# Chapter 10: Signaling Networks

Basic Introduction

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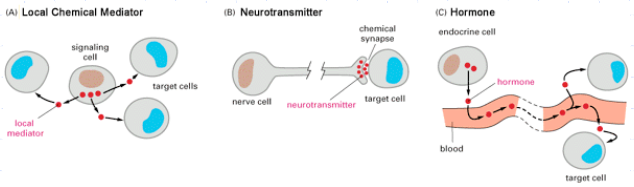
## Overview

- Introduction
- Basic structure & operations
- References
  - Book: R.A. Weinberg, The Biology of Cancer (2007)
  - Review: Yarden & Sliwkowski Nat Rev Mol Cell Biology 2: 127-137, 2001

# Introduction

## Cells Communicate Signals

- Cells need to coordinate functions:
  - Growth, resource usage, defenses…
- Cells encode messages in ligands
  - Grow, proliferate,
- Ligands are processed by pathways
  - Receive signal and transduce it to handlers (e.g., transcribe genes)
  - Pathway is organize as a cascade of processing activities
- Pathways form signaling networks
  - Interact with each other (crosstalk)

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# Origins: Bacteria

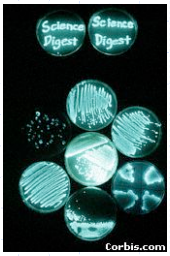
- Bacteria coordinate social functions using signals
- E.g., salmonella signals a “call to arms”

  Veterinarian Jean Guard-Petter… found that the food pathogen Salmonella enteritidis uses acyl-homoserine lactone, or AHL, as its chemical "call to arms."

  "This chemical tells cells to rewrite their genetic programming," says Petter. "It enhances their ability to grow as much as a hundredfold and … increase virulence…"

  http://www.ars.usda.gov/is/AR/archive/jan00/bact0100.htm

- Vibrio Harveyi communities shine together

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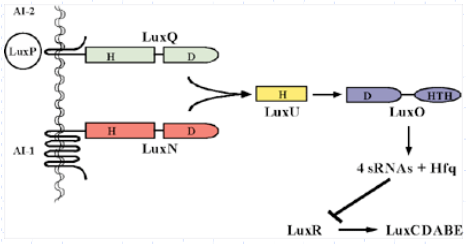
# Bacteria Chat With Each Other

Bonnie Bassler: http://www.nyas.org/ebriefreps/ebrief/000544/presentations/player.html

- Bacteria use quorum sensing to control social behaviors
  - Mating, releasing toxins, attacking a common target…
- Bacteria communicate/sense quorum
  - Two pathways transduce signals from receptors to regulation
  - Use phosphorylation (ATP-ADP) to activate cascade
  - Crosstalk regulates pathways interactions & reuse

AI-1 is an acyl homoserine lactones (AHL)

Waters and Bassler. 2006. Genes and Develop. 20:2754

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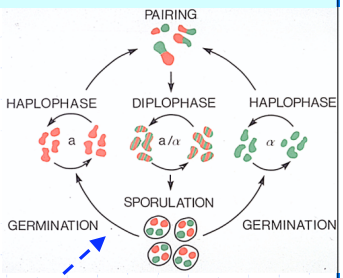
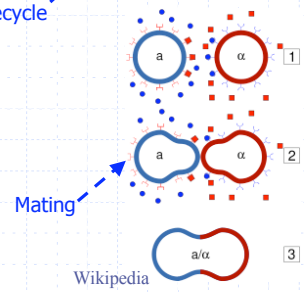
# The Social Life of A Budding Yeast

- Three mating types:
  - Haploids: a, α and diploid: a/α
  - Can switch haploid type (select its "gender")
- Multiplying:
  - Budding
  - Mating: a+α
- Only a/α can sporulate to survive
- How does the yeast coordinate mating?
  - Signal/sense presence through pheromones




Lifecycle




Mating

Wikipedia

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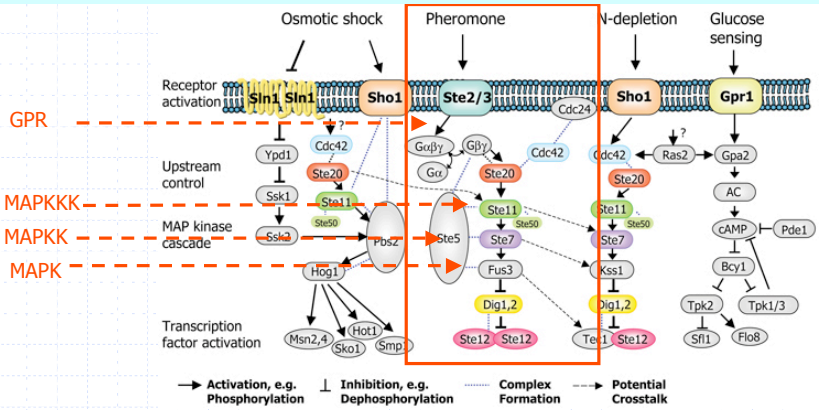
# The Yeast Signaling Network




- Pathway mechanisms:
  - Pathway transduces signals to invoke responses
  - Receptors detect signals and trigger activation
  - Transduction through a cascade of phosphorylation reactions (GTP-GDP)
  - Activation/repression; feedback mechanisms (will see feed-forward later)
  - Crosstalk (why does mating need cross-talking?)

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# Multi-cellular Organisms Require More Complex Signaling

- Cells need to communicate:
  - Coordinate: growth, resource use, defenses…
  - Share information: state changes…
- Pathways transduce signals into response actions
- Pathways interact, forming signaling networks

Table 1 | **The scope of the human cellular signalling network**

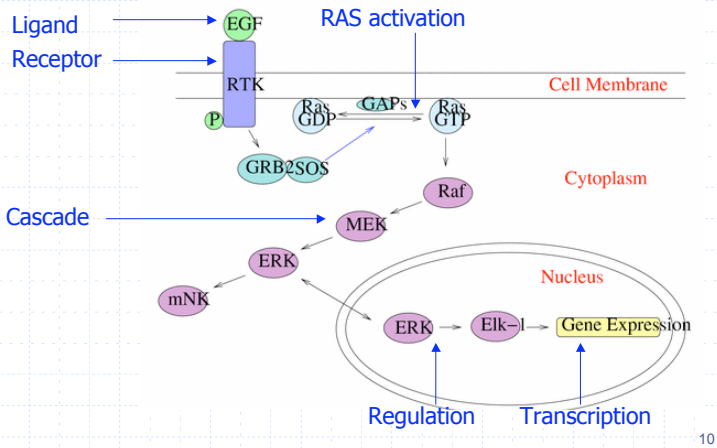
| Network component | Number | References |
|---|---|---|
| Cells | $10^{14}$ | 12 |
| Cell types | 200 | 13 |
| Genes | 25,000 | 14,19 |
| Percentage of genes with splice variants | 40–60 | 21 |
| Average number of exons per alternatively spliced gene | 8 | 14 |
| Maximum number of exons per alternatively spliced gene (taken from *TTID* that encodes titin) | 234 | 14 |
| Average number of splice variants per gene across the genome | 2.5 | 19 |
| Percentage of alternatively spliced genes with signalling function | 75 | 24 |
| Average number of post-translational modifications per protein (current estimates) | 2.5 | (see below*) |
| Genes for transcription factors | 1,850 | 19 |
| Genes for protein kinases | 518 | 15 |
| Genes for protein phosphatases | 150 | 16–18 |
| Genes for receptors | 1,543 | 14 |
| Genes for GPCRs (for endogenous ligands) | 367 | 33 |

*See the Human Proteomics Initiative in the online links box.

Papin et al (2005) Nat Rev Mol Cell Biol 6:99

# Receptor Tyrosine Kinase (RTK) Pathway

- Receptor binds ligand activates RAS
- RAS uses GDP-GTP phosphorilation to activate cascade
- Cascade activates transcription

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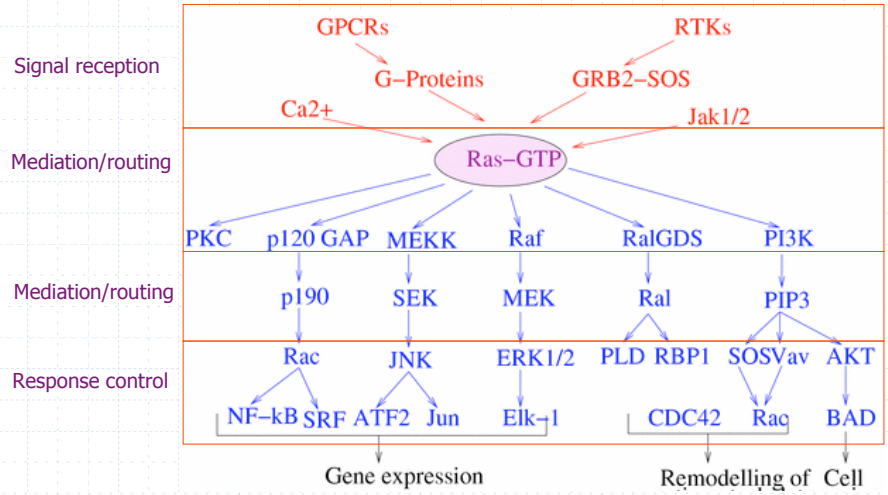
# A Simplified View of Signaling

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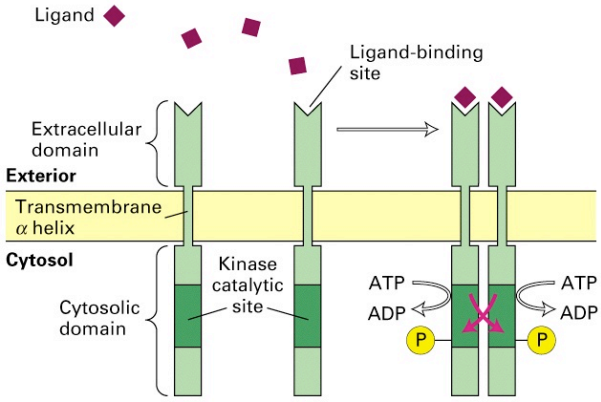
# RTKs: Activation Through Dimerization




Lodish et al. Fig. 20-21

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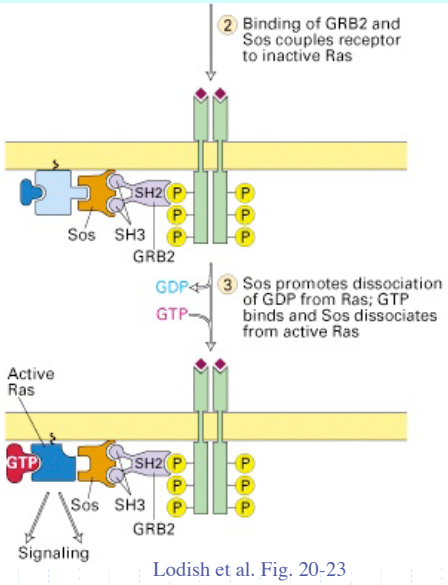
# Receptor Complex Activates RAS




Lodish et al. Fig. 20-23

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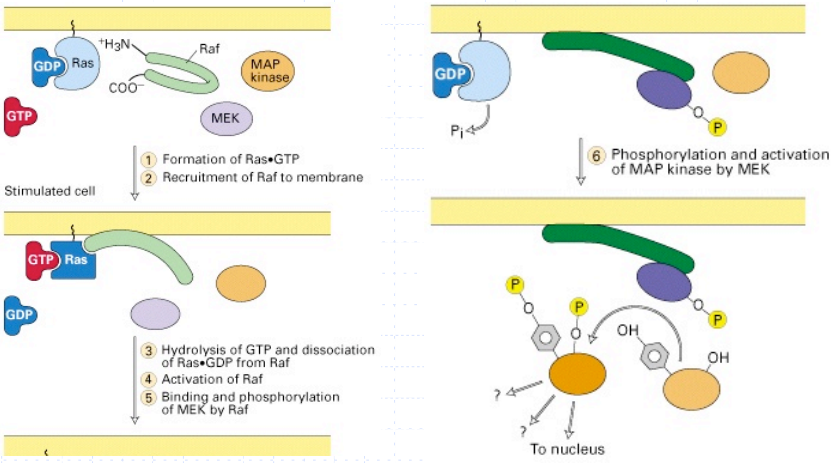
# RAS Activates Cascade




Lodish et al. Fig. 20-28

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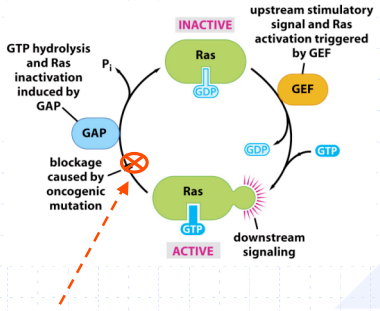
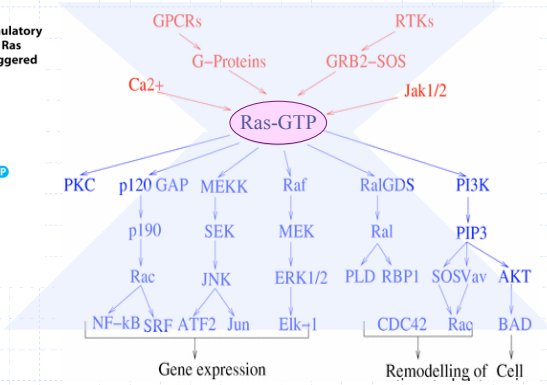
# RAS Is A Central GDP-GTP Switch




Ras is stuck ➔ cancer

- Ras routes the signal to respective cascades
- Ras mutations are present in 20-30% of cancers

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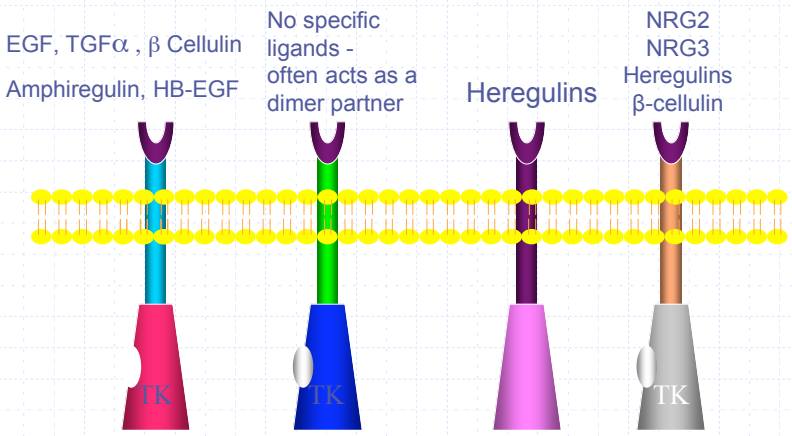
# The ErbB Family of RTKs




Adopted from Paul Shapiro

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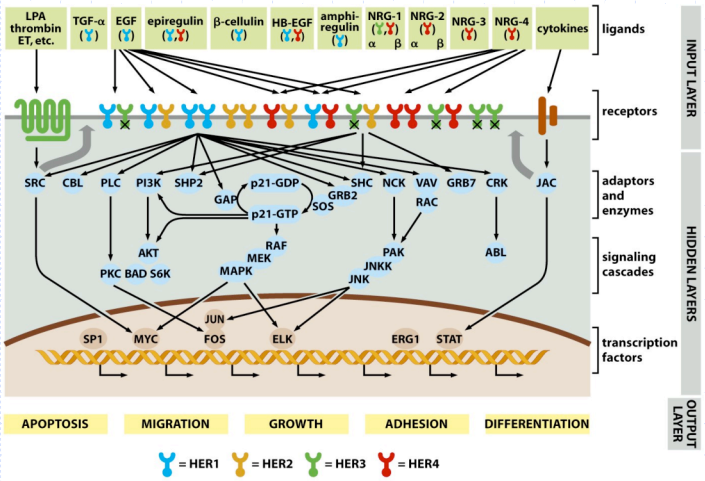
# The ErbB Network

Figure 5.1 *The Biology of Cancer* (© Garland Science 2007)
From Yarden & Sliwkowski Nat Rev Mol Cell Biology 2: 127-137, 2001




- ERBB network manages growth signals: “grow”, “proliferate”, “die”…
- Failures of the ERBB network account to numerous cancers

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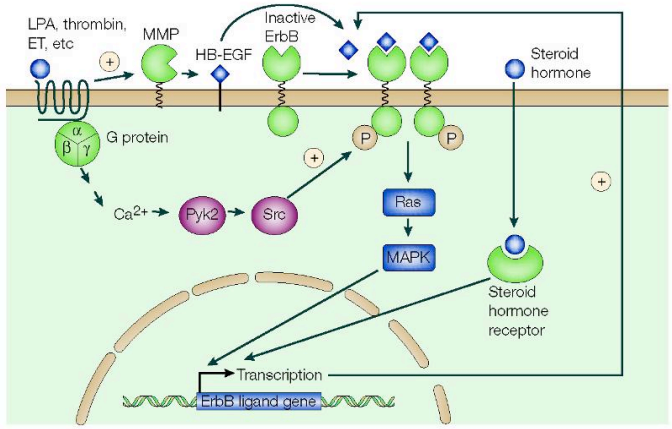
# Pathways Form A Network

- Interact through crosstalk
- Share resources, regulate functions…




Yarden & Sliwkowski (2001) Nat Rev Mol Cell Biol 2:127

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# Network Failures May Result in Cancer

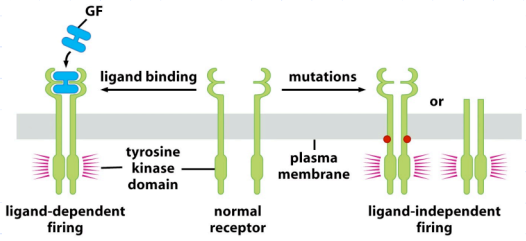


Table 5.2 Tyrosine kinase GF receptors altered in human tumors[a]

| Name of receptor | Main ligand | Type of alteration | Types of tumor |
|---|---|---|---|
| EGF-R/ErbB1 | EGF, TGF-α | overexpression | non-small cell lung cancer; breast, head and neck, stomach, colorectal, esophageal, prostate, bladder, renal, pancreatic, and ovarian carcinomas; glioblastoma |
| EGF-R/ErbB1 | | truncation of ectodomain | glioblastoma, lung and breast carcinomas |
| ErbB2/HER2/Neu | NRG, EGF | overexpression | 30% of breast adenocarcinomas |
| ErbB3, 4 | various | overexpression | oral squamous cell carcinoma |
| Flt-3 | FL | tandem duplication | acute myelogenous leukemia |
| Kit | SCF | amino acid substitutions | gastrointestinal stromal tumor |
| Ret | | fusion with other proteins, point mutations | papillary thyroid carcinomas, multiple endocrine neoplasias 2A and 2B |
| FGF-R3 | FGF | overexpression; amino acid substitutions | multiple myeloma, bladder and cervical carcinomas |

[a]See also Figure 5.17.

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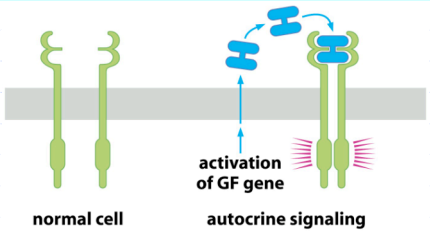
# Network Failures May Result in Cancer




*The Biology of Cancer* (© Garland Science 2007)

Table 5.3 Examples of human tumors making autocrine growth factors

| Ligand | Receptor | Tumor type(s) |
|---|---|---|
| HGF | Met | miscellaneous endocrinal tumors, invasive breast and lung cancers, osteosarcoma |
| IGF-2 | IGF-1R | colorectal |
| IL-6 | IL-6R | myeloma, HNSCC |
| IL-8 | IL-8R A | bladder cancer |
| NRG | ErbB2[a]/ErbB3 | ovarian carcinoma |
| PDGF-BB | PDGF-Rα/β | osteosarcoma, glioma |
| PDGF-C | PDGF-α/β | Ewing's sarcoma |
| PRL | PRL-R | breast carcinoma |
| SCF | Kit | Ewing's sarcoma, SCLC |
| VEGF-A | VEGF-R (Flt-1) | neuroblastoma, prostate cancer, Kaposi's sarcoma |
| TGF-α | EGF-R | squamous cell lung, breast and prostate adenocarcinoma, pancreatic, mesothelioma |
| GRP | GRP-R | small-cell lung cancer |

[a]Also known as HER2 or Neu receptor.

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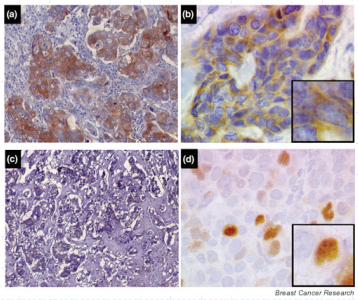
# ERBB Expression In Cancers

Table 1 | **Expression of ErbBs and their ligands in cancer**

| Molecule | Nature of dysregulation | Type of cancer | Notes | References |
|---|---|---|---|---|
| ***Ligands*** | | | | |
| TGF-α | Overexpression | Prostate | Expressed by stroma in early, androgen-dependent prostate cancer and by tumours in advanced, androgen-independent cancer | 52 |
| | Overexpression | Pancreatic | Correlates with tumour size and decreased patient survival; may be due to overexpression of Ki-Ras, which also drives expression of HB-EGF and NRG1 | 108 |
| | Overexpression | Lung, ovary, colon | Correlates with poor prognosis when co-expressed with ErbB1 | 51 |
| NRG1 | Overexpression | Mammary adeno-carcinomas | Necessary, but not sufficient for tumorigenesis in animal models | 109 |
| ***Receptors*** | | | | |
| ErbB1 | Overexpression | Head and neck, breast, bladder, prostate, kidney, non-small-cell lung cancer | Significant indicator for recurrence in operable breast tumours; associated with shorter disease-free and overall survival in advanced breast cancer; may serve as a prognostic marker for bladder, prostate, and non-small-cell lung cancers | 110,111 |
| | Overexpression | Glioma | Amplification occurs in 40% of gliomas; overexpression correlates with higher grade and reduced survival | 35 |
| | Mutation | Glioma, lung, ovary, breast | Deletion of part of the extracellular domain yields a constitutively active receptor | 54 |
| ErbB2 | Overexpression | Breast, lung pancreas, colon oesophagus, endometrium, cervix | Overexpressed owing to gene amplification in 15–30% of invasive invasive ductal breast cancers Overexpression correlates with tumour size, spread of the tumour to lymph nodes, high grade, high percentage of S-phase cells, aneuploidy and lack of steroid hormone receptors | 56 |
| ErbB3 | Expression | Breast, colon gastric, prostate, other carcinomas | Co-expression of ErbB2 with ErbB1 or ErbB3 in breast cancer improves predicting power | 64,65 |
| | Overexpression | Oral squamous cell cancer | Overexpression correlates with lymph node involvement and patient survival | 112 |
| ErbB4 | Reduced expression | Breast, prostate | Correlates with a differentiated phenotype | 66 |
| | Expression | Childhood medullo-blastoma | Co-expression with ErbB2 has a prognostic value | 67 |

(TGF-α, transforming growth factor-α; NRG1, neuregulin-1; HB-EGF, heparin-binding epidermal growth factor.)




Examples of HER-2 and p21WAF1/CIP1 immunoreactivity in infiltrating ductal carcinoma of the breast. (a) A tumour showing HER-2 overexpression, with (b) a concomitant predominance of cytoplasmic p21WAF1/CIP1staining (c) A HER-2-negative tumour, with (d) a predominance of nuclear p21WAF1/CIP1 expression compared with cytoplasmic p21WAF1/CIP1

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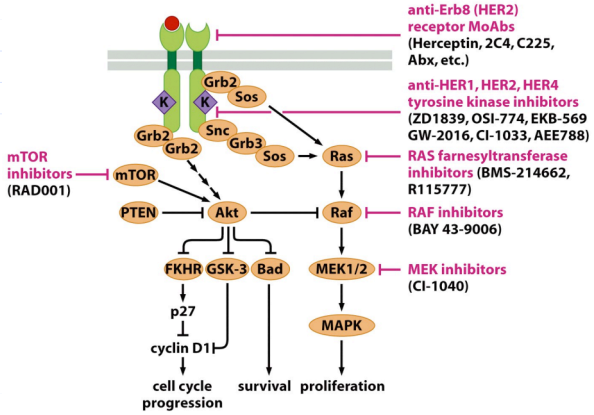
# Cancer Therapy Can Target Signaling Pathways




- Inhibit tumor growth by blocking pathway elements
  - Antibodies can block ectodomain of receptor (e.g, Herceptin)
  - Small ligands can block active sites of cascade components
- Can computational models help (e.g., predict side-effects)?

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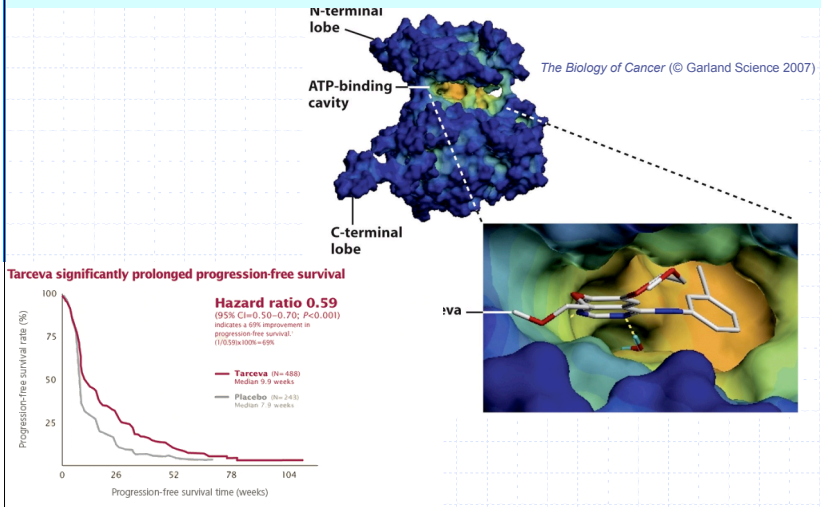
# Example: Blocking The EGF Receptor




- Success = increase binding strength
- Can this be computed?

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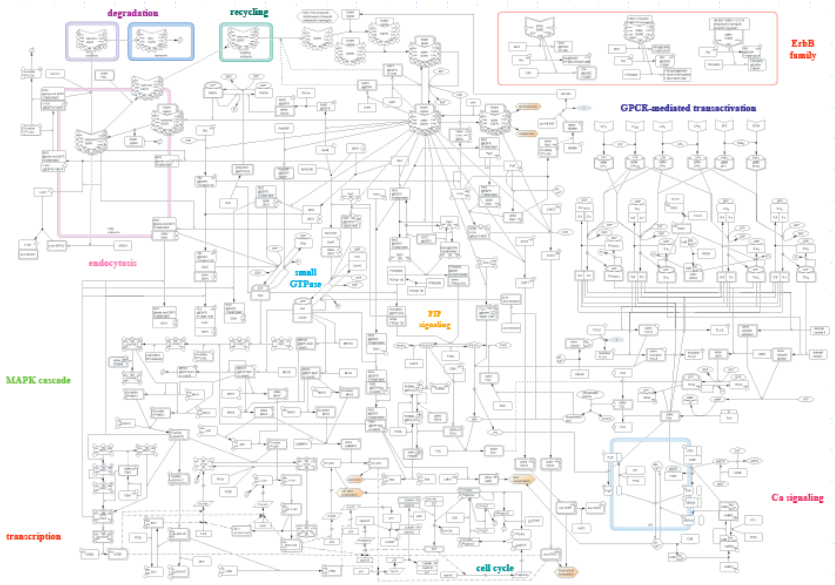
# Network Architecture

# The EFGR (ERBB) Network

Kanae Oda et al A, comprehensive pathway map of epidermal growth factor receptor signaling; Molecular Systems Biology 2:2005.0010

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# Are There Organizing Principles?

- Pathways share components
  - E.g., RAS, HER2
- Components share structural & operational motifs
  - E.g., signal reception through dimerization
  - Cascade transduction through phosphorylation
- Layered organization
- Regulation through activation/repression
  - Similar to regulatory network
- Cascading reactions
  - Similar to metabolic network

Grand Challenge: genome-scale computational models

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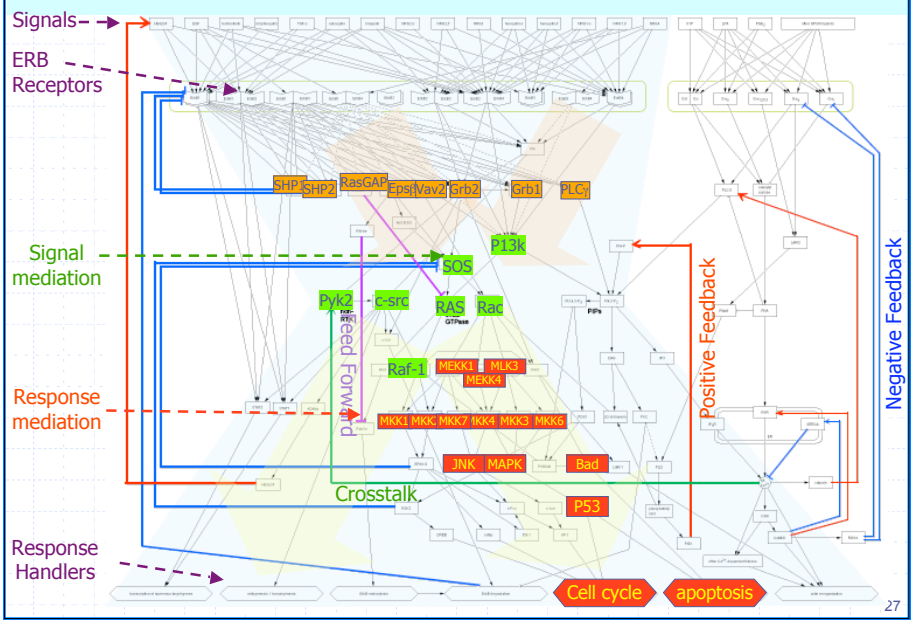
# An Architectural View

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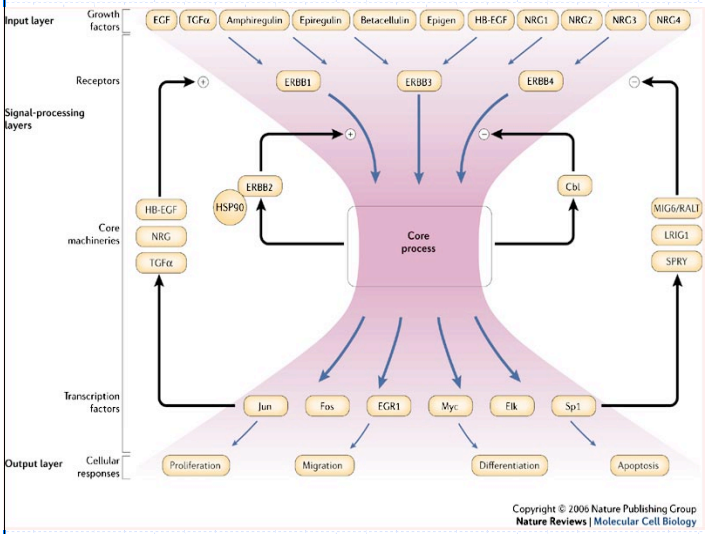
# A System View

Ami Citri and Yosef Yarden, EGF–ERBB signalling: towards the systems level
Nature Reviews Molecular Cell Biology 7, 505-516 (July 2006)






The system maintains two steady states (bistability) based on ligand binding. The fail-safe (robustness) action of the system is conferred by structural modularity and functional redundancy, along with rich and stringent controls.

ERBB2 regulates input through heterodimerization with any of the three ERBB input modules. ERBB2 is chaperoned and catalytically suppressed by heat-shock protein-90 (HSP90). A ubiquitin ligase involved in receptor degradation, Cbl, controls an important negative-feedback loop.

Several activation-dependent control loops fine-tune bistability. These include transcription of ERBB ligands (positive regulation) and newly synthesized negative regulators such as MIG6/RALT and SPRY. EGF, EGR1, NRG1/2/3/4, neuregulin-1/2/3/4; and TGFalpha.

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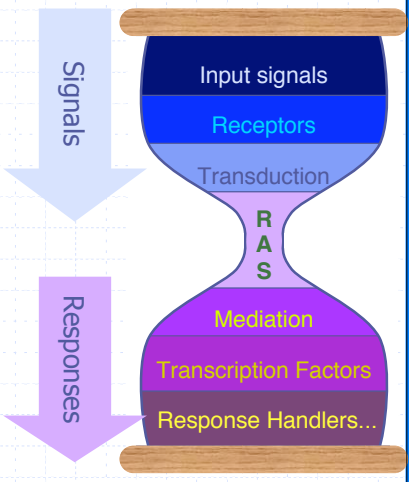
# An Hourglass Architecture???

- Scalability:
  - map m signals to n functions
  - n is combinatorial
- Layering:
  - Combine layers components into pathways
- Hourglass:
  - Minimize signal routing abstractions -- phosphorylaztion
- Evolutionary extensibility:
  - Add new signals & functions
  - E.g, duplication & speciation

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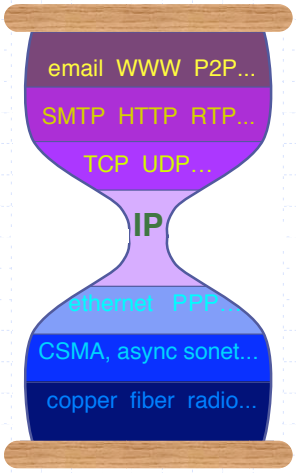
# An Internet-Like Occam Design ????

- Scalability:
  - map n applications to m link types
  - n is combinatorial
- Layering:
  - Combine layer abstractions into stack
- Hourglass:
  - Minimize routing abstractions (IP)
- Evolutionary extensibility:
  - Add new link types & applications




Henning Schulzrinne slide
www.cs.columbia.edu/~coms6181/slides/1/internet.ppt

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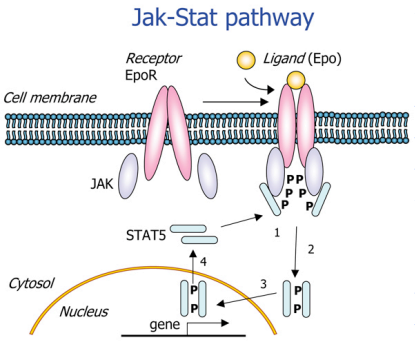
# Modeling & Analysis

## Stoichiometric Approach To Signaling Net Topology

- Papin & Palsson:
  - Biophysical J. Vol 87, July 2004; JAK-STAT network (type of TRK)
  - J. Theoretical Biology 227 (2004) 283-297
- Key idea: model signaling reactions much like metabolic nets
  - Compute topological features (crosstalk)

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# The Model

- Key step: construct network of signaling reactions
- Extract stoichiometric matrix
  - Rows represent reactions; columns represent pathway elements
  - Account for co-factor/by-product phosphorylation reactions
- Compute extreme pathways
- Classify input/output topology of EP

| Signaling Pathway Type | Number of Pathways |
|---|---|
| Single-Input, Single-Output | 37 |
| Multiple-Input, Single-Output | 110 |

Table 1
Reaction listing for the prototypic signaling network in Fig. 2

| Name | Chemical equation |
|---|---|
| bindLR | L + R → LR |
| LRpS | LR + ATP + S → ADP + S_p + LR_in |
| SpT | S_p + T → T_p + S |
| TpW | T_p + W → W_p + T |
| bindL2R2 | L2 + R2 → L2R2 |
| L2R2pS2 | L2R2 + ATP + S2 → ADP + S2_p + L2R2_in |
| S2pT2 | S2_p + T2 → S2 + T2_p |
| T2pW2 | T2_p + W2 → T2 + W2_p |
| bindL3R3 | L3 + R3 → L3R3 |
| L3R3pS3 | L3R3 + ATP + S3 → ADP + S3_p + L3R3_in |
| S3pT3 | S3_p + T3 → S3 + T3_p |
| T3pW3 | T3_p + W3 → T3 + W3_p |
| bindL4R | L4 + R → LR |
| bindL5R2 | L5 + R2 → L2R2 |
| L3R3pS | L3R3 + ATP + S → ADP + S_p + L3R3_in |
| LRpS2 | LR + ATP + S2 → ADP + S2_p + LR_in |
| L2R2pT | L2R2 + ATP + T → ADP + T_p + L2R2_in |
| S2pW | S2_p + W → S2 + W_p |
| T3pW | T3_p + W → T3 + W_p |
| S2T3pWW2 | S2_p + T3_p + W + W2 → S2 + T3 + W2_p + W_p |
| SWpW2W3 | S_p + W_p + W2 + W3 → W2_p + W3_p + W + S |
| formW2W3 | W2_p + W3_p → W2_W3_pp |
| formWW2W3 | W_p + W2_p + W3_p → WW2W3_ppp |
| form2WW3 | 2 W_p + W3_p → 2W_W3_ppp |

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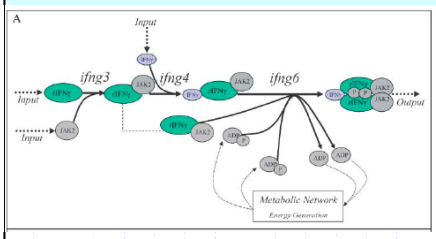
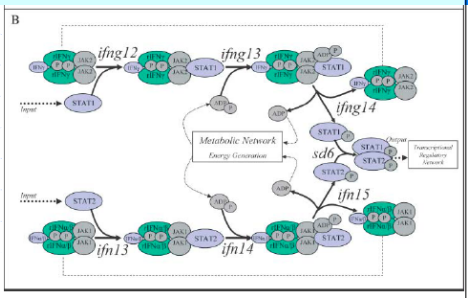
# EP Characterize Topological Features




Sample Extreme Pathways

FIGURE 3 Examples of extreme signaling pathways of the JAK-STAT signaling network. Pathway 119 (*A*) corresponds to the binding of the interferon γ ligand to its receptor and the subsequent formation of an activated receptor-ligand complex. Pathway 1 (*B*) corresponds to the activation of the STAT1-STAT2 heterodimer by the interferon γ and interferon α/β bound receptors. The names of the reactions are in italics above the reaction arrows (see supplementary material).

- What do extreme pathways tell us about topology?
- Define crosstalk as input/output sharing
  - Two pathways may have disjoint/overlapping/identical inputs/outputs
  - Classify EP into 9 categories of interactions

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# Crosstalk

| Outputs \ Inputs | ● ● | ● | ● | Total |
|---|---|---|---|---|
| ● ● | 63.89 | 21.12 | 14.76 | 99.77 |
| ● | 0.00 | 0.00 | 0.00 | 0.00 |
| ● | 0.05 | 0.15 | 0.04 | 0.24 |
| Total | 63.94 | 21.27 | 14.80 | |

FIGURE 4 Cross talk in the JAK-STAT signaling network of the human B-cell. There are 10,731 pairwise combinations of the 147 extreme pathways. Each value is the percentage of all pairwise combinations that belong to the corresponding category.

- Crosstalk occurs at input more than output
- Overlaps are slightly more likely than identity

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# Redundant Pathways Provide Robustness

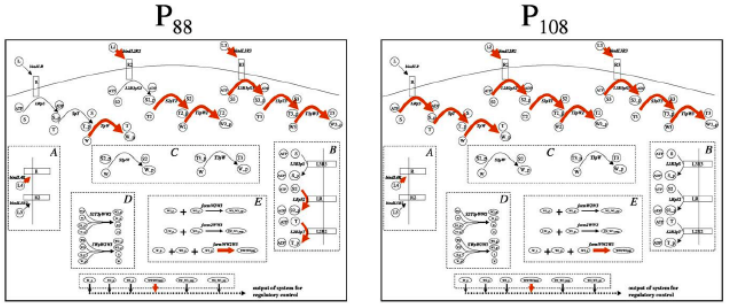


Fig. 7. Completely redundant extreme pathways. Pathways 88 and 108 have identical inputs and outputs and yet use different internal reactions.

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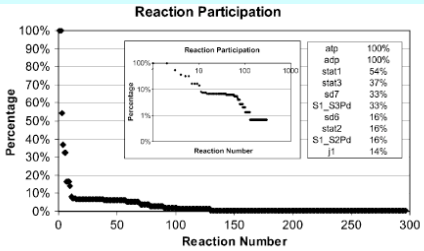
# Reaction Participation & Correlated Reactions




| | |
|---|---|
| atp | 100% |
| adp | 100% |
| stat1 | 54% |
| stat3 | 37% |
| sd7 | 33% |
| S1_S3Pd | 33% |
| sd6 | 16% |
| stat2 | 16% |
| S1_S2Pd | 16% |
| j1 | 14% |

FIGURE 5 Reaction participation in JAK-STAT signaling network. The percentage of the extreme signaling pathways in which the corresponding reaction participates is indicated on the y axis. The inset table only lists the reaction participation values greater than 20% (abbreviations in supplementary material). The inset plot is the reaction participation plotted on a log-log scale.

- Reaction participation statistics indicates hourglass topology (?)
- Correlated reactions describe "motifs"

**TABLE 4 Representative correlated reaction sets**

| Set number | Reaction name | Stoichiometry/Exchange |
|---|---|---|
| 1 | atp | INPUT |
| | adp | OUTPUT |
| 2 | pr11 | rPRL + j2 → rPRLj2 |
| | pr12 | rPRLj2 + PRL → rlPRLj2 |
| | pr13 | rlPRLj2 + rPRLj2 + 2 atp → 2 adp + 2PRLj2 |
| | PRL | INPUT |
| | rPRL | INPUT |
| | 2PRLj2 | OUTPUT |
| 3 | pr14 | 2PRLj2 + stat5A → 2PRLj2s5A |
| | pr15 | 2PRLj2s5A + atp → PRLj2s5Ap |
| | pr16 | PRLj2s5Ap → 2PRLj2 + stat5AP + adp |
| 4 | l4 | INPUT |
| | rl4 | INPUT |
| 5 | il4i | 2l4j1 + stat6 → 2l4j1_s6 |
| | il4j | 2l4j1_s6 + atp → l4j1s6p |
| | il4k | l4j1s6p → 2l4j1 + adp + stat6P |
| 6 | il4l | 2l4j3 + stat6 → 2l4j3_s6 |
| | il4m | 2l4j3_s6 + atp → l4j3s6p |
| | il4n | l4j3s6p → 2l4j3 + adp + stat6P |

Each set of reactions always appears together in a set of extreme pathways for the JAK-STAT signaling network. The complete list of correlated reaction sets, as well as the reaction and component abbreviations, are provided in the supplementary material.

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# Conclusions

## Notes

- Grand challenge: computational modeling of signaling nets
  - Genome-scale network model
  - Structural and dynamic modeling
  - Analyze perturbations & failure modes
  - Model & analyze network evolution
  - …
- Integrative system models:
  - Signaling/regulation, signaling/metabolic…
- High-speed genome-scale data collection

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