

AIMS

- ➔ Take a close look at similarity within Src-family and other members of NRTK
- ➔ Phosphorylation site mapping : Models vs. Reality
- ➔ Comparison with receptor tyrosine kinase

CYTOPLASMIC NON-RECEPTOR TYROSINE KINASE FAMILY

***CELLULAR
LOCALISATION***

Soluble components

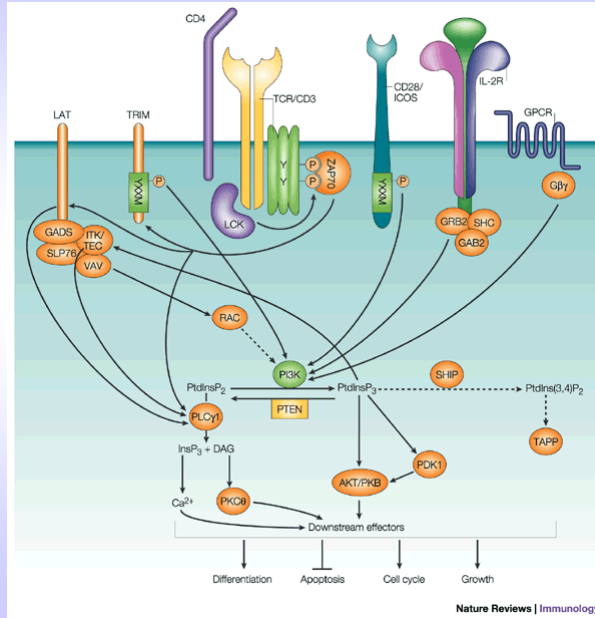
Membrane-associated

***FUNCTIONAL
ROLE***

Cell proliferation-differentiation-death

Receptors that recruit NRTKs:
TCR, BCR; IL-2R, Ig-receptors,
erythropoietin and prolactin receptors

Signalling in T cells mediated by src kinases



Cytoplasmic Tyrosine Kinases

SRC, YES, FYN,
LYN, LCK, BLK,
HCK, FGR, YRK

FRK (MKK3), BRK
SRM, SAD

BTK, ITK, TEC
MKK2 (BMX)
TXK

CSK,
CTK (MCK1)

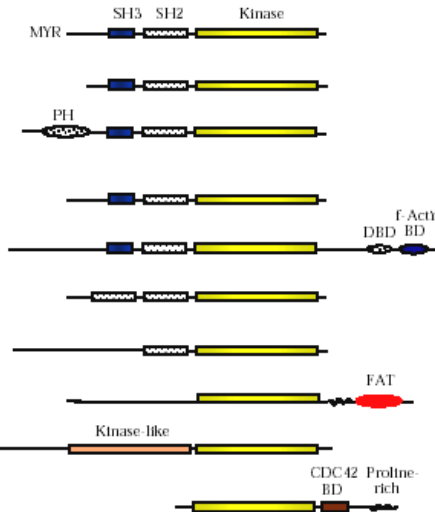
ABL, ARG

ZAP70, SYK

FAK, PYK2

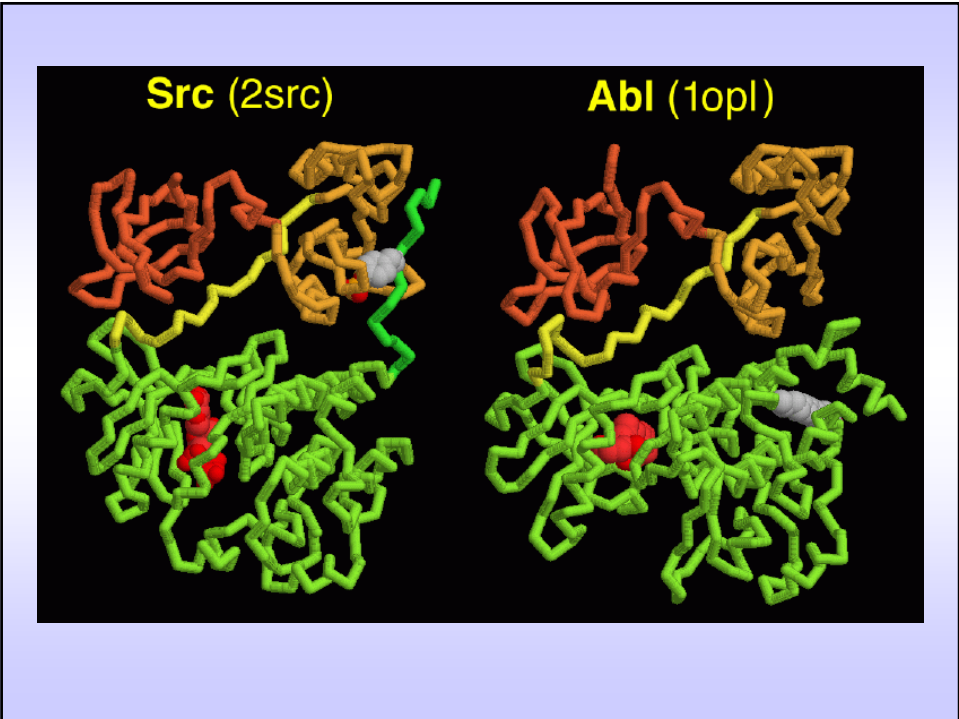
JAK1, JAK2,
TYK2, JAK3

ACK, ACK2



src-related

others



PHOSPHORYLATION SITE MAPPING
Prediction vs. reality

cSrc

| MAPPED | REAL |
|----------------------------|---|
| <p>Y 416 (ProSite)</p> | <p>Y 416 (activation loop) Y 527 (CSK site)</p> |

Abl- Phosphorylation sites

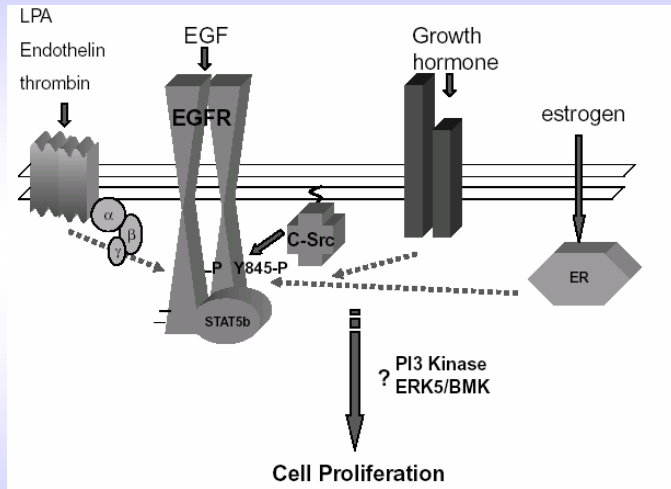
| Real | NetPhos | ProSite | ScanSite stringency |
|-------|---------|---------|------------------------|
| Y 125 | | | |
| Y 134 | | | + |
| Y 204 | + | | |
| Y 245 | | | + |
| Y 251 | | | |
| Y 272 | | | + |
| Y 276 | + | | + |
| Y 412 | | | |

Phosphorylation Mapping

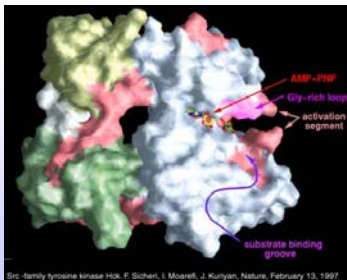
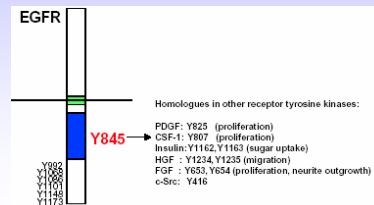
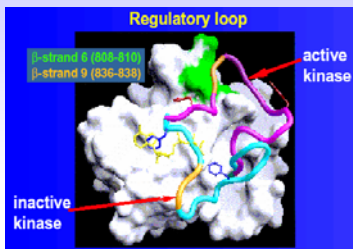
Problems to be aware of:

- ➔ Models might miss phosphorylation sites to bias in the mathematics underlying the program
- ➔ How can you be sure to have mapped all P- sites and exclude the false positives from mapping?? (look at 3D if available → buried residues unlikely to be accessible for kinases)
- ➔ Experimental models: in vivo vs. In vitro use of fusion proteins
→ influence on structure and accessibility of residues ?

Receptor Tyrosine Kinase-EGFR



EGFR and c-Src synergism



-Members of both families are overexpressed in ~70% of human breast cancers

-Tyr 845 shares high degree of homology among all tyrosine kinases

-Tyr 845 phosphorylation is mediated by cSrc...a unique characteristic

Receptor (Her2/Her3) and non-receptor (c-Src) tyrosine kinase domain similarities

| | | |
|--------------------|-----|---|
| EGFR.kin_dom | 1 | PHVSRLLGICLTSTVOLVTCQMPVGGCLLDVYVREHKDNHGSQYLLNWCVOIAKGMNVLED |
| HER2/ErbB2.kin_dom | 1 | FVYSRLLGICLTSTVQLVTCQMPVGGCLLDHVREHRCRLEGSDLLNWCVOIAKGMNVLED |
| HER3/ErbB3.kin_dom | 1 | AEIVRLLGICPGSSTQLVTCYVDFGSLLDHVREHRCRLEGFQLLNWCVOIAKGMNVLEE |
| SRC.kin_dom | 1 | EKLIVQLIAVYVSEEPYIVVTEHMSRSGSLDLELKGETGRVRLFCQVMAAQLASGMAMVGR |
| EGFR.kin_dom | 60 | RRLVHRDLAARNVLVKTPQHVKKTDFGLAKLLGAEKEKYHAEGGKVPKWMMALESILHR |
| HER2/ErbB2.kin_dom | 60 | VRLVHRDLAARNVLKSPNHVKKTDFGLARLLIDEIYHADGGKVPKWMMALESILRR |
| HER3/ErbB3.kin_dom | 60 | HGIVHRDLAARNVLKSESCVQVADFGVADLPPDKKLLYSEARKPIKWMMALESIFHG |
| SRC.kin_dom | 61 | MNYVHRDLAARNLVGENLVCKVADFGLARLLEQNEYTARQCAKPKPKWMALESILVY |
| EGFR.kin_dom | 119 | IVTHQSDVWSYGVTVWELMTFGSKPYDGPASEISSLLEKGERLPOPPICITIDVYIMV |
| HER2/ErbB2.kin_dom | 119 | RIVTHQSDVWSYGVTVWELMTFGAKPYDGPAREIPDLLEKGERLPOPPICITIDVYIMV |
| HER3/ErbB3.kin_dom | 119 | KIVTHQSDVWSYGVTVWELMTFGAEPYAGIRLAEVDPDLLEKGERLPOPPICITIDVYIMV |
| SRC.kin_dom | 119 | RIVTHQSDVWSYGVTVWELMTFGSKPYDGPAREIPDLLEKGERLPOPPICITIDVYIMV |
| EGFR.kin_dom | 178 | KCWMIDAEKSRPRFRELVEFS |
| HER2/ErbB2.kin_dom | 178 | KCWMIDAEKCRPRFRELVEFS |
| HER3/ErbB3.kin_dom | 178 | KCWMIDENIRPTFKELANEE |
| SRC.kin_dom | 178 | QCWRKEEERPTFELVCAE |

The ErbB signalling network →

