Micro 204

TCR Signal Transduction

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October 15, 2018

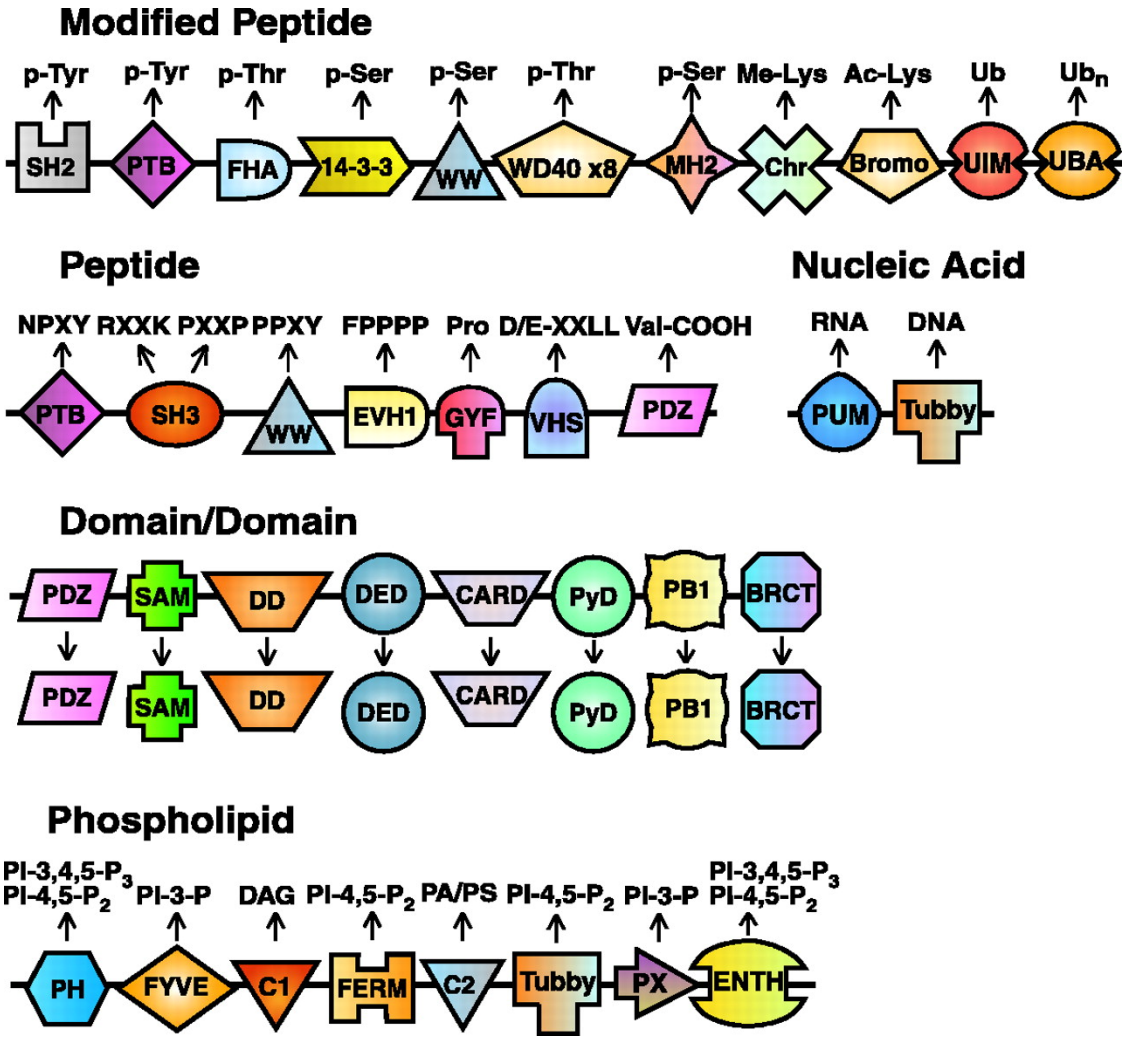
Signal Transduction: What is it?

Definition: *Signal transduction is the transmission of information from outside of the cell or within the cell to convey information that could induce or affect a change in the cellular status.*

How is it accomplished? (a few examples)

Changes in protein or lipid phosphorylation
Other protein modifications (i.e., ubiquitylation, sulfenylation glycosylation, etc.)
Changes in protein localization
Changes in protein:protein interactions
Changes in protein:lipid interactions
Assembly of macromoleculear complexes
Disassembly of protein complexes
Protein degradation
Changes in lipid turnover
Generation of second messengers
Ionic fluxes
Signal dampening or amplification
Feedback loops, positive or negative

Modular Protein Interaction Domains Are Involved in Signaling Pathways

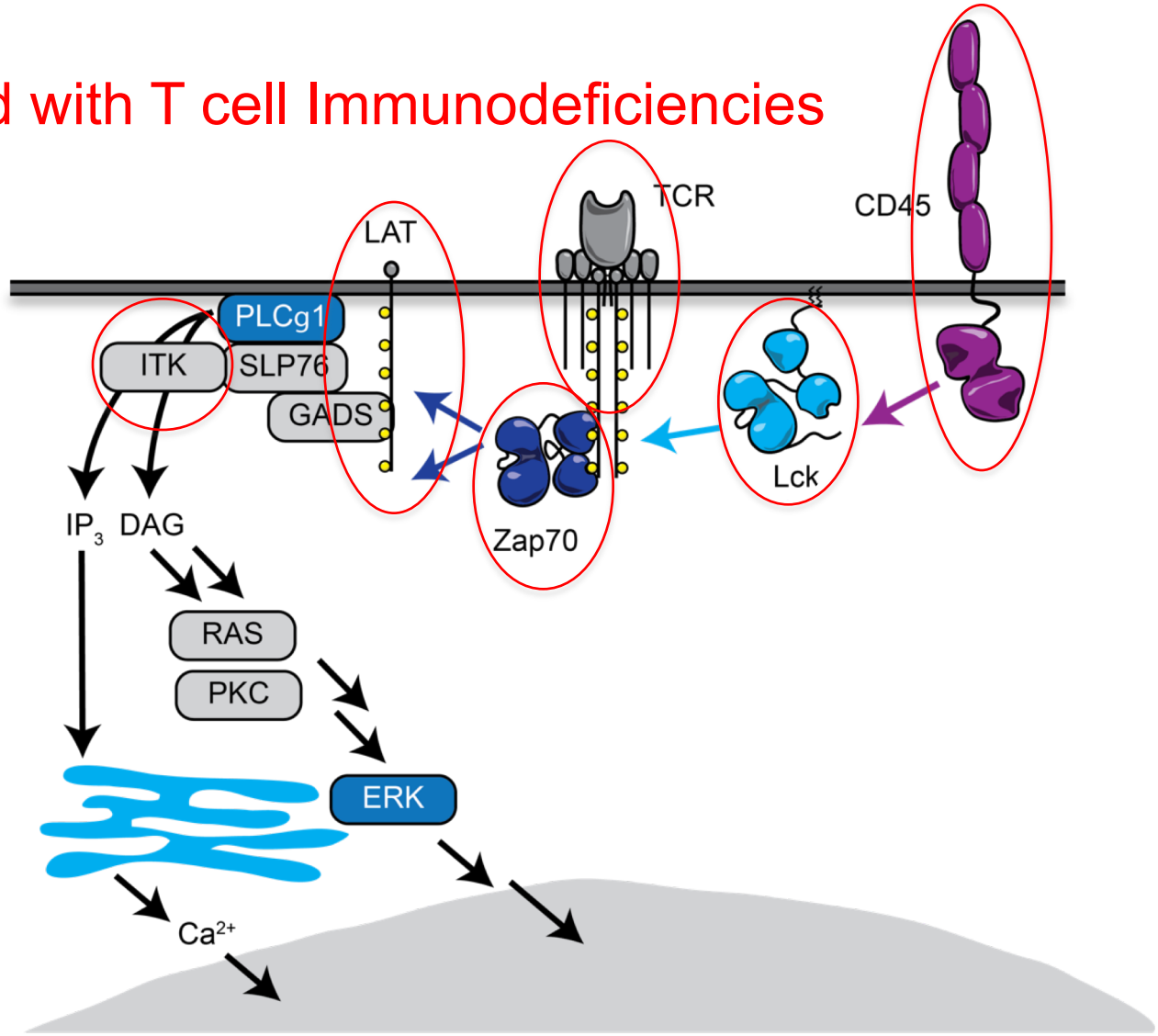


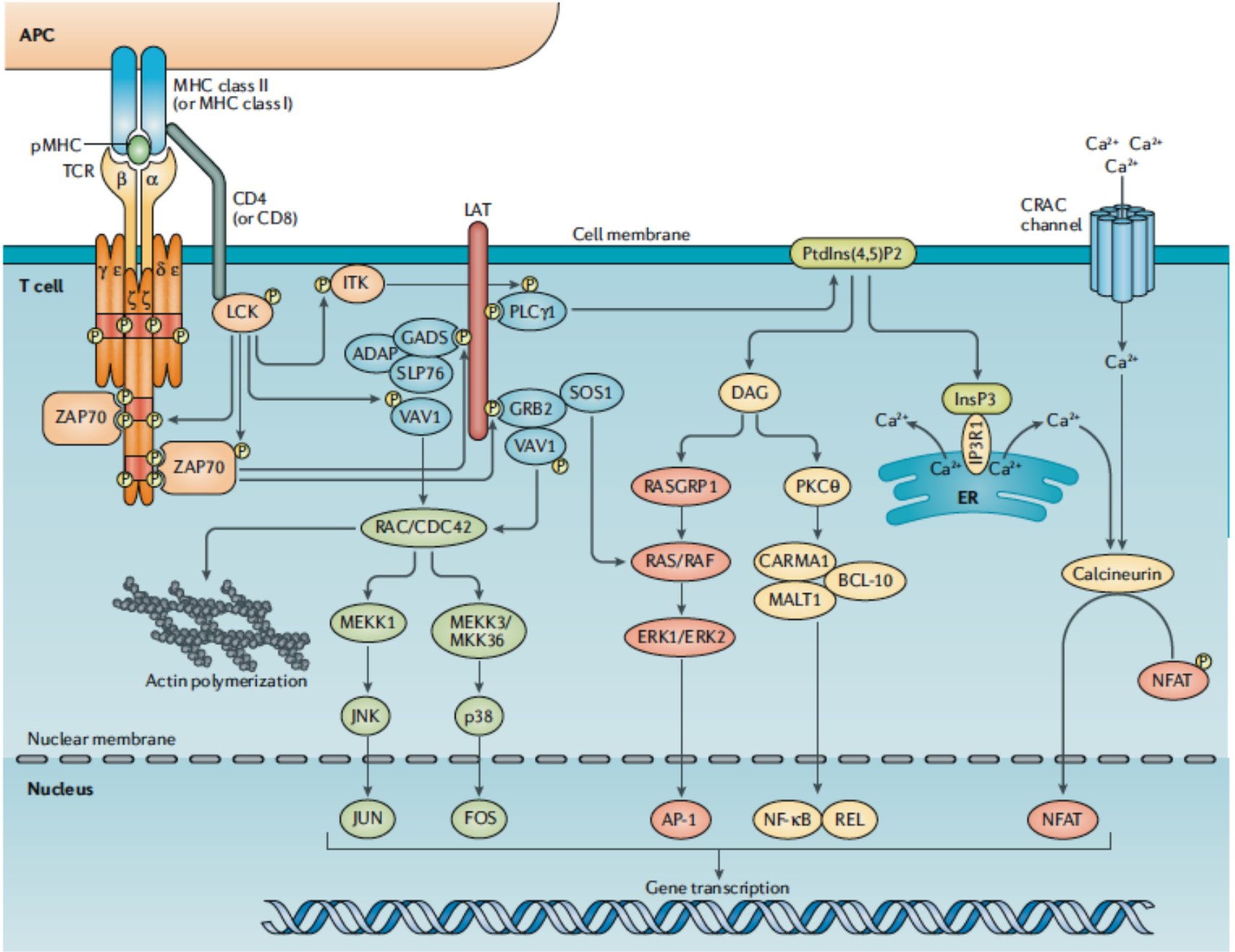
T Pawson, P Nash Science 2003;300:445-452

Early TCR signaling events

Associated with T cell Immunodeficiencies

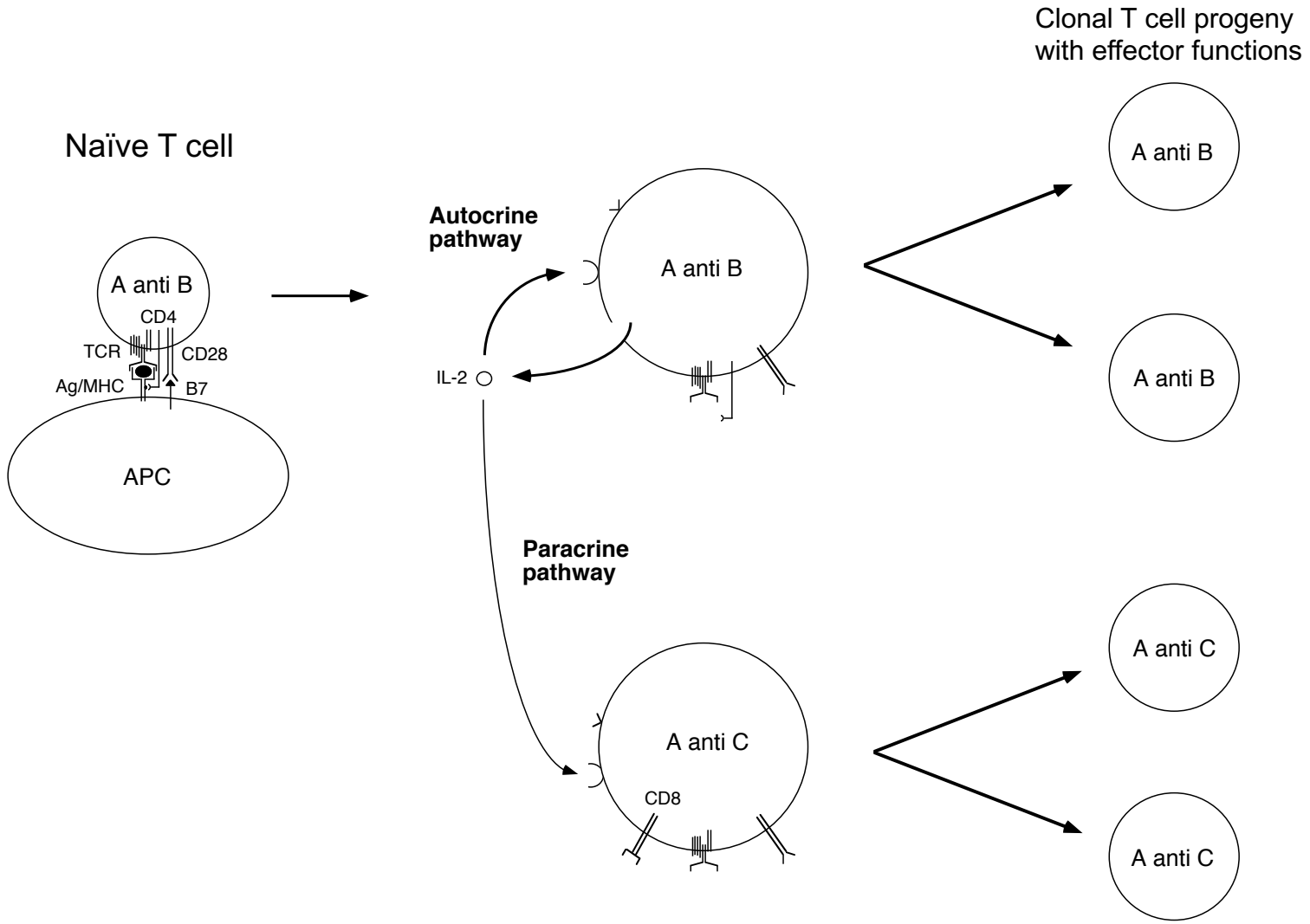
Others:
MAGT1
Orai1
STIM1

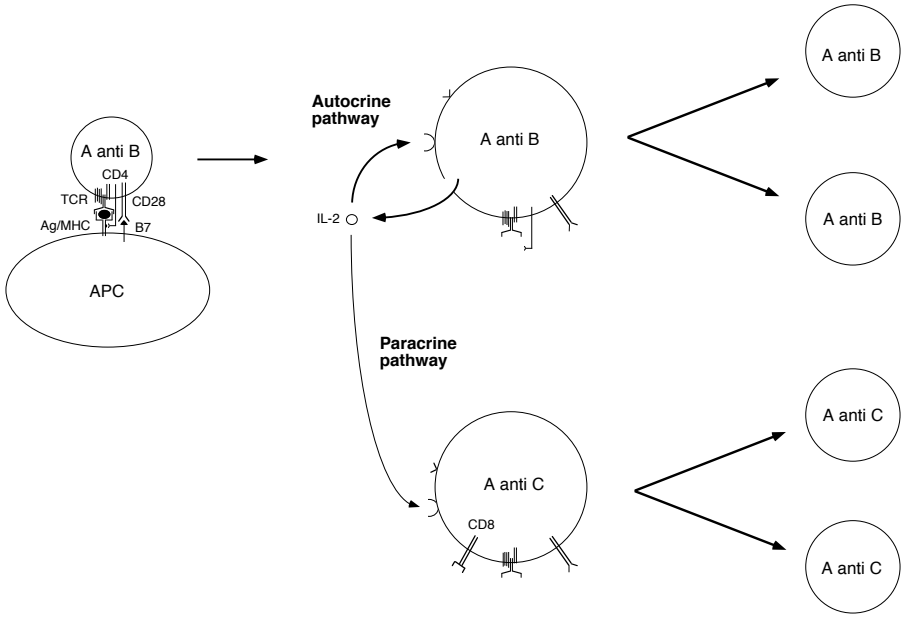




Gaud, et al., Nat. Rev. Immunol., 2018

TCR Signaling is Required for the Activation, Clonal Proliferation and Differentiation of Naïve T Cells

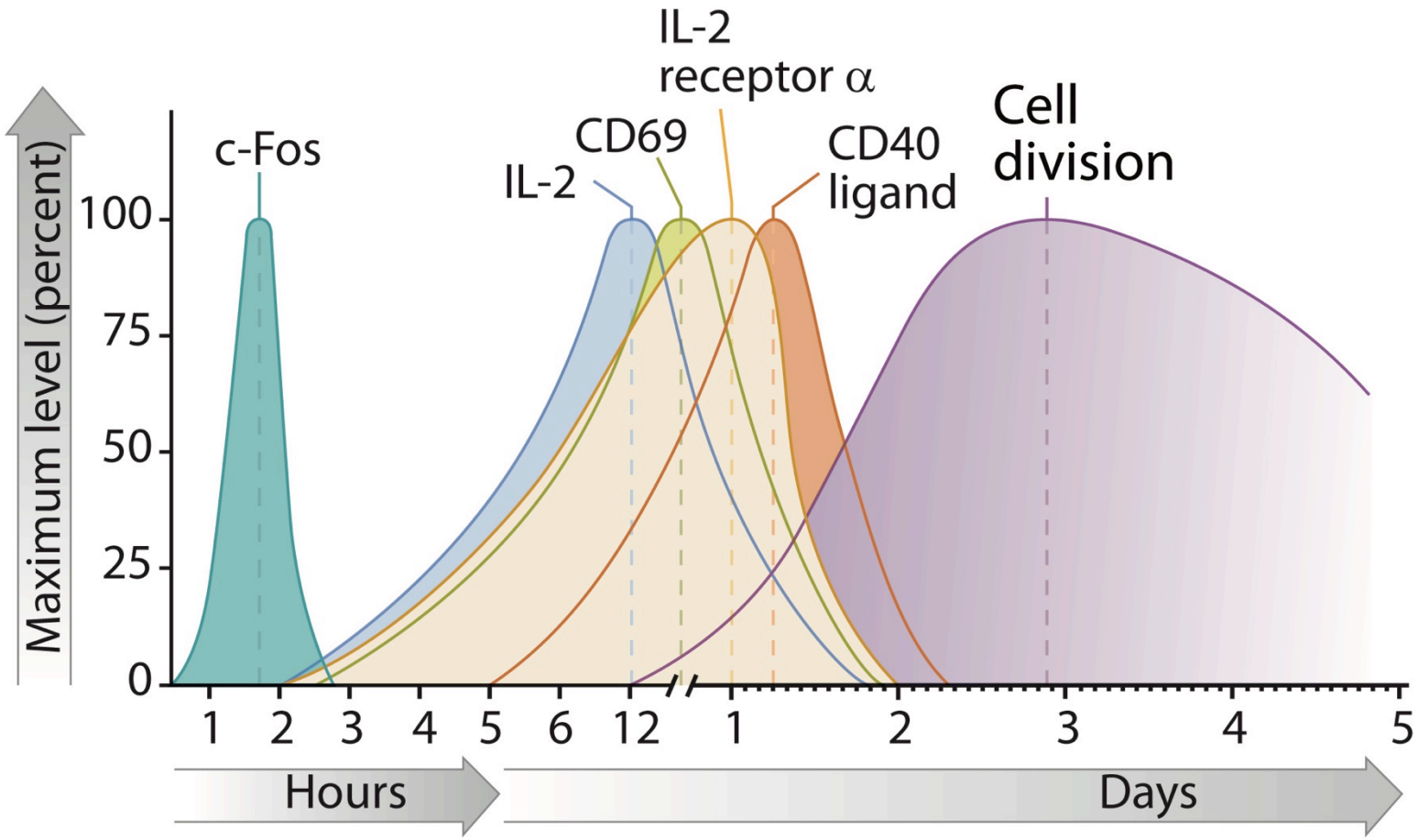




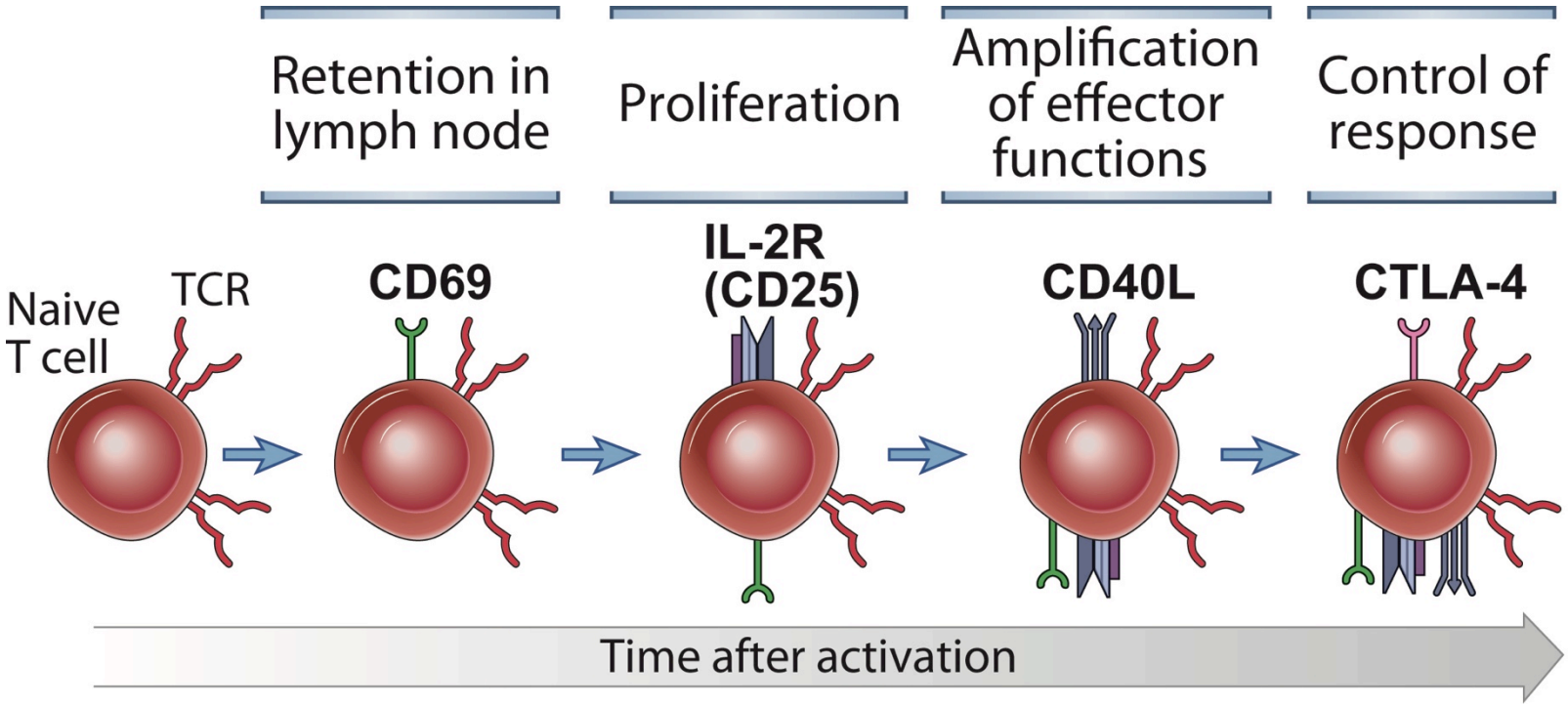
Key Points:

1. T cell activation occurs during a complex cell-cell interaction
2. Stimulation of a quiescent G_O T cell results in cell cycle progression as well as enormous metabolic and synthetic changes and cell enlargement
3. Activation is initiated by the TCR, but also requires multiple other receptors involved in intercellular interactions (i.e., costimulation via CD28)
4. Key event is the production of lymphokines and the cellular response to them
5. End result is the clonal expansion and acquisition of effector functions

Genes Expressed After T Cell Activation: Time Scale



Molecules Expressed After T Cell Activation

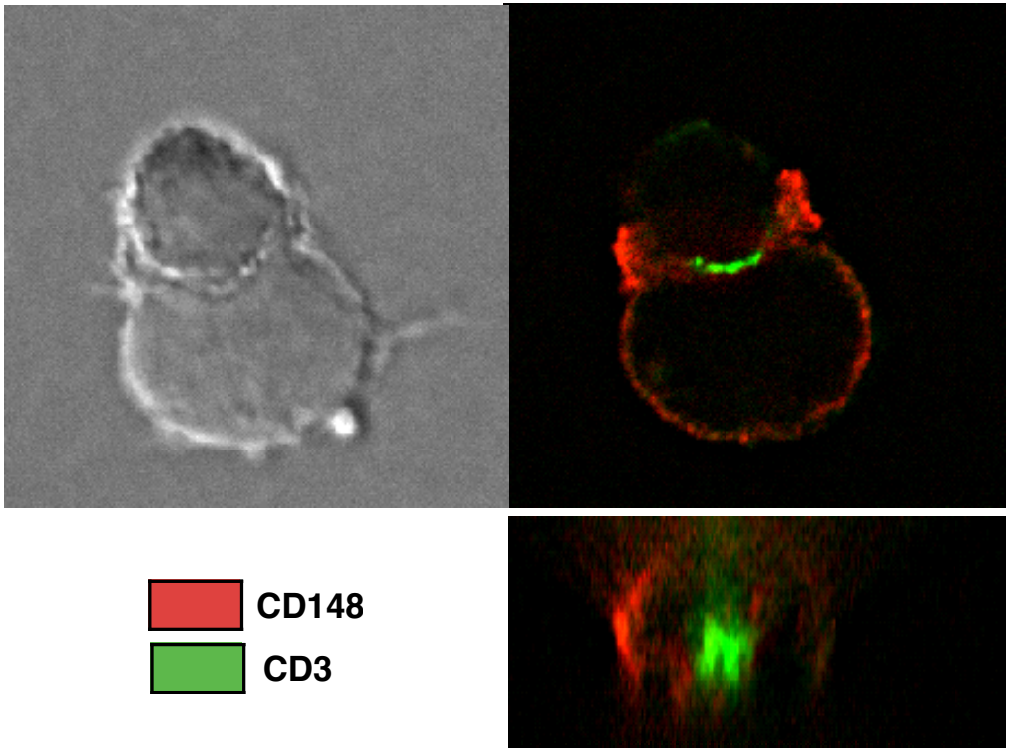


Consequences of Antigen Receptor Signaling

- Biochemical changes
  - Protein phosphorylation and other modifications
  - Phospholipid turnover
  - Ionic changes
  - Protein relocalization
  - Proteolysis
- Cytoskeletal Reorganization/Cell Polarization
  - Directed cytotoxicity
  - Directed cytokine secretion
- Gene Transcription
  - Cytokines and their receptors
  - Cell surface molecules
  - Antibody production
- Effector Functions
  - Cytokine secretion
  - Cytotoxicity
  - Antibody class switching
- Cell proliferation
- Apoptosis

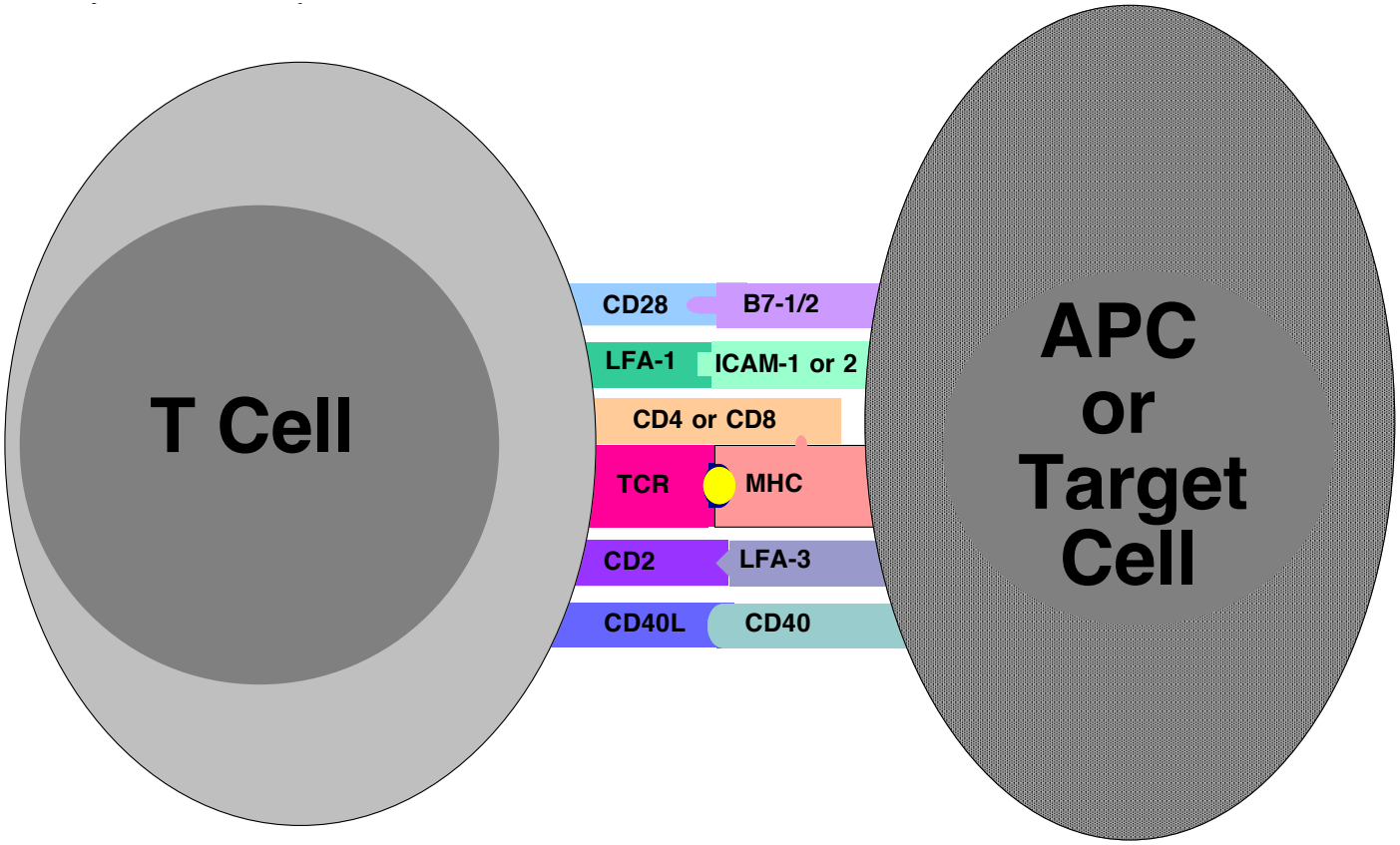
Immunologic Synapse:
A spatial and temporal segregation of cell surface molecules

Jurkat:APC conjugates with SAg



Lin and Weiss, J. Cell Biol., 2003

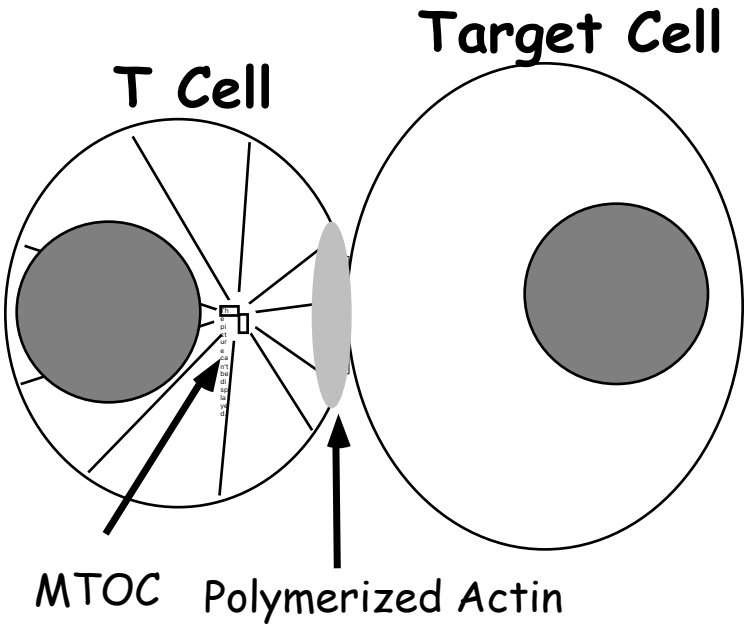
Complexity of the T Cell : Antigen Presenting Cell Interaction



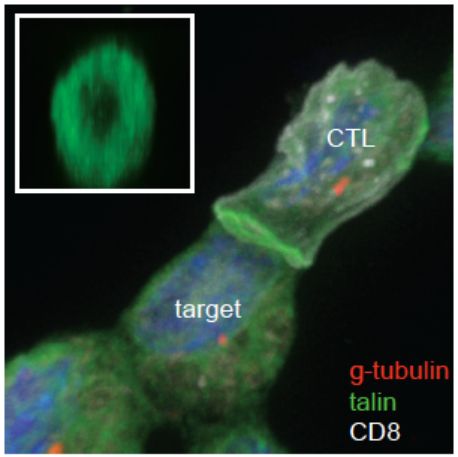
Intermolecular Interactions

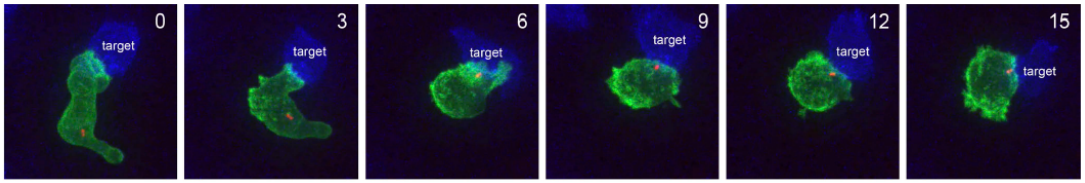
Antigen recognition:	TCR – pMHC
Coreceptor function:	CD4/CD8 - MHC
Adhesion:	LFA-1 – ICAM1
	CD2 – LFA-3
Costimulation:	CD28 – B7-1/2
Differentiation:	CD40L – CD40
	Cytokines

T Cells Polarize During Antigen-Specific Recognition



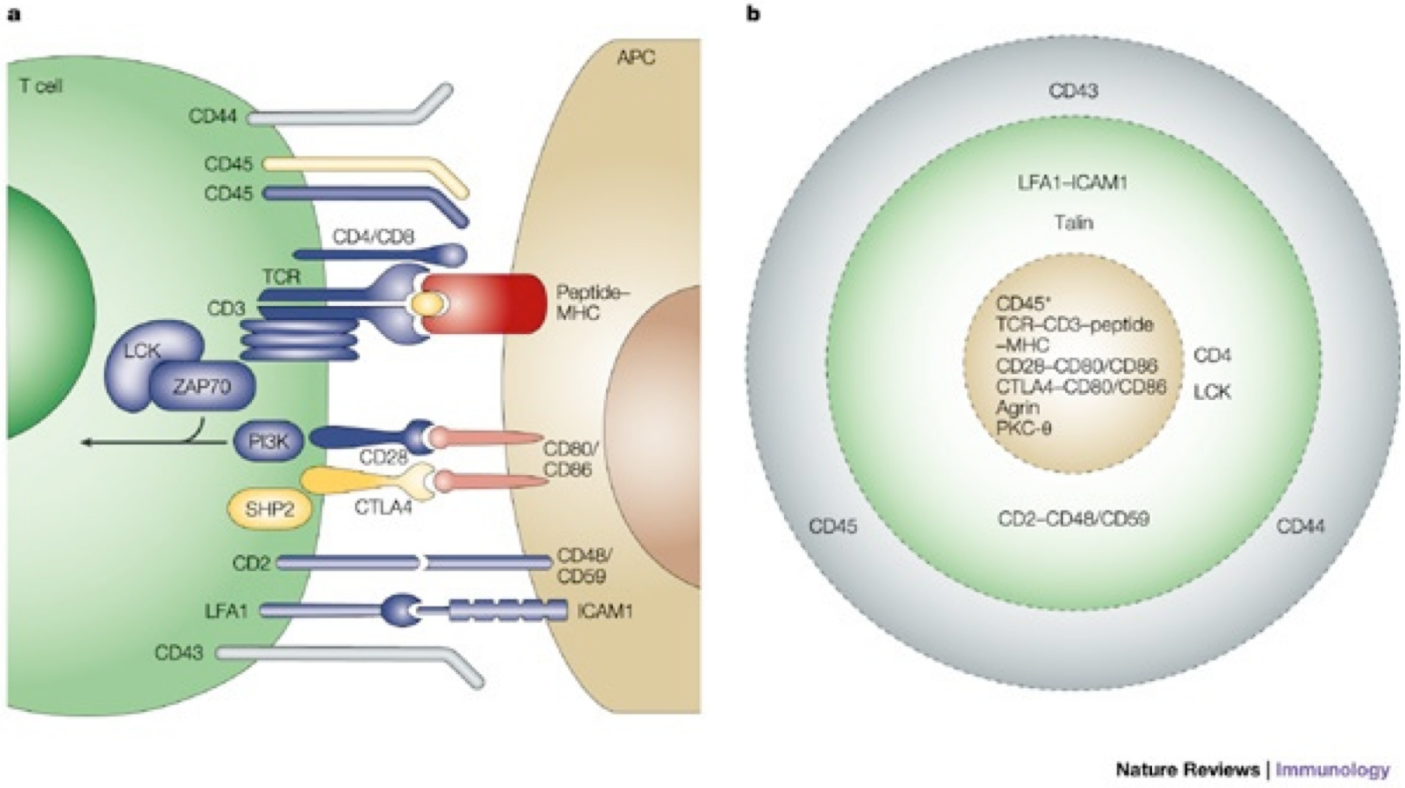
1. T cell cytoskeleton polarizes; APC does not
2. MTOC and microtubules polarize
3. Actin cytoskeleton polymerizes and polarizes
4. Requires TCR signals: Rho and Rac GTPases
5. Polarization of cytoskeletal elements contribute to polarized secretion of cytolytic granules or lymphokines





Jenkins, et al. eLIFE, 2014

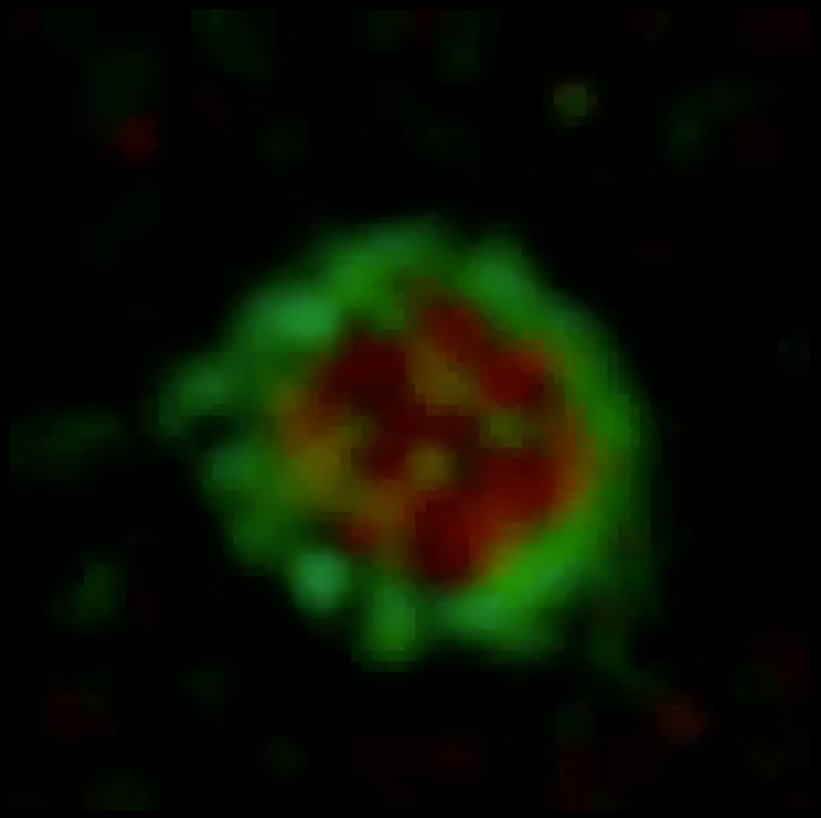
Immunologic Synapse:
A spatial and temporal segregation of cell surface molecules



Avi Kupfer - deconvolution IF microscopy of T cell/APC interactions - organized, bulls-eye type of structure

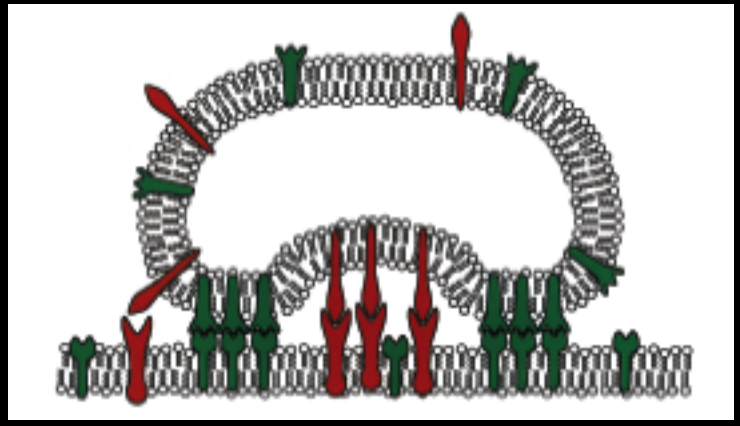
Non-activating, altered, peptides do not support the formation of these structures

Formation of an Immunological Synapse Involves Coordinated Spatial Organization



Red= ICAM-1

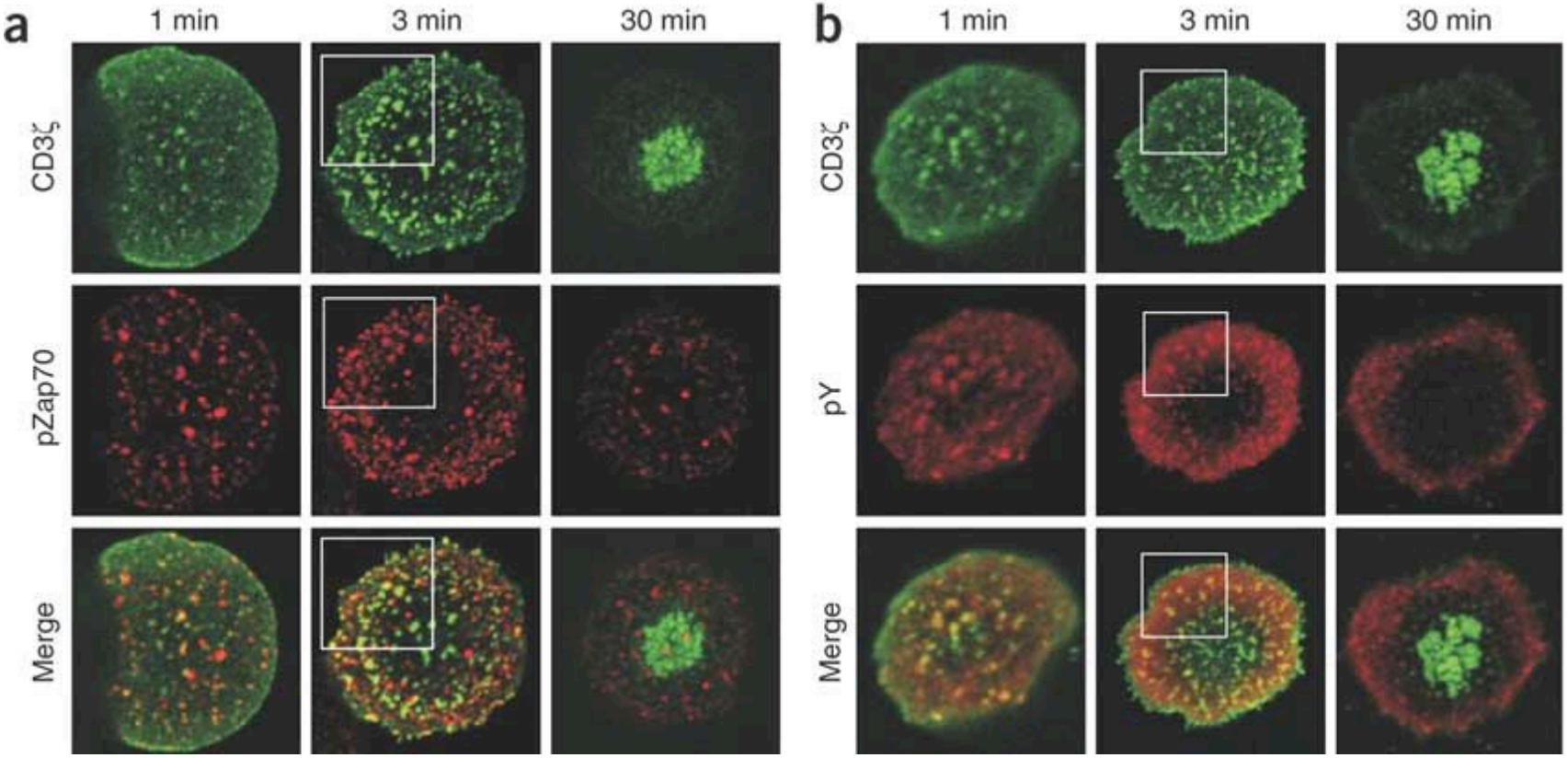
Green=MHC/pep



Grakoui et al. (1999) Science *285*:221.

Signaling Occurs in TCR Microclusters at the Periphery of the Synapse

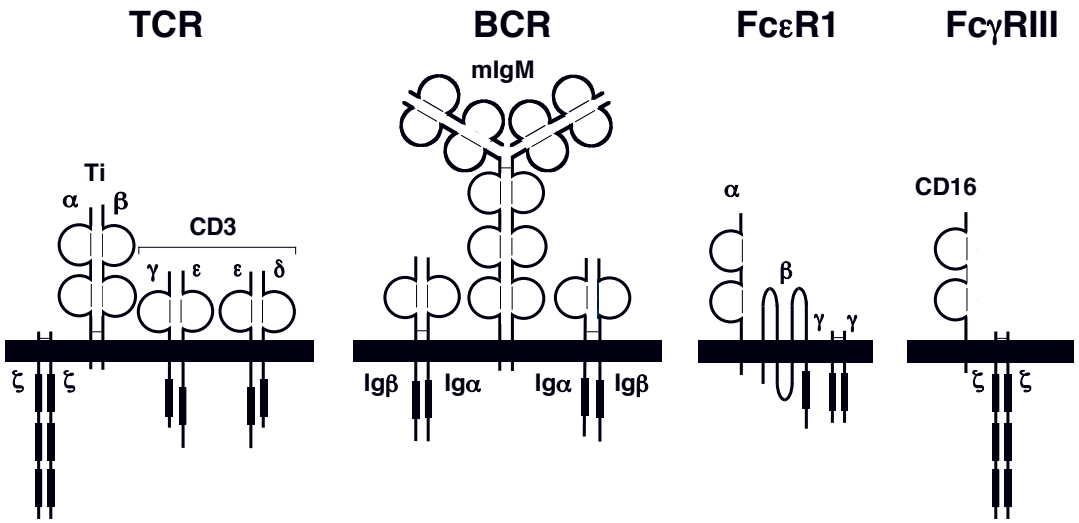
Yokosuka et al., Nature Immunol. 6, 1253, 2005



Function(s) of the Immunological Synapse?

1. **Signal Initiation**: *probably not*
   - P-tyr and Calcium responses precede mature synapse formation
2. **Signal enhancement**: *perhaps*
   - But no biochemical correlate and mature synapse does not appear to be required (CD2-AP KO signals better than wt but does not form synapse)
   - Peripheral microclusters appear to be most active signaling complexes
3. **Directed secretion**: *fits well*
   - Directed secretion by CTL and Th cells makes teological sense and has been well documented
4. **Receptor downmodulation**: *likely*
   - Modeling and CD2-AP KO data supports this
   - New data on TCR microclusters also supportive
   - Some of the central TCR clusters are in internal structures and may even be in extracellular vesicles

How does ligand binding induce TCR signaling?



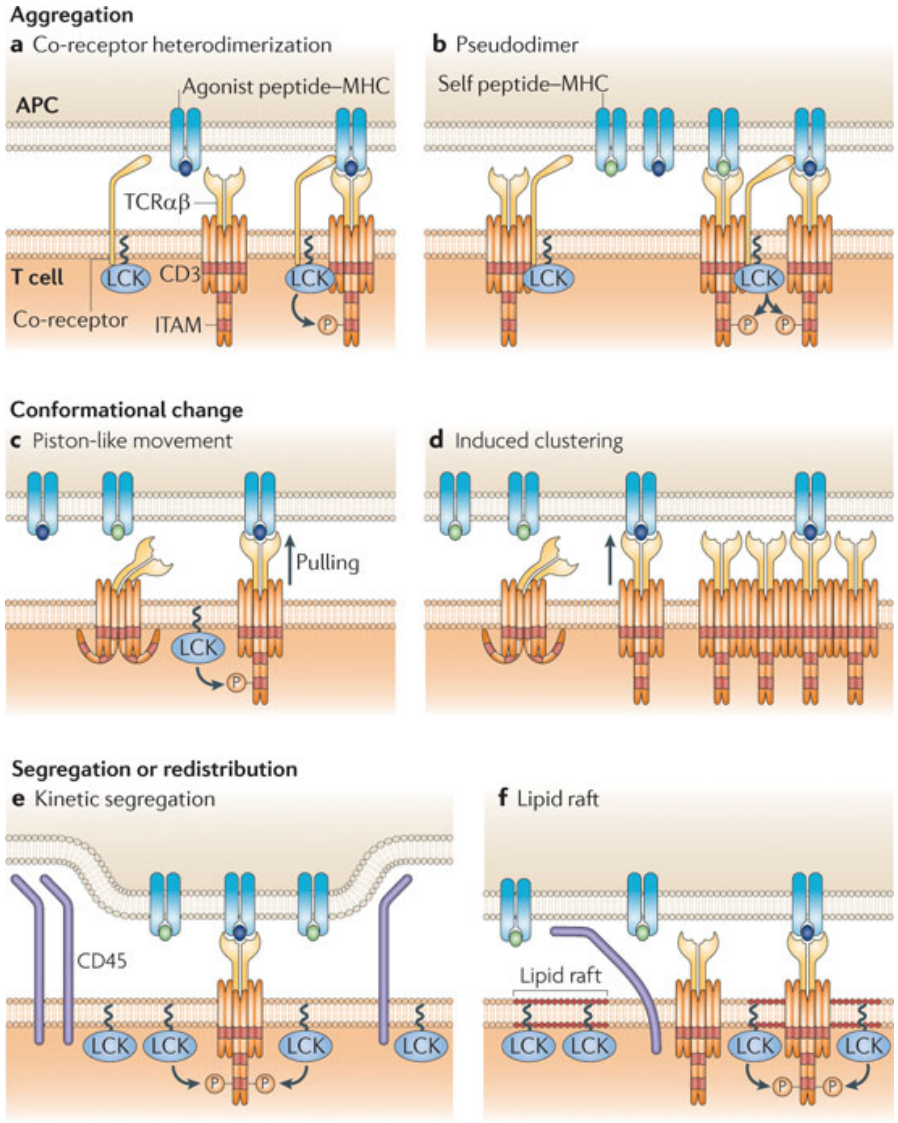
ITAMs
(Immunoreceptor Tyrosine-Based Activation Motif)

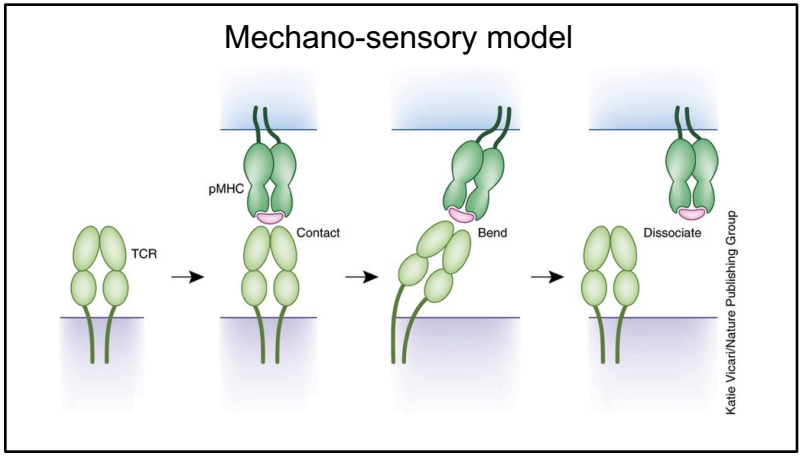
hζ1	N Q L Y N E L N L G R R E E – Y D V L
hζ2	E G L Y N E L Q K D K M A E A Y S E I
hζ3	D G L Y Q G L S T A T K D T – Y D A L
hCD3γ	D Q L Y Q P L K D R E D D Q – Y S H L
hCD3ε	N P D Y E P I R K G Q R D L – Y S G L
hCD3δ	D Q V Y Q P L R D R D D A Q – Y S H L
hDAP12	E S P Y Q E L Q G Q R S D V – Y S D L
rIgE FcR γ	D A V Y T G L N T R N Q E T – Y E T L
rIgE FcR β	D R L Y E E L – H V Y S P I – Y S A L
mIg α	E N L Y E G L N L D D C S M – Y E D I
mIg β	D H T Y E G L N I D Q T A T – Y E D I
BLV gp30	D S D Y Q A L L P S A P E I – Y S H L
EBV LMP-2	H S D Y Q P L G T Q D Q S L – Y L G L
SIV Nef	G D L Y E R L L R A R G E T – Y G R L
KSHV	L Q D Y Y S L H D L C T E D – Y T Q P
Consensus	– – – Y – – L – – – – – – – – Y – – L

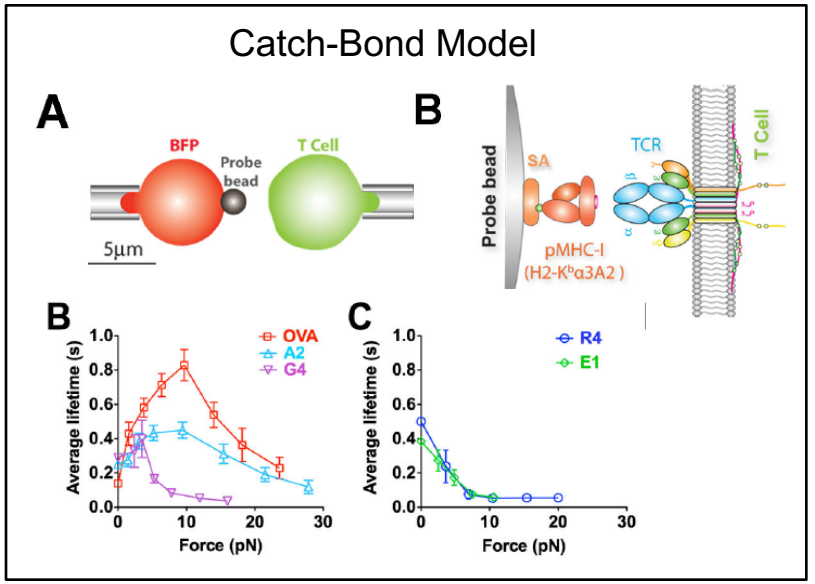
1. *How do TCRs transmit their ligand occupancy state across the plasma membrane?*

2. *Do TCRs undergo conformational changes when they bind ligand?*

Models of TCR triggering





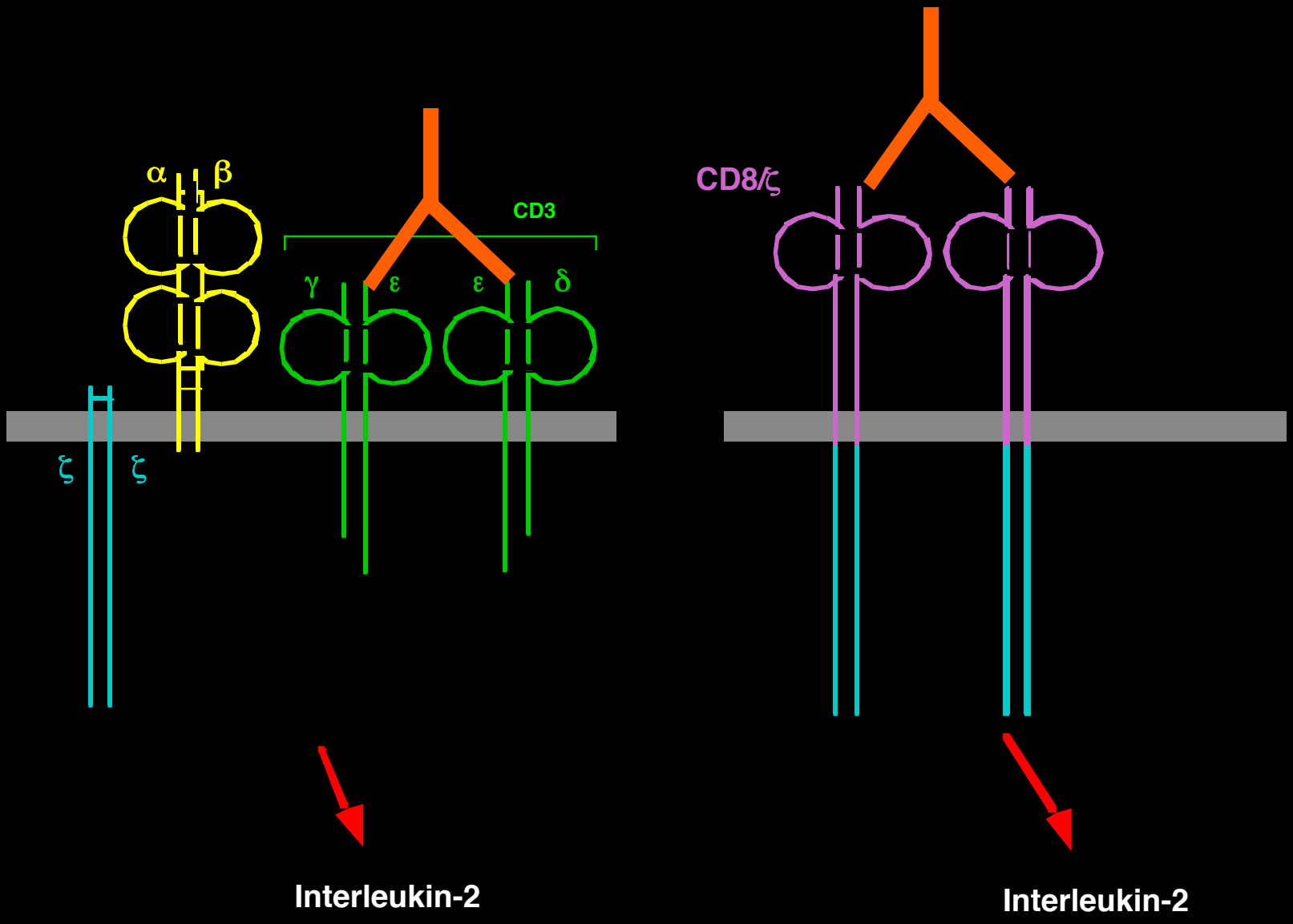


Van der Merwe & Duschek, Nature Rev. Immunol
Chakraborty and Weiss, Nature Immunol., 2014;
Liu, et al., Cell, 2015

Support for the Oligomerization Model

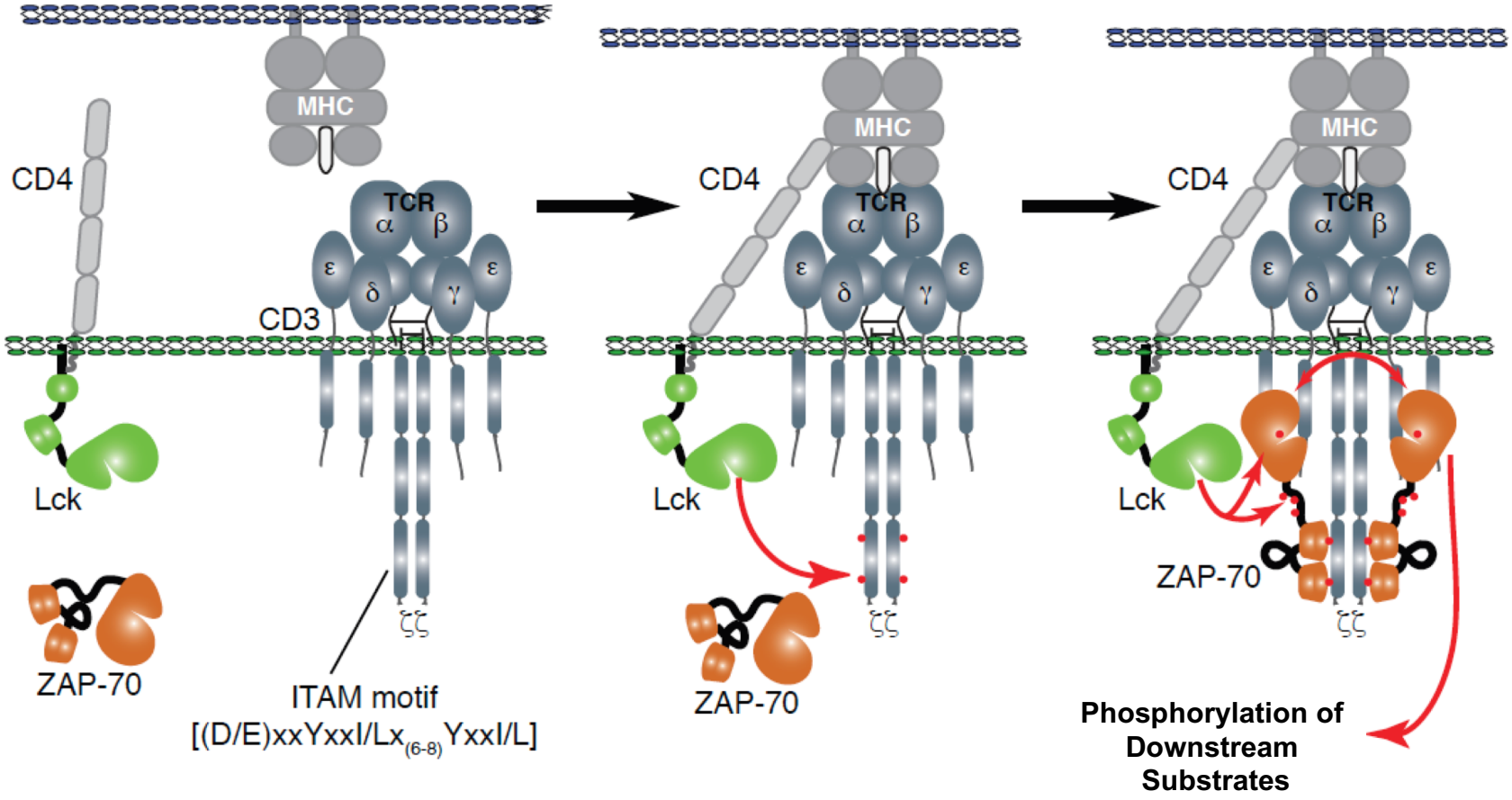
- Bivalent (but usually not univalent Fab) mAbs can induce T cell responses
- Many different anti-TCR mAbs can induce TCR signaling
- The more multivalent the mAb or pMHC (i.e., tetramer), the better
- Chimeric receptors (ITAM containing chimeras) signal
- Hetero-oligomerization with coreceptors (ie. CD4) increases sensitivity to peptide/MHC molecule

mAbs Against the TCR/CD3 Complex or Chimeric Receptors can Induce Similar Signals and Responses



A Hetero-Oligomerization Model:

CD4 and TCR are Brought Together During Antigen Recognition

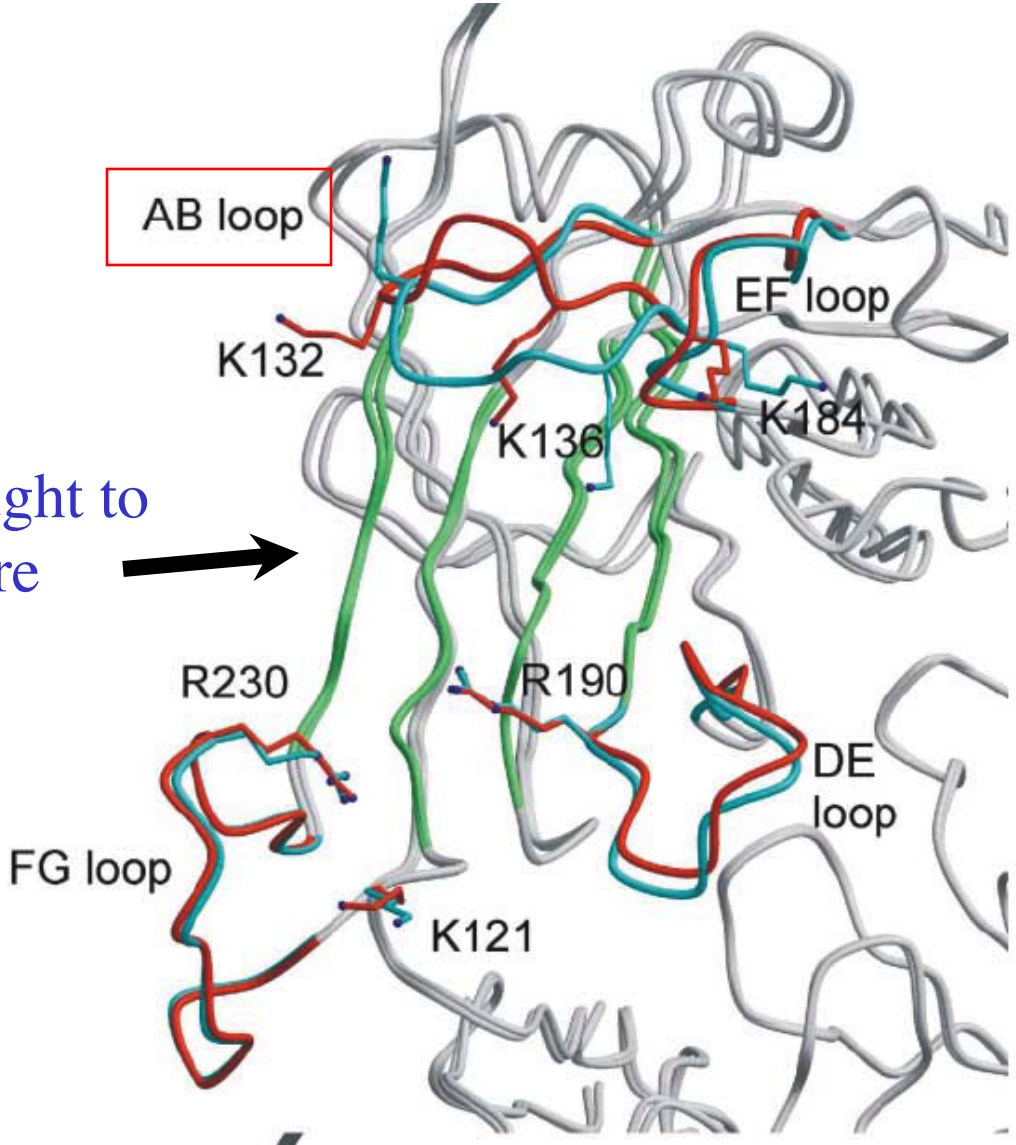


Weiss, Cell, 1993
Wang, et al., CSHL, 2009

Some data support a conformational change

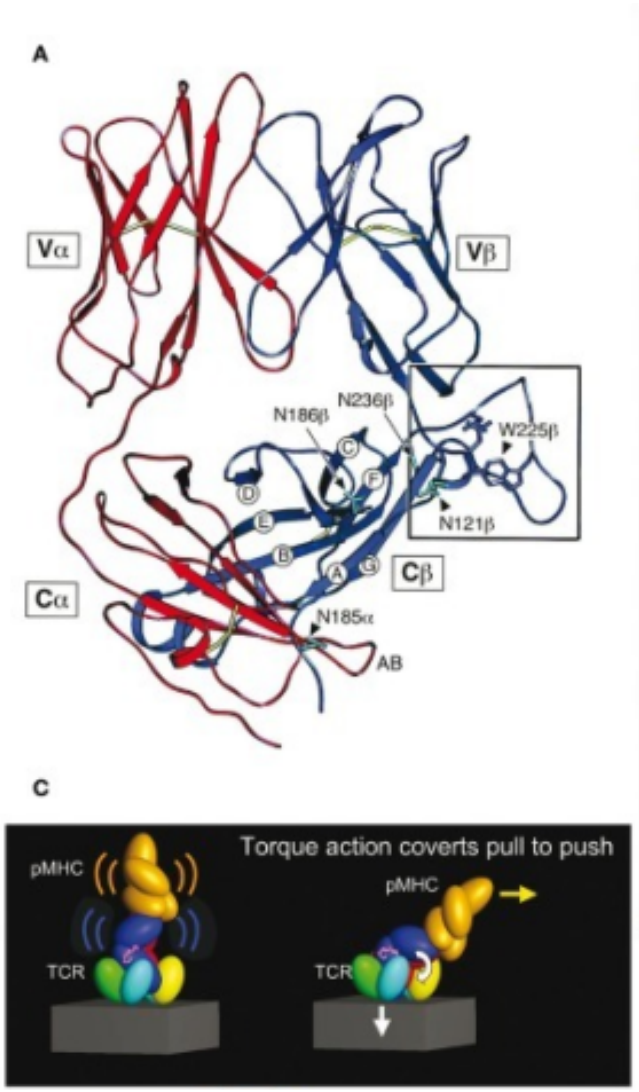
Conformational Change in the TCRα Constant Region A-B Loop in the LC13 TCR

Kjer-Nielsen, et al., Immunity, 2003



Ligated - red
Unligated - cyan

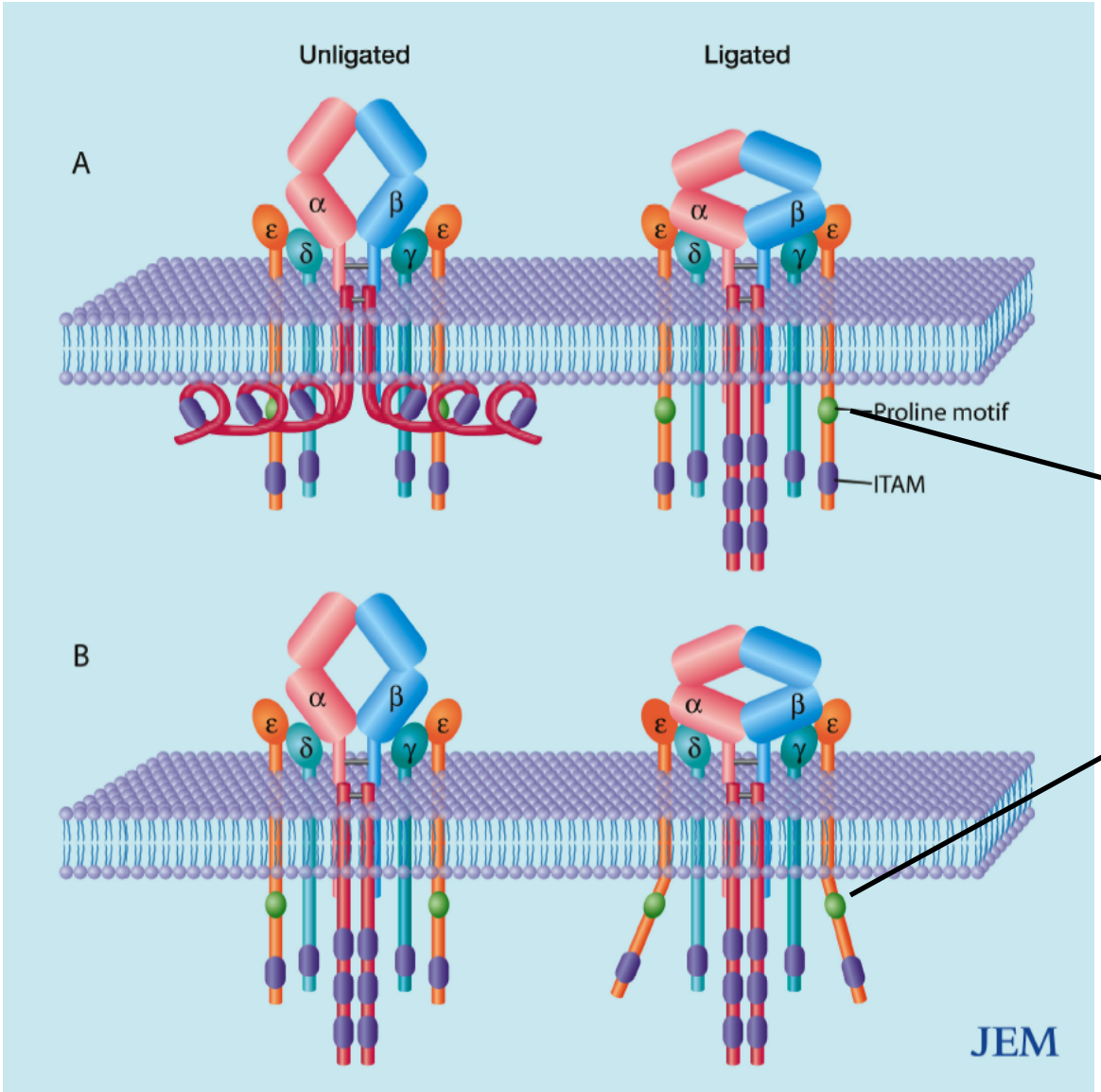
The TCR as a Mechanosensor



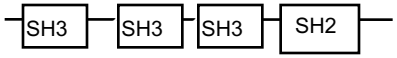
Kim, et al, Front. Immunol. 2012

A CD3ε Proline Motif May Become Accessible During a Putative TCR Conformational Change: *Models*

(Alarcon, et al, *Cell*, 2002;
Gil, et al, *J. Exp. Med.*, 2005;
Levin and Weiss, *J. Exp. Med.*, 2005)



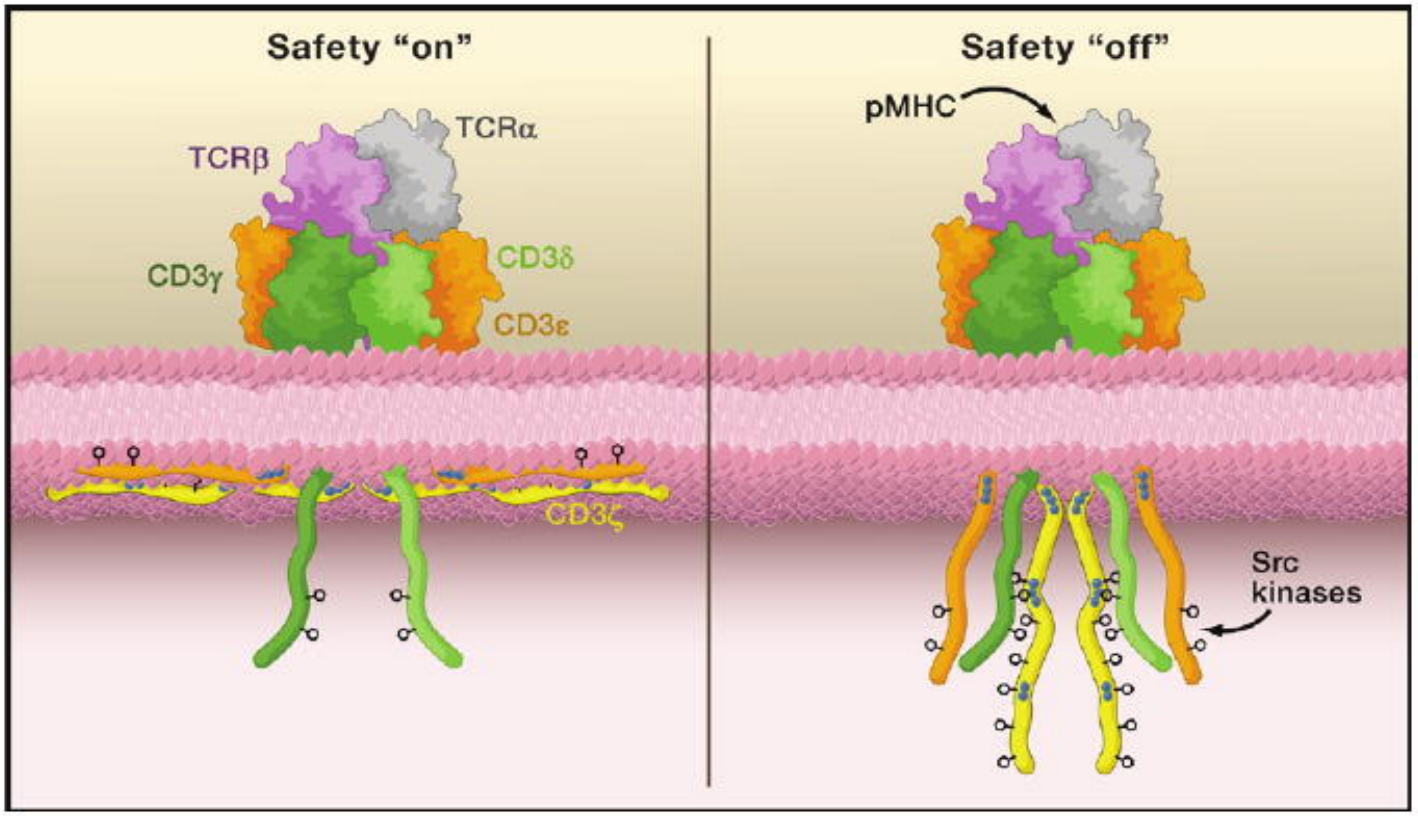
Nck



actin cytoskeleton
Pak kinase
????

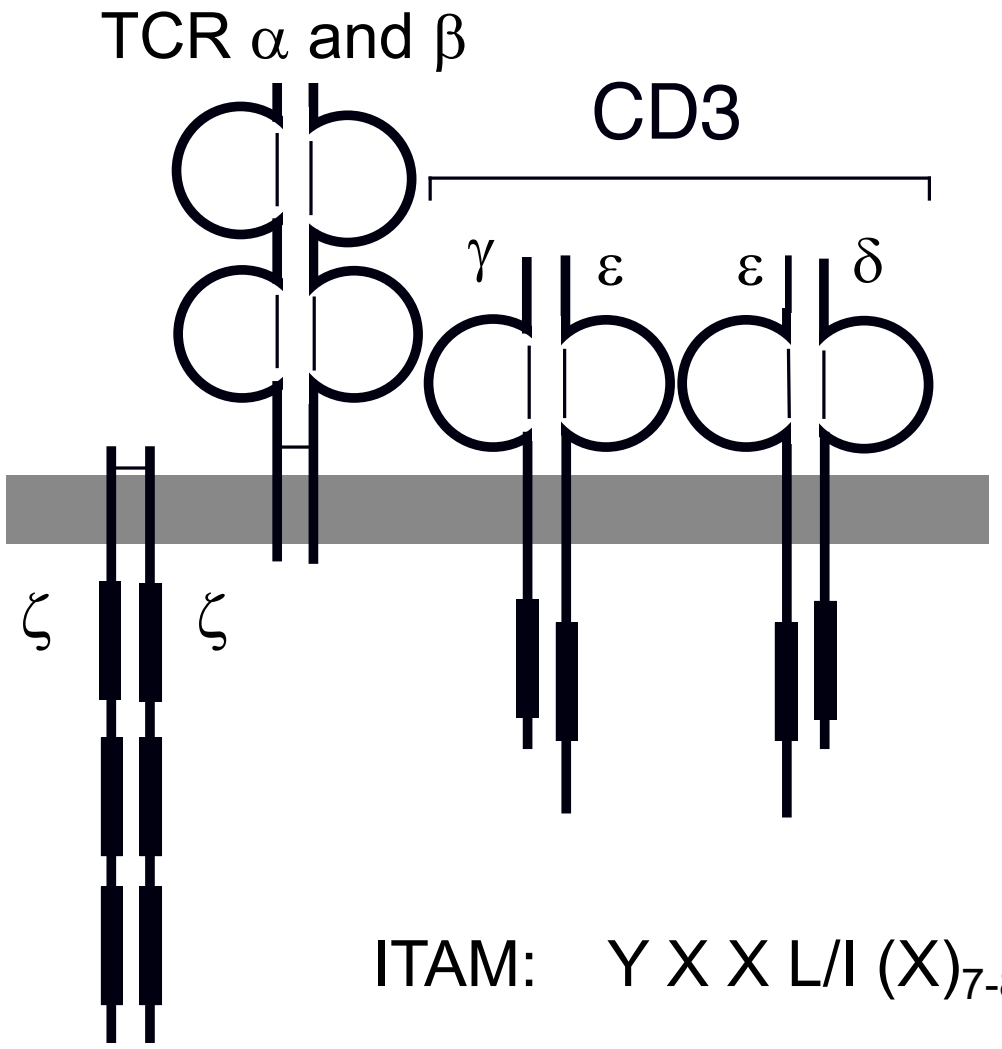
Non-Phosphorylated Tyrosines in ITAMs May be Sequestered by Hydrophobic Interactions with Lipids in Plasma Membrane

Liu, et al., Cell, 2008



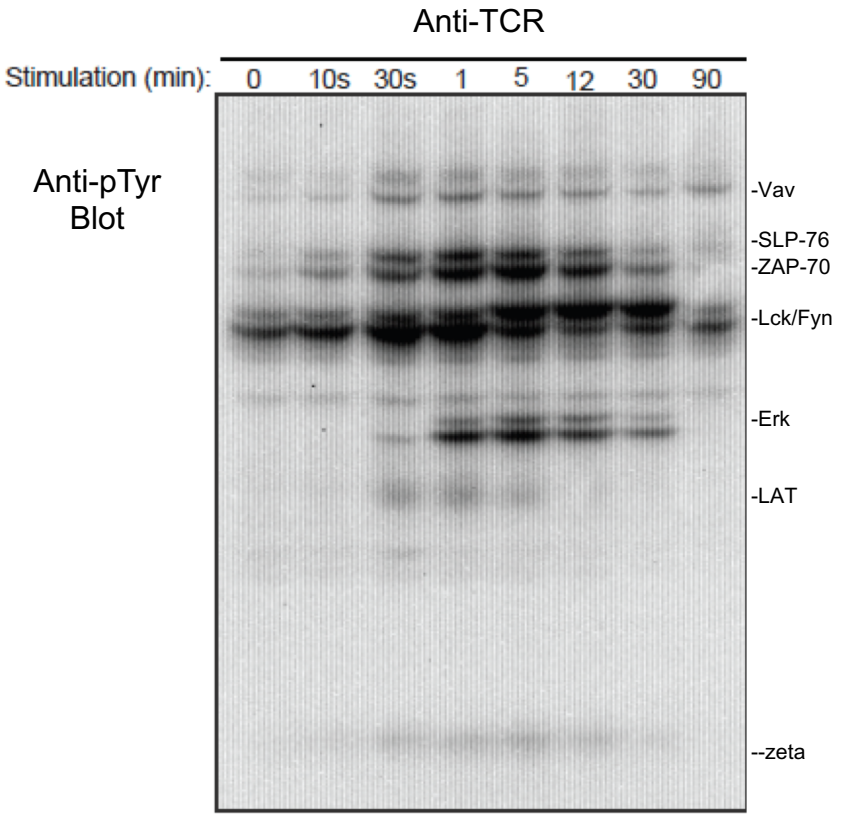
Preview by Kuhns and Davis, Cell, 2008

The TCR is an Oligomer and Utilizes ITAMs to Initiate Signal Transduction



ITAM: Y X X L/I $(X)_{7\text{-}8}$ Y X X L/I

TCR Cross-linking Induces Rapid Protein Tyrosine Phosphorylation

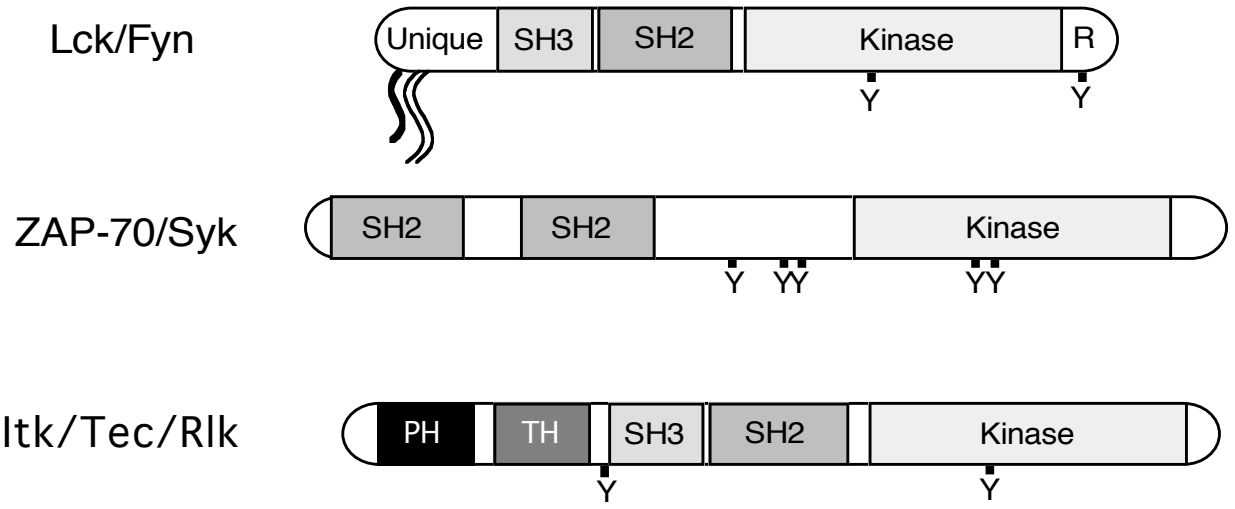


Jurkat T cell Leukemic Line stimulated with anti-TCR mAb

TCR signaling is initiated by protein tyrosine phosphorylation which can:

Induce conformational changes
Promote recruitment to the membrane
Promote protein-protein interactions
Activate enzymes (kinases, phosphatases, PI3-kinase, PLC-γ1)
Inactivate enzymes
Promote ubiquitination

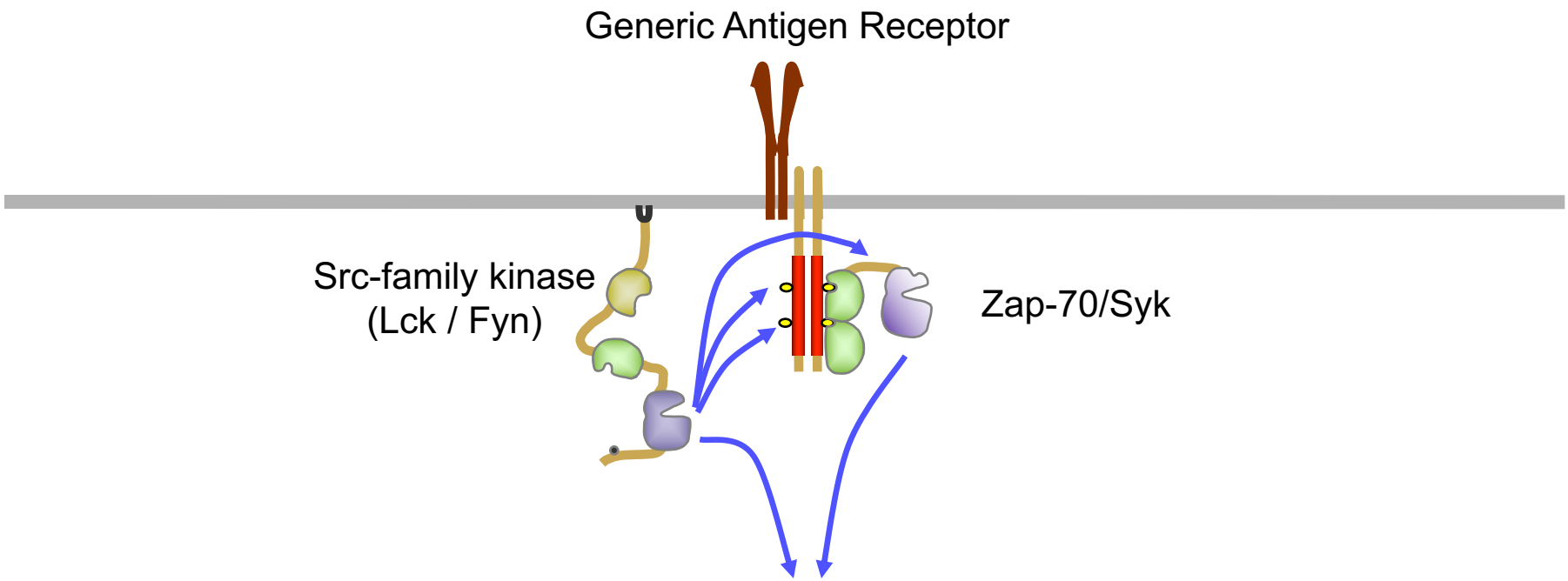
Three Families of Cytoplasmic Protein Tyrosine Kinases Are Involved in TCR Signal Transduction



Protein Interaction modules

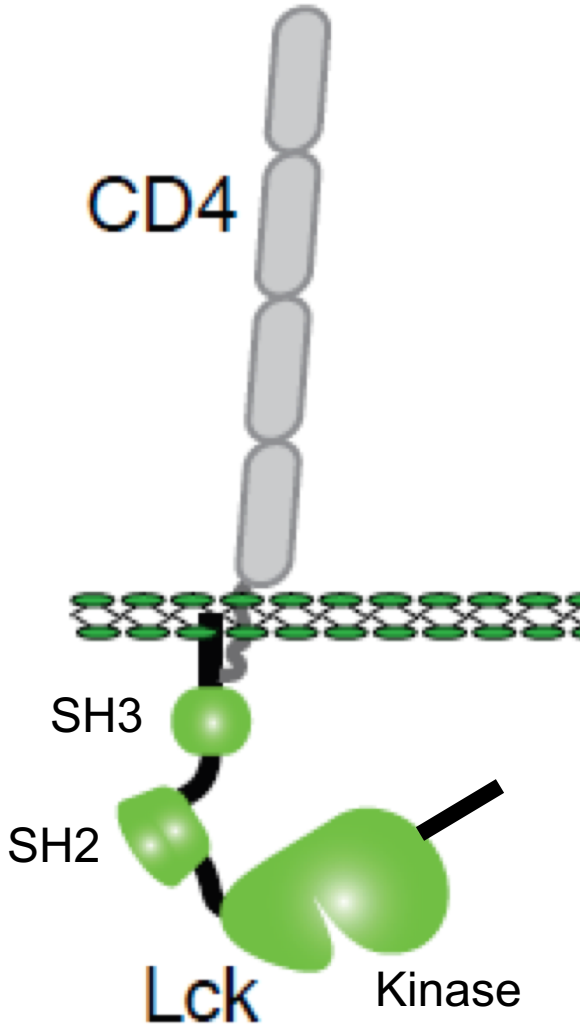
SH2 Domains - Interact with tyrosine phosphorylated sequences
SH3 Domains - Interact with proline-rich sequences
PH Domains - Interact with membrane phospholipids
TH Domains - Unique to Tec family kinases - proline rich

SFKs Are Critical Initiators of Antigen Receptor Signaling, So their Regulation is Important



Tyrosine phosphorylation, MAPK activation (P-Erk)
Intra-cellular calcium increase…

Lck, the Kinase that Initiates TCR signaling



Associates non-covalently with **CD4** and **CD8** via C-C rich motifs

Src Kinase Family Member

Peripheral Membrane Protein

- Palymitoylated
- Myristoylated
- Enriched in lipid rafts

Lipid rafts, GEMs, DIGs

Cholesterol-rich microdomains in the plasma membrane
(25 to 70 nm in diameter)

Enriched in GPI-linked receptors and glycosphingolipids

Enriched in many signaling molecules, including:
PIP2 and PIP3, several GTPases including Ras,
Src kinases, some heterotrimeric G-proteins

Operationally defined based on detergent solubility

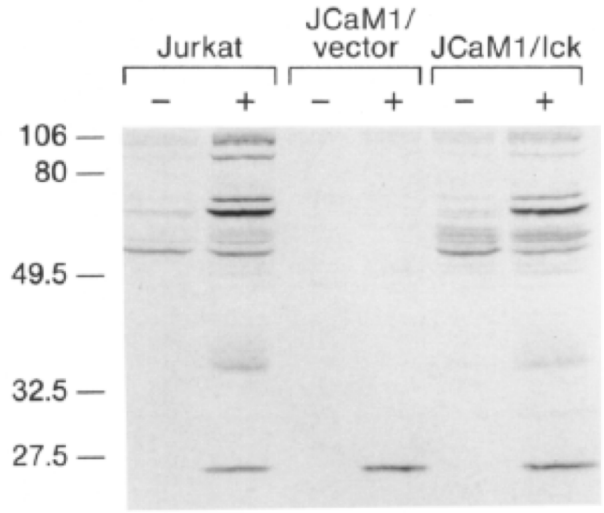
Some of Lck (50% or less) partitions into lipid rafts
(reversibility and dynamic changes?)

May be a focal point of signal initiation or sequestration

- *A topic of considerable controversy!*

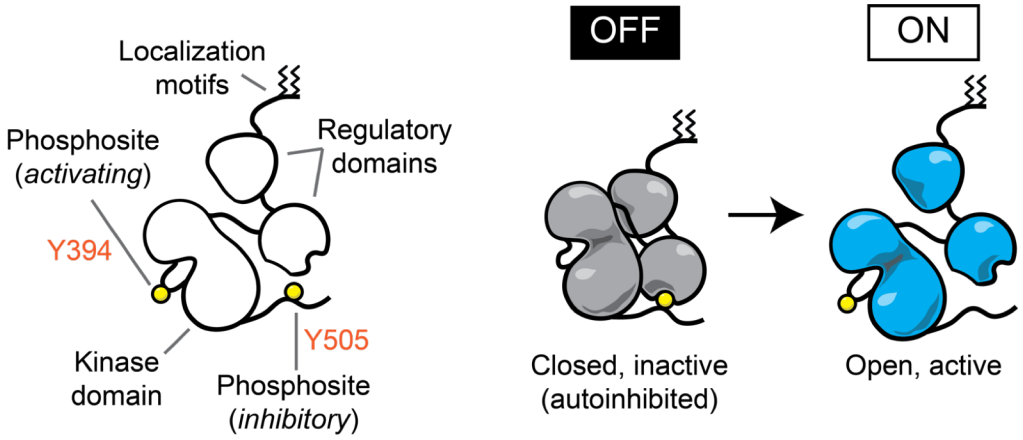
Lck is Involved in TCR Signaling

- A pool of active Lck exists in resting T cells and may not increase following TCR stimulation
- Repositioning Lck may be most critical.
- KO in mice results in a relative block in thymocyte development.
- Lck-deficient T cells have a TCR signaling defect (ITAMs are not phosphorylated, nor are distal events induced).
- CD4-Lck or CD8-Lck associations are required for optimal antigen responsiveness.



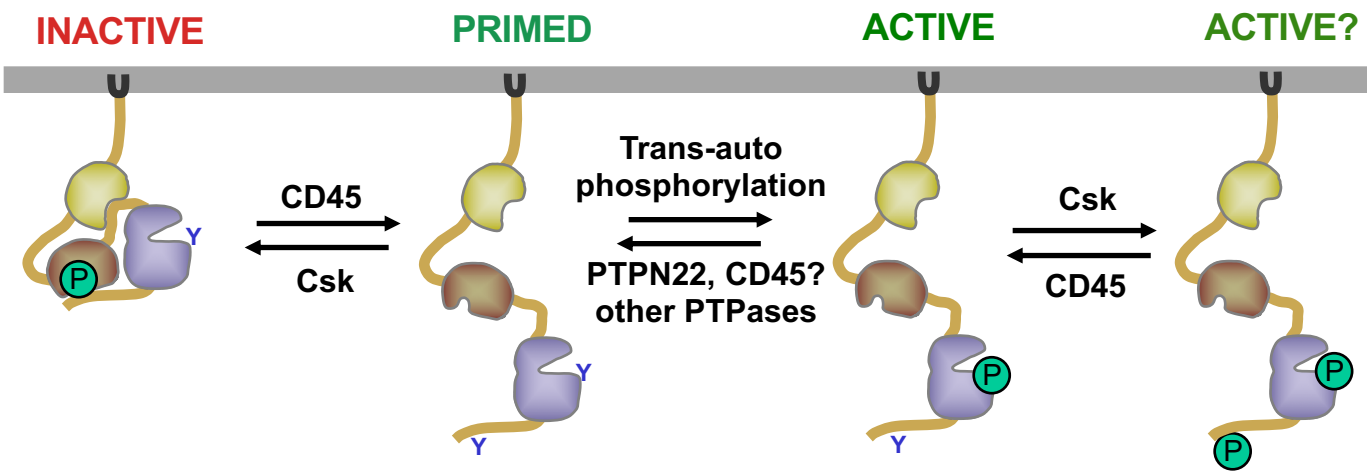
JCaM1 is a Jurkat mutant that does not signal. Lck reconstitution restored early and late TCR signaling events (Straus and Weiss, Cell, 1992)

Regulating Src Family Kinases

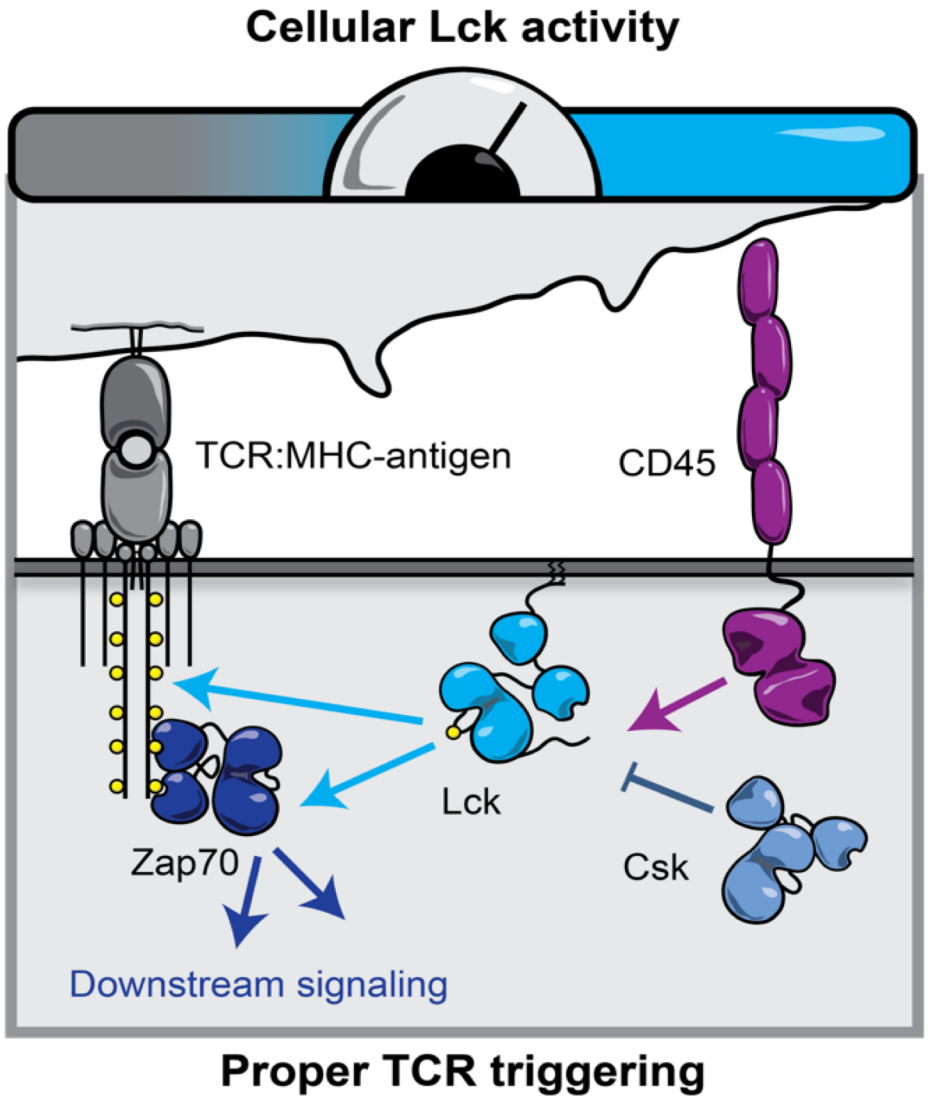


- Lck and Fyn are Src family kinases found in T cells
- Conformation determines activity
- Phosphorylation regulates conformation & therefore activity

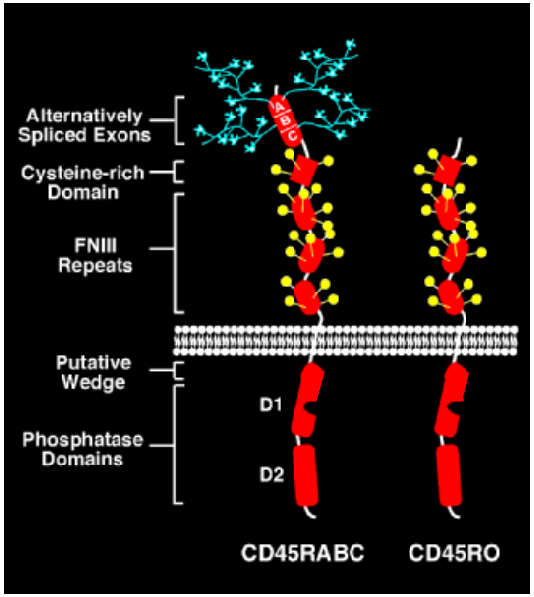
Various activation states of SFKs in T cells

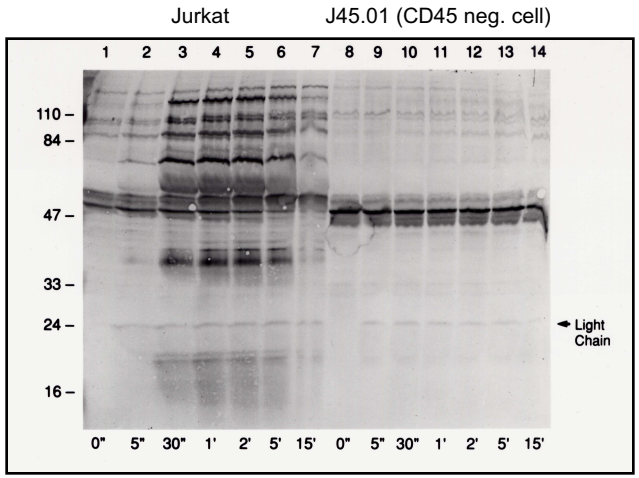


A dynamic interplay of CD45 and Csk is important in setting the level of Lck activity



CD45, a Tyrosine Phosphatase Plays a Positive and a Negative Regulatory Role for TCR Induced Tyrosine Phosphorylation

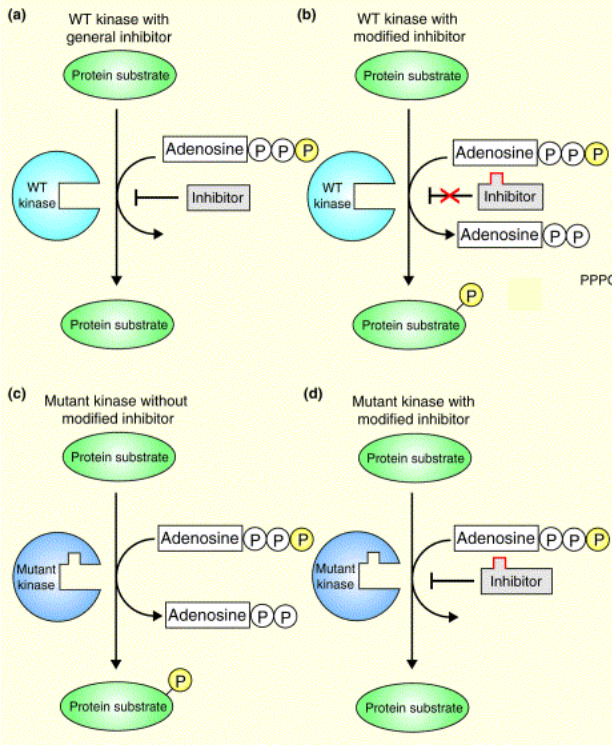




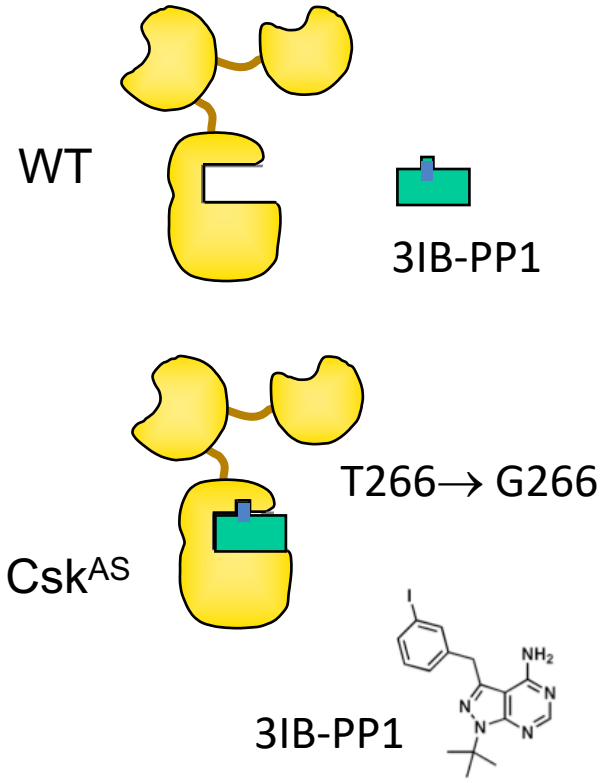
Koretzky, et al, PNAS, 1991

- Receptor-like protein tyrosine phosphatase expressed on all nucleated hematopoietic expressed at very high levels (25 μM)
- Distinct isoforms expressed in a cell-type and developmental-stage specific manner
- Required for T cell and B cell antigen receptor signaling and for normal development - dephosphorylates negative regulatory tyrosine in Src kinases.
- Mutations associated with SCID and autoimmunity in humans and mice
- Jurkat and T cell clone deficient in CD45 does not signal
- Contains tandemly duplicated phosphatase domains, but only domain 1 is functionally active.

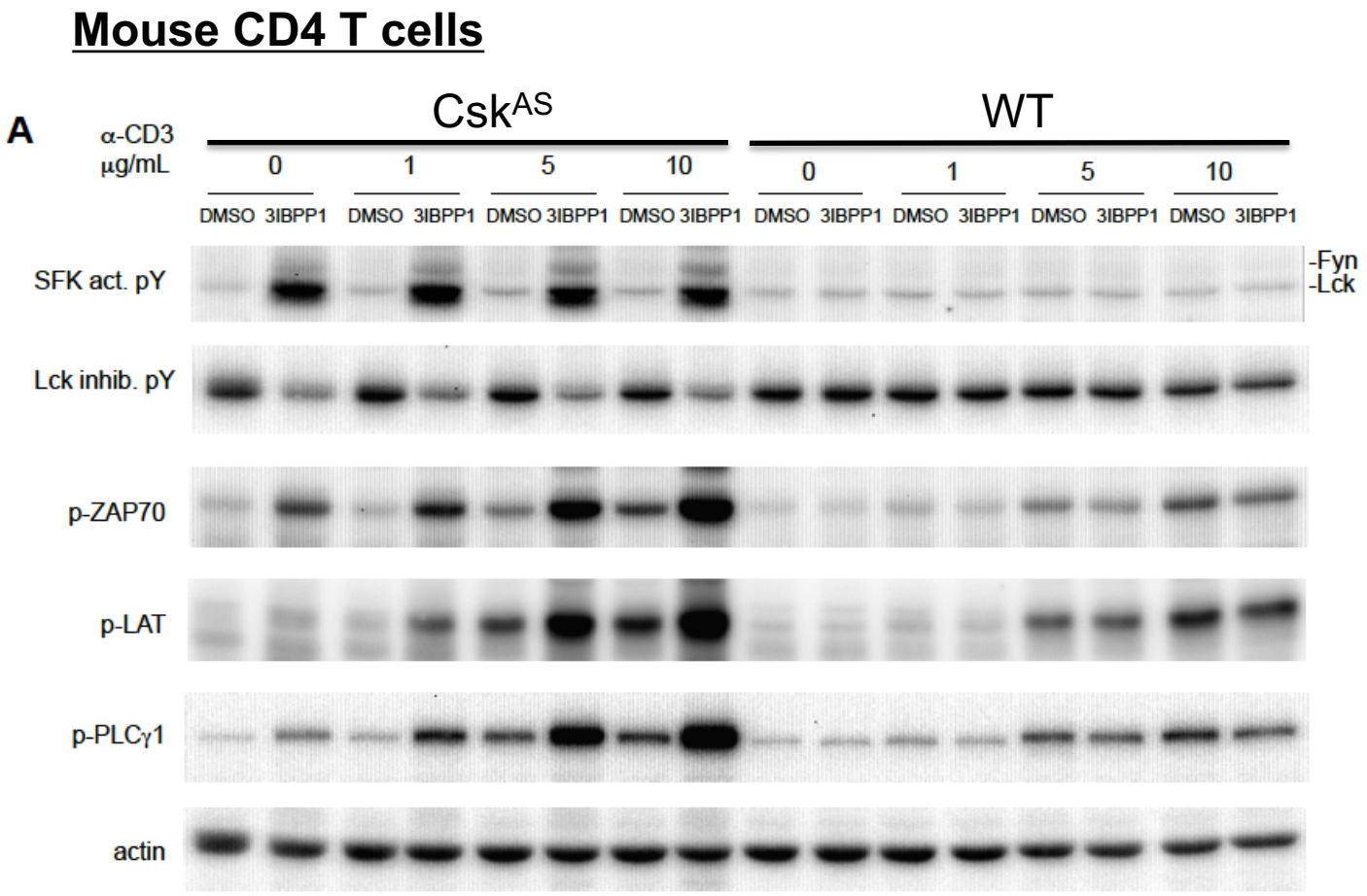
A unique analog-sensitive allele of Csk can be rapidly and specifically inhibited



Alaimo et al, Curr Op Chem Bio, 2001

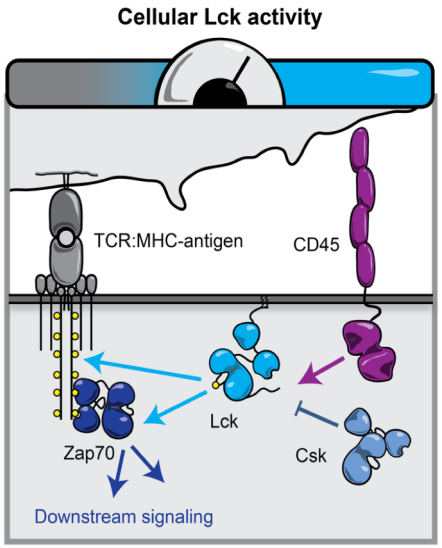


Synergy of Csk inhibition and TCR stimulation occurs downstream of Lck phosphorylation

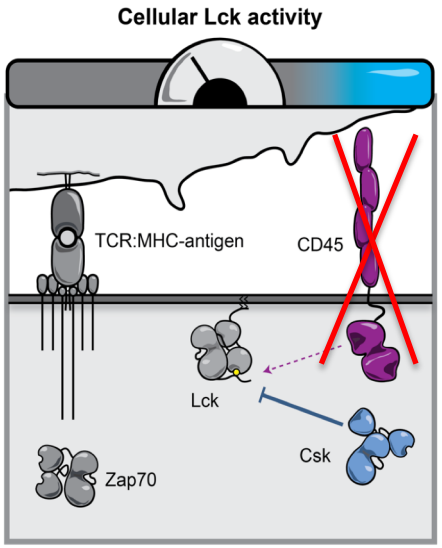


Manz B, Tan Y-X, et al., Elife, 2015

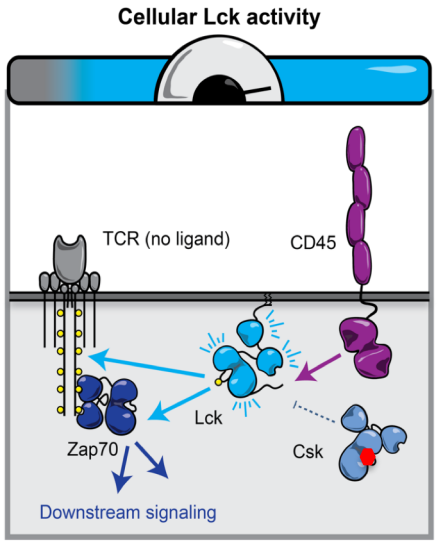
Lck activity is required and sets a threshold for T cell activation



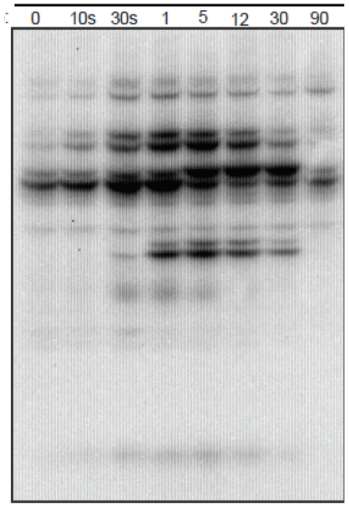
Proper TCR triggering

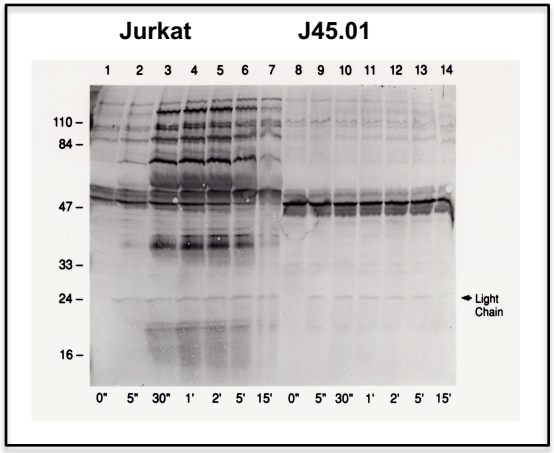


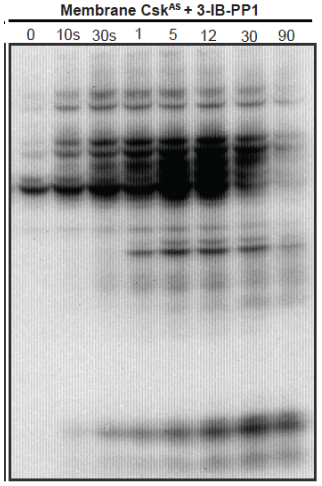
Decreased Lck activity - Ignorance



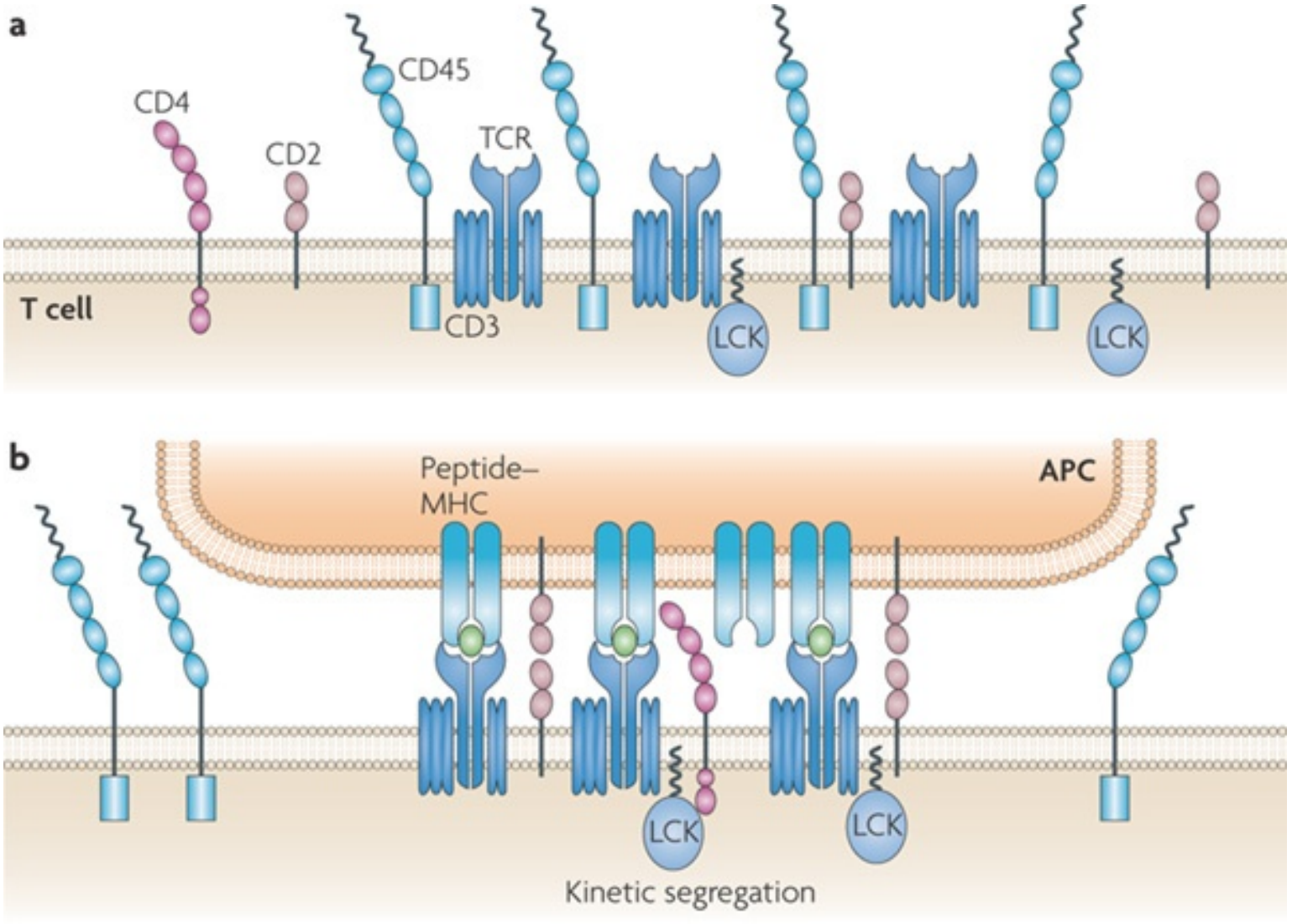
Increased Lck activity - Increased triggering







Kinetic Segregation Model: Separation of CD45 PTP via Large EC Domain Segregates PTP activity from pTyr Events



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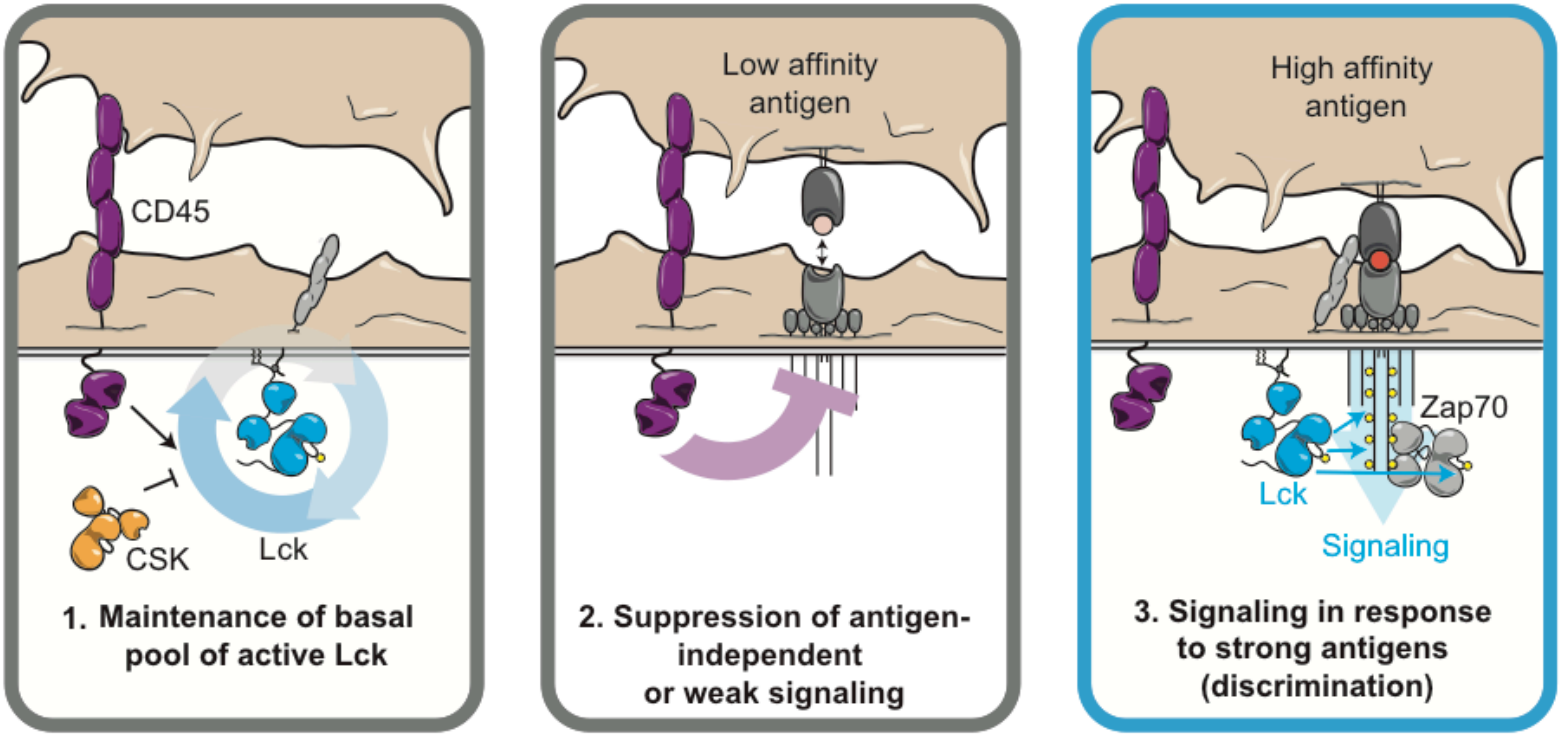
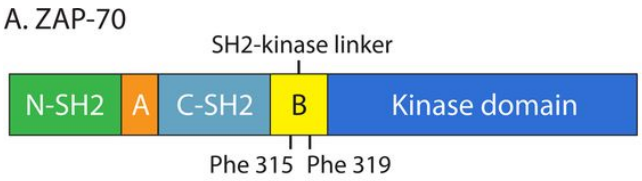
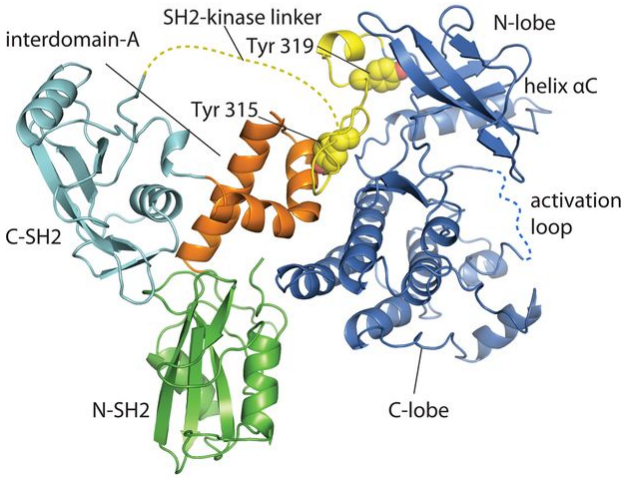
TCR ligand discrimination can be influenced by the relative positiveand negative regulators of Lck and by ligand potency



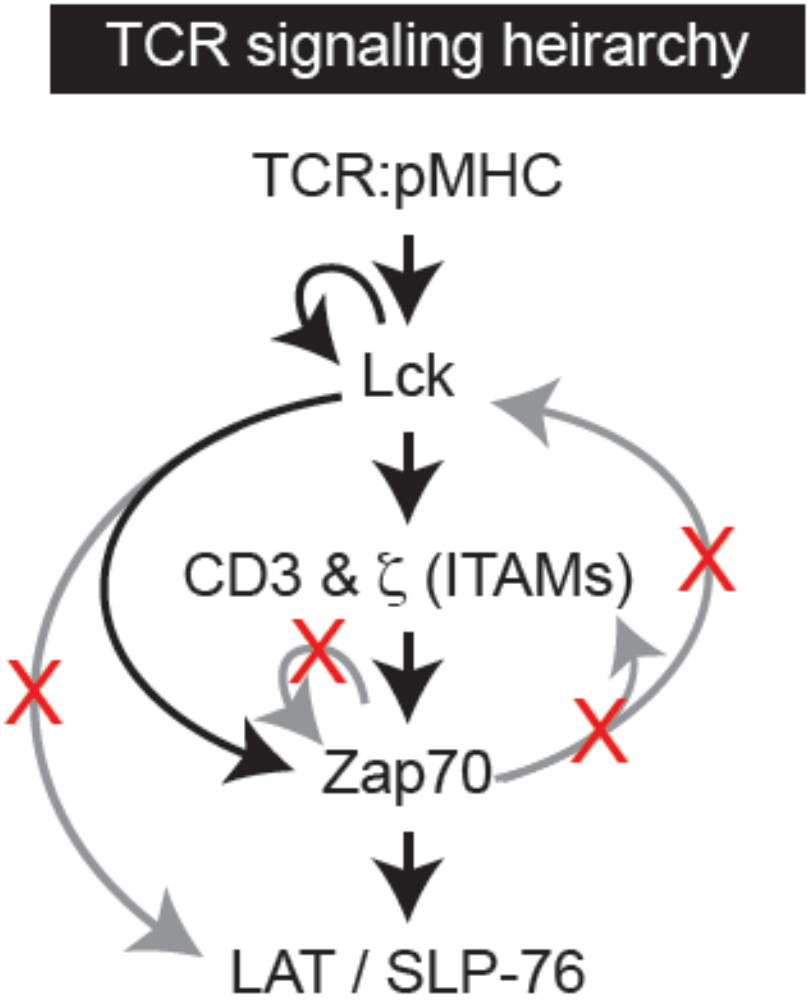
ZAP-70 in TCR Signaling



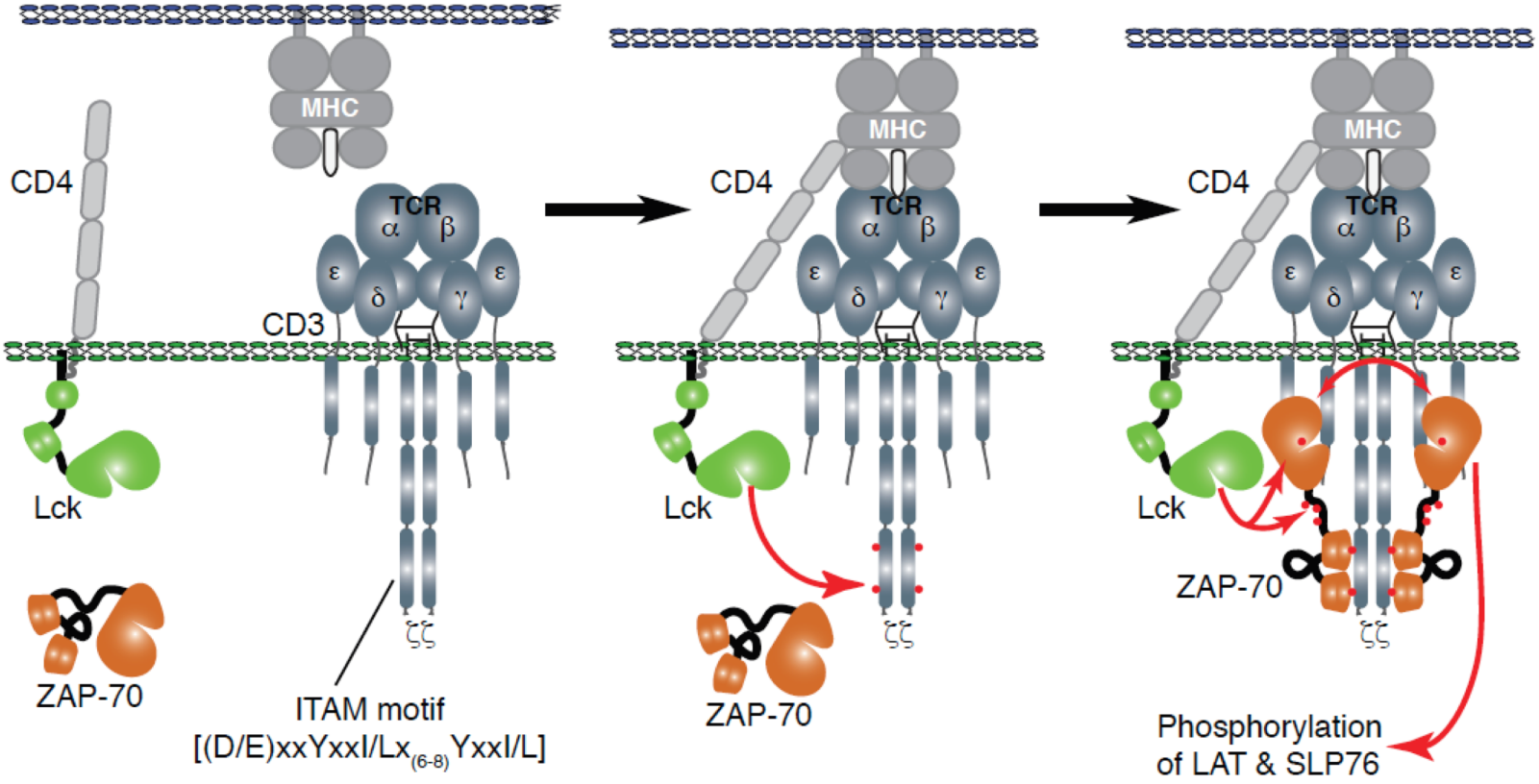


- **Expressed only in T cells and NK cells**
- **Cooperative binding of tandem SH2 domains to doubly phosphorylated ITAMs**
- **Substrates: LAT, Slp-76**
- **ZAP-70 LOF mutations:**
  - **Cell line - loss of TCR signaling**
  - **Mouse KO - developmental arrest (DP -> SP)**
  - **Human SCID - No CD8 T cells**
    - **CD4 T cells with TCR signaling defect**

Why did Zap70 evolve?
Why isn't Syk good enough?

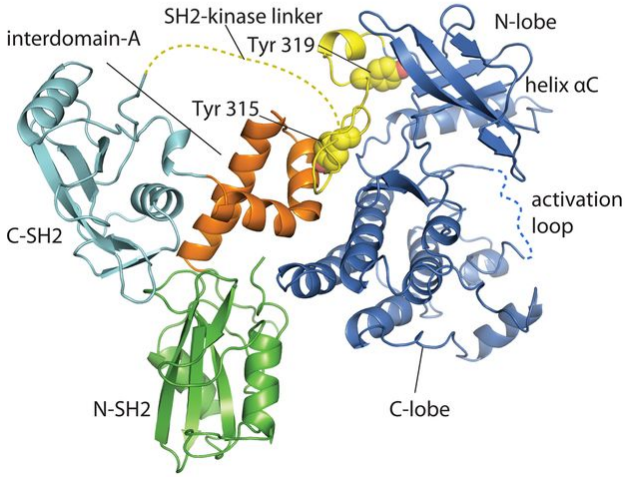

TCR signaling heirarchy
TCR:pMHC
Lck
CD3 & ζ (ITAMs)
Zap70
LAT / SLP-76

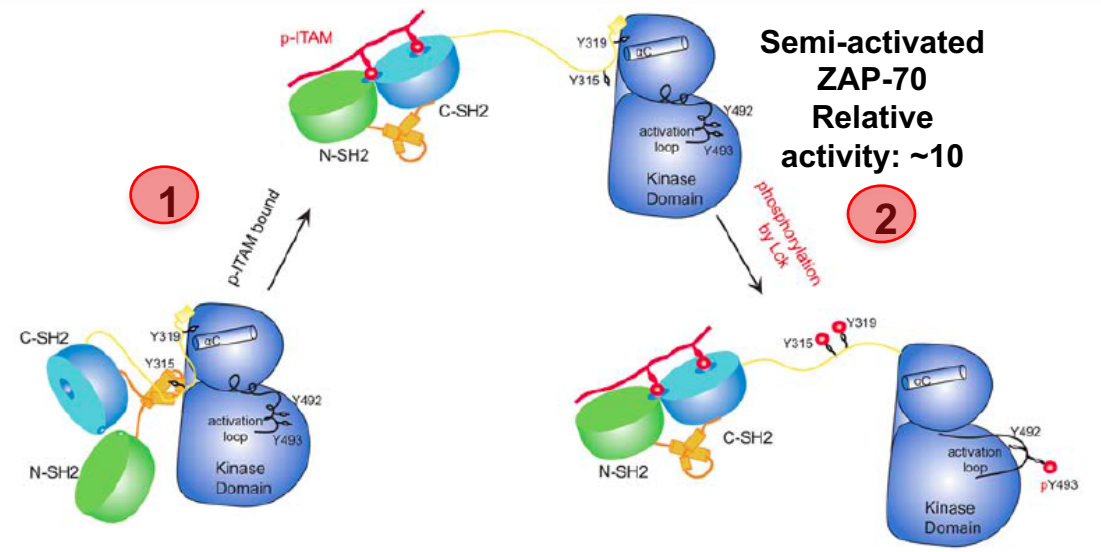
Model requiring TCR and coreceptor (CD4 or CD8) function: Zap70 and Lck evolved to enforce a requirement for MHC recognition by the TCR



Weiss, Cell, 1993
Wang, et al, CSHL, 2009

Two Allosteric Changes Involving ITAM Binding and Lck-Mediated Phosphorylation are Necessary for ZAP-70 activation





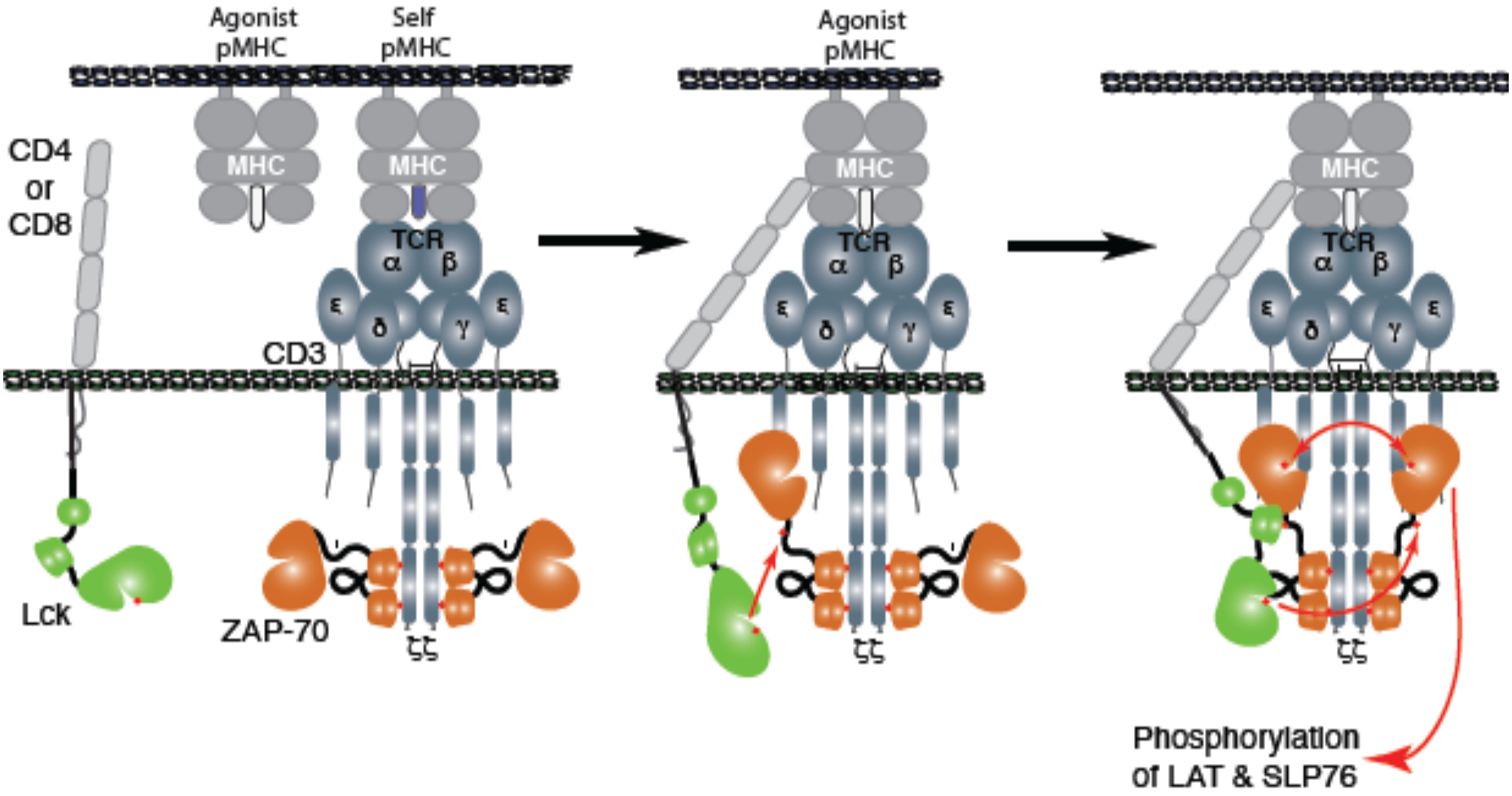
Autoinhibited ZAP-70 Relative activity: 1

Semi-activated ZAP-70 Relative activity: ~10

Fully Active ZAP-70 Relative activity: 100

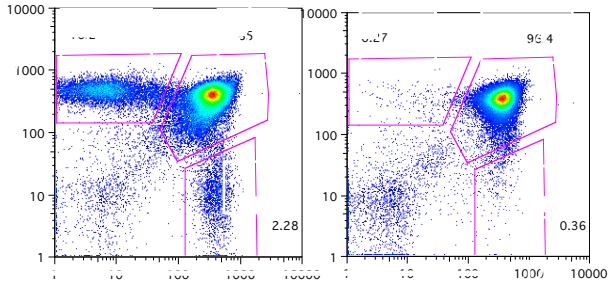
Deindl, et al., 207
Yan, et al., Mol. Cell. Biol., 2013

Lck phosphorylation of and its binding to ZAP-70 that is already bound to the TCR-ζ chain may be Critical for Activation of the TCR



Chakraborty and Weiss, Nat. Immunol., 2014

ZAP-70 in Disease

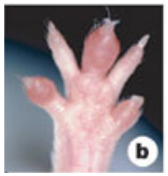


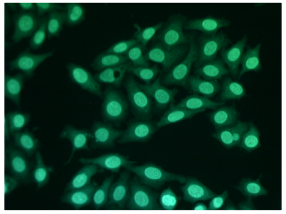
ZAP-70 deficiency

Human (CD8 T cell-deficient SCID)
Mouse (T cell-deficient SCID)

Spontaneous mouse model of arthritis

Hypomorphic *skg* allele – alters development
(Sakaguchi, et al., Nature, 2005)



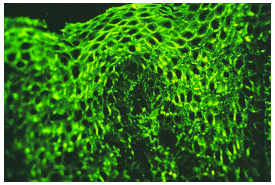


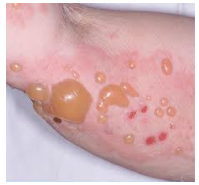
Catalytic domain ENU mouse mutants - hyper-IgE and autoimmunity

Level of TCR signaling alters development (Siggs, et al, Immunity, 2007)

SH2 + catalytic domain mutants – human autoimmunity

Bullous pemphigoid, anti-Factor VIII antibodies,
Glomerulonephritis, IBD (Chan, et al., J Exp Med, in press)

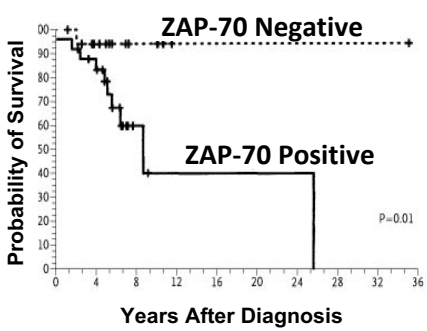




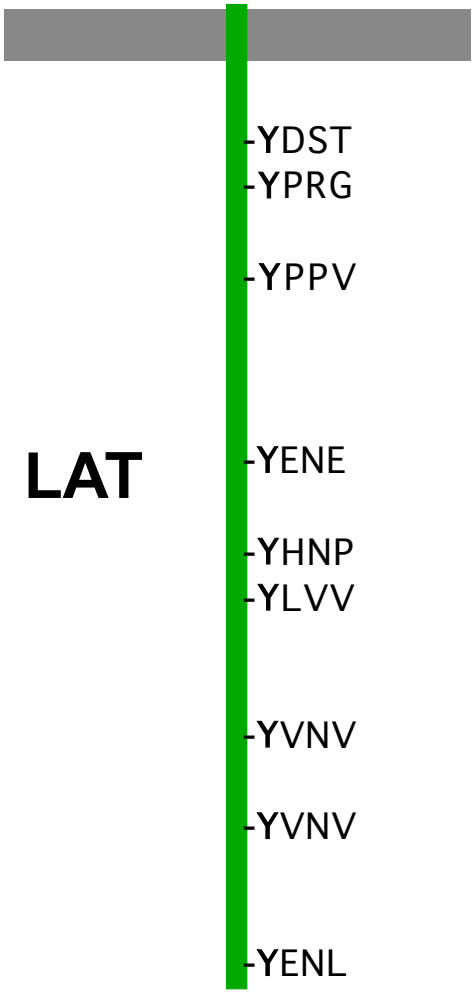
Aberrant expression in B cell CLL

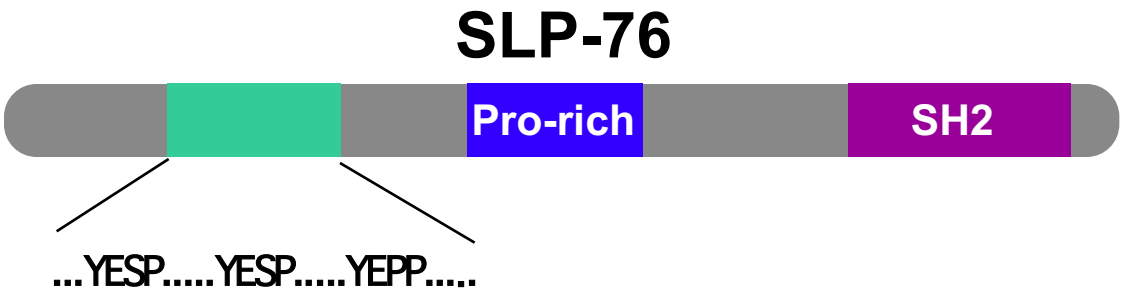
ZAP-70+ CLL: Poor prognosis

(Rassenti, et al., NEJM 2004)



Two Adapters Are ZAP-70 Substrates: Critically Required for TCR Downstream Signaling

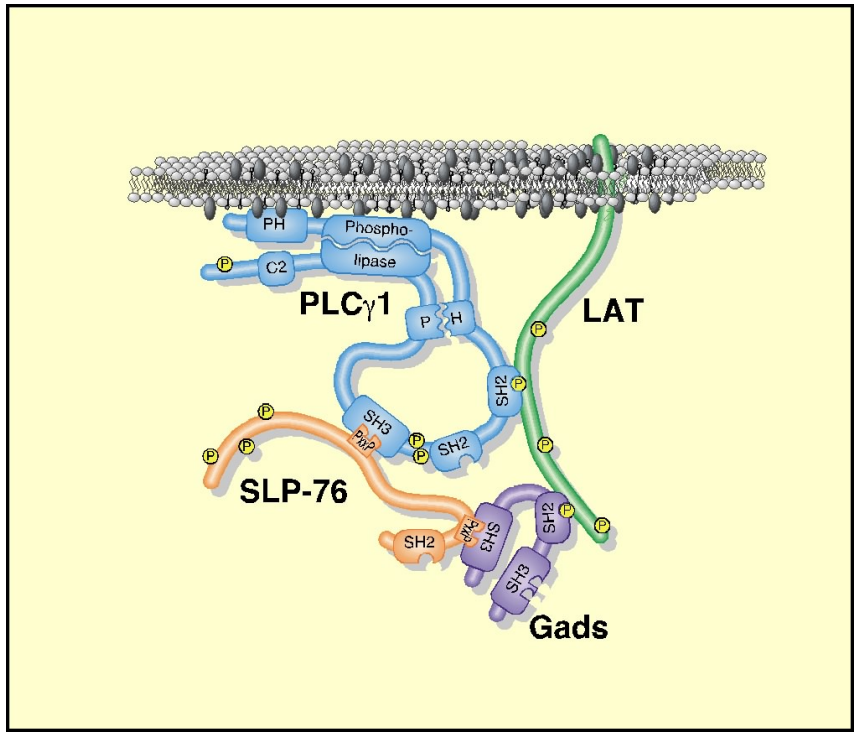




LAT function: Lipid raft recruitment of effectors

SLP-76 function: Organization of signaling complex

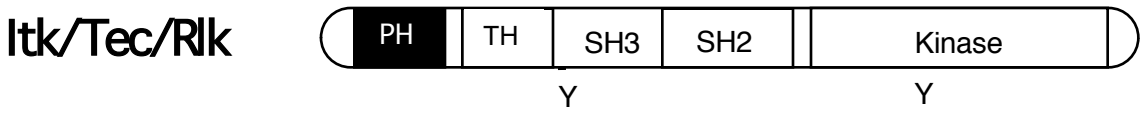
Assembly of LAT and SLP-76 into a Signalosome in Lipid Rafts Is required for Multiple Downstream TCR Signaling Pathways



Events requiring LAT & SLP-76

PLCγ1 activation
Ras activation
Rac activation
HPK1 activation

Tec Kinases in T cells

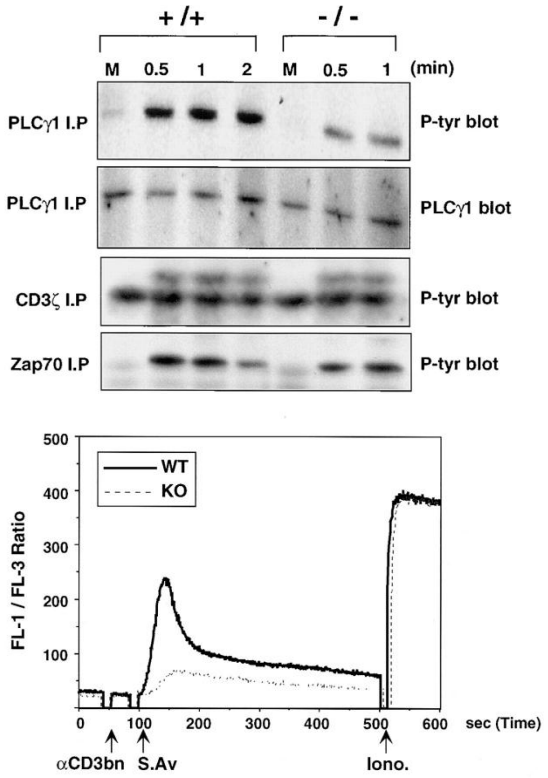


Itk

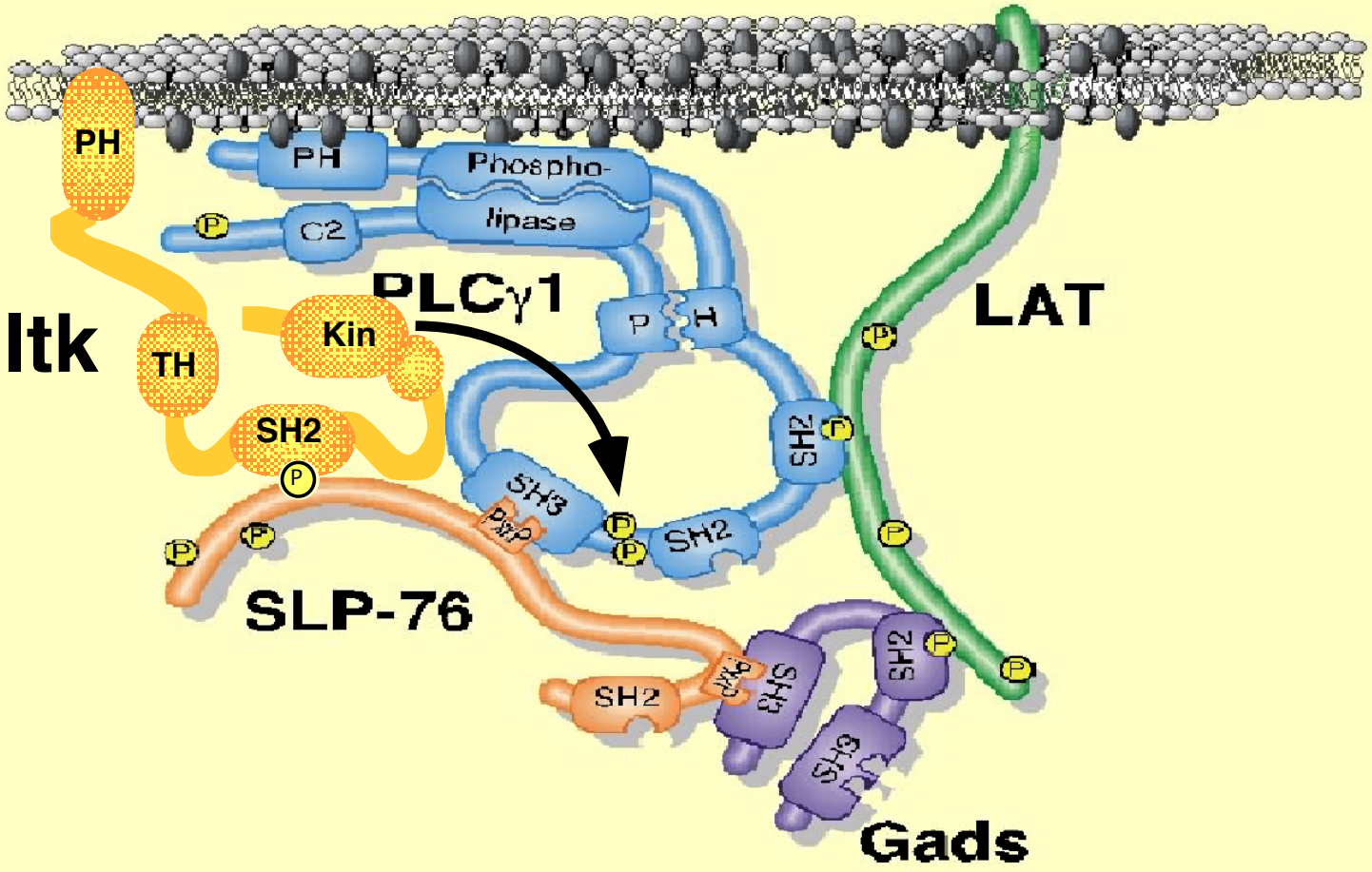
- Expressed predominantly in T cells
- PH domain binds to PIP3 which recruits Itk to membrane
- Kinase domain is phosphorylated and activated (by Lck)
- TH and SH3 domains interact in basal state to inhibit kinase
- Autophosphorylation of Y180 adjacent to SH3 domain allows Itk to unfold, rendering SH2 and SH3 domains accessible
- Likely to phosphorylate and activate PLC γ1
- KO in mice results in mild defect in PLC activation and Ca2+ increase (redundant roles of other family members, Rlk and Tec, in T cells)

Defective PLC γ1 phosphorylation and Ca2+ increase in Itk null T cells

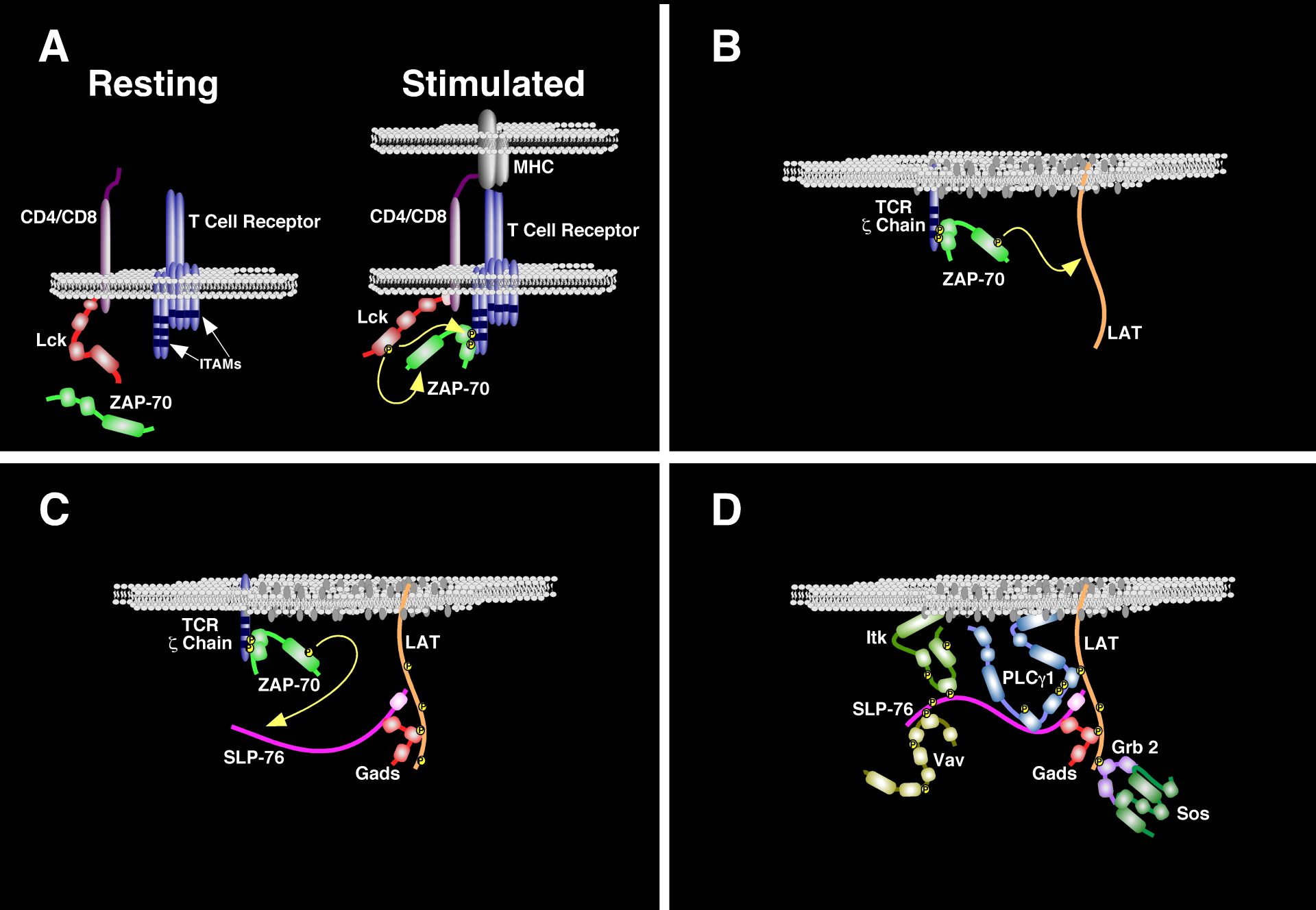
Liu, et al.., JEM, 1998



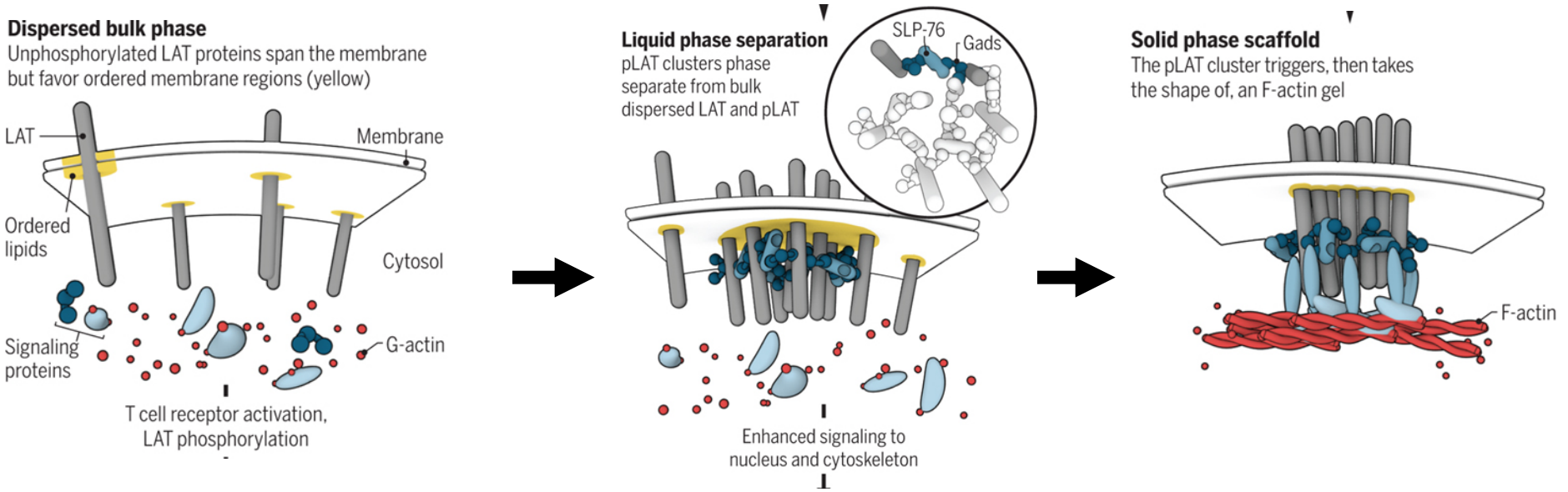
Itk is recruited to the Signalosome and Phosphorylates PLCγ1



Sequential Events in TCR Signal Transduction

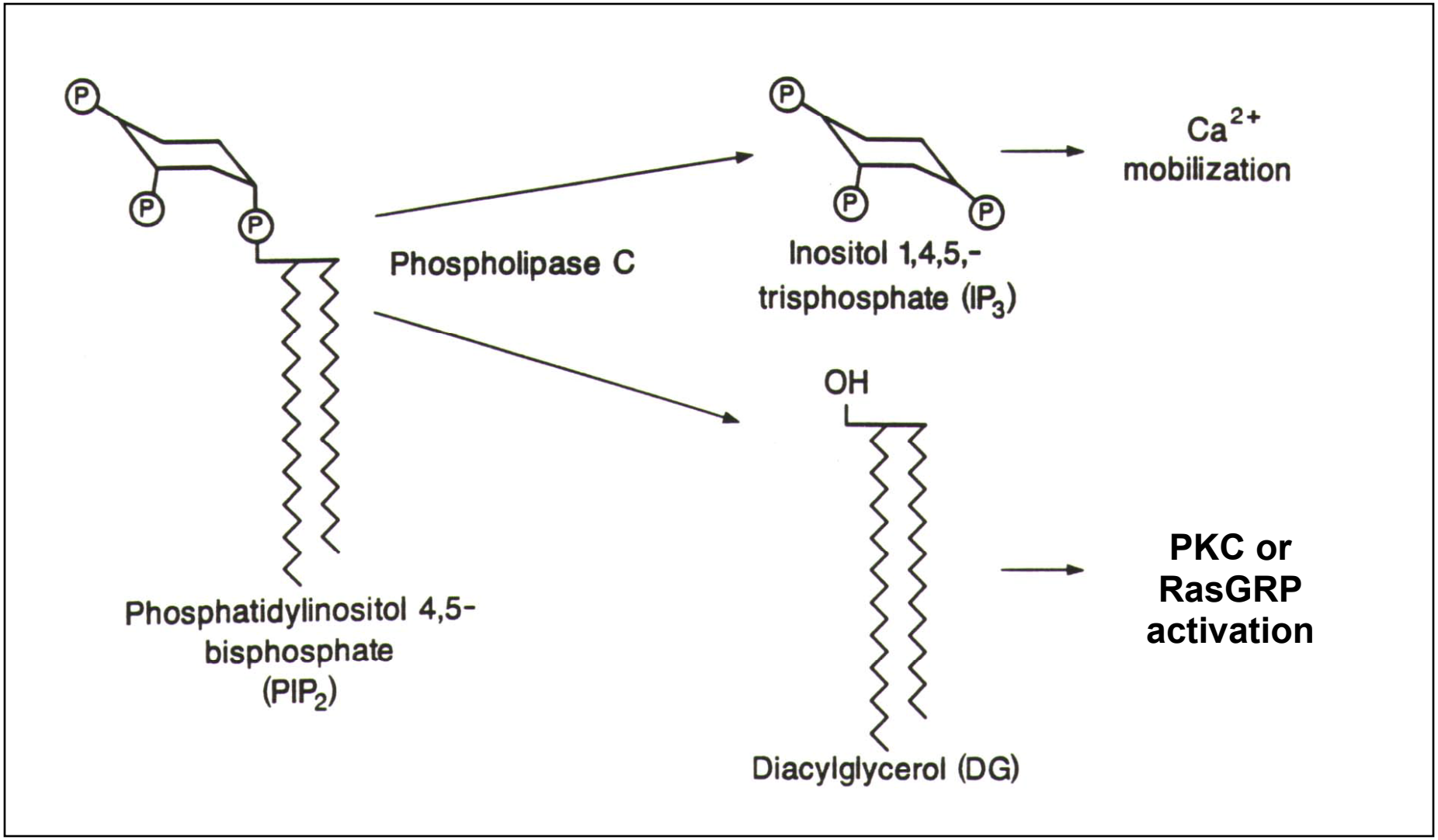


Dynamic In Vitro Assembly of Signaling Components



Dustin and Muller, Science, 2016
Su, et al., Science, 2016

PLC Activation Leads to Second Messenger Generation and Mobilization of Calcium as well as PKC and RasGRP Activation



Evidence for the Importance of the Phosphatidylinositol Pathway in T cells

1. Activating and synergistic effects of calcium ionophores and phorbol esters

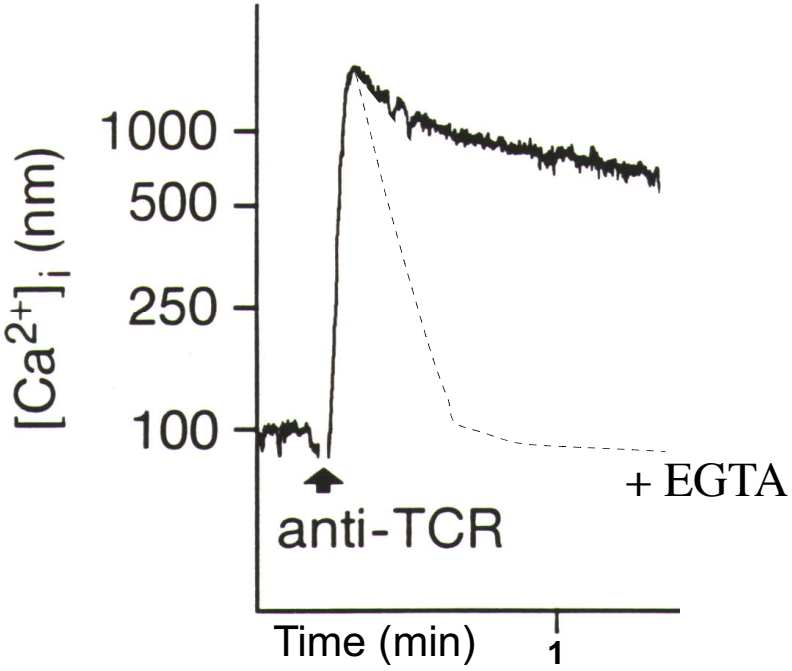
2. Inhibitory effects of calcium chelators and PKC inhibitors

3. Positive effects of activating alleles of calcineurin and PKC or Ras)

4. Ability of heterologous G-protein coupled receptors that activate PLC beta but not Src or Syk PTKs to induce IL-2 gene activation

5. T cell activation defects in PKC theta or RasGRP1 mice

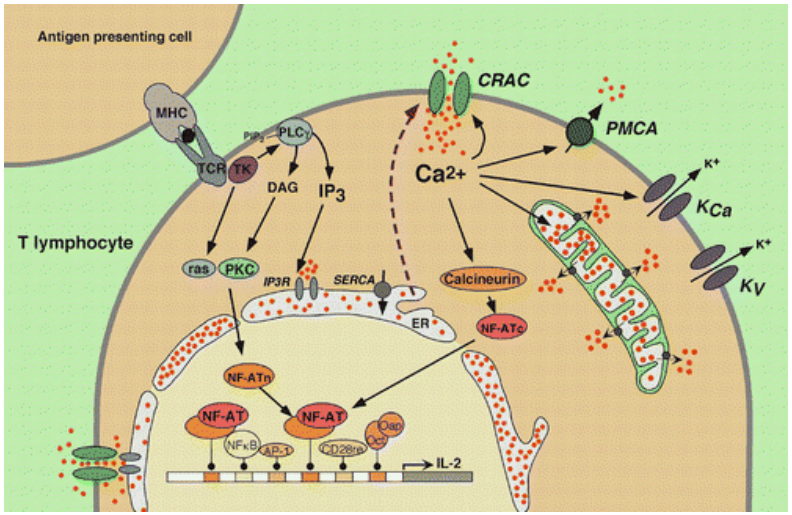
The Phosphatidylinositol Pathway: Calcium Mobilization after TCR Stimulation



Initial release from Intracellular stores (results from IP3 interacting with receptors)

Depletion of intracellular stores results in a transmembrane flux of calcium, Icrac

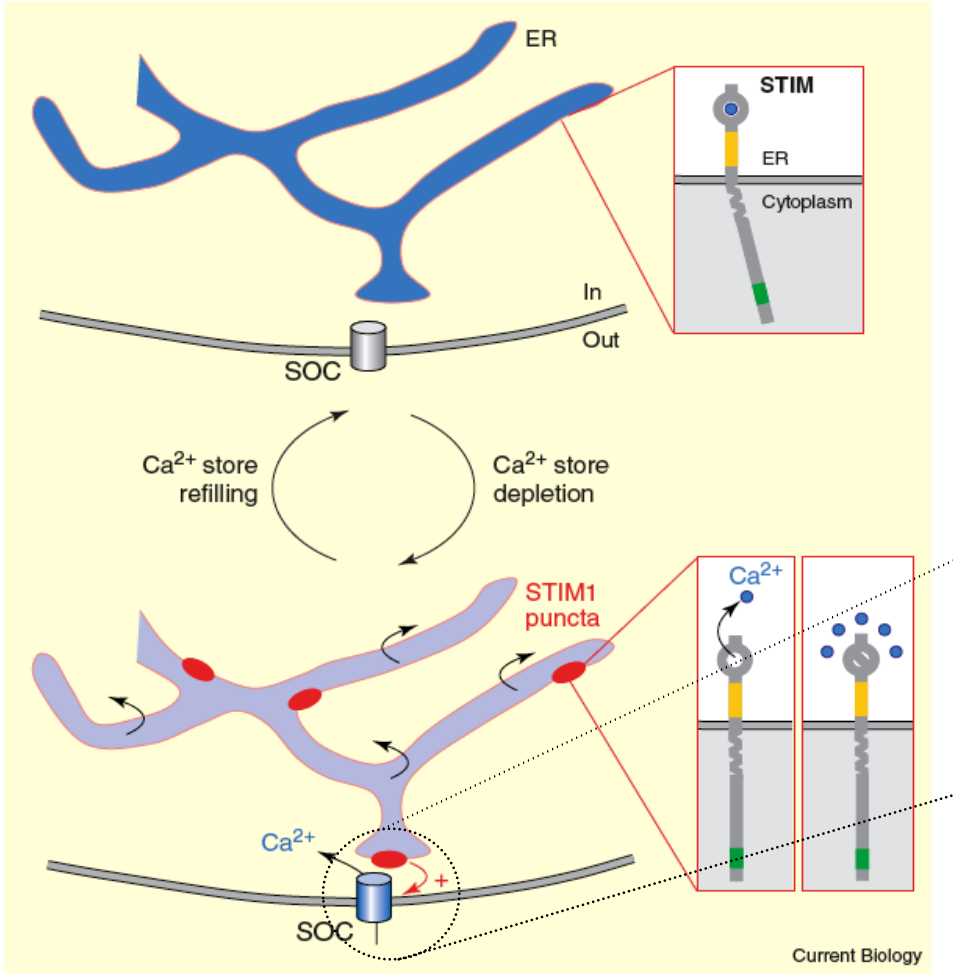
Lewis Annu. Rev. Immuno. 2001



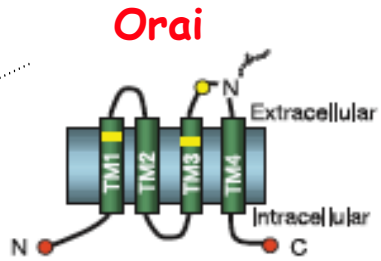
Downstream Consequences:

- Activation of Calcineurin (Ca/CaM-dependent serine/threonine phosphatase), the target of Cyclosporin A and FK506
- Activation of Ca/CaM kinase

STIM is the ER Calcium Sensor and Orai is a Component of Icrac



Depletion of intracellular stores results in altered function and distribution of ER resident protein STIM. STIM migrates in "puncta" towards plasma membrane where it regulates the recently identified Icrac channel, Orai, in the plasma membrane to allow a transmembrane flux of calcium



Orai initially identified as a consequence of tracking down the basis of unusual calcium pathway-defective SCID

Marchant, Current Biology, 2005

Prakriya, et al.,, Nature, 2006

The Phosphatidylinositol Pathway: PKC activation after TCR Stimulation

PKC - a large family of serine/threonine kinases

Multiple isozymes in T cells

DAG-regulated, phorbol ester responsive

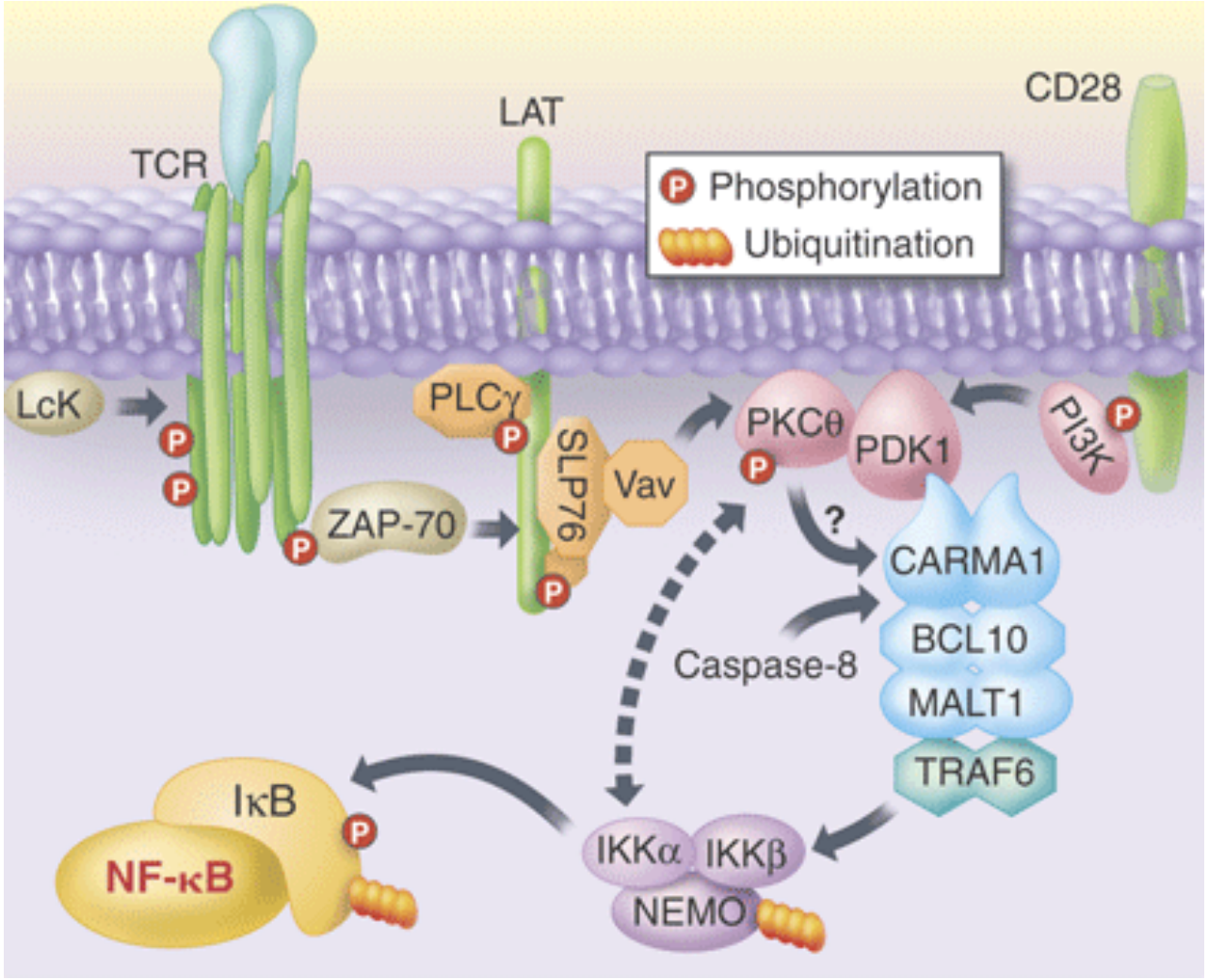
Some are calcium responsive

PKC theta localizes to cSMAC, and KO mice have selective defect in NF-kB activation in T cells

Function:

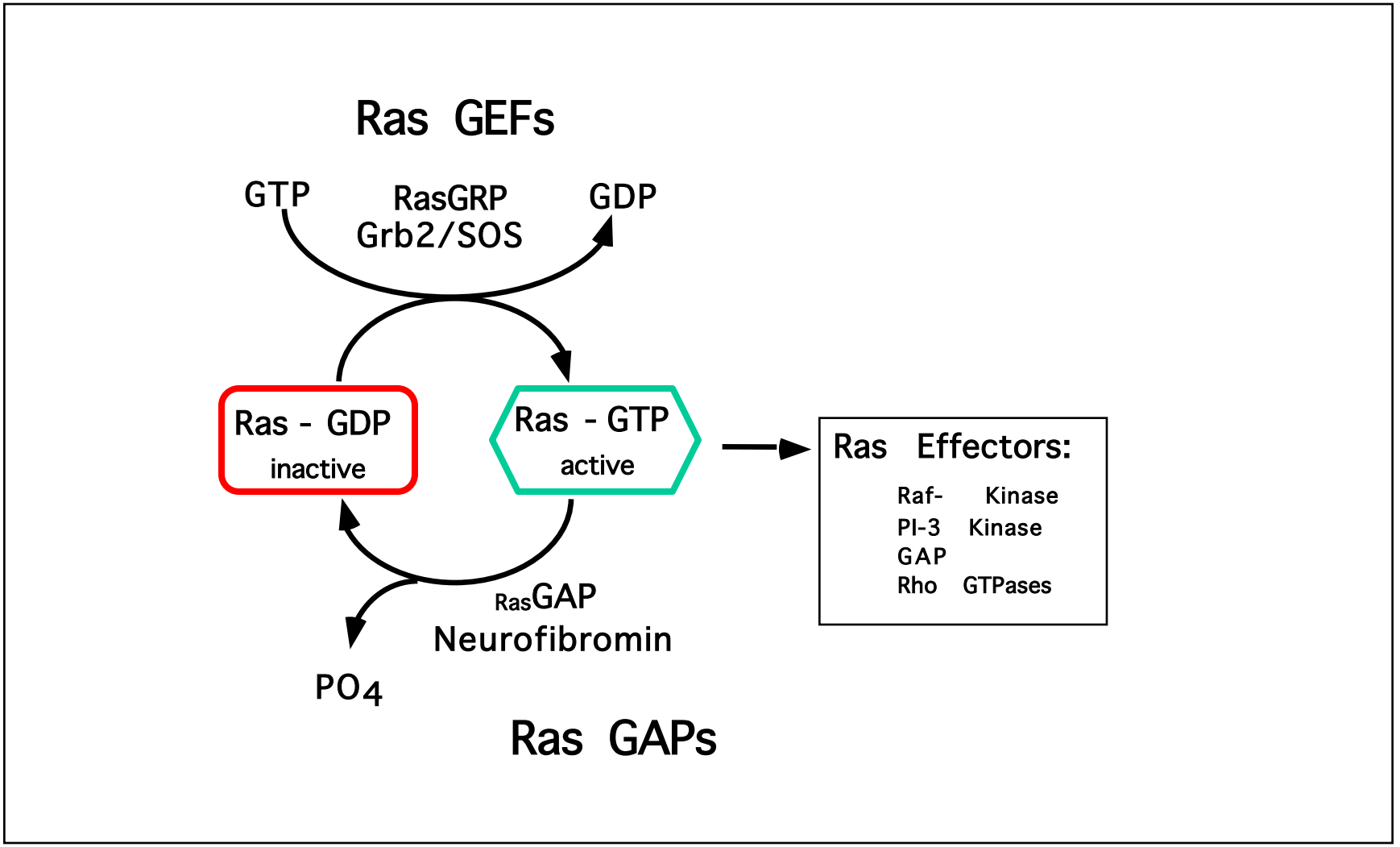
- TCR downregulation
- NFkB activation
- Activation of RasGRP

TCR- and CD28-mediated activation of PKC and PI3K Signaling Leads to NFkB Activation

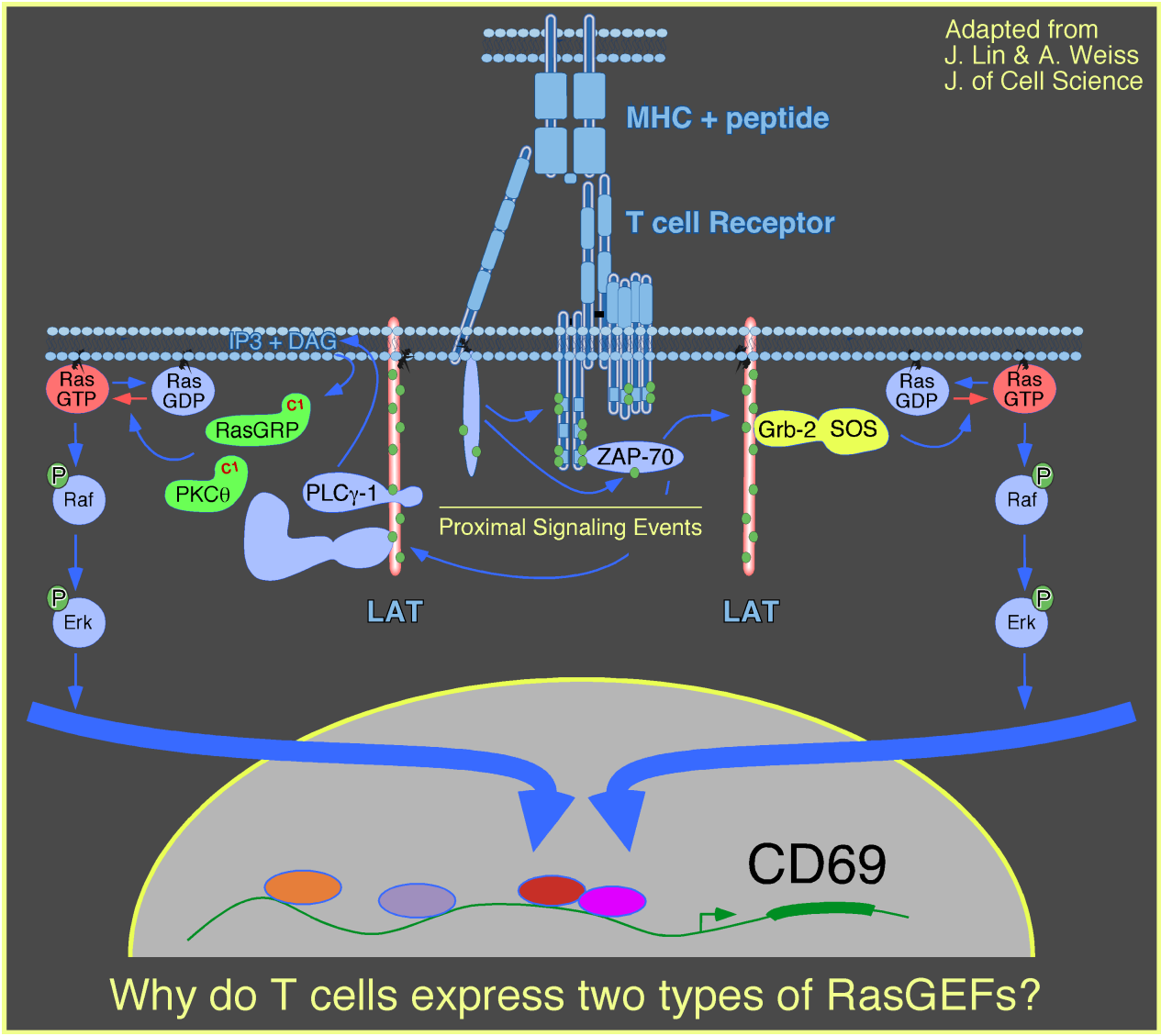


van Oers and Chen, Science, 2005

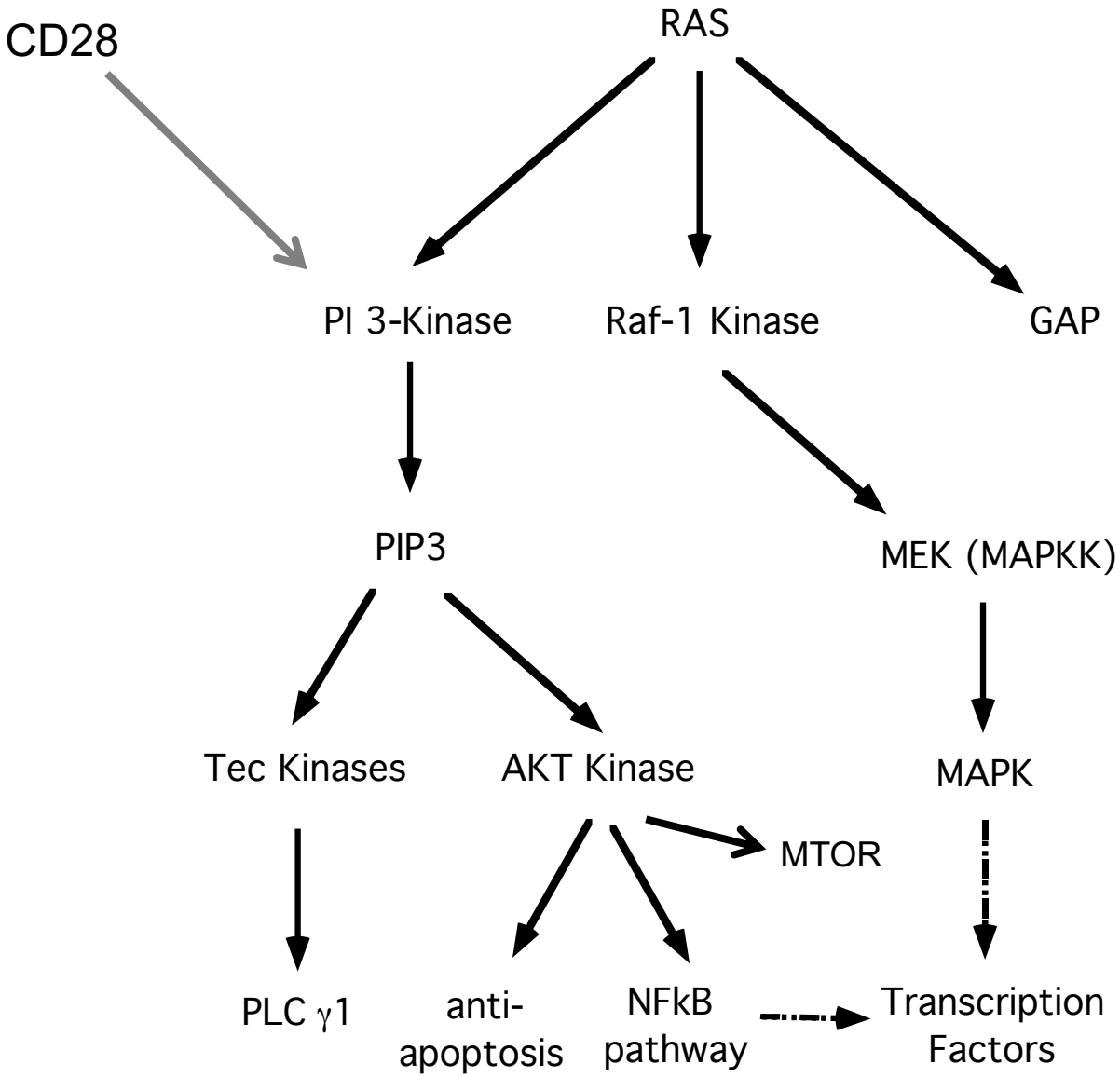
TCR Stimulation Activates Ras Via Guanine Nucleotide Exchange Factors (GEFs)



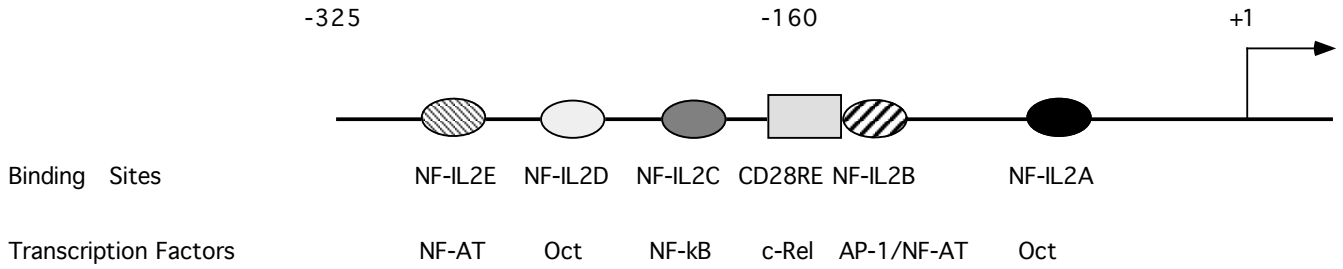
The TCR Uses Two GEFs to Activate Ras



Ras Activates Multiple Signaling Pathways



IL-2 Gene Regulation



Transcriptional Regulation

- IL-2 gene is inactive in naive T cells
- Site of for signal integration (TCR and costimulatory signals)
- Transcriptional Activation
  - Cascade of Events:
    - Immediate-early genes are involved (i.e., Jun and Fos)
      - delayed temporal expression of IL-2 transcripts
      - requirement for protein synthesis
    - Nuclear translocation of preformed-inactive factor (NFAT)
      - requires calcium flux
      - requires calcineurin activation
      - dephosphorylation of inactive cytoplasmic NFAT
- Post-transcriptional Regulation:
  - mRNA stabilization via AU-rich 3' untranslated region

Activation of Transcription Factors in T Cells