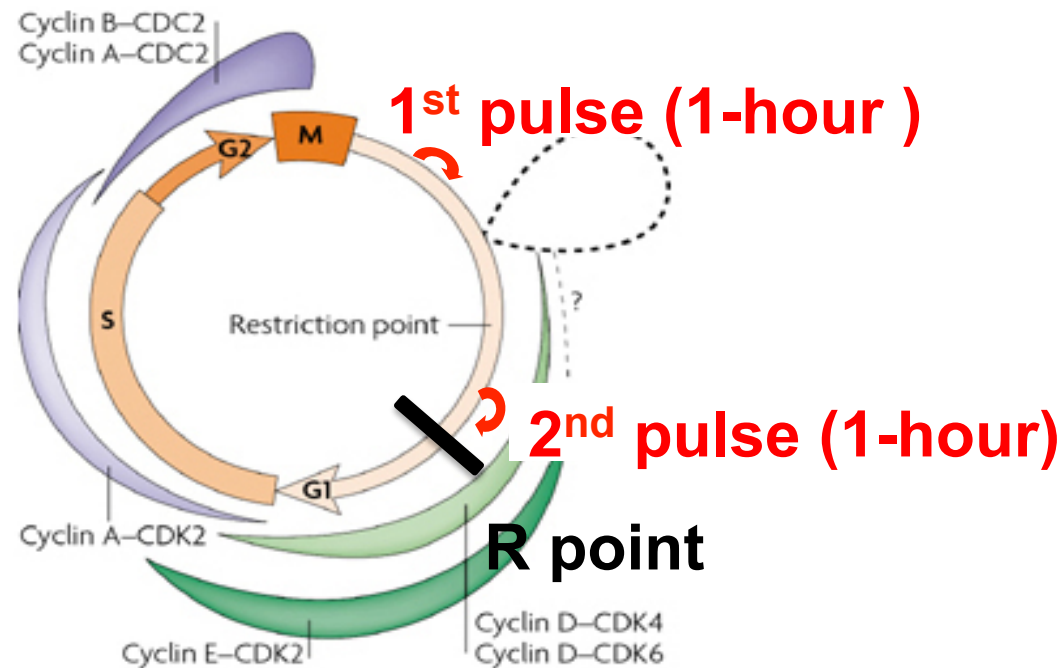
R crossing requires continuous (>6 hours) presence of growth factors



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

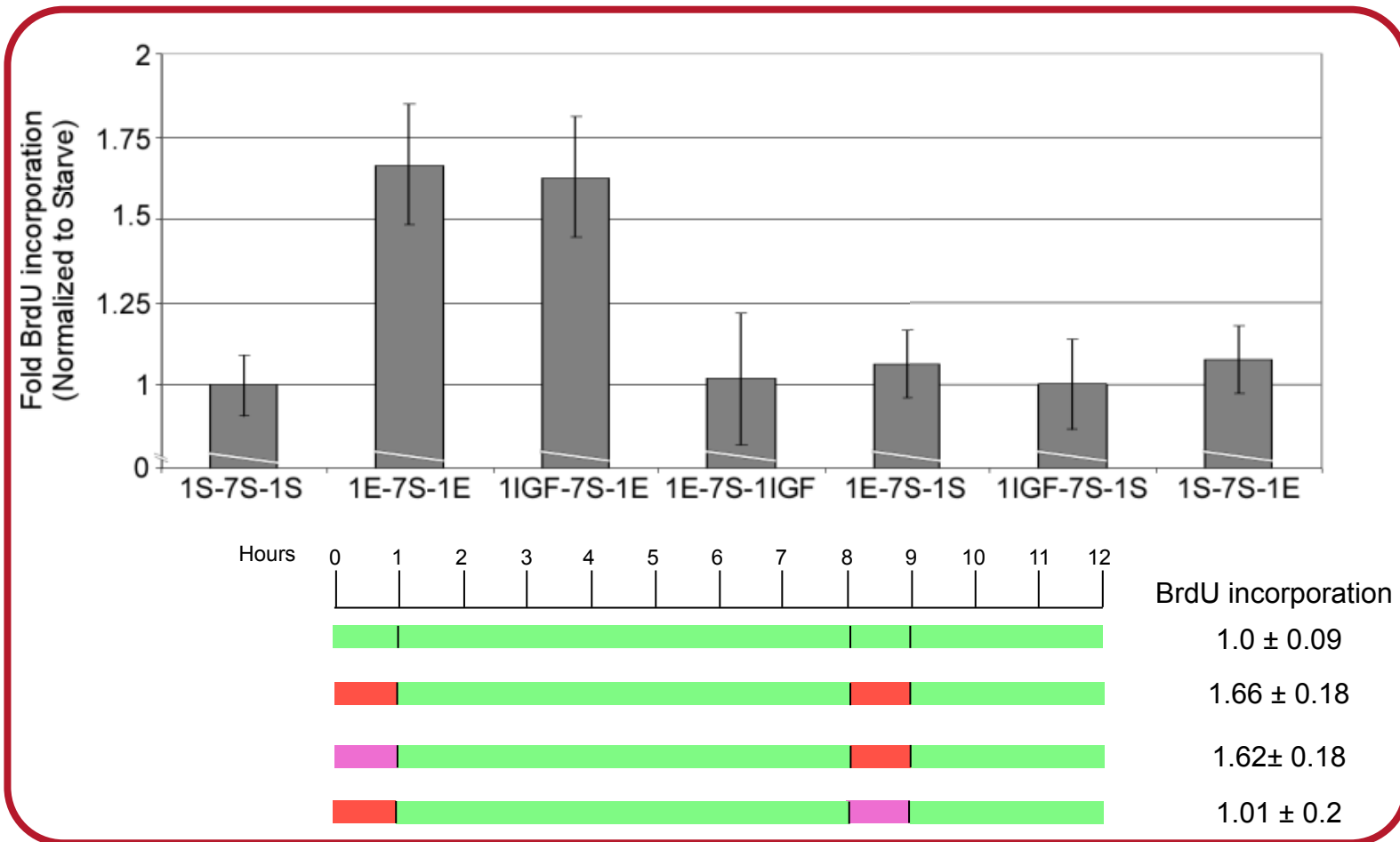
Steven M. Jones<sup>1†</sup> and Andrew Karsenti<sup>1†\*</sup>

NATURE CELL BIOLOGY | VOL 3 | FEBRUARY 2001



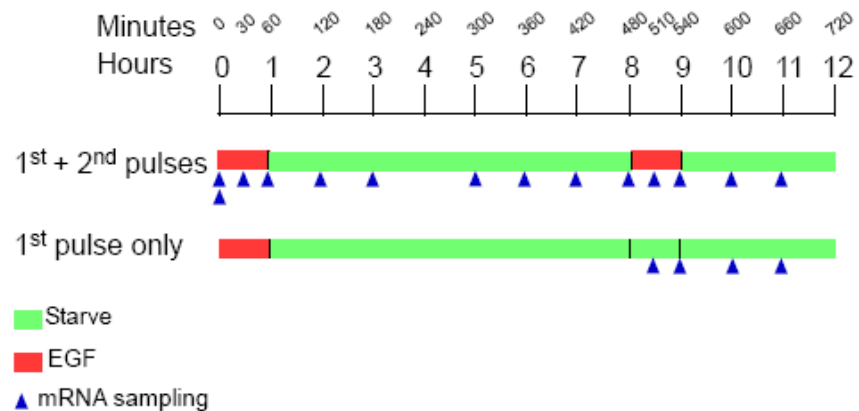
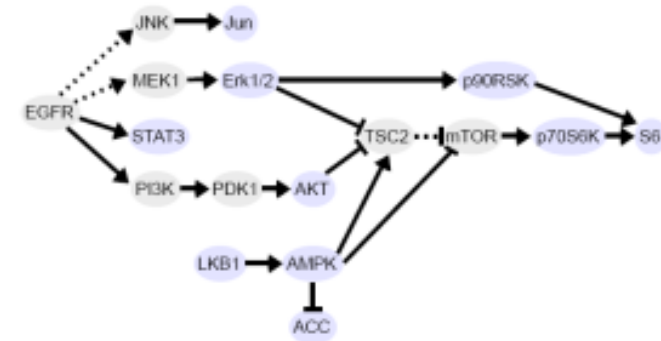
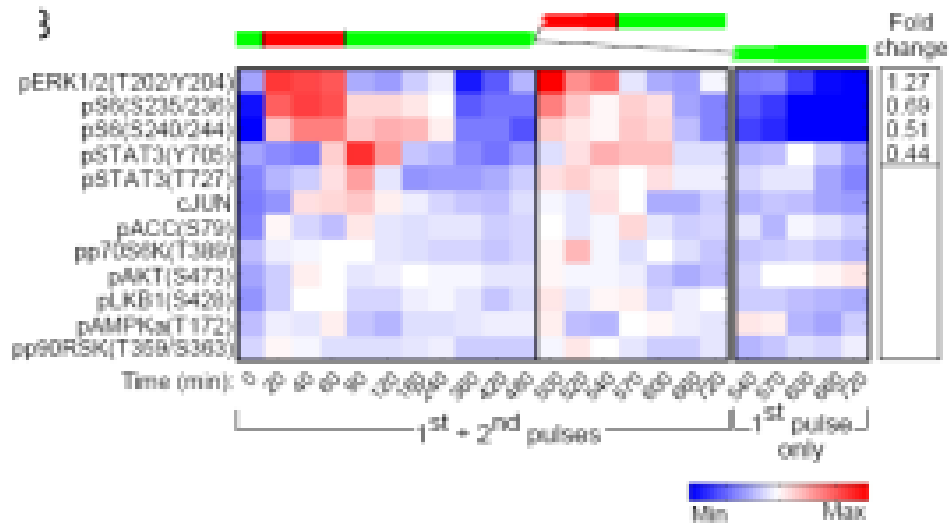
R-crossing is enabled by two short pulses of growth factors

# IGF1 may replace EGF in the 1<sup>st</sup>, not the 2<sup>nd</sup> pulse

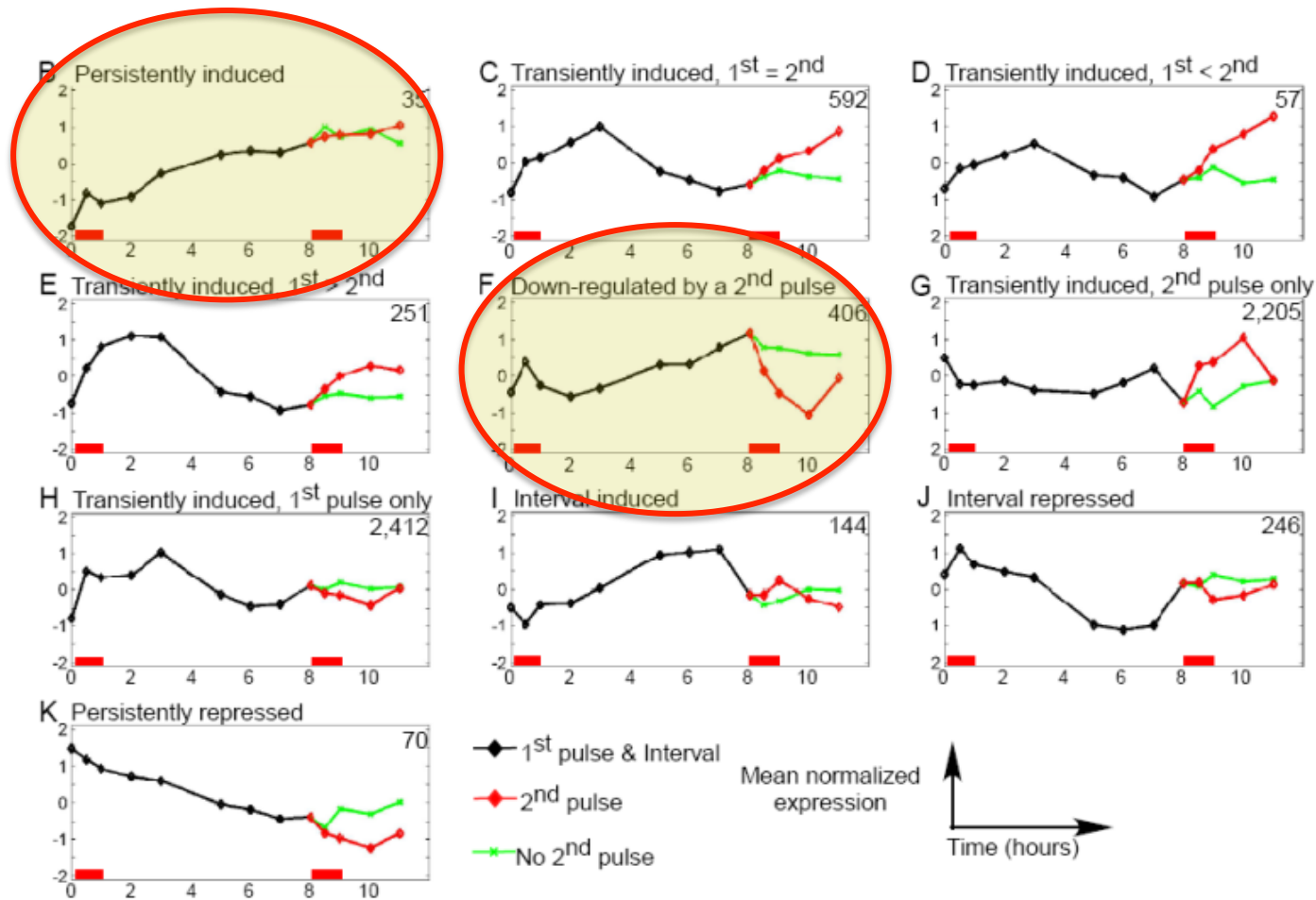


**IGF-1 can substitute 1st pulse, but not 2<sup>nd</sup> pulse EGF.**

# RPPA and Transcriptomic Analyses of the Two-Pulses

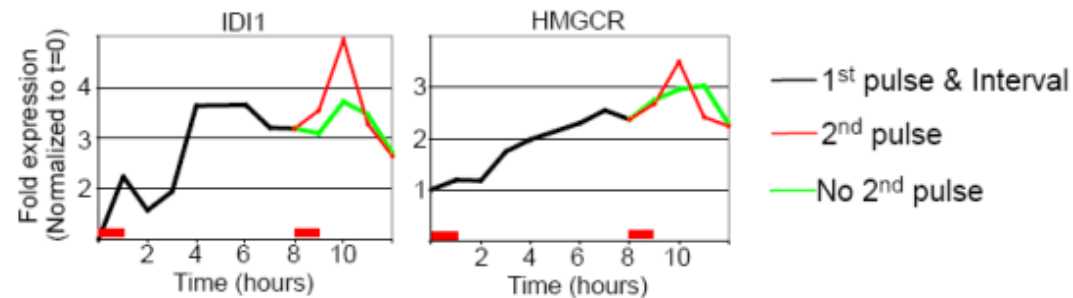
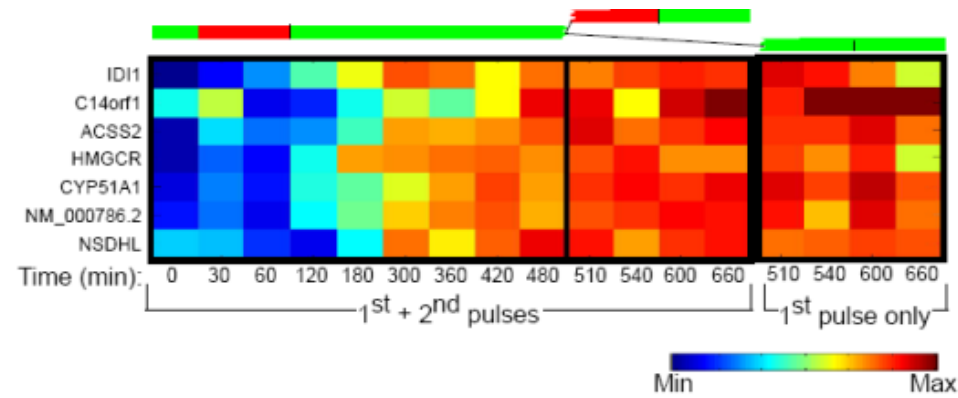


# 10 expression profiles are induced by EGF (two pulses)

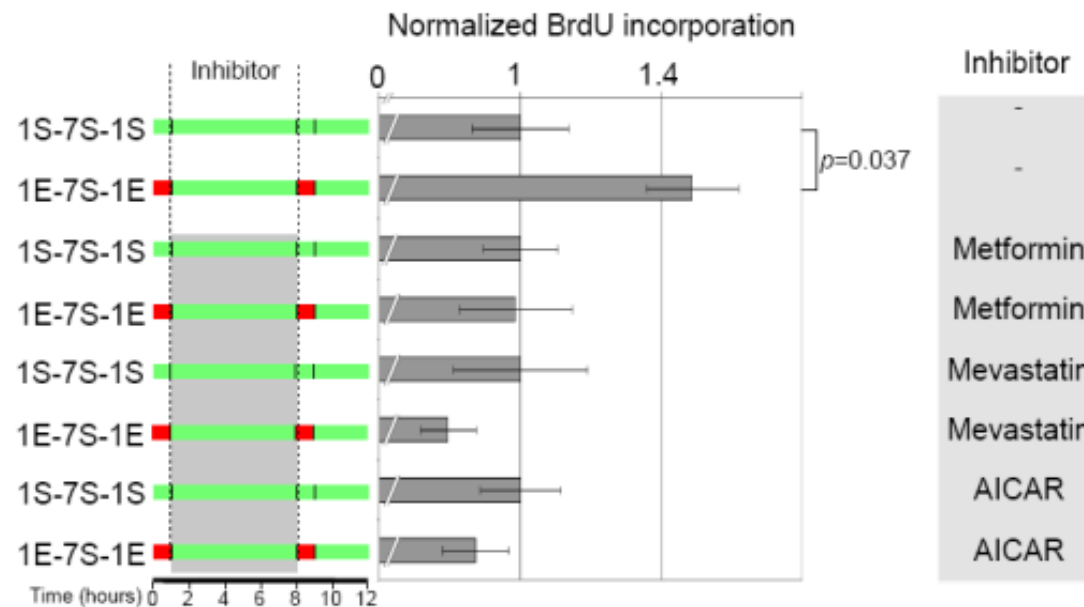




# The Persistently Induced module is enriched for metabolic genes



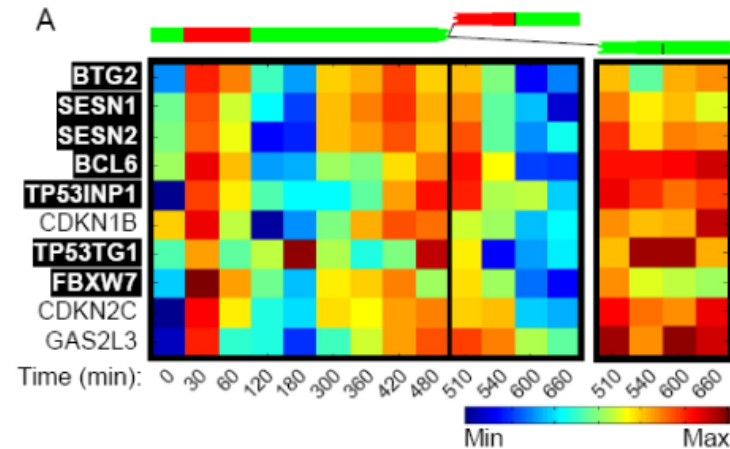
# Induction of metabolic processes is essential for R-crossing



**Lipid metabolism and membrane biogenesis initiate at the 1<sup>st</sup> pulse and might be essential for R crossing**

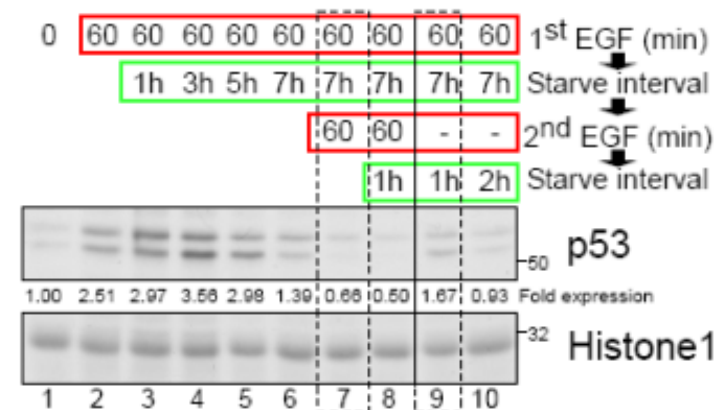
# The module “Down-regulated by 2<sup>nd</sup> Pulse” comprises several p53 regulated genes

The module includes well-established p53 target genes

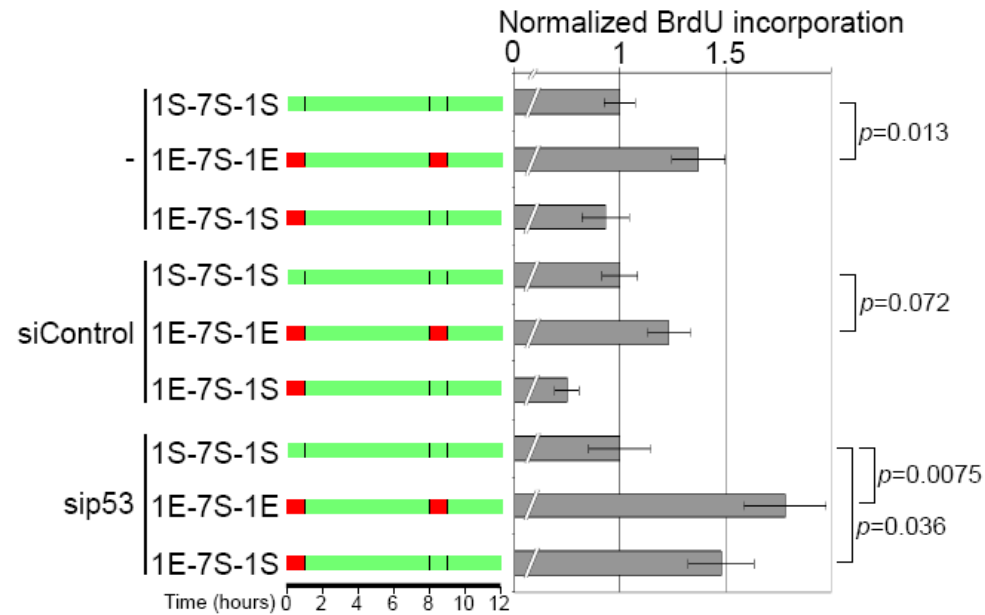


And

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

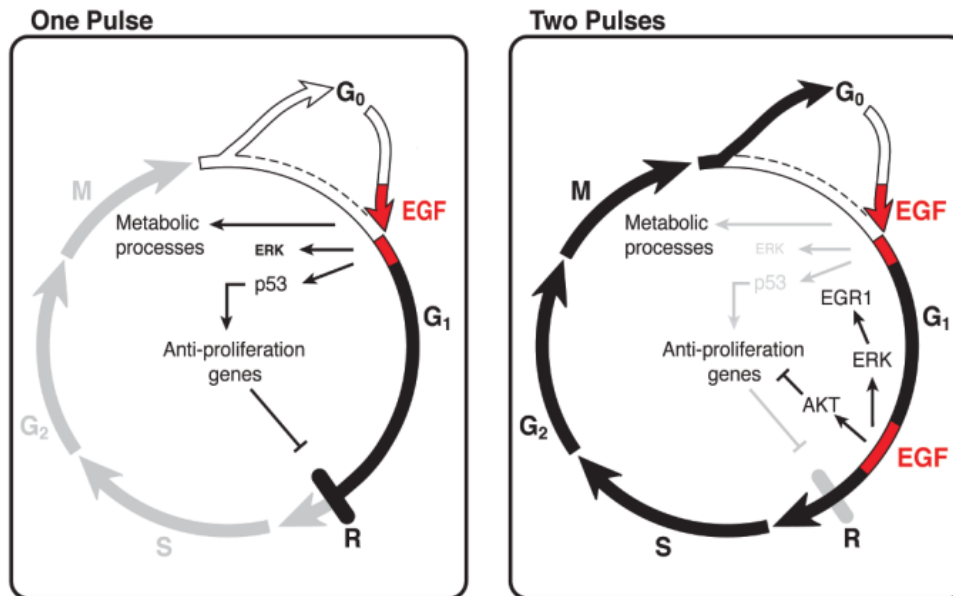


# Knockdown of p53 enables R-crossing in the absence of a second pulse





# 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

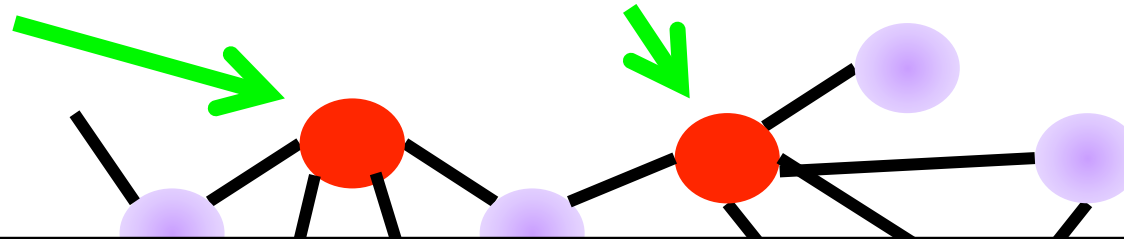
❑ In the absence of p53 (e.g., cancer cells), this filtering mechanism is defective

# **Back to Complexity: Lessons from Graph (Network) Theory**

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

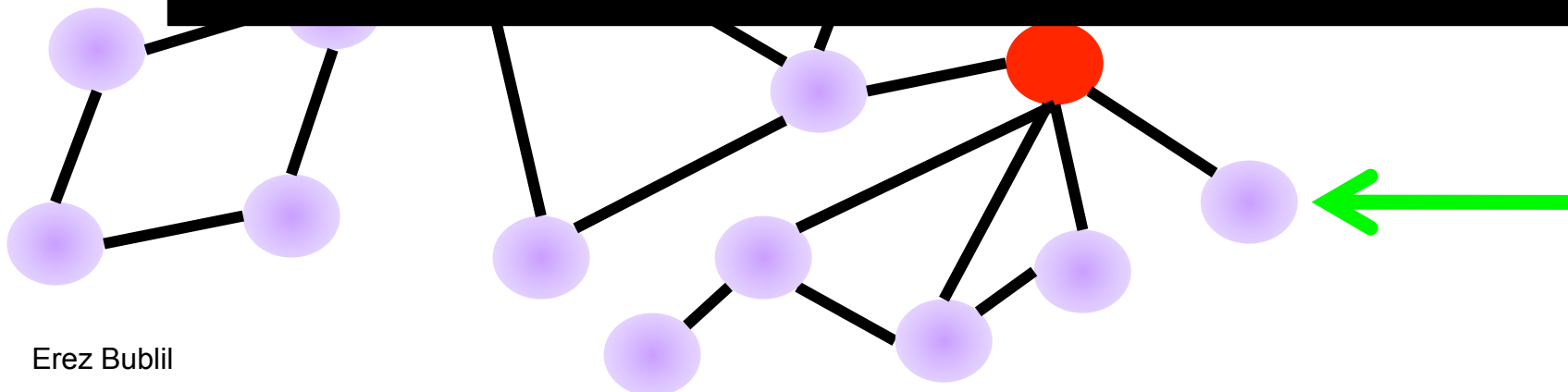
1. A. Wagner, 2001; 2. Carlson & Doyle, 2000; 3.  
2. I.B. Weinstein, 2002; 4. Barbasí & Oltavi; 2001

# Centrality-Lethality Principle

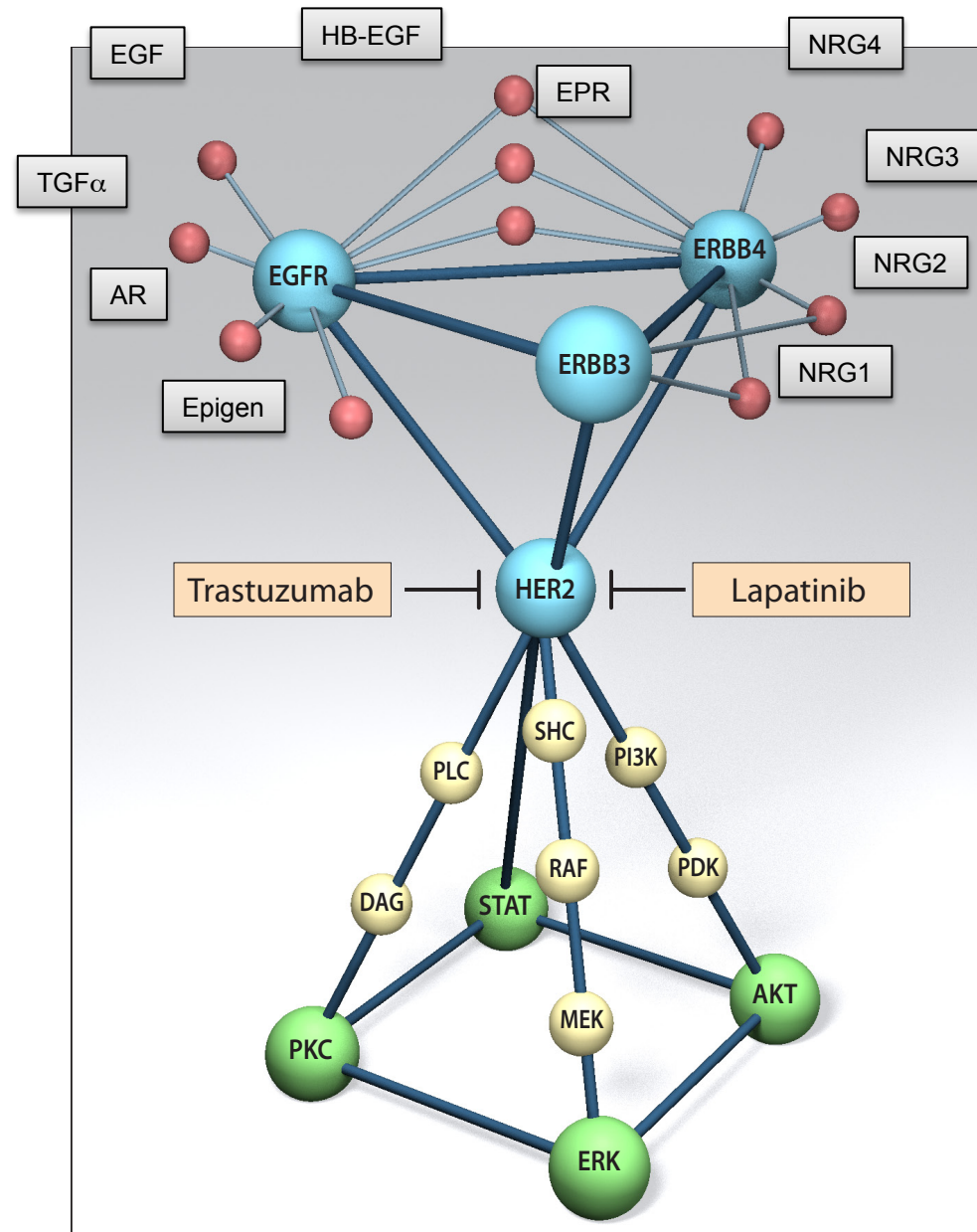


Pre-requisites for effective pharmacological interventions:

1. An essential hub
2. An uncommon perturbation
3. Simultaneous inhibition of the relevant feedback loops



# HER2<sup>+</sup> Breast Tumors: excessive reliance (addiction) on heterodimers

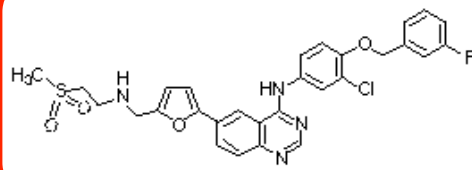


I. Bernard Weinstein



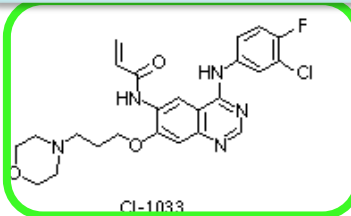
# Uncommon Perturbation #1: Double-hit drugs (e.g., Lapatinib)

Bi-specific

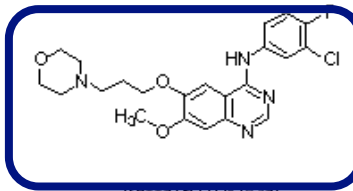


GW2016

Irreversible

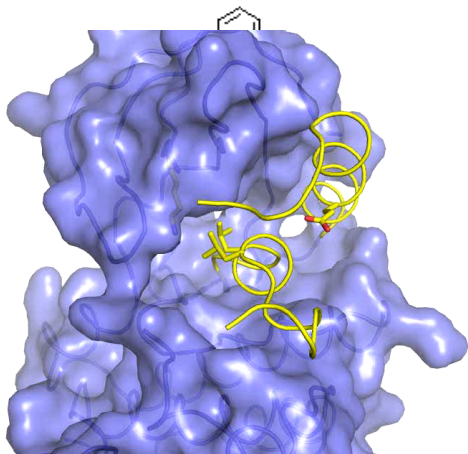


CI-1033



Erlotinib (ZD1875)

Mono-specific:  
Erlotinib



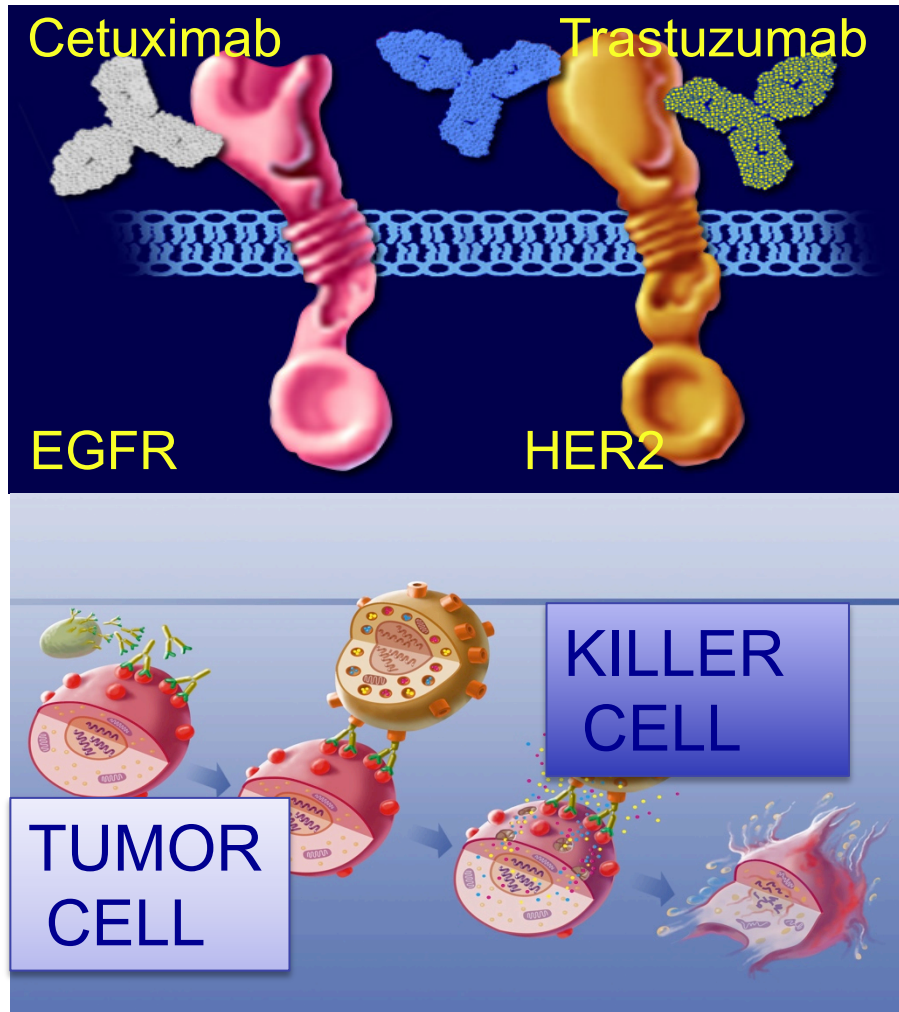
17-AAG

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



TUMOR  
CELL

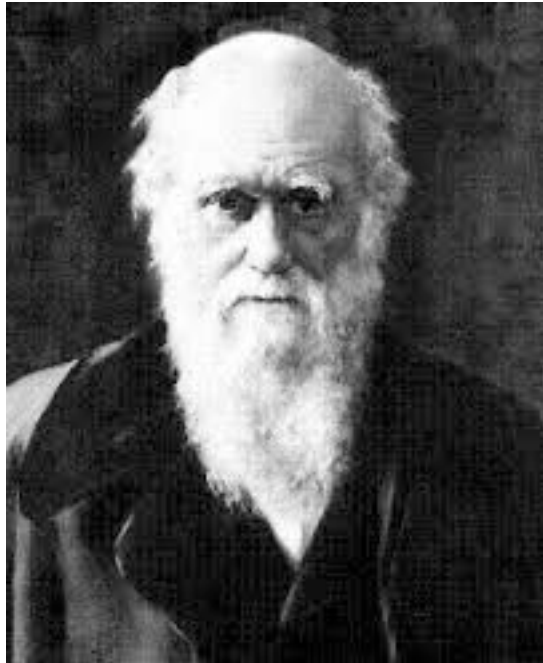


# Our Toughest Enemies

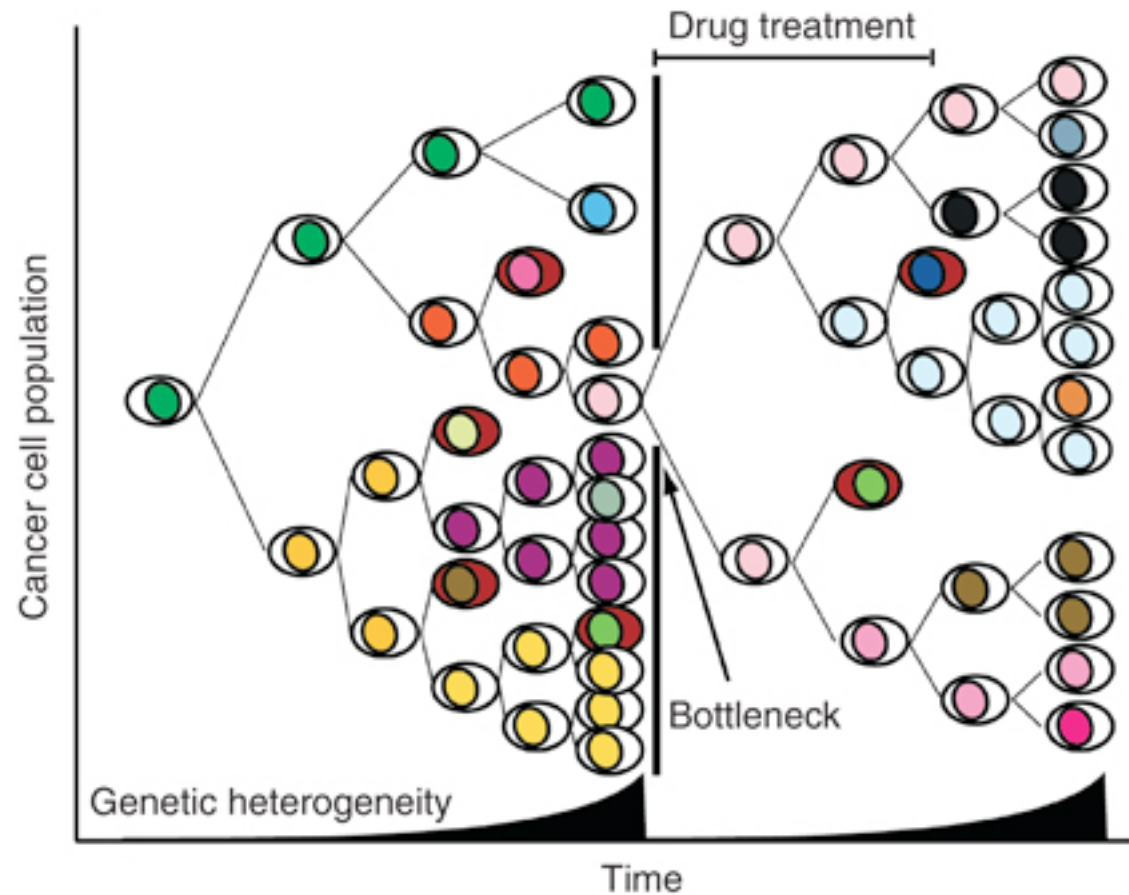
- ✓ **RAS and p53:** In combination, mutations in RAS and p53 are shared by >75% of human tumors, but both oncogenes are hardly druggable.
- ✓ **Micro-metastases:** These small clusters of malignant cells evade all imaging technologies and remain most deadly.
- ✓ **Secondary (evolving) resistance to drugs:** Patients under treatment often develop resistance due to secondary mutations and other mechanisms.
- ✓ **Tumor heterogeneity:** Intra-tumor and inter-metastases genetic heterogeneity underlie resistance to drugs and robust spreading throughout the body.

# TUMOR HETEROGENEITY

## Darwinian Bottlenecking Due to Treatment or Metastasis



**Charles Robert Darwin**  
(1809 – 1882)

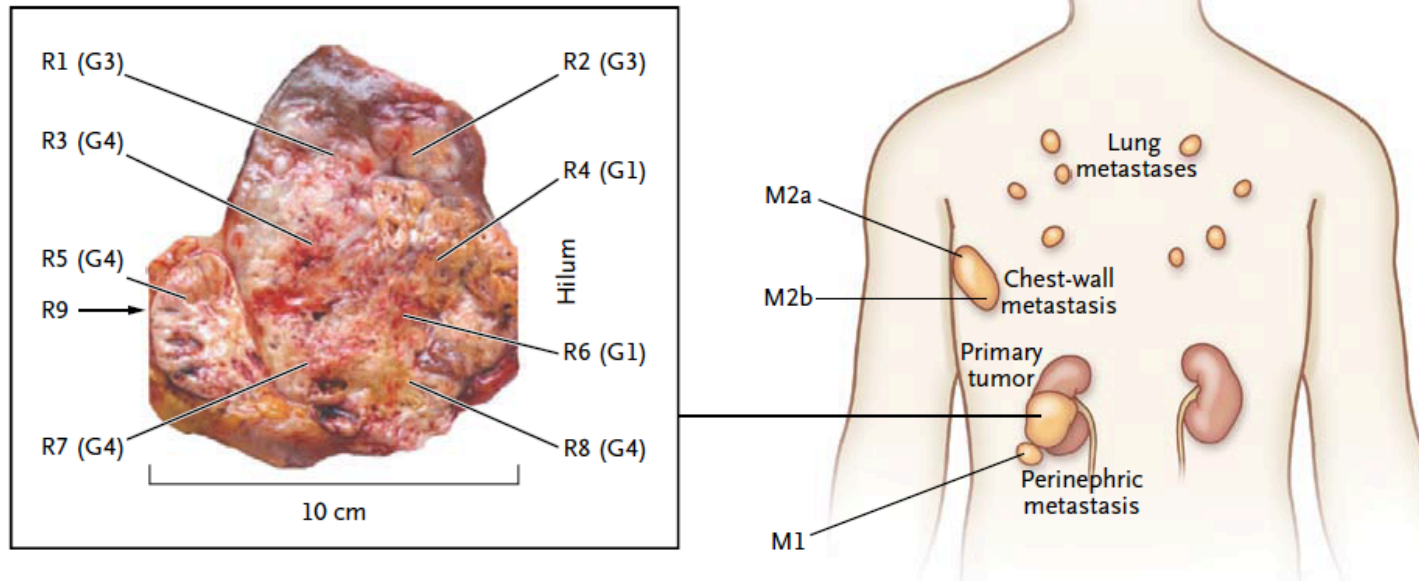


**Intratumor Heterogeneity and Branched Evolution  
Revealed by Multi-region Sequencing (M. Gerlinger et  
al., 2012, NEJM)**

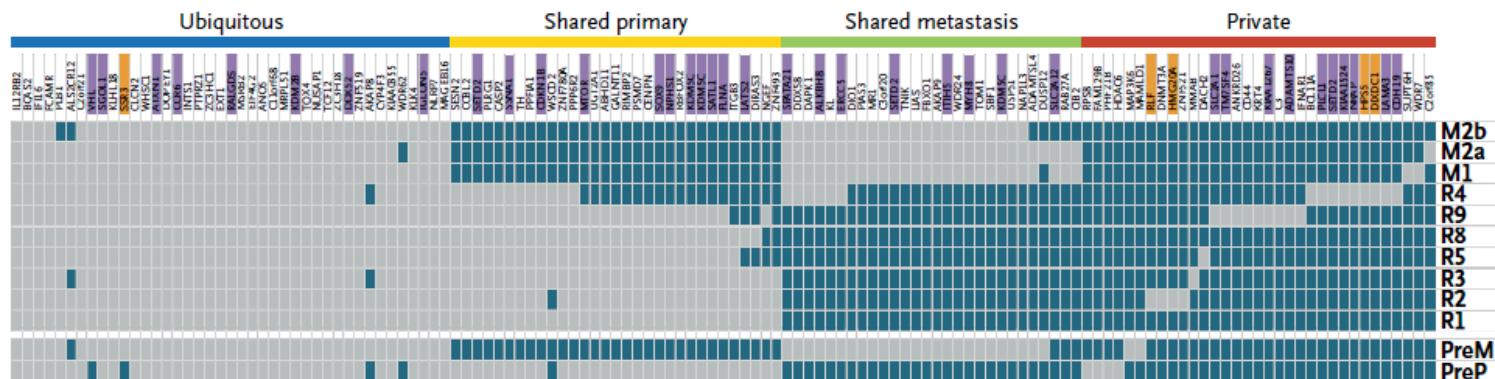
- **Used a primary RCC tumor and several metastases**
- **Performed exome sequencing, analysis of chromosome aberrations and ploidy profiling**
- **Observed branched evolutionary growth with 65% of all somatic mutations not shared by all tumor regions**

# Intra-tumour genetic heterogeneity

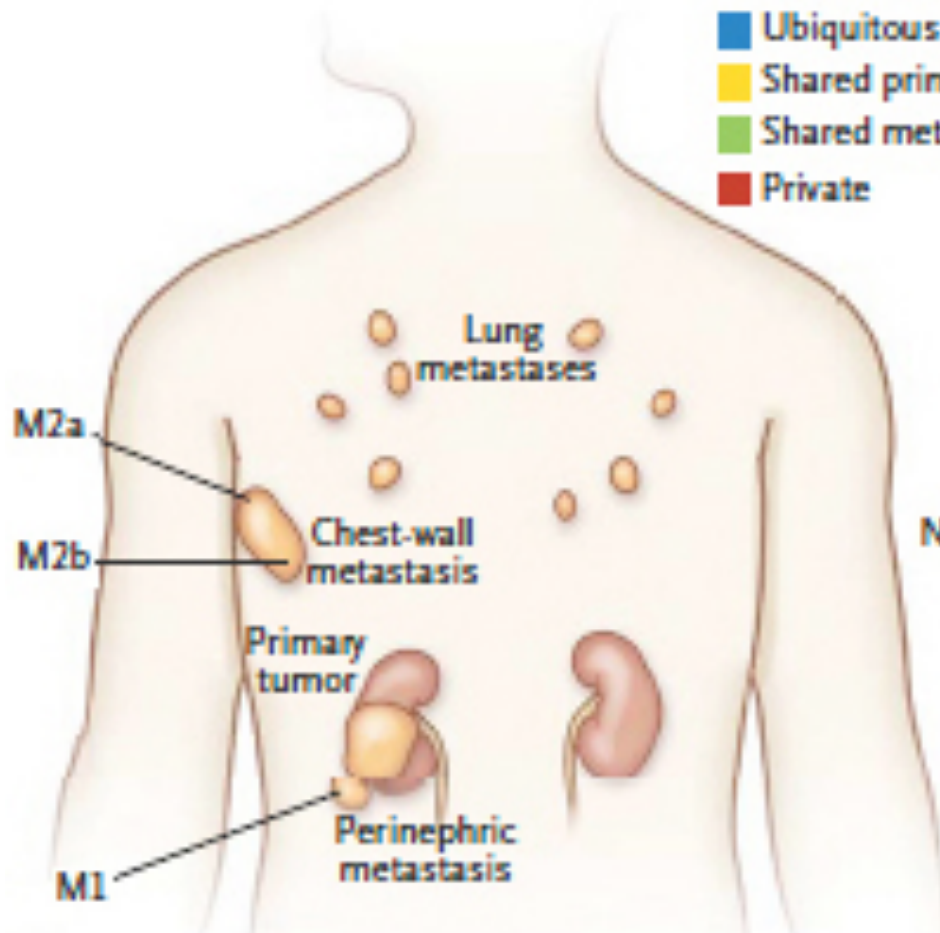
**A Biopsy Sites**



**B Regional Distribution of Mutations**

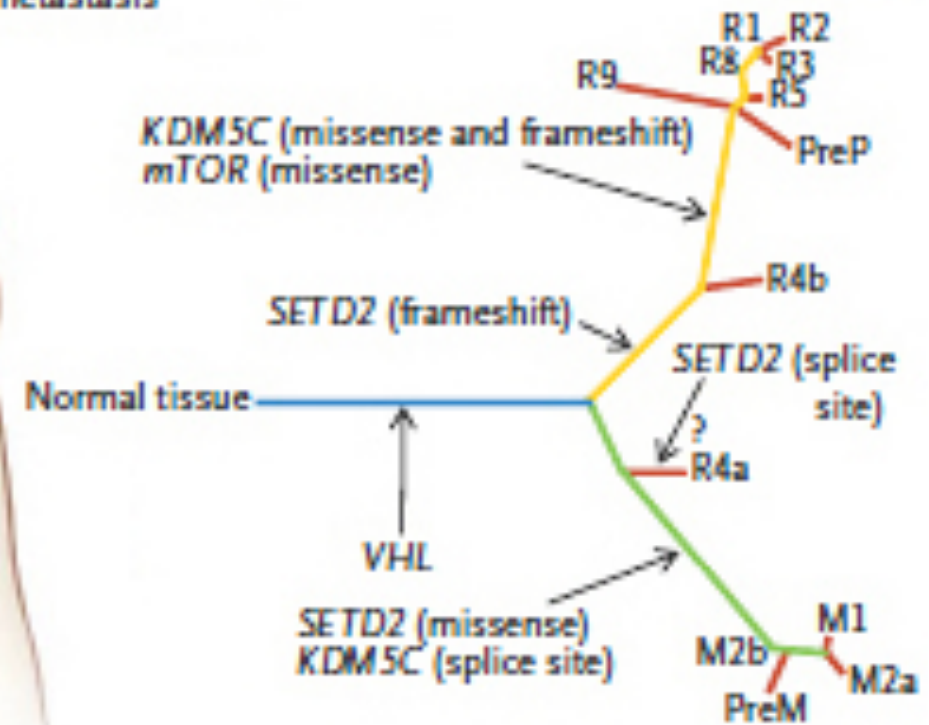


# Phylogenetic Relationships of Tumor Regions



- Ubiquitous
- Shared primary
- Shared metastasis
- Private

## Phylogenetic Relationships of Tumor Regions



## Messages: Network Biology Provides a Conceptual Framework for Signal Transduction Therapies

- ❑ 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)
- ❑ Growth factors employ a pulsatile mode of regulation, which filters noise and ensures commitment to S-phase entry
- ❑ 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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