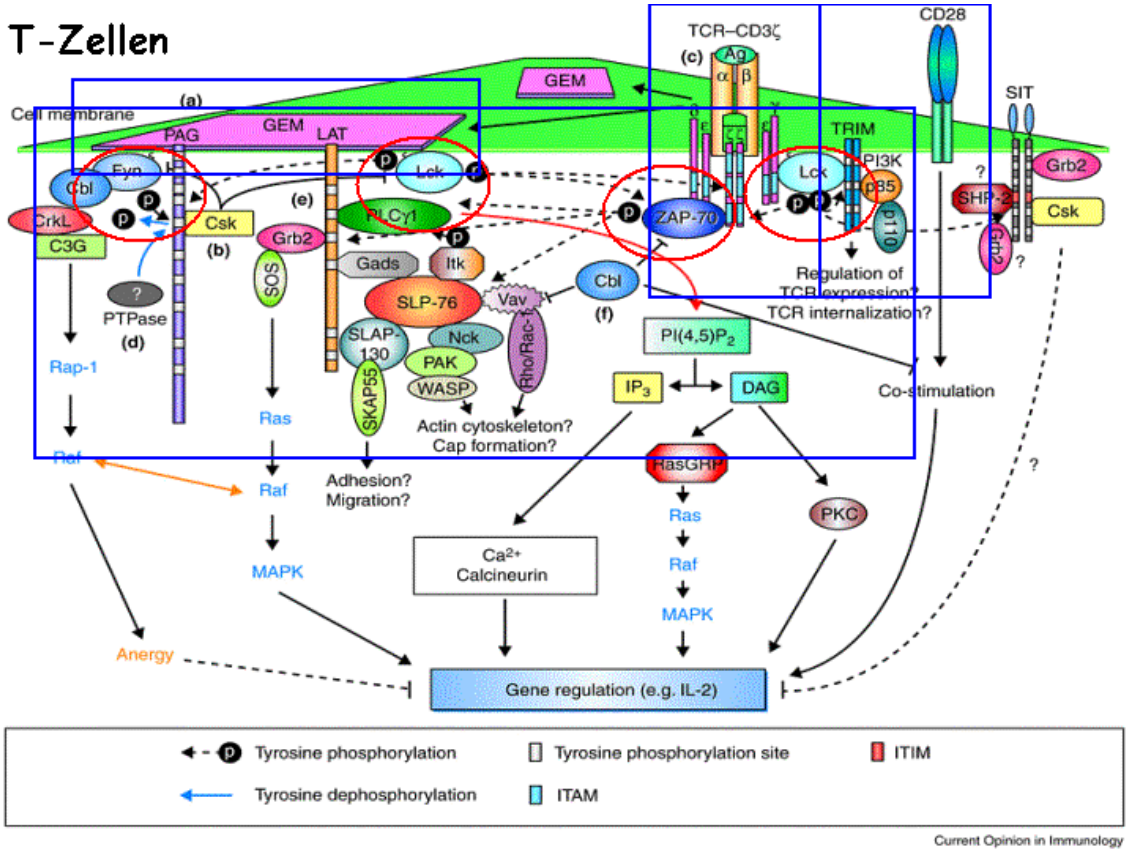
Signaltransduktion in Lymphocyten



Priv.-Doz. Dr. Michael Stassen

INSTITUTE FOR
IMMUNOLOGY
MAINZ

Signaltransduktion

- Möglichkeiten der interzellulären Kommunikation
- Signalweiterleitung in die Zelle („Transduktion“)
- Rezeptoren als Signalumwandler
- Phosphorylierung und Dephosphorylierung

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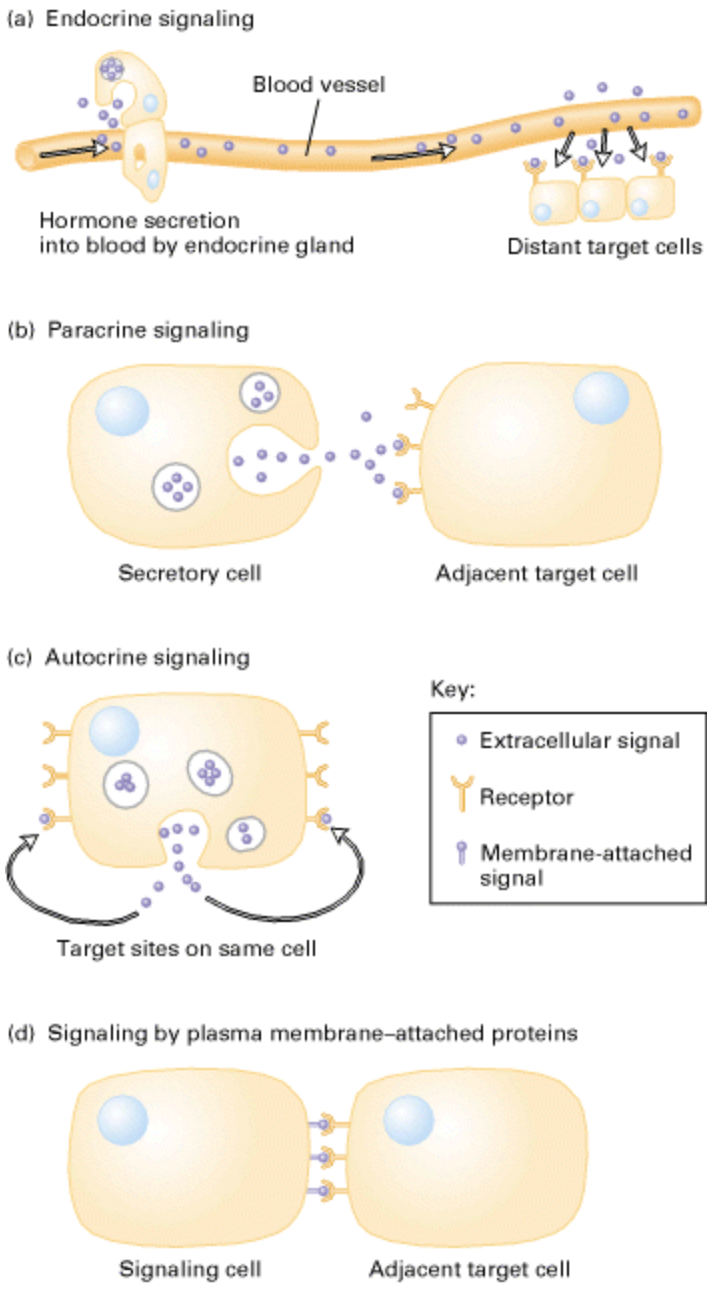
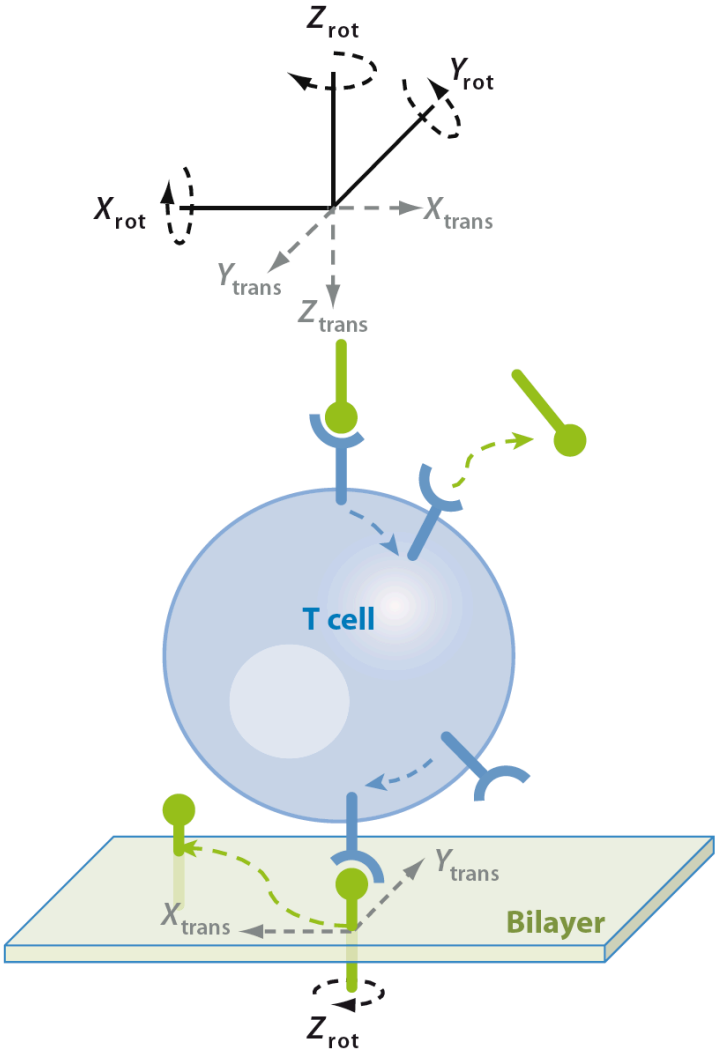
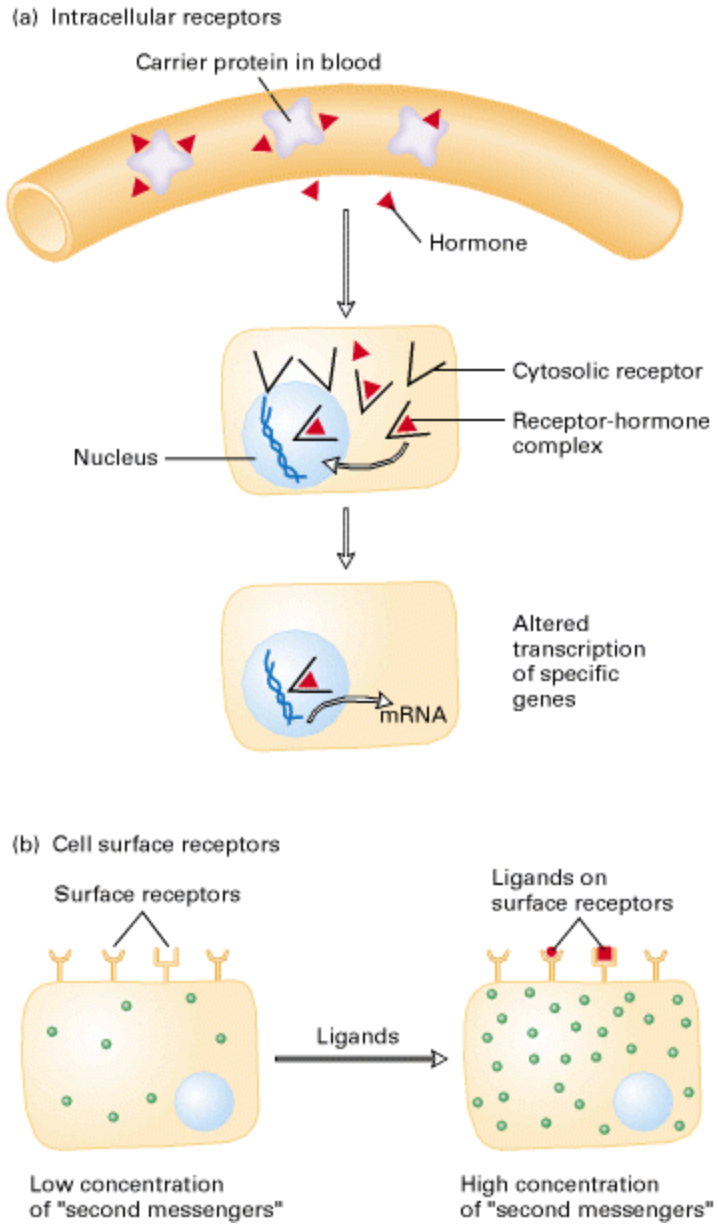


Lodish et al. *Molecular Cell Biology*, W.H. Freeman and Comp.



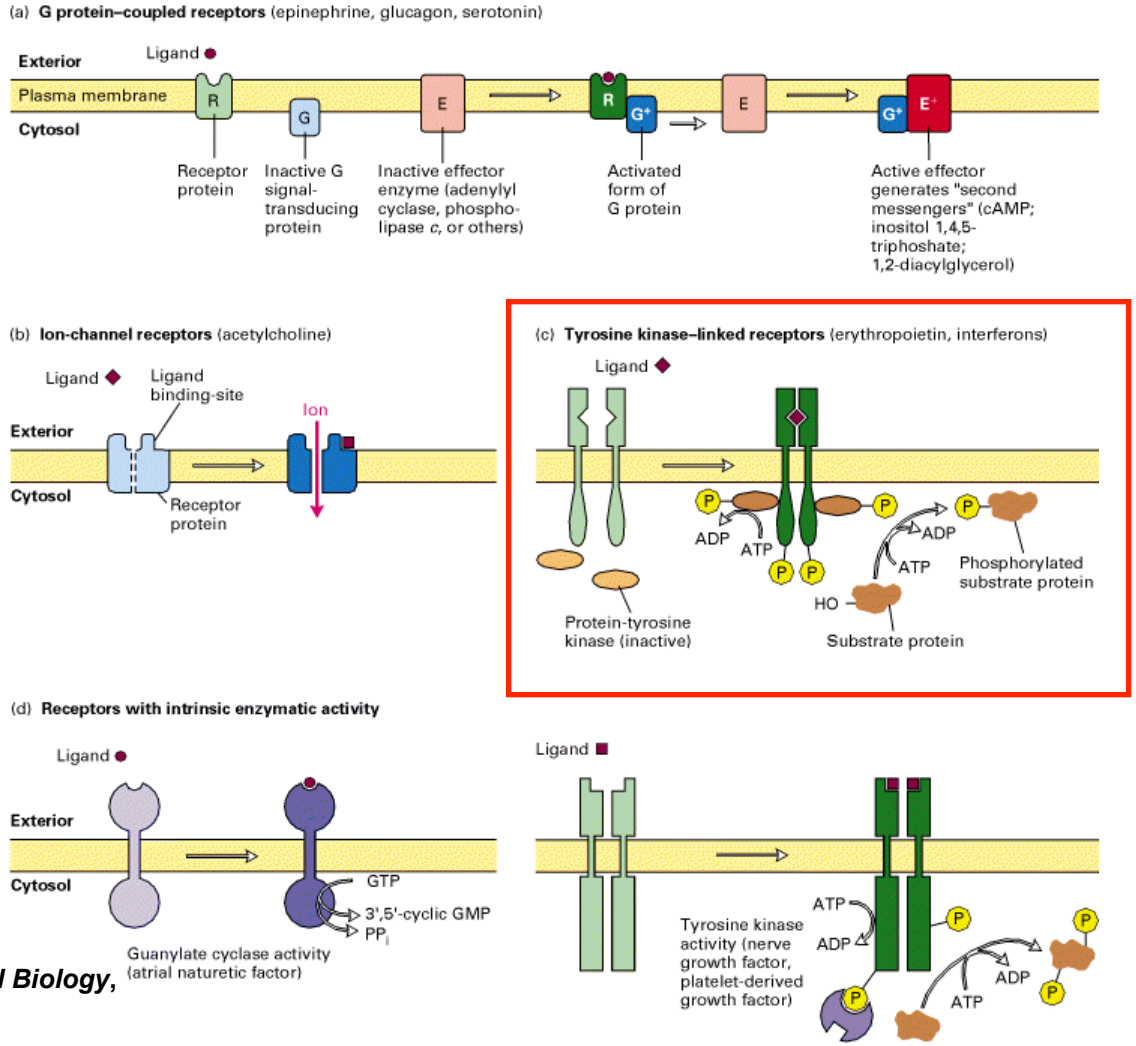
Fooksman et al., *Annu Rev Immunol* 2010



Lodish et al. *Molecular Cell Biology*, W.H. Freeman and Comp.

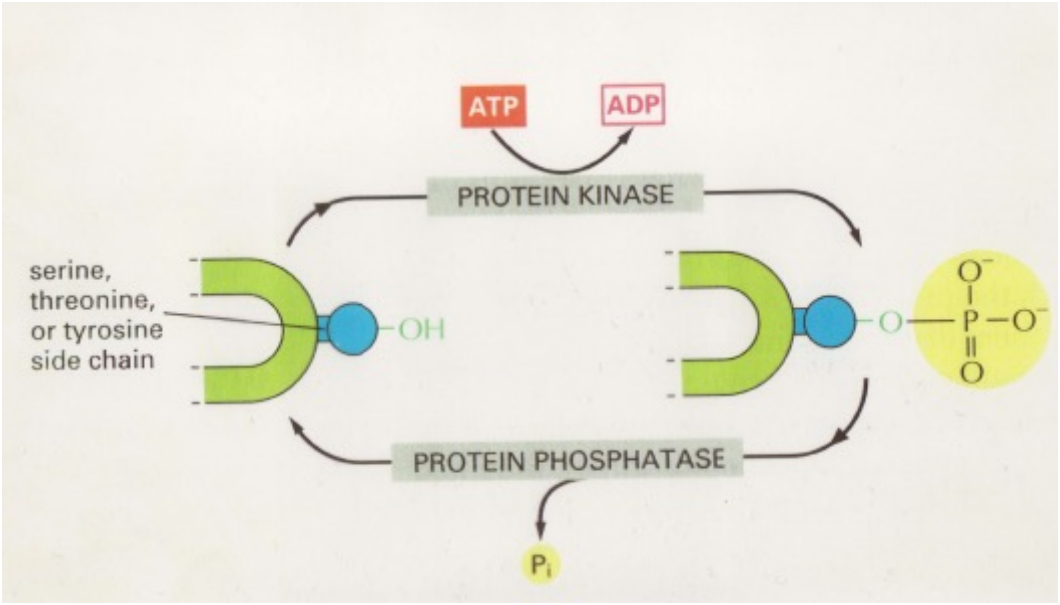
Einteilung membranständiger Rezeptoren:

1. **G-Protein gekoppelte Rezeptoren**
2. **Ionen Kanäle**
3. **Rezeptoren mit intrinsischer Enzymaktivität (S/T-Kinasen, Y-Kinasen, Phosphatasen)**
4. **Rezeptoren ohne intrinsische Enzymaktivität; sind mit Protein –Tyr-Kinasen assoziiert (Cytokinrezeptor-Superfamilie)**



Lodish et al. *Molecular Cell Biology*, W.H. Freeman and Comp.

Phosphorylierung / Dephosphorylierung als wichtiges Mittel der Signalweitergabe



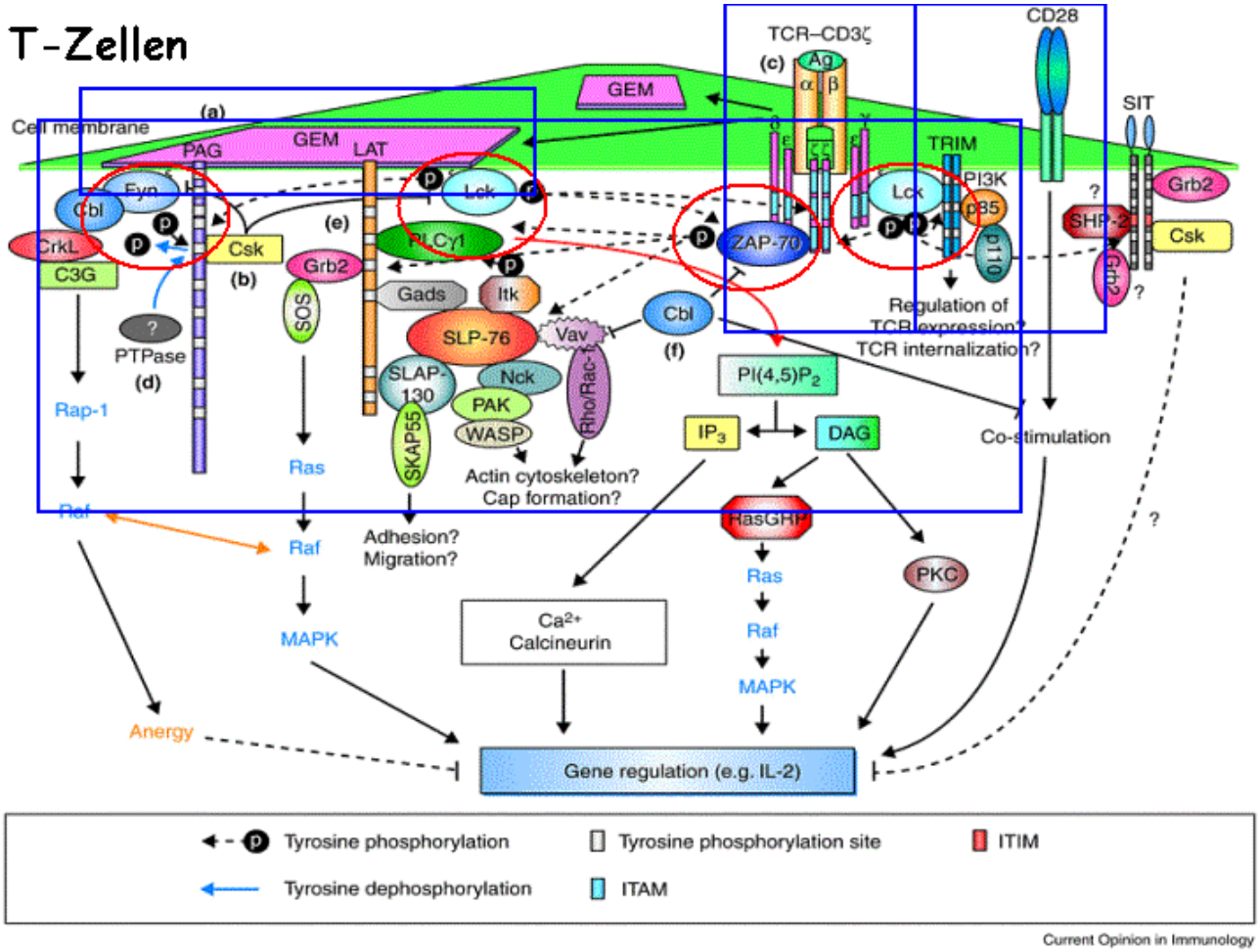
Etwa 1/3 aller Proteine in Säugerzellen sind phosphoryliert

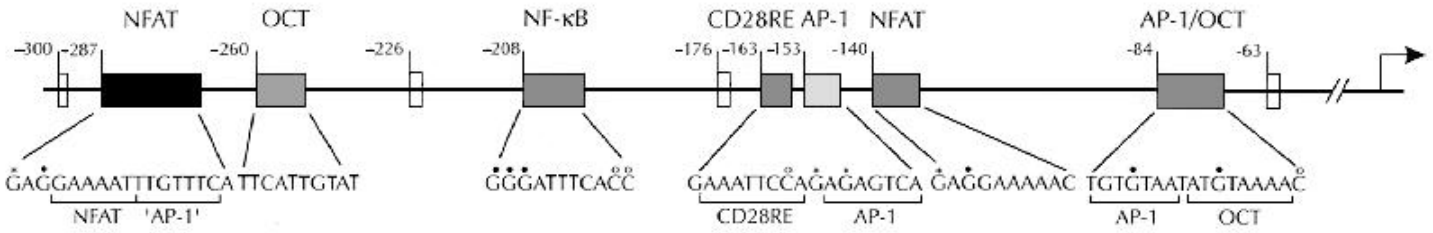
Phosphorylierung ist häufigste posttranslationale Modifikation

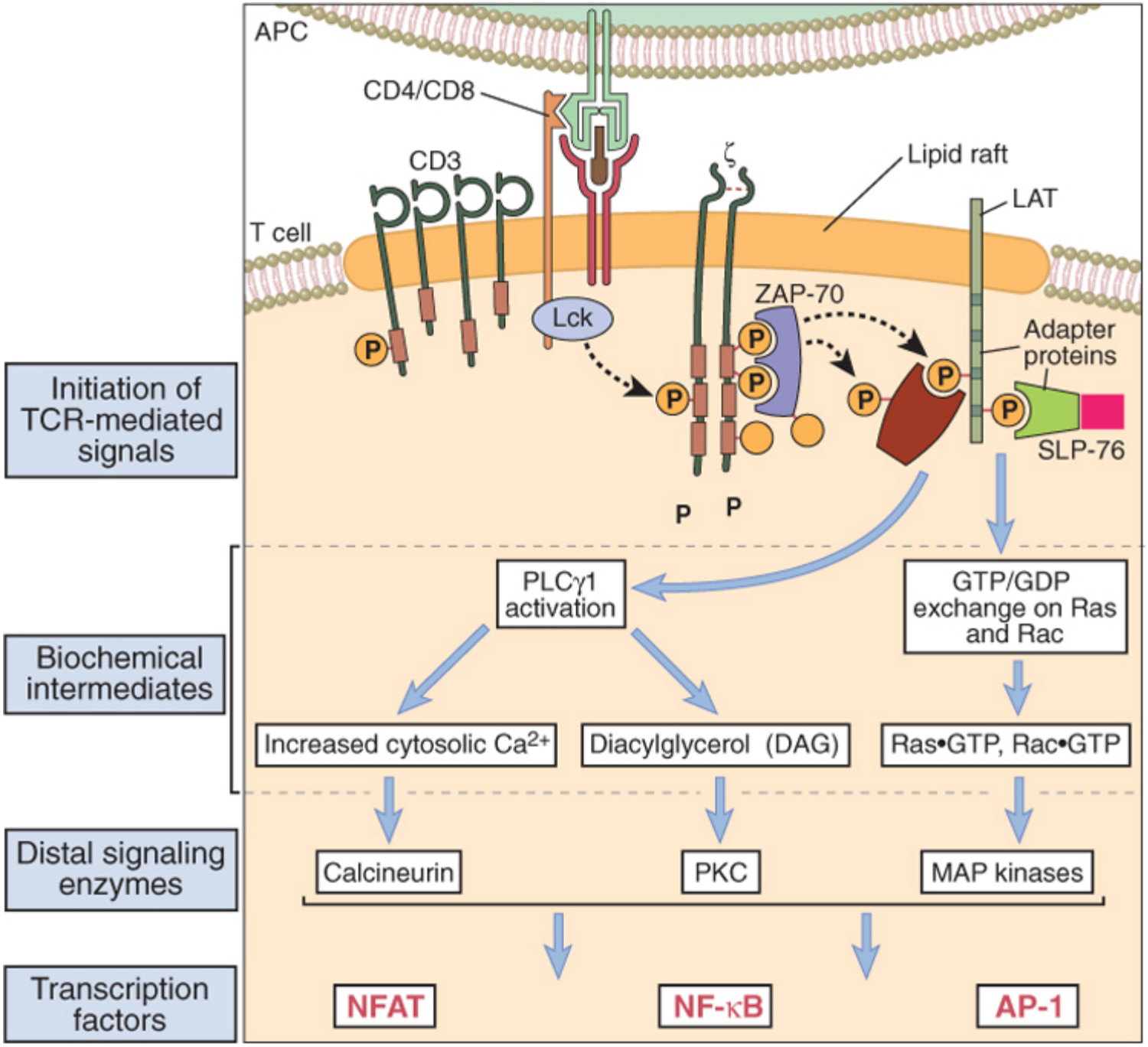
Verhältnis P-Ser : P-Thr : P-Tyr = 1800 : 200 : 1

Etwa 200 Kinasen (2-3% des Genoms) und 100 Phosphatasen sind bekannt

T-Zellen







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Geladene Aminosäuren

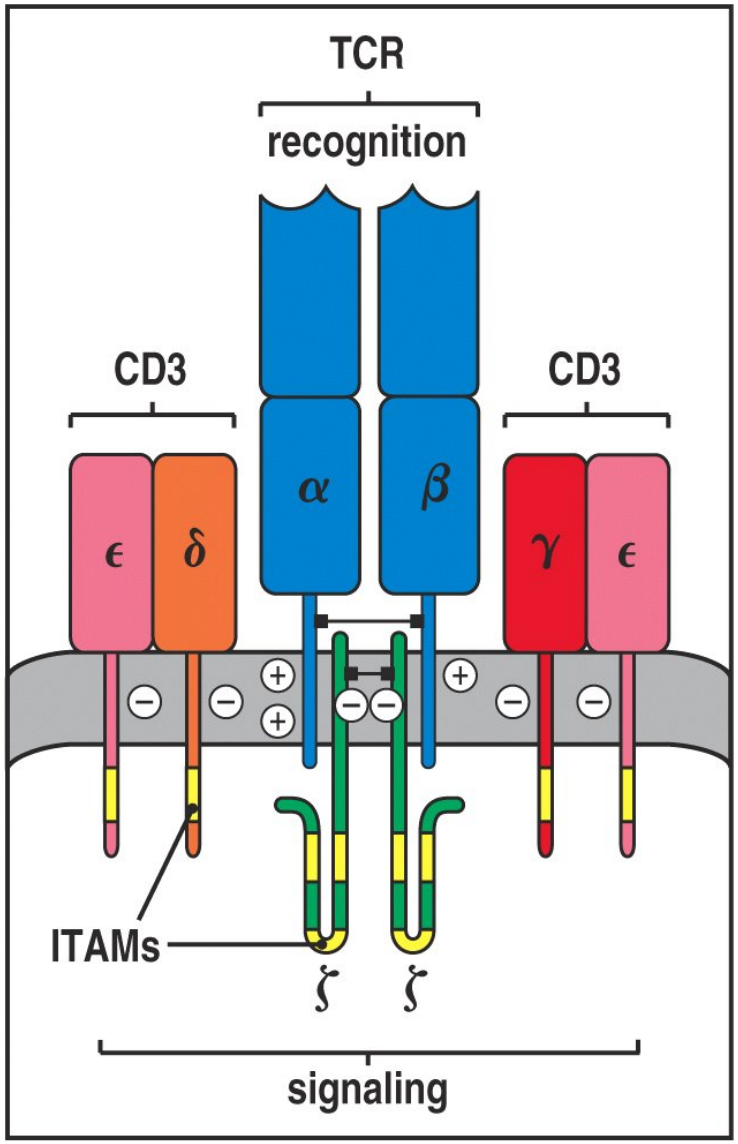
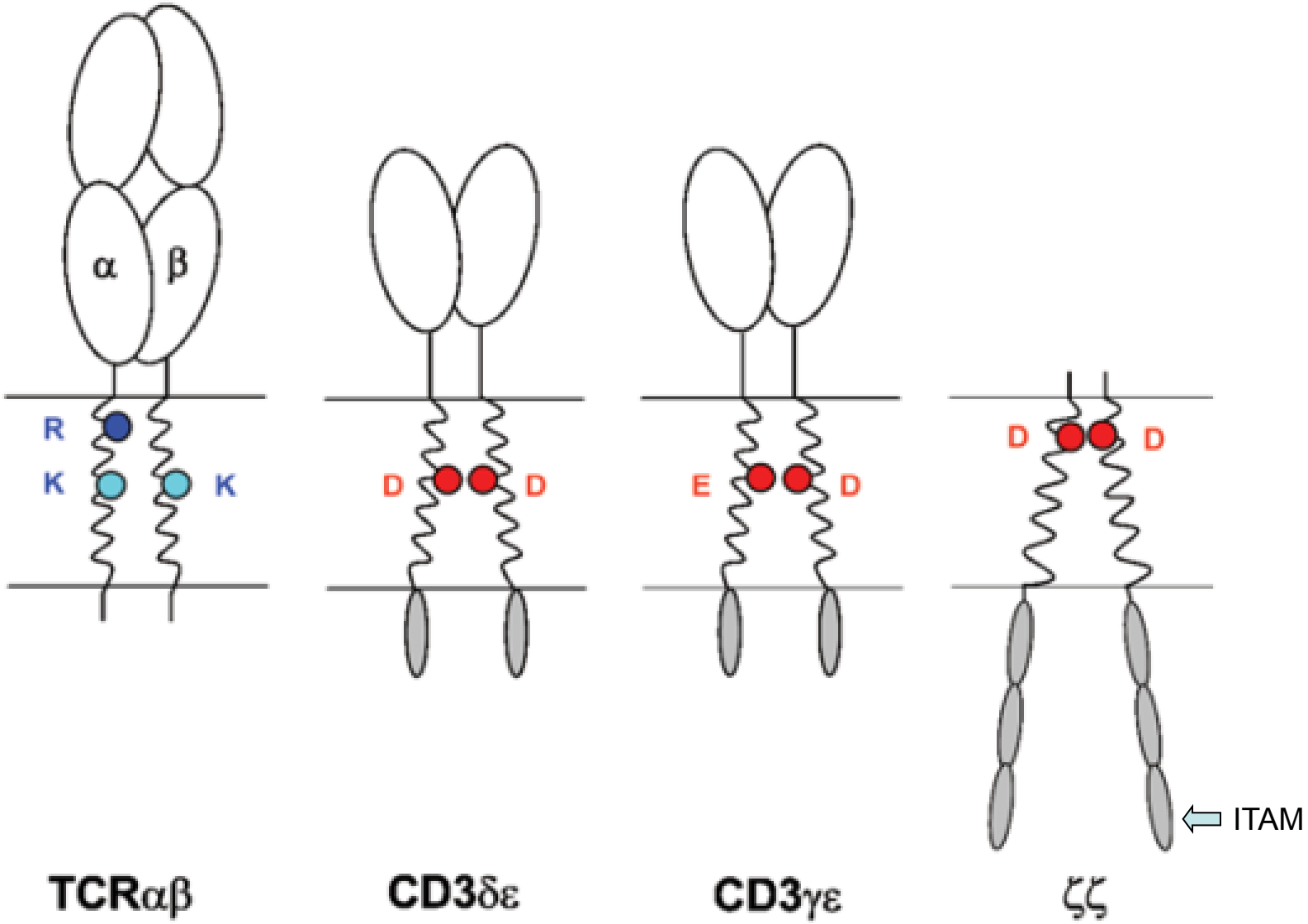


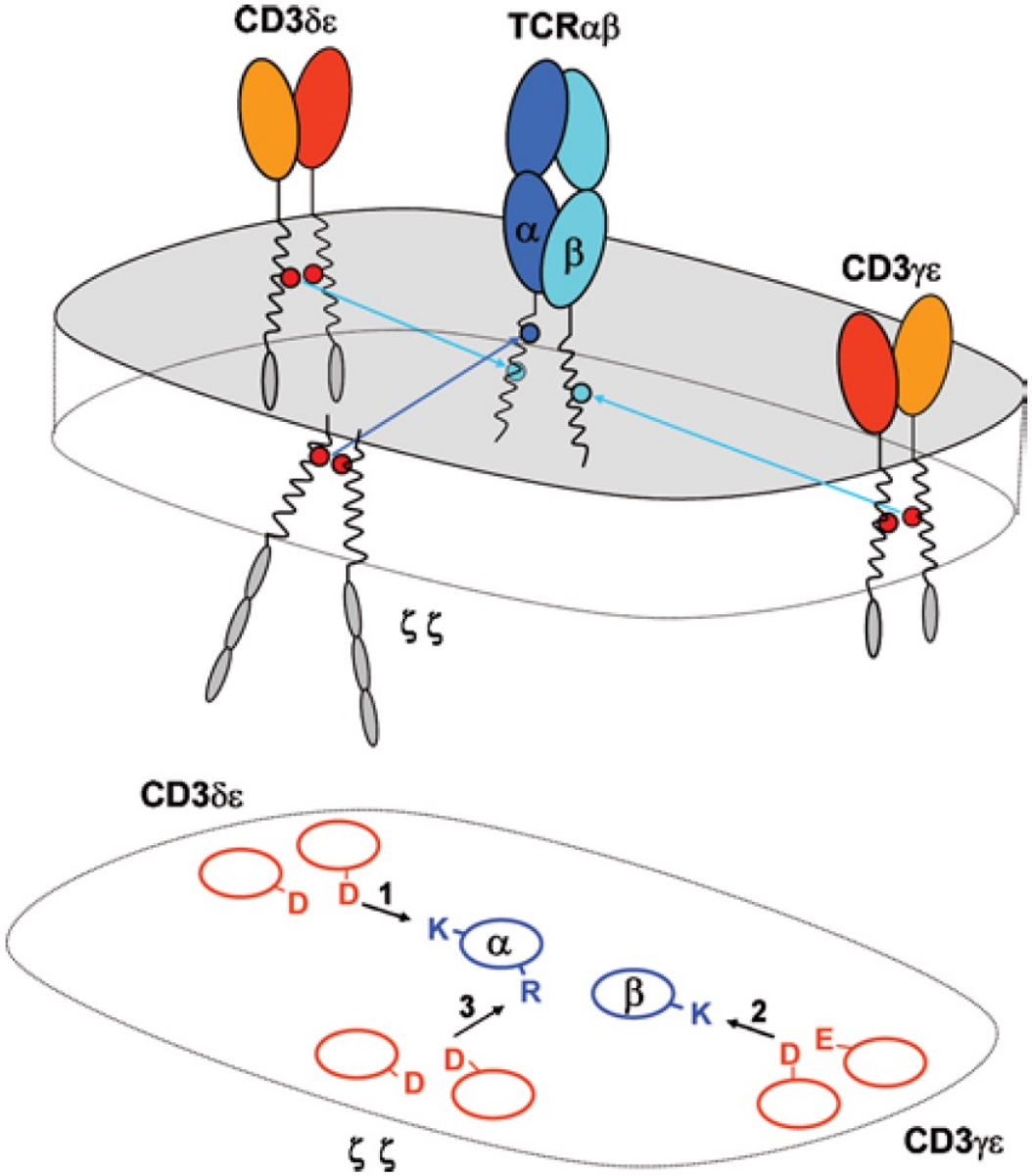
Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

ITAM: Immunoreceptor tyrosine-based activation motive

Tyr-X-X-Leu/Val-$X_{7\text{-}11}$-Tyr-X-X-Leu/Val



Call & Wucherpfennig, *Annu. Rev. Immunol*. 2005



Call & Wucherpfennig, *Annu. Rev. Immunol*. 2005

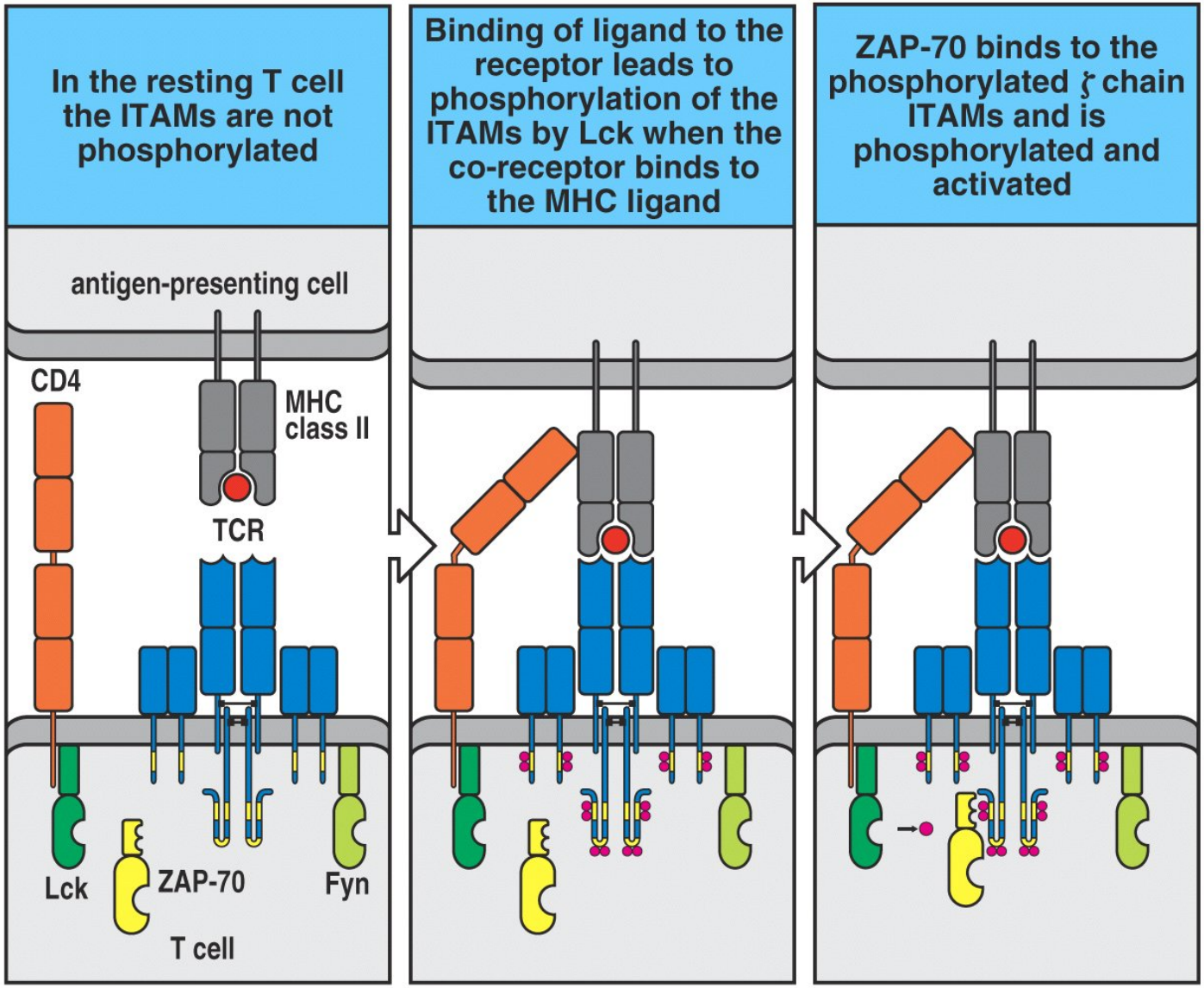


Figure 6-12 Immunobiology, 6/e. (© Garland Science 2005)

Lck, Fyn, ZAP-70: Rezeptor-assoziierte Tyrosin-Kinasen (RTKs)

Wie wird Auslösung der proximalen TZR Signale reguliert ?

1. Regulation der src-Kinase Lck

(Leukocyte-specific protein tyrosine kinase)

Exkurs: Protein-Protein Wechselwirkungen durch SH2-und SH3-Domänen

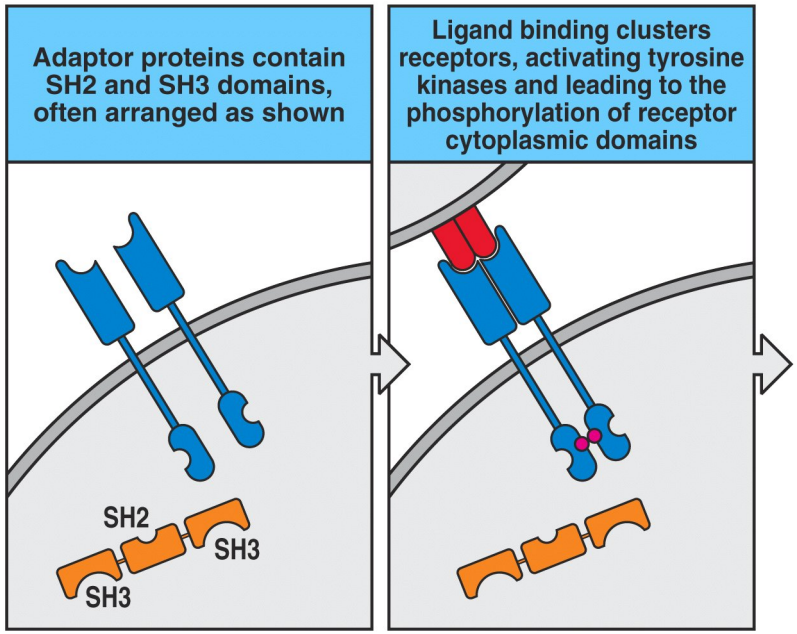


Figure 6-6 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

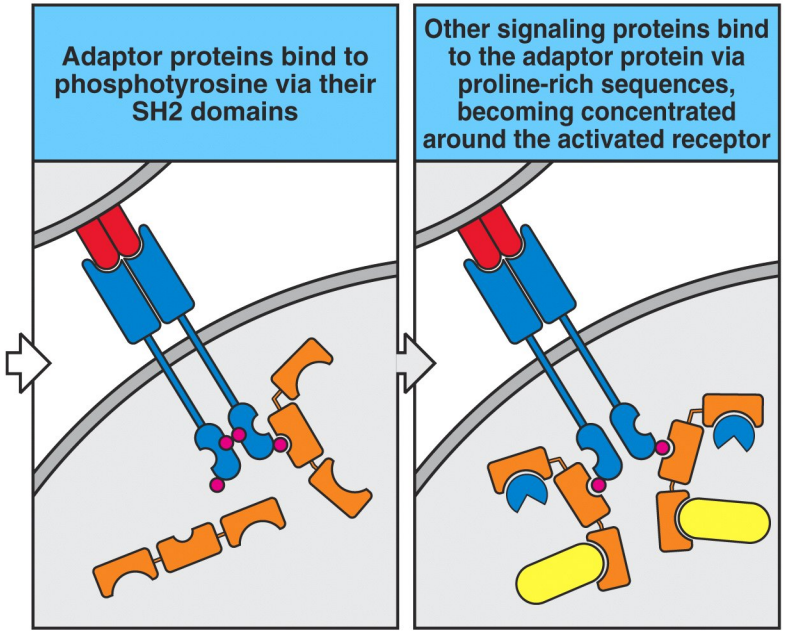


Figure 6-6 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

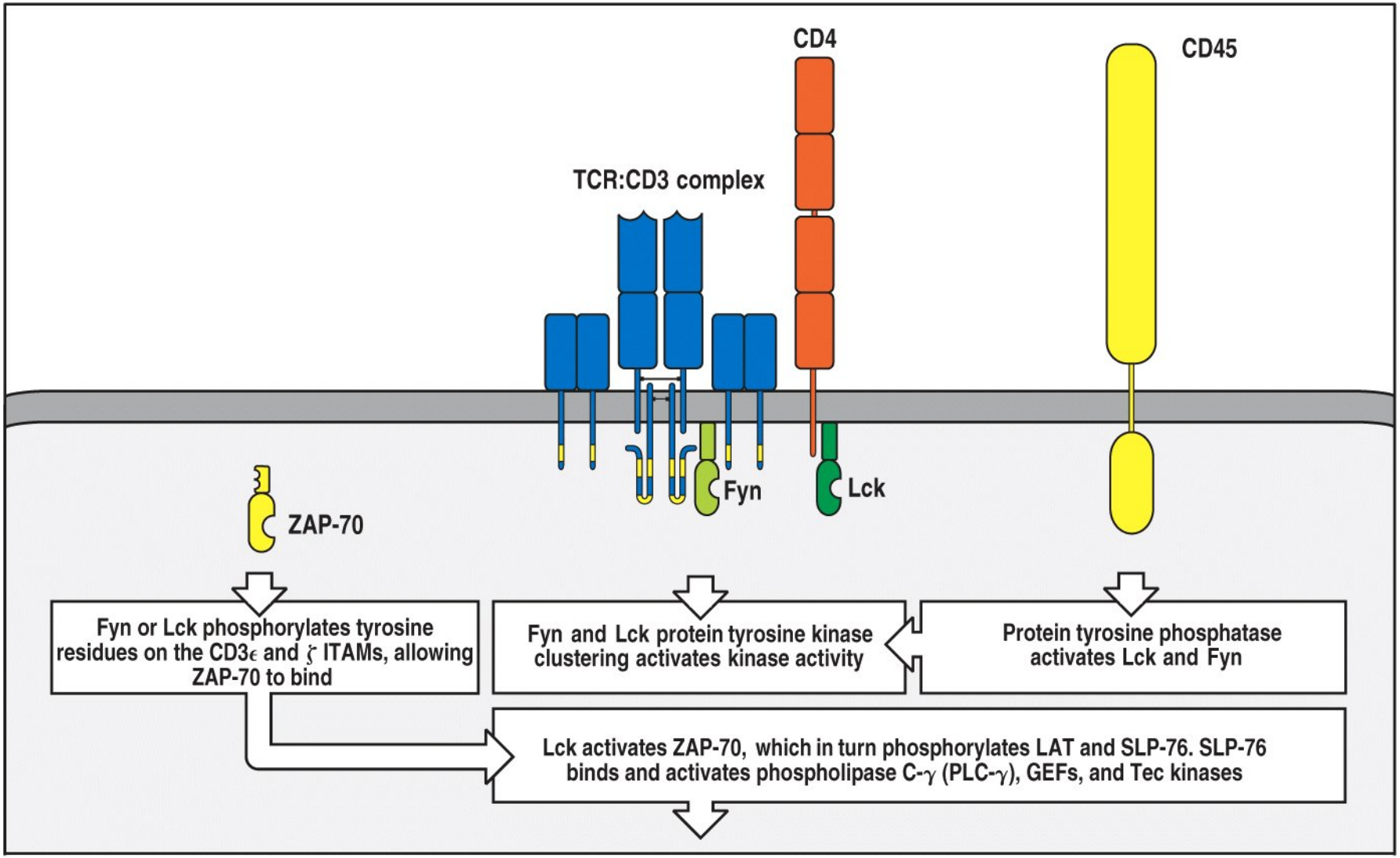


Figure 6-16 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Regulation von lck durch csk (c-*terminal src kinase*) und CD45

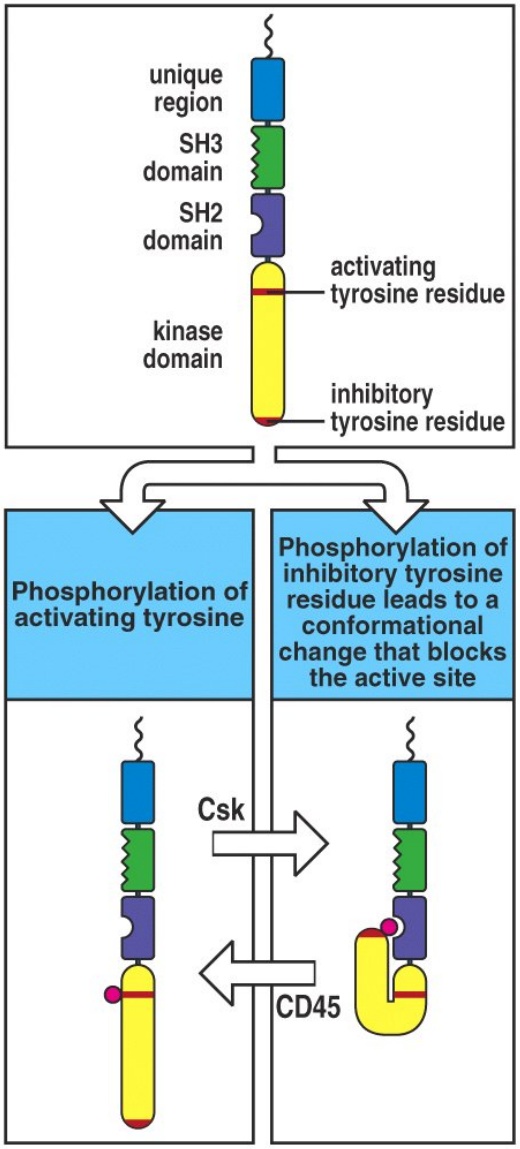


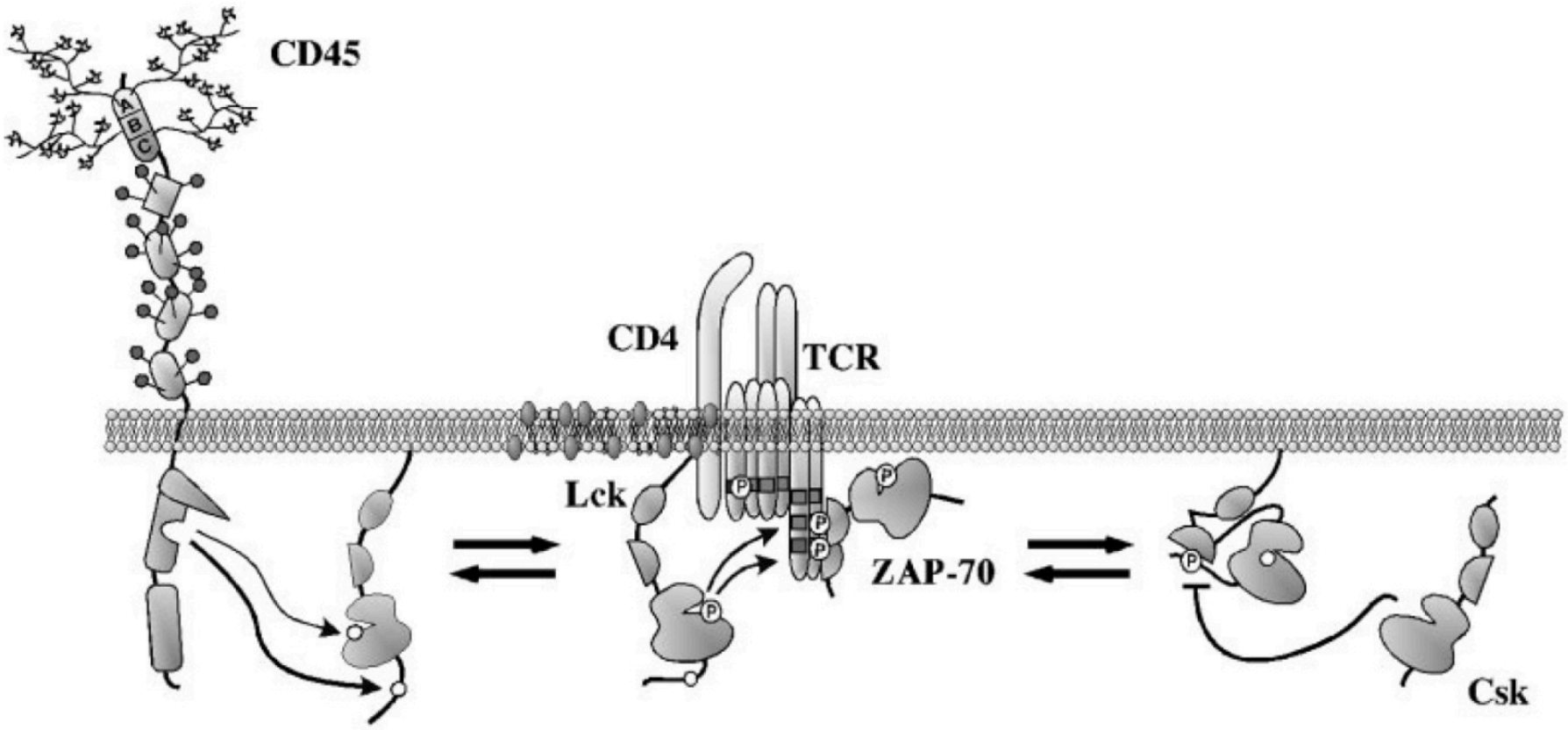
Figure 6-11 Immunobiology, 6/e. (© Garland Science 2005)

Domänenstruktur von lck

Aktive lck

Inaktive lck

Regulation von lck durch csk (*c-terminal src kinase*) und CD45



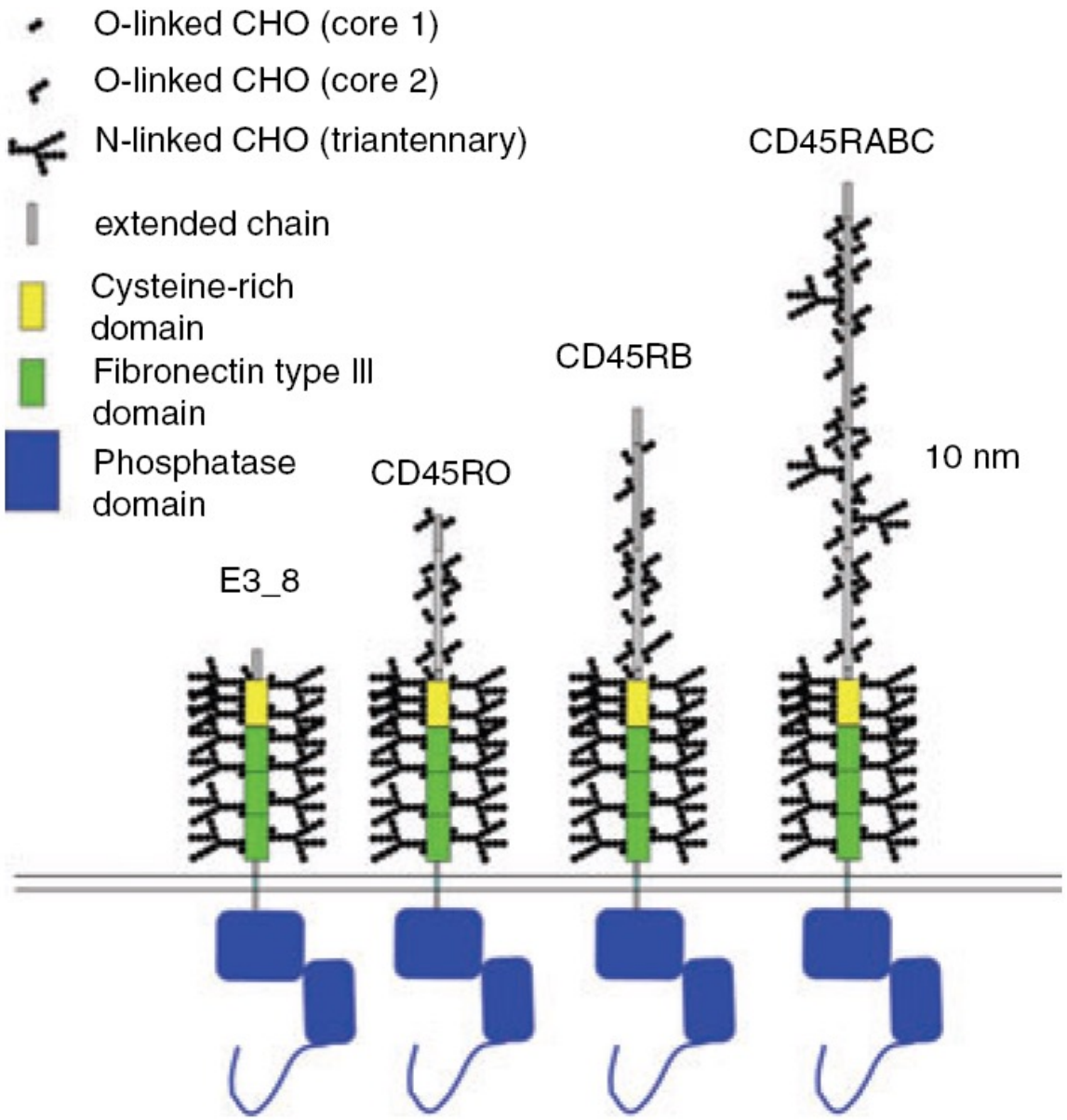
Lck Primed
Y394 (Autokatalytisches Y)
Y505 (Inhibitorisches Y)

Lck Active
Y394 Ⓟ
Y505

Lck Inactive
Y394
Y505 Ⓟ

auto-transphosphorylation

Hermiston et al. *Annu. Rev. Immunol*. 2003



The CD45 isoform family

Holmes, *Immunology*, 2005

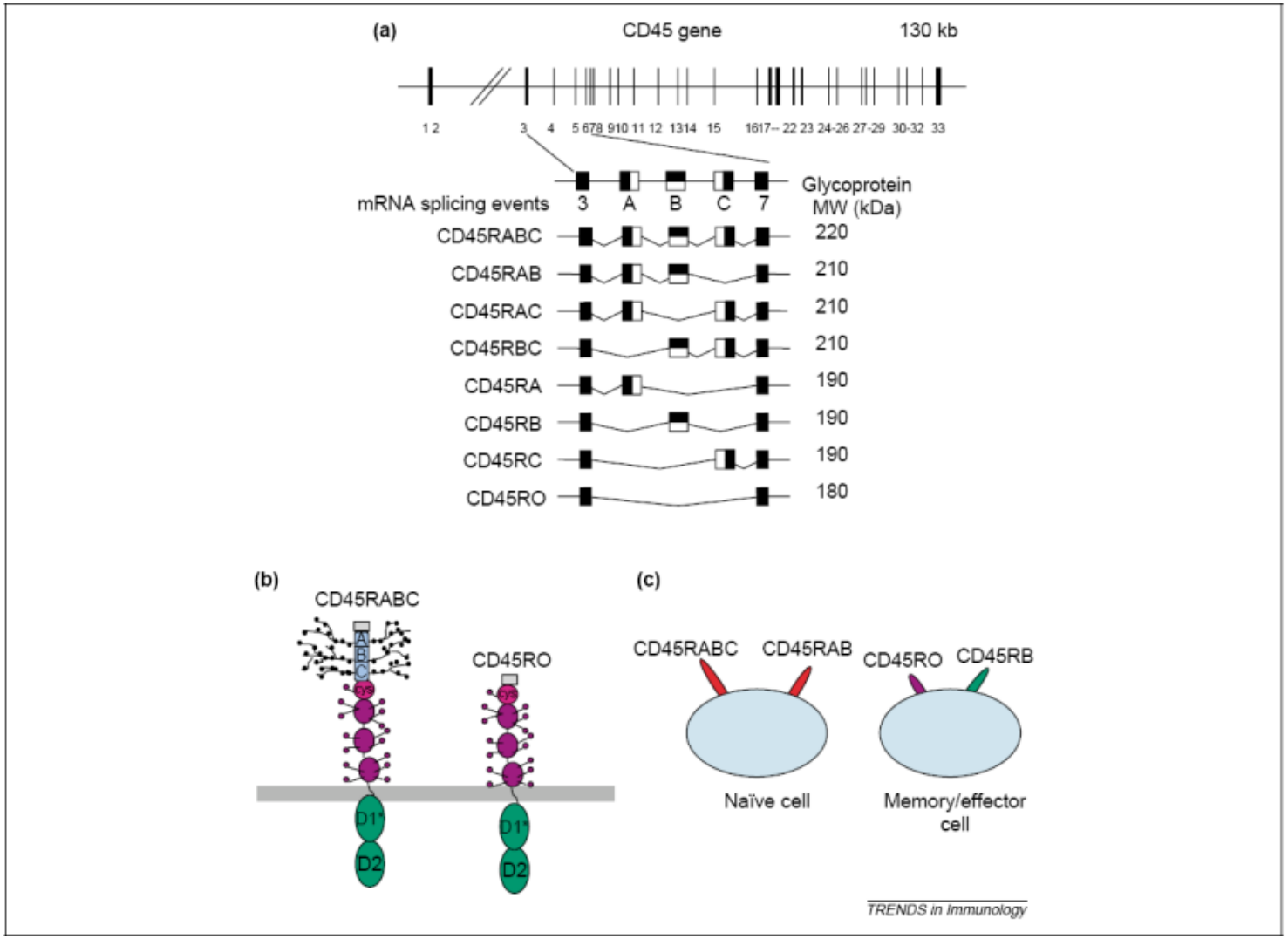
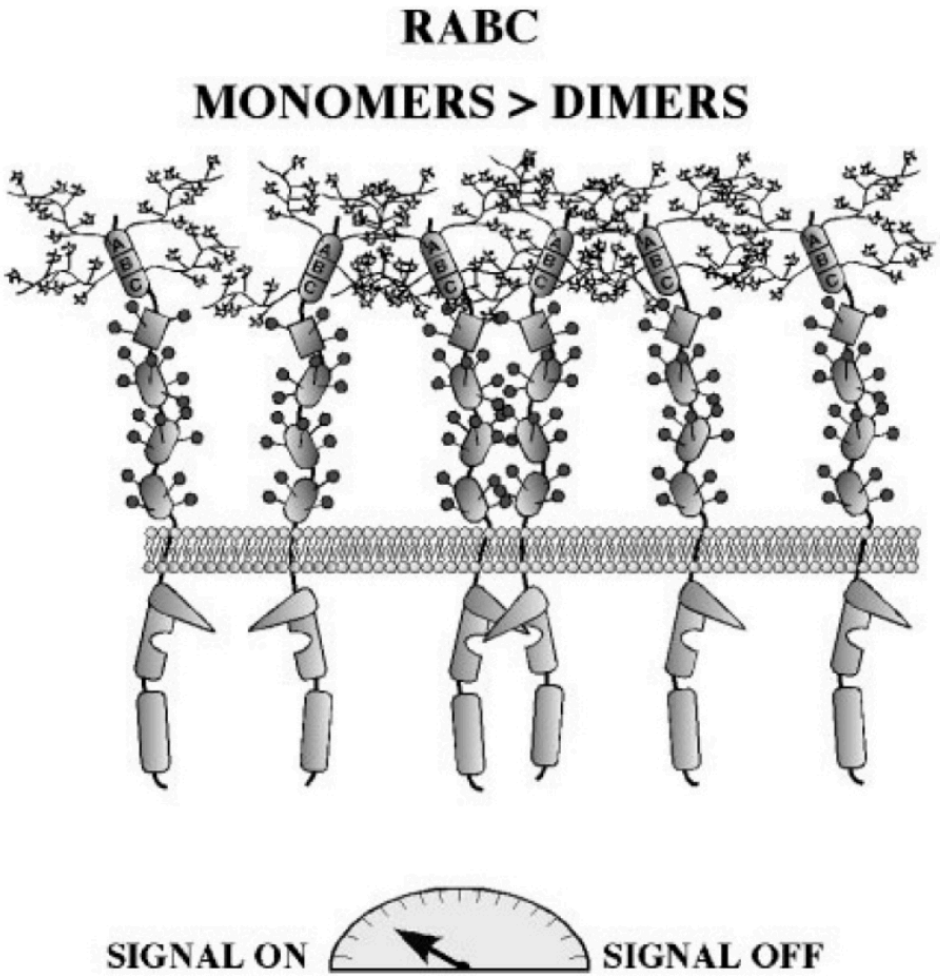
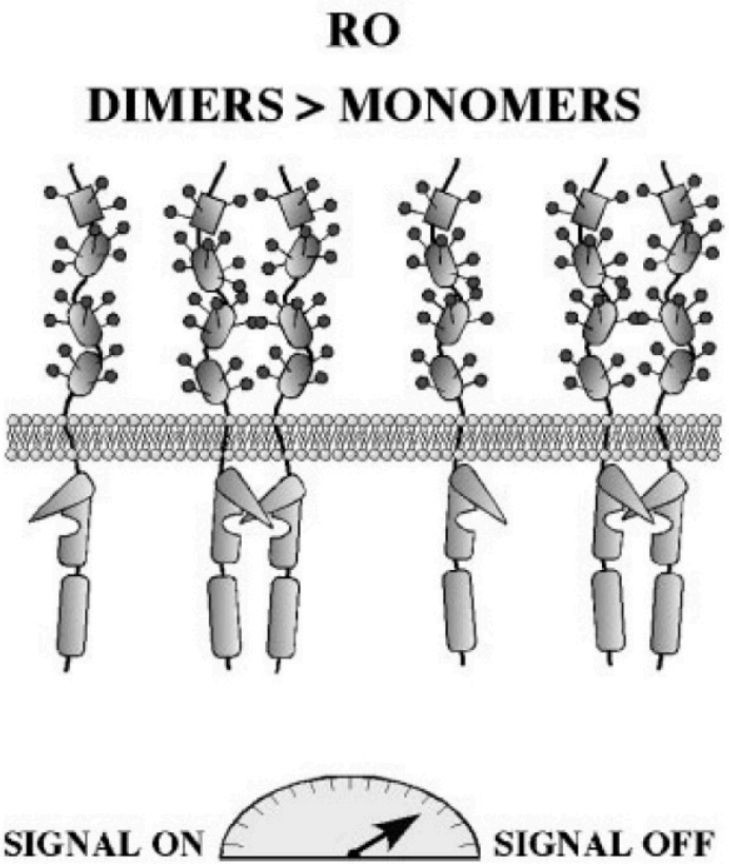


Figure 1. Gene and protein structure of CD45. **(a)** Exon–intron structure of the gene, including a very large intron (~50 kb) between exons 2 and 3. Alternative splicing of exons 4 (A), 5 (B) and 6 (C) can generate eight isoforms. **(b)** The highest (CD45RABC) and lowest (CD45RO) MW isoforms are shown. Anti-CD45R (restricted) antibodies to the A, B and C exons (blue) detect any isoform containing the respective exon, whereas anti-CD45RO detects an isoform lacking the A, B and C exons. All isoforms have a common extracellular part derived from exons 7–15 that encodes a cysteine rich domains (cys, pink) and three fibronectin-like domains (purple), a transmembrane segment (exon 16) and intracytoplasmic phosphatase domains (green; D1* catalytically active and D2 inactive). O-glycosylation of the A, B and C exons (solid dark lines with filled circles) and N-glycosylation of the fibronectin-like domains (short lines and purple circles) are shown. **(c)** As well as detecting specific isoforms, anti-CD45R antibodies identify subsets of leucocytes. For example, anti-CD45RA antibodies identify naïve T cells expressing high MW isoforms (CD45RABC and CD45RAB), whereas anti-CD45RO antibodies identify memory cells expressing low MW (CD45RO and CD45RB) isoforms.

Tchilian & Beverley, *TRENDS in Immunology*, 2006

Was reguliert die Regulatoren ?





Naive T-Zelle —Mehrere Tage→ Effektor-/Gedächtniszelle

Hermiston et al. *Annu. Rev. Immunol.* 2003

Aktivierung des T-Zell Rezeptors führt zur Aggregation von „Lipid Rafts“ und zur Ausbildung der Immunologischen Synapse

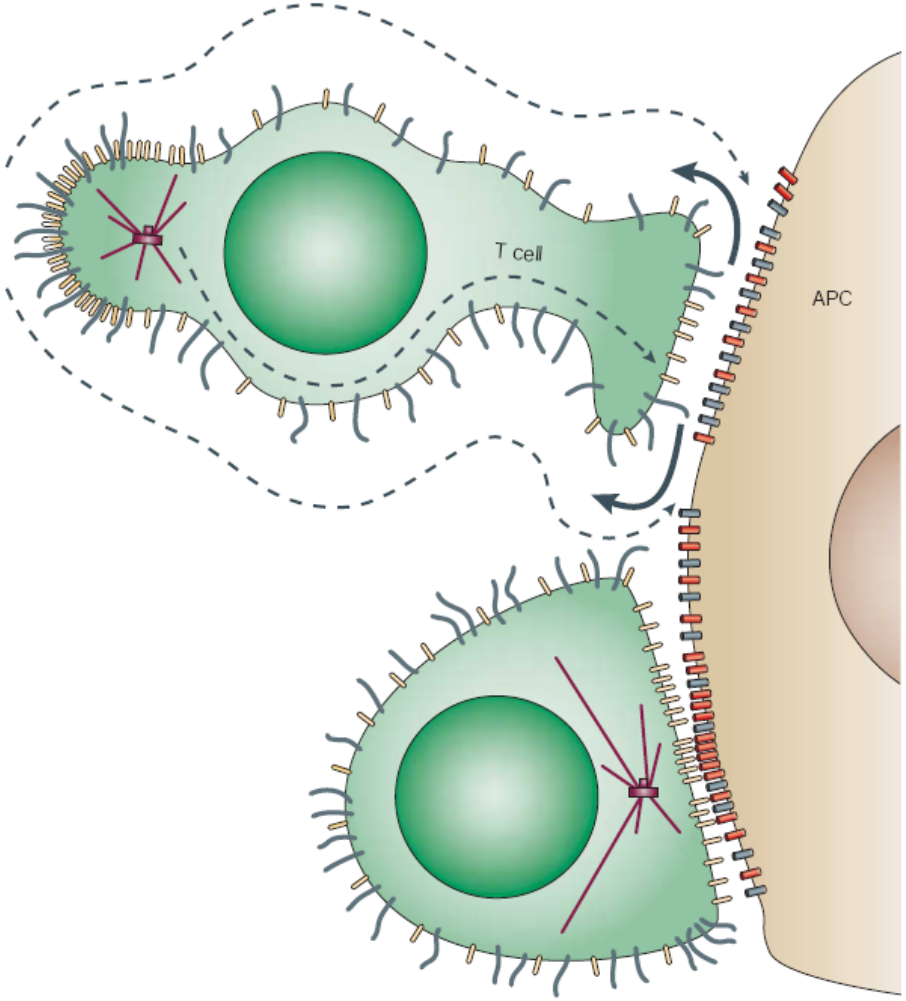


Figure 3 | **Morphological and cytoskeletal changes in the initial engagement and formation of a stable T-helper-cell synapse.** After initial engagement of the T-cell receptor (TCR) with its cognate peptide–MHC complex a T cell stops migrating and the microtubule organizing centre (MTOC) is reoriented from the uropod to beneath the immunological synapse. TCR molecules (yellow) are recruited into the synapse and other cell-surface molecules (for example, CD43) are excluded. Stimulatory (red) and non-stimulatory (grey) peptide–MHC complexes are present in the synapse as indicated.

Huppa & Davies, *Nature Reviews Immunology*, 2006

Lipid rafts („Flöße“): reich an Cholesterol, Glykolipiden, gesättigten Fettsäuren
Bilden dynamische Strukturen
Extraktion von Cholesterol inhibiert T-Zell Rezeptor Signal

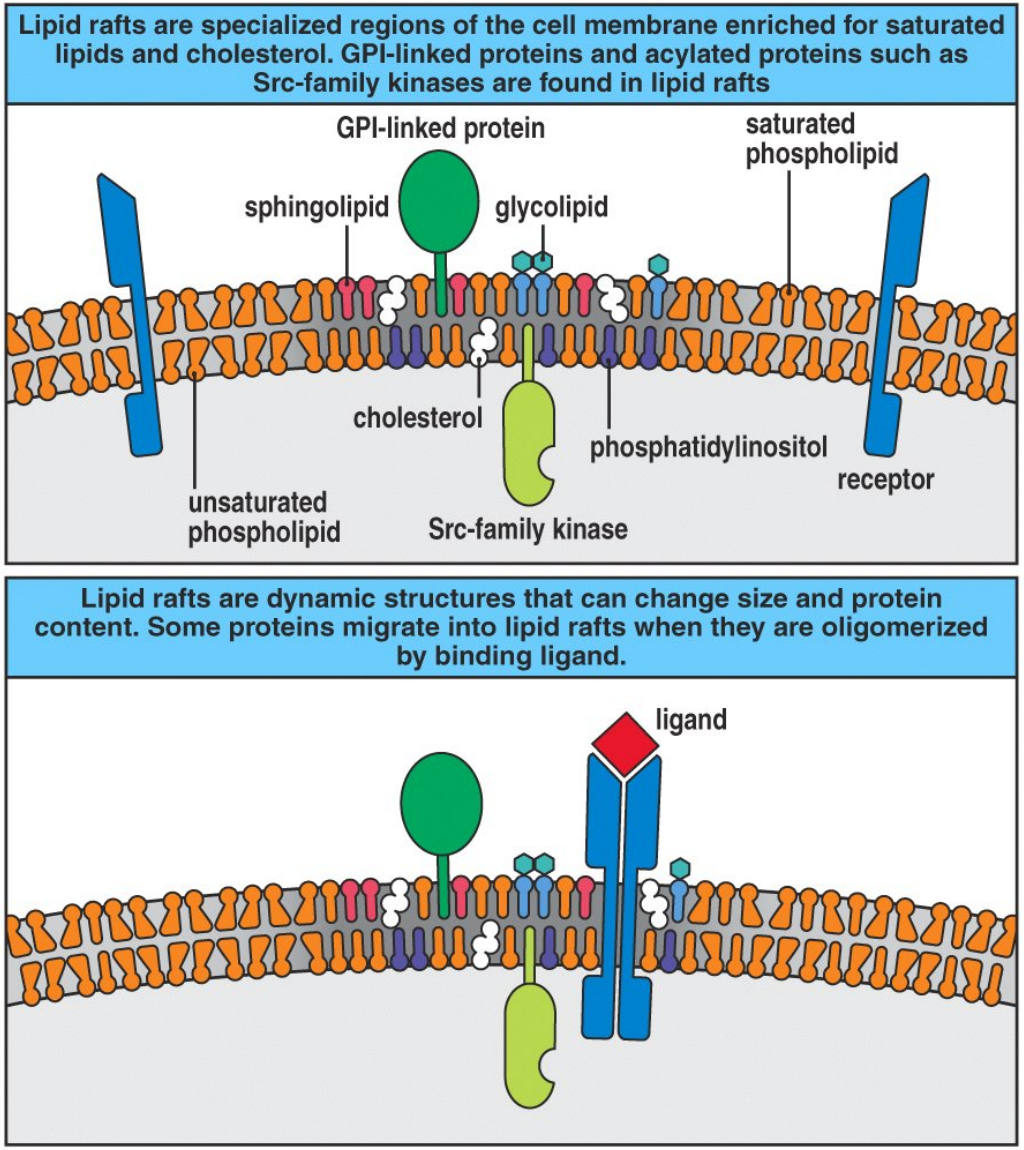


Figure 6-3 Immunobiology, 6/e. (© Garland Science 2005)

Integrin-vermittelter Kontakt zwischen T-Zelle und APC

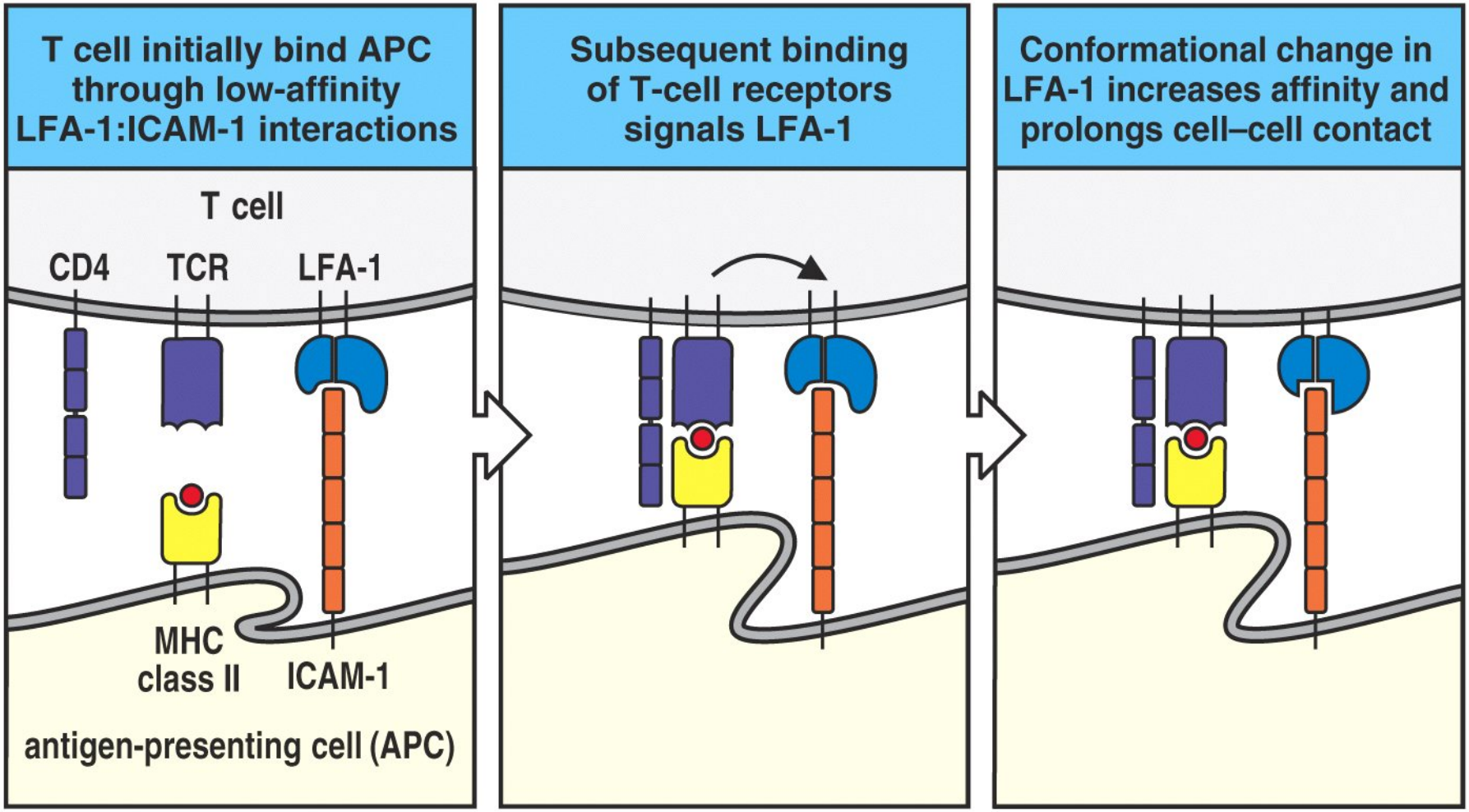
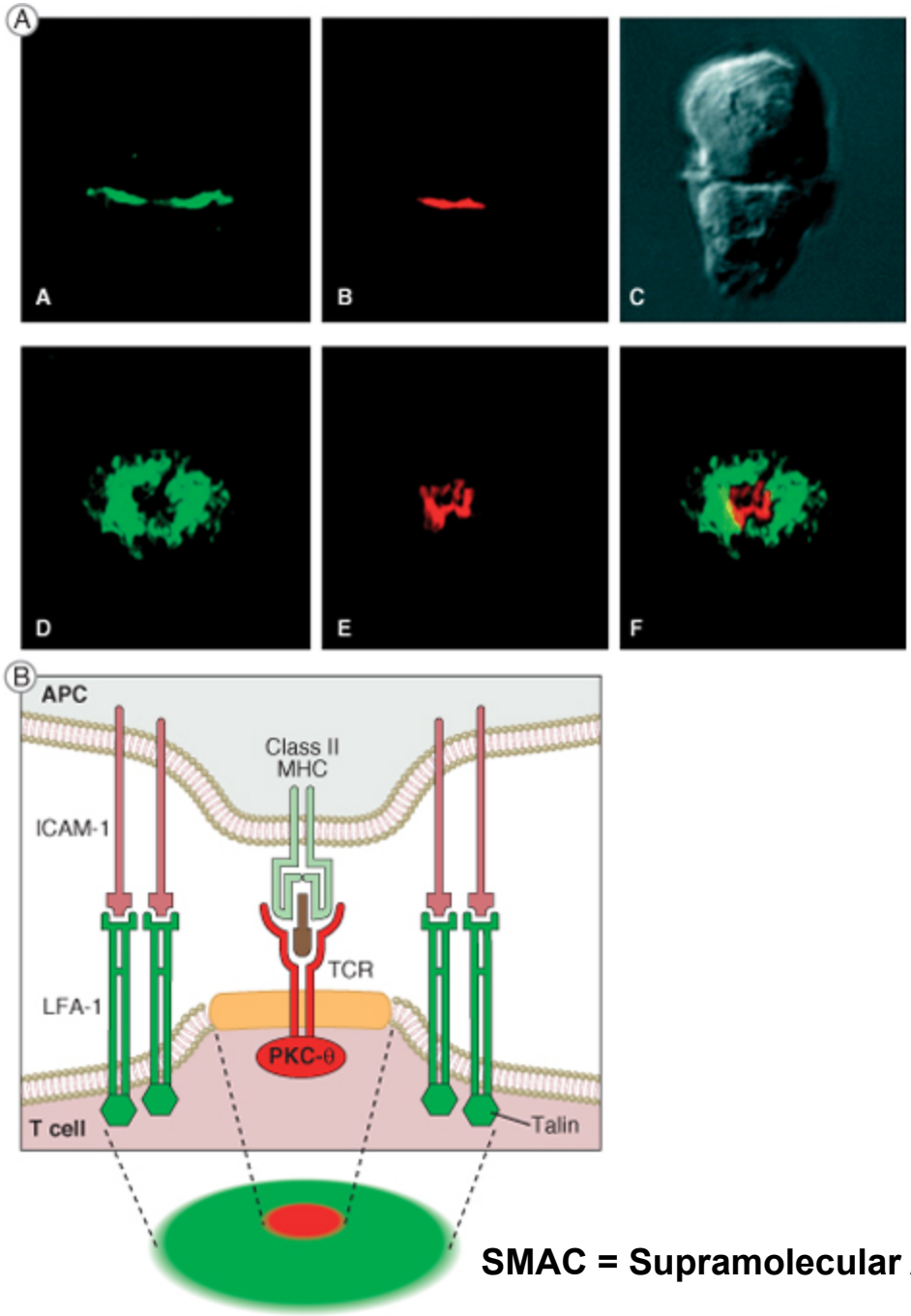


Figure 8-9 Immunobiology, 6/e. (© Garland Science 2005)

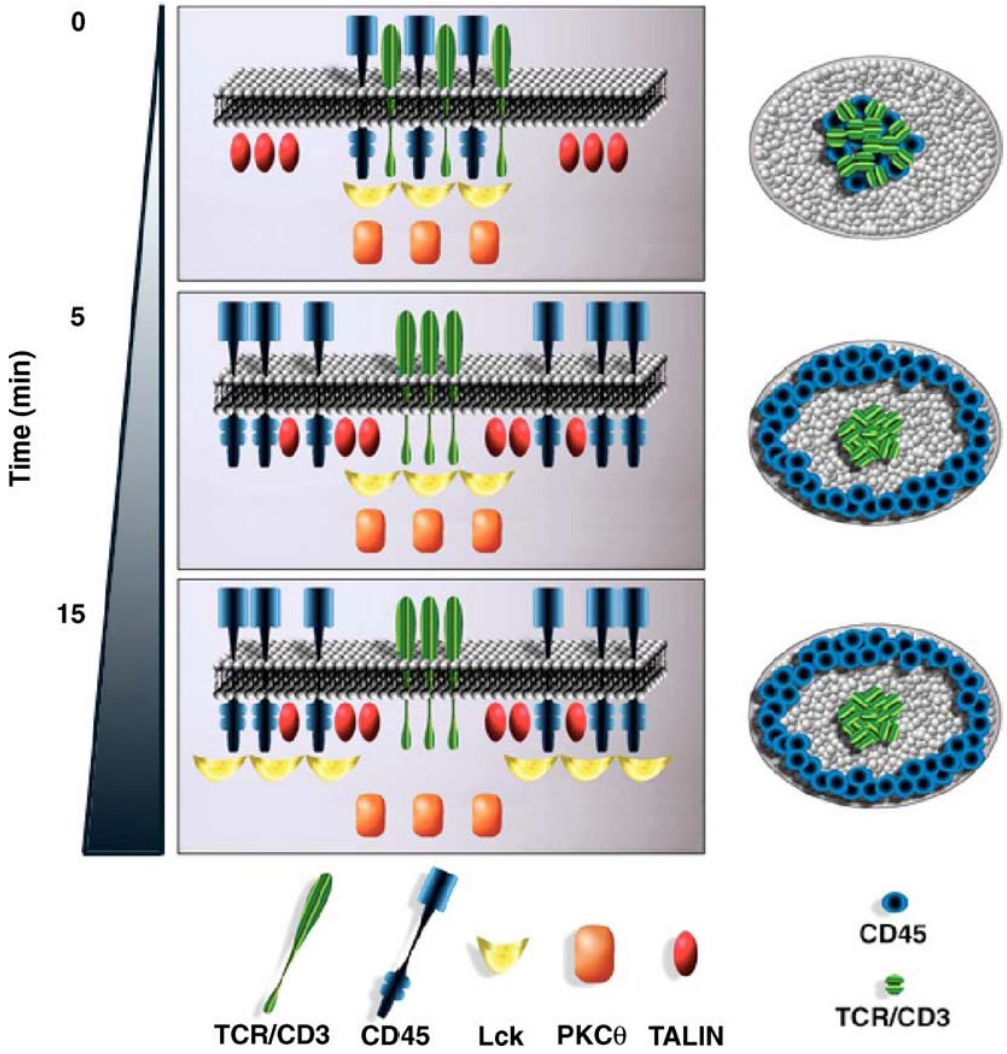
LFA-1: Lymphocyte function-associated antigen (ein Integrin)
ICAM-1: Intercellular adhesion molecule (ein Adhesionsmolekül)

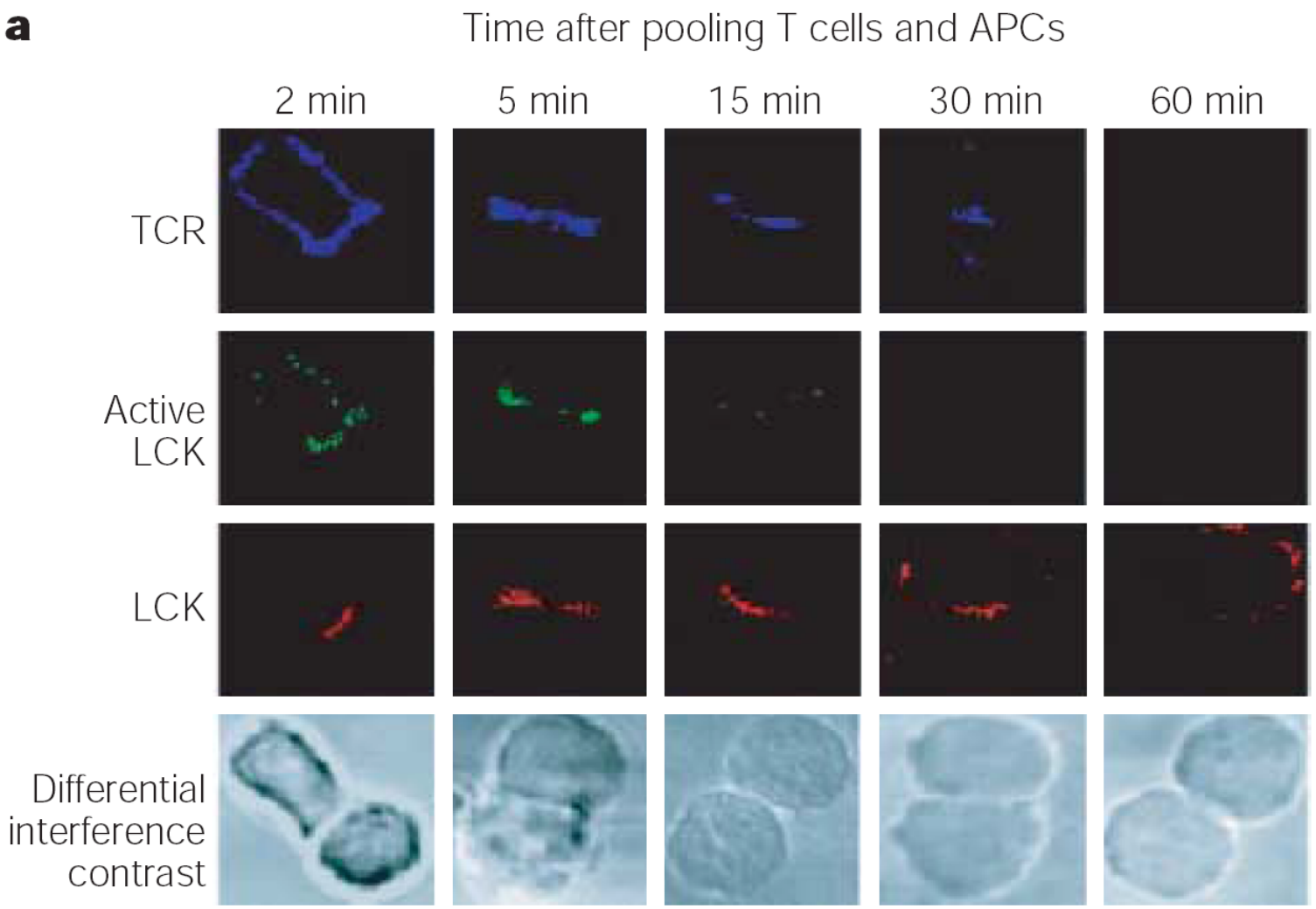


SMAC = Supramolecular Activation Cluster

Aktivierung des T-Zell Rezeptors führt zur Aggregation von „Lipid Rafts“ und zur Ausbildung der Immunologischen Synapse (SMAC = Supramolecular Activation Clusters)

CD45 wird ausgeschlossen





Huppa & Davies, *Nature Reviews Immunology*, 2006

Zusammenfassung der proximalen Ereignisse am T-Zell Rezeptor

Hemmung

Aktivierung

Expression und Dimerisierung kleiner CD45 Isoformen in aktivierten T-Zellen (enzymatisch inaktiv)

CD45 → **CD45 Monomer enzymatisch aktiv große Isoformen auf naiven T-Zellen**

Lck
(Leukocyte-specific protein tyrosine kinase)

Csk
(c-terminal src kinase)
Regulation durch Subzelluläre Lokalisation, Phosphatasen

Trennung vom TZR bei Bildung der immunologischen Synapse

Auto-trans-Phosphorylierung
Phosphorylierung ITAMs
Rekrutierung von ZAP-70
Phosphorylierung ZAP-70
(Zeta-assoziiertes Protein 70)

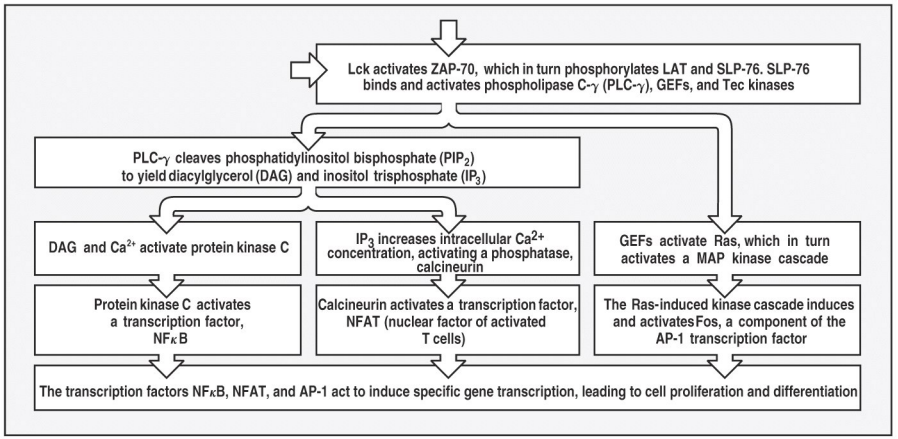


Figure 6-16 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

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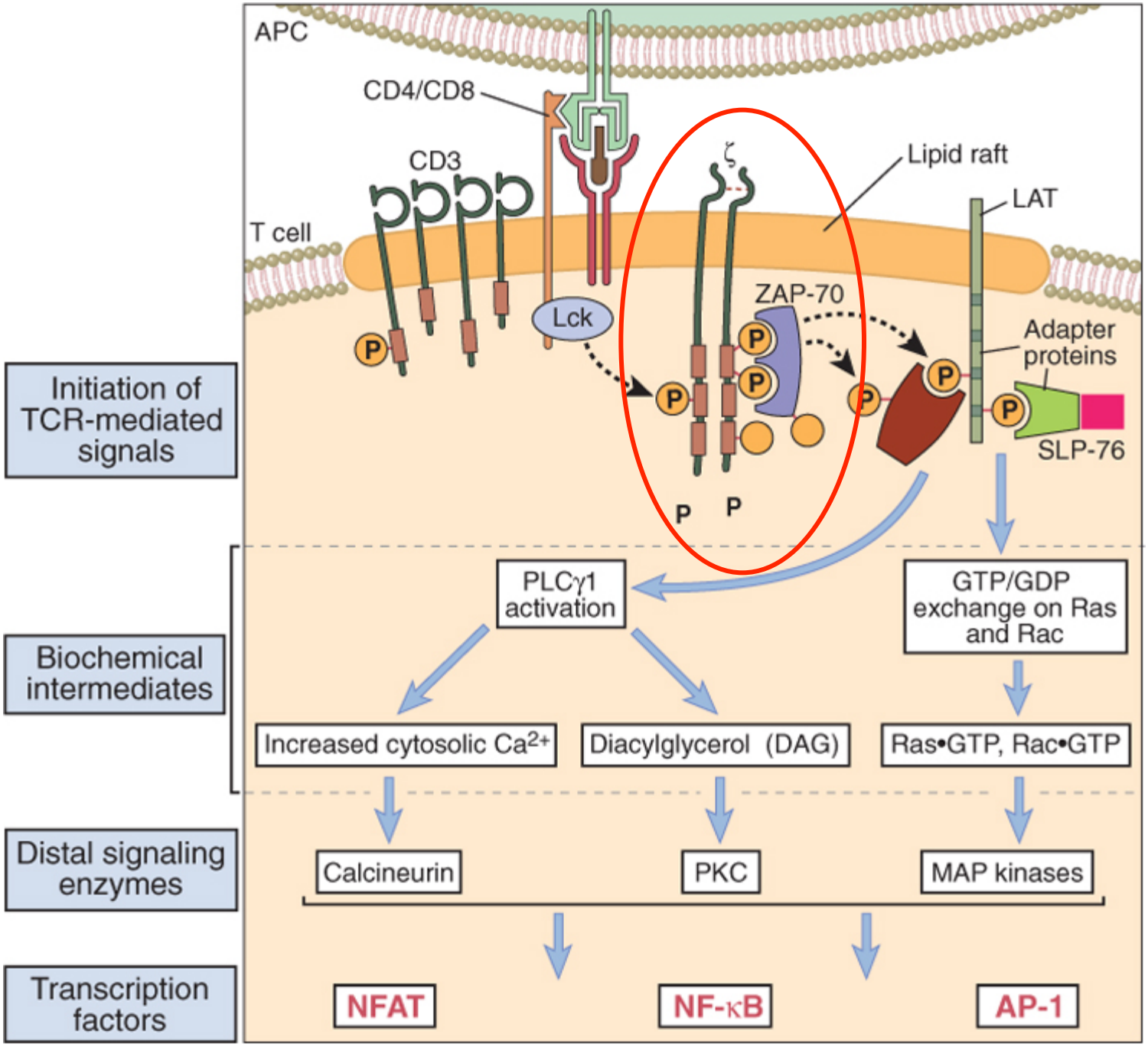
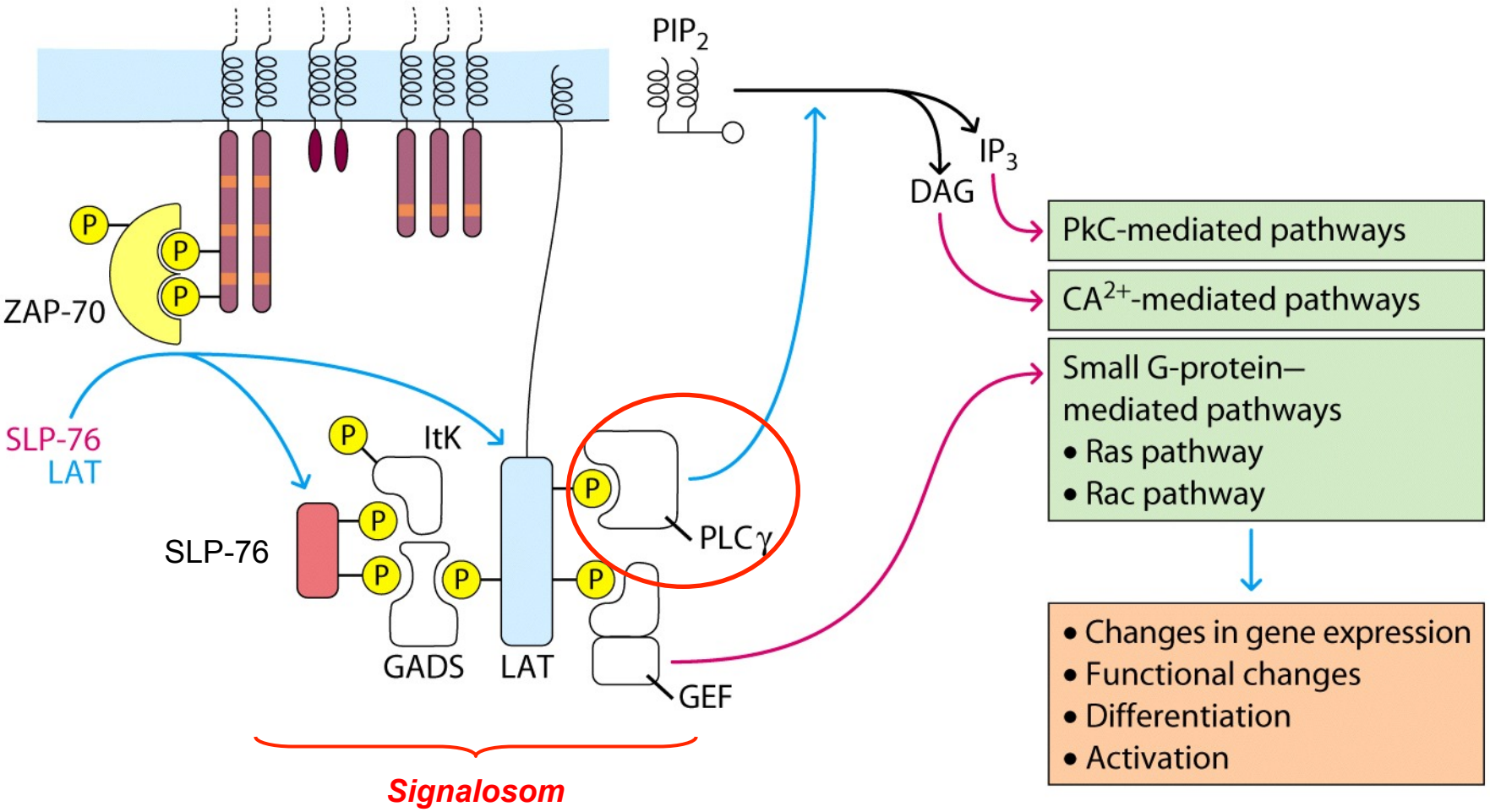
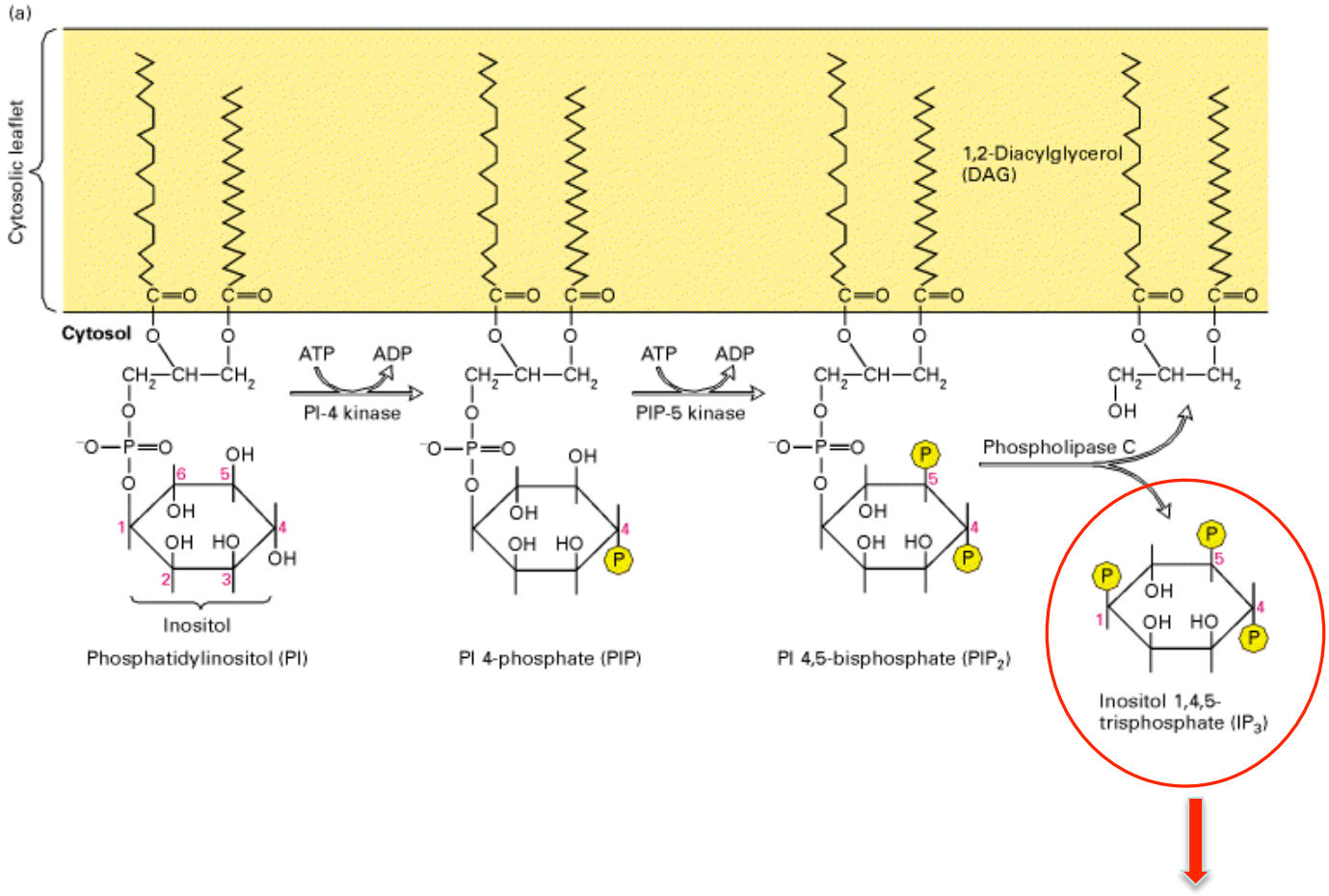


Die Bildung des „Signalosoms“ am T-Zell Rezeptor



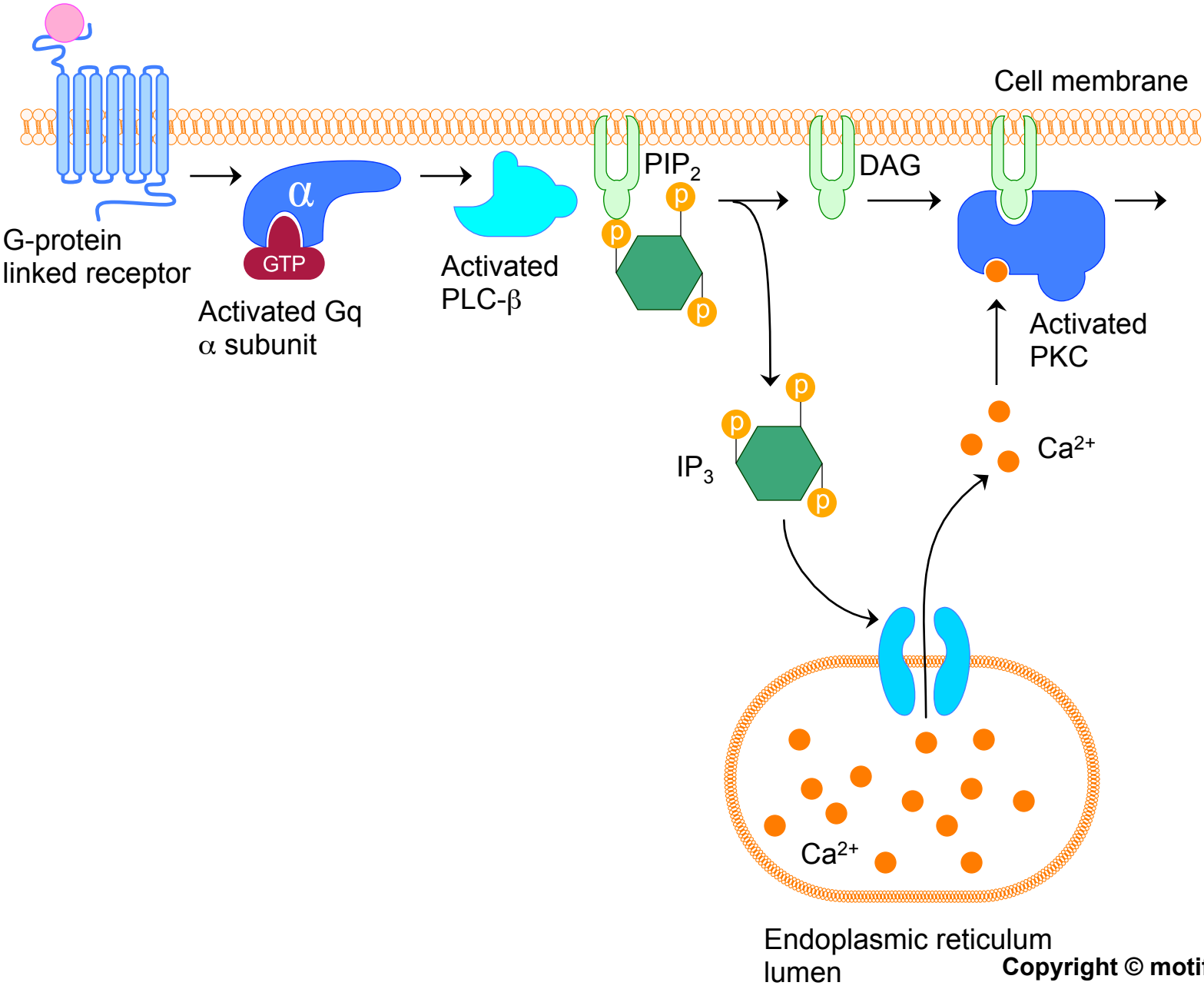
ZAP-70: Zeta-assoziiertes Protein 70; Aktivierung durch src-vermittelte Phosphorylierung
LAT (linker of activation in T cells), SLP76, GADS: Adaptermoleküle
SLP-76: SH2 domain containing leukocyte phosphoprotein of 76 kDa.
Itk (Familie der Tec-Kinasen): Phosphoryliert PLC-γ

Aktive Phospholipase Cγ erzeugt 2 wichtige Signalmoleküle:
Inositol-tris-phosphat und Diacylglycerin

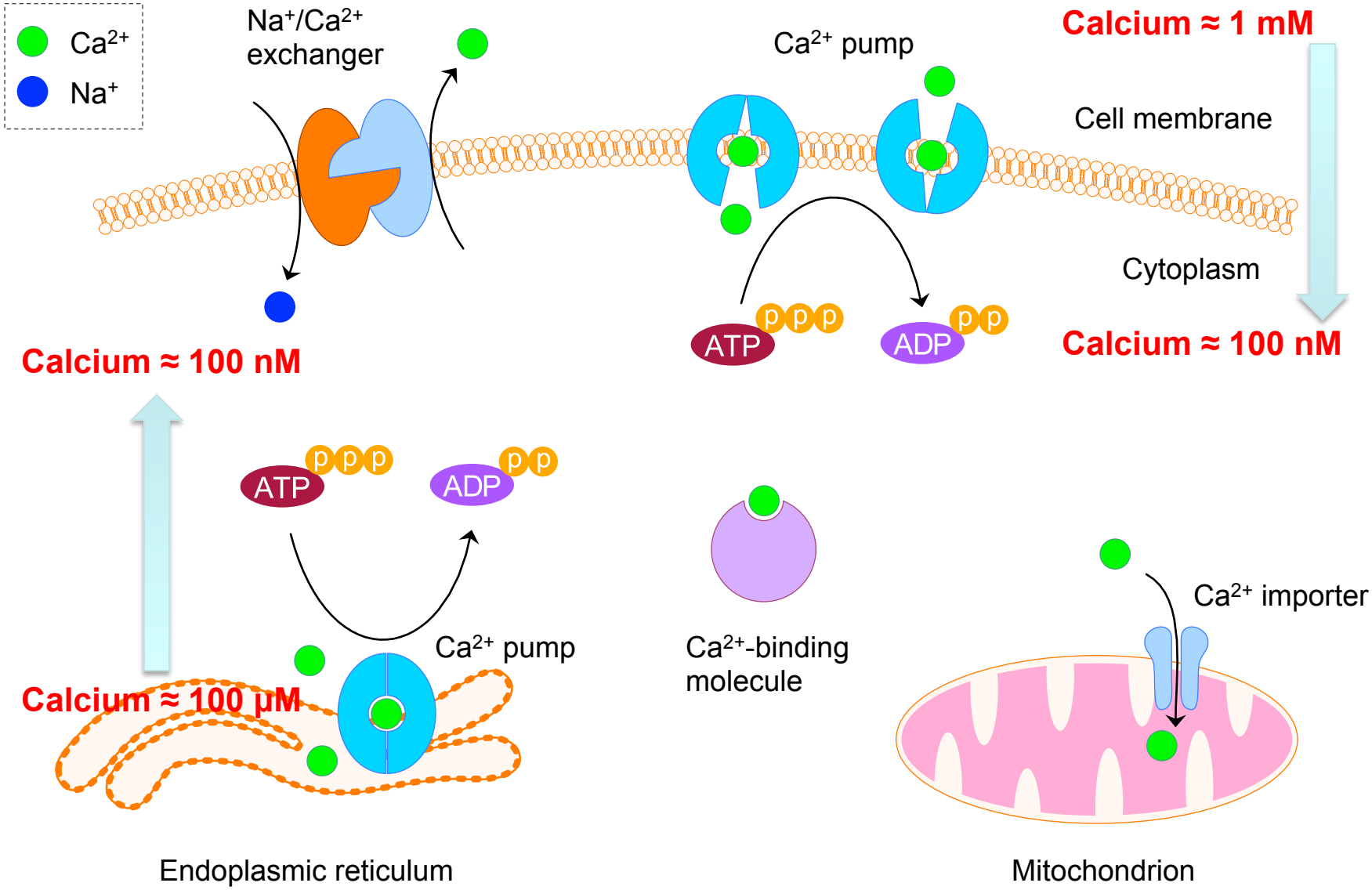


Bindet an IP_3-Rezeptor in ER Membran:
Ligandenaktivierter Calciumkanal

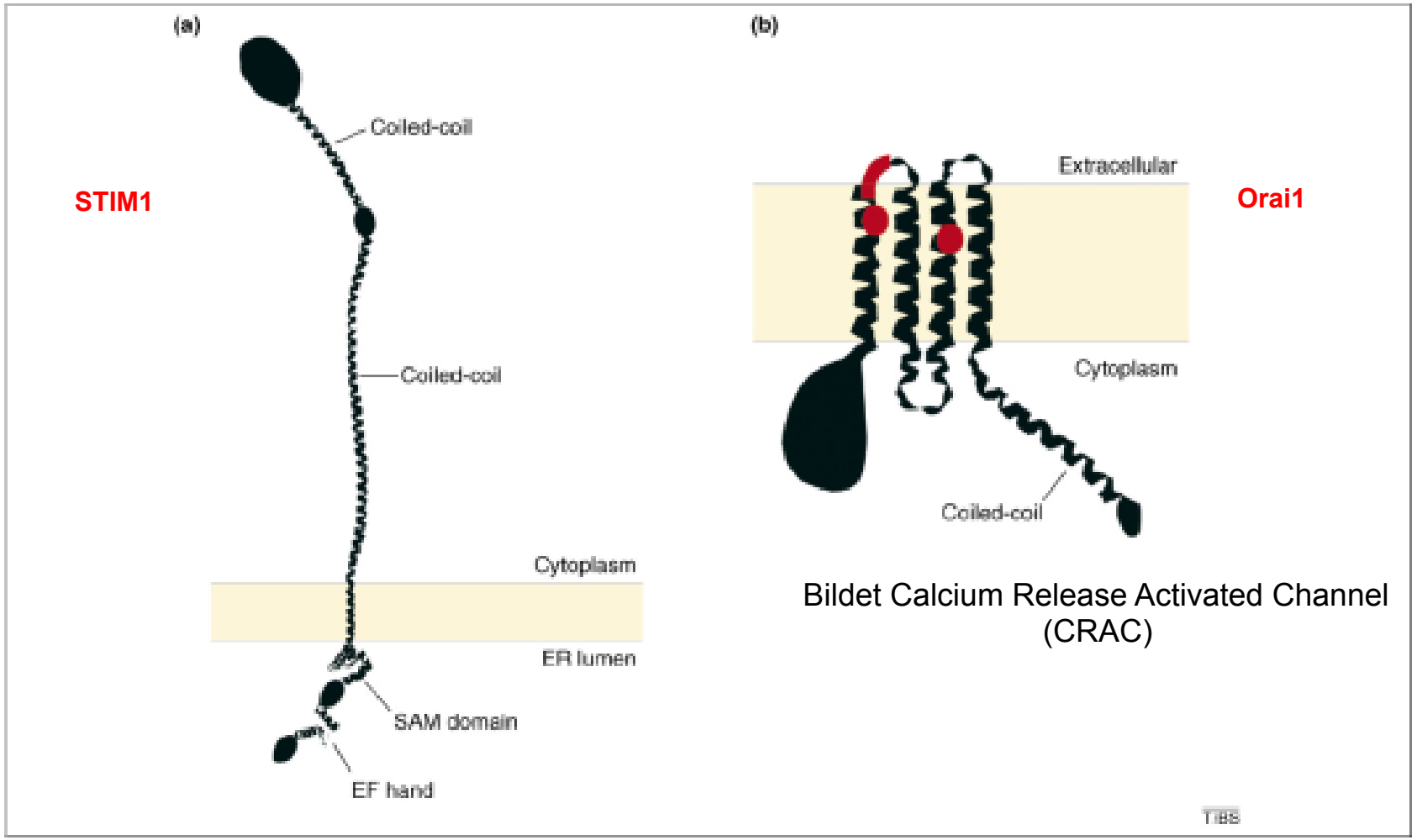
Exkurs: Aktivierung von Phospholipase Cβ über G-Protein gekoppelte Rezeptoren



Mechanisms of maintaining calcium level in the cytoplasm



Koppelung des Ca-Signals zwischen ER und Plasmamembran: STIM1 and Orai1



Bildet Calcium Release Activated Channel (CRAC)

Figure 3. STIM1, Orai1 and the activation I_{CRAC}. (a) The predicted features of human STIM1. Although the structure of STIM1 has not been solved, it is known that it is an ER protein anchored by a single transmembrane segment. The luminal portion contains a functional EF-hand [12,13,23,63] paired with a second vestigial EF-hand sequence that does not bind Ca^{2+}, and a predicted sterile α-motif (SAM) domain. The cytoplasmic portion features predicted coiled-coil regions. Here, the EF-hand, predicted SAM domain and predicted coiled-coil regions are represented pictorially based on known structures; other regions of the protein are represented as black ovals. The cytoplasmic region of STIM1 can potentially span the distance between ER and closely apposed plasma membrane, either to interact directly with Orai or to recruit other proteins into an active CRAC channel complex. (b) Human Orai1. The correct transmembrane topology of Orai1 is presented but, again, the protein structure has not been solved. Orai1 is an intrinsic plasma membrane protein with four transmembrane segments. The approximate locations of glutamic acid residues 106 and 190, which are crucial determinants of ion selectivity [19,20,24], are marked by red circles in transmembrane segments 1 and 3. The position of the acidic residues in the TM1–TM2 loop that affect ion permeation [20,24] is marked with a red block. There is a small predicted coiled-coil region near the C terminus [18].

Koppelung des Ca-Signals zwischen ER und Plasmamembran: STIM1 and Orai1

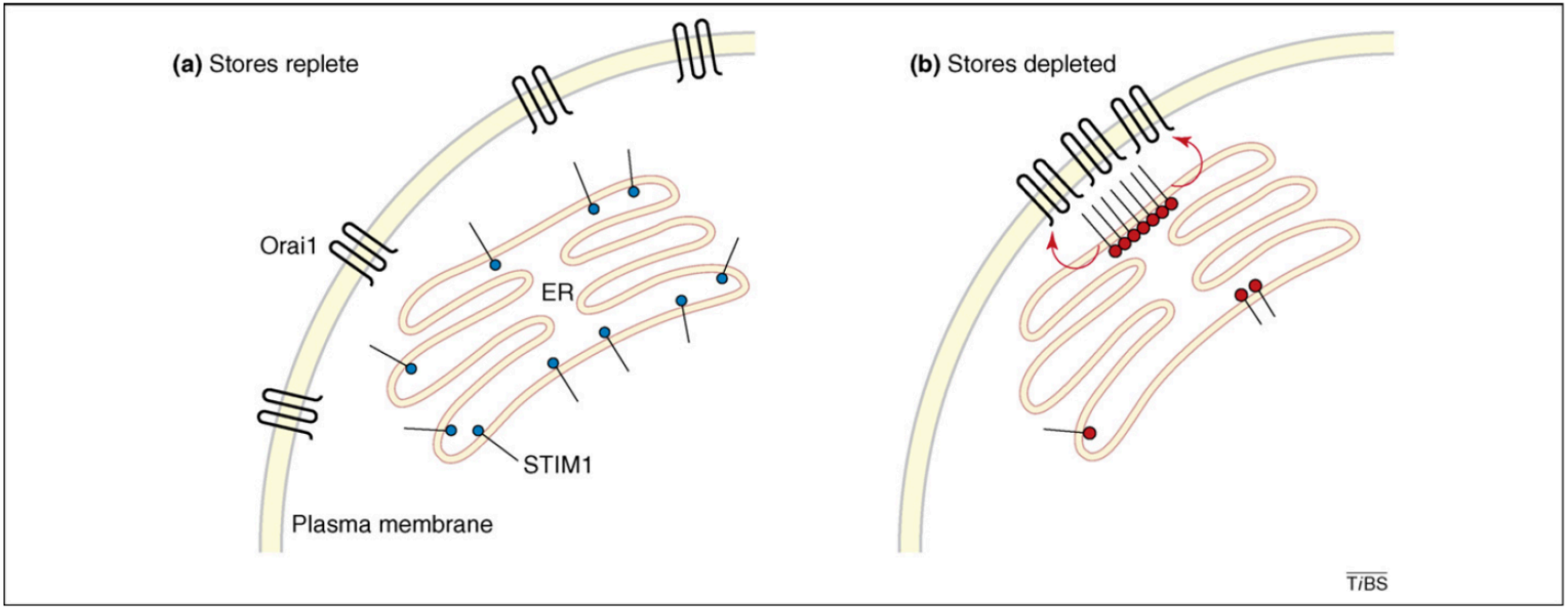
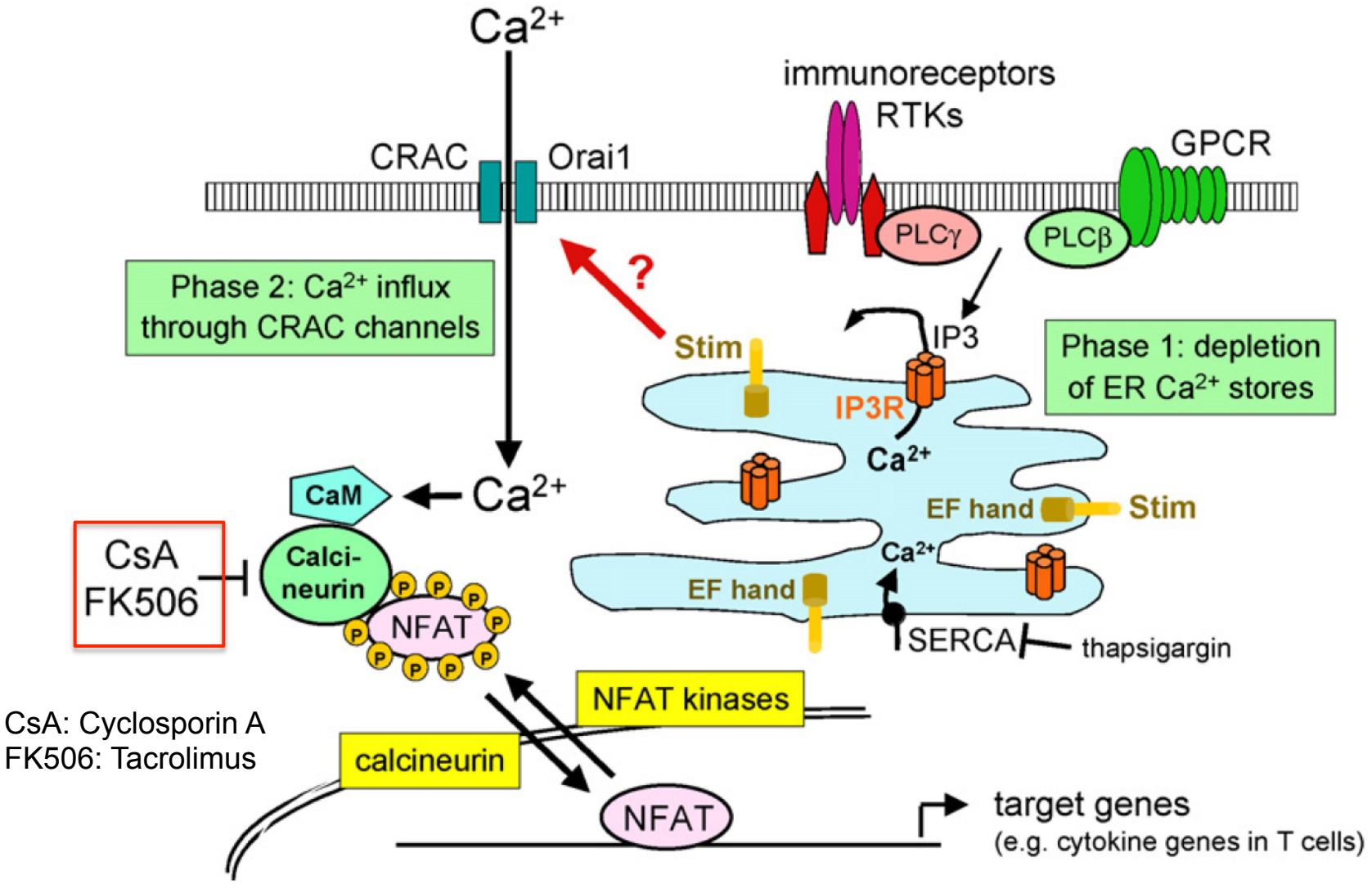


Figure 4. The current model of STIM1–Orai1 signalling. **(a)** STIM1, an ER Ca^{2+} sensor, is distributed throughout the ER in a resting cell. Free Ca^{2+} concentration in the ER lumen is sufficiently high that the EF-hand site is occupied by Ca^{2+} and, therefore, the STIM1 luminal domain is in its resting conformation (grey circles). **(b)** Upon depletion of luminal Ca^{2+}, bound Ca^{2+} dissociates from the EF-hand, which elicits a change in conformation of the STIM1 luminal domain (red circles) and alteration of its protein–protein interactions. STIM1 then moves laterally in the ER and forms punctate accumulations, some immediately subjacent to the plasma membrane. A signal from STIM1 (red arrow) activates Ca^{2+} influx at these sites. Orai1 is a CRAC channel subunit and, like STIM1, it is localized at sites of Ca^{2+} influx following depletion of ER Ca^{2+} stores. In the simplest version of the model, the gating signal is transferred directly from STIM1 to an Orai1 multimer, but other possibilities have not been ruled out.

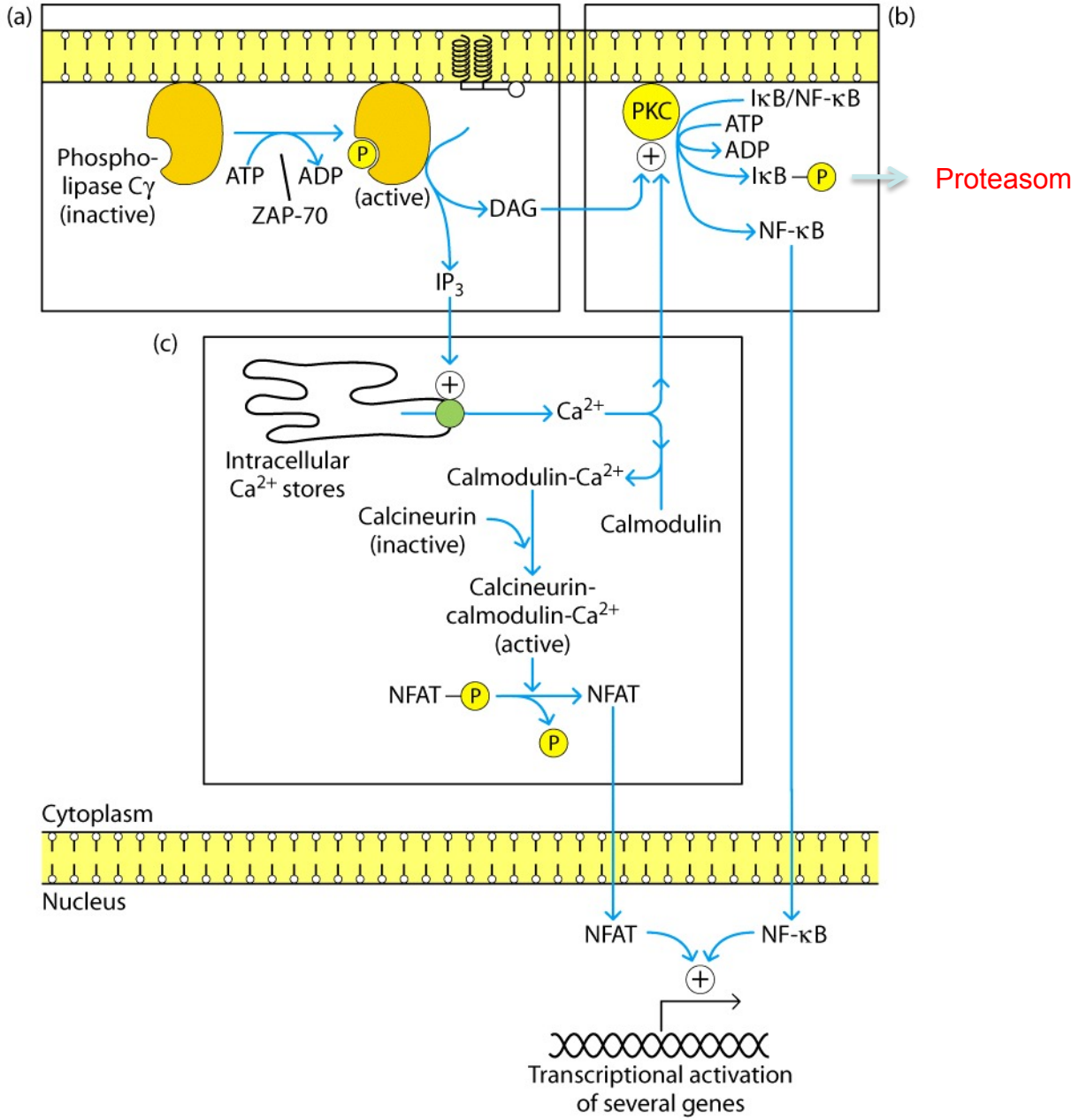
Aktivierung des „Nuclear Factor of Activated T Cells“ durch das Calcium-Signal



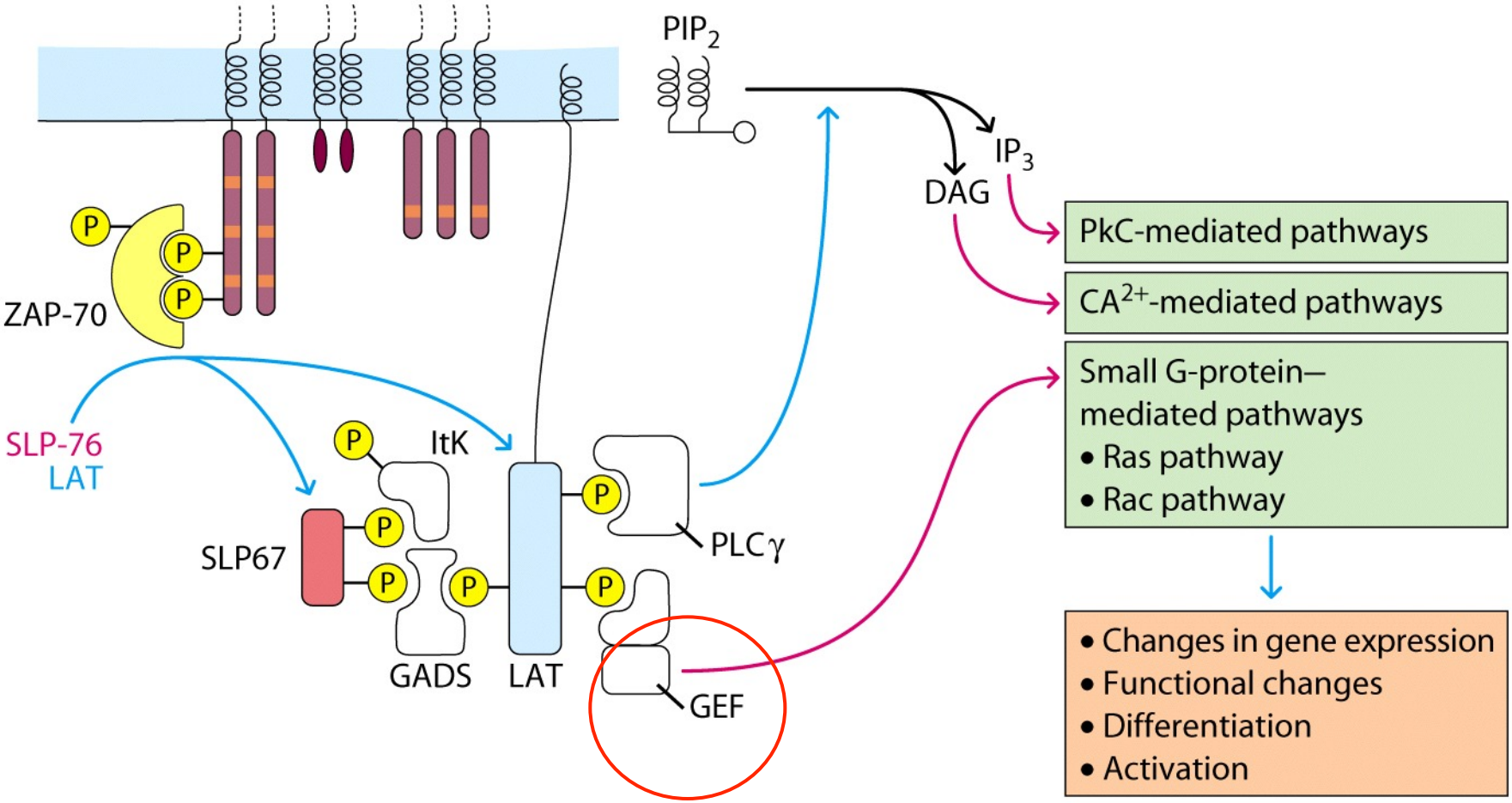
CsA: Cyclosporin A
FK506: Tacrolimus

Fig. 2. Schematic view of the NFAT activation cycle.

Aktivierung des „Nuclear Factor Kappa B“ durch das Calcium/DAG-Signal

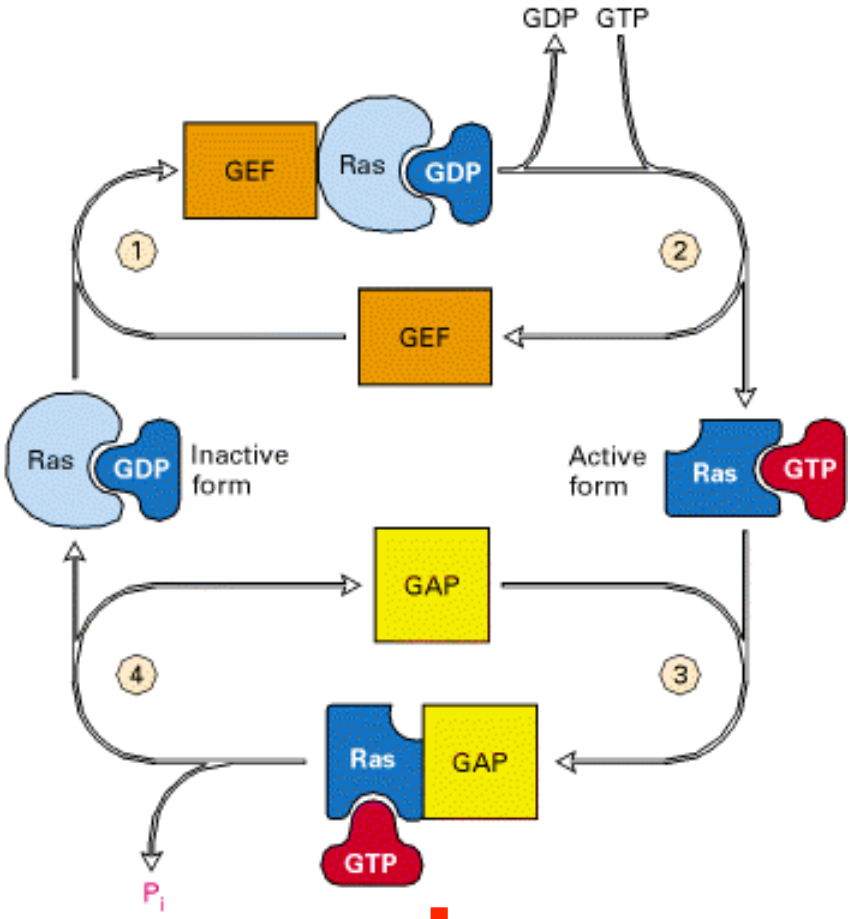


Die Bildung des „Signalosoms“ am T-Zell Rezeptor



ZAP-70: Zeta-assoziiertes Protein 70; Aktivierung durch src-vermittelte Phosphorylierung
LAT (linker of activation in T cells), SLP67, GADS: Adaptermoleküle
Itk (Familie der Tec-Kinasen): Phosphoryliert PLC-γ

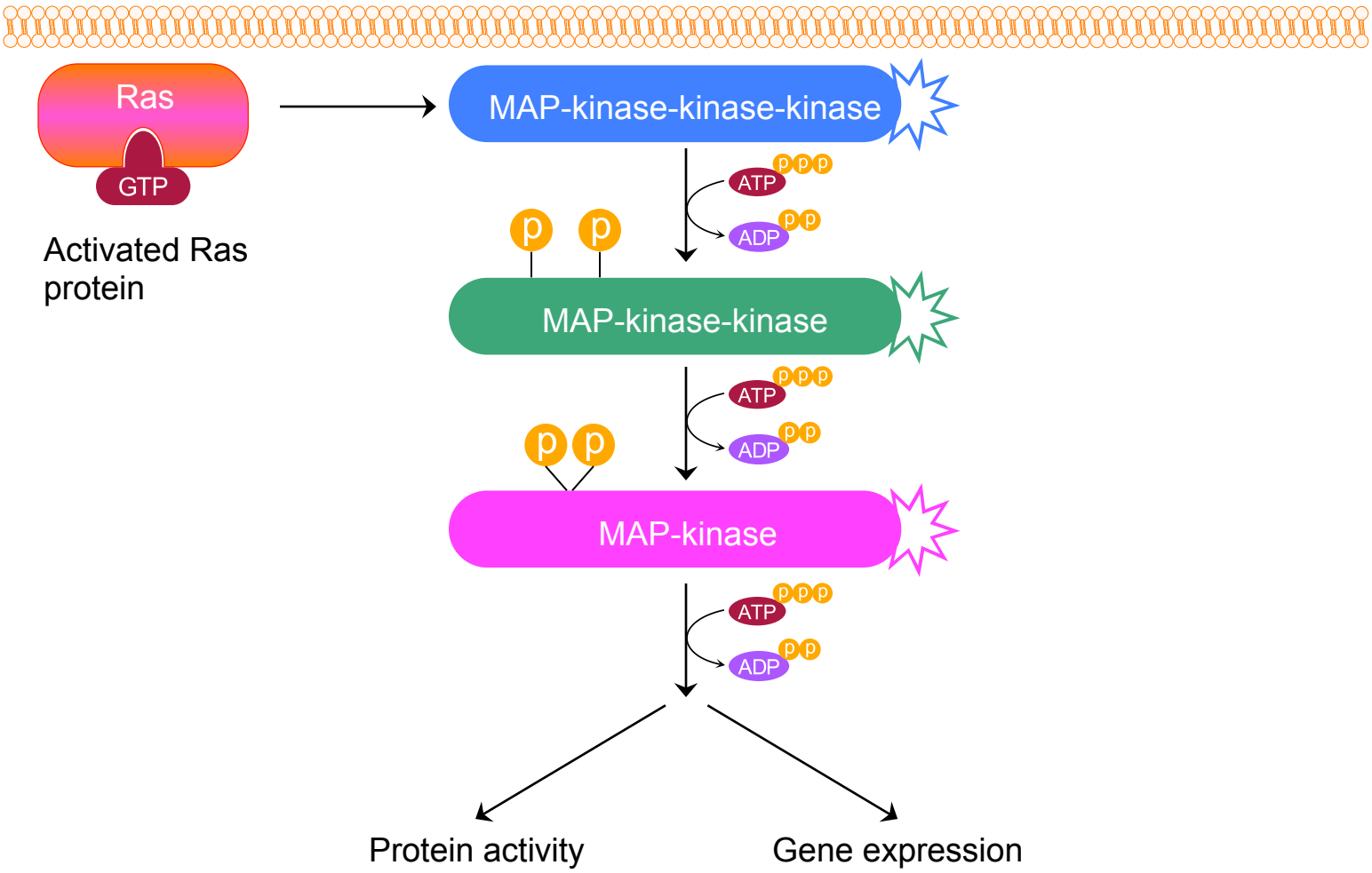
Kleine GTP-bindende Proteine (G-Proteine) als Schalter der MAP-Kinase Kaskade



GEF: Guanosine exchange factor
GAP: GTPase activating protein

Raf-1 (MAPKKK)
MAP-Kinase Kaskade

MAP-kinase phosphorylation pathway activated by Ras



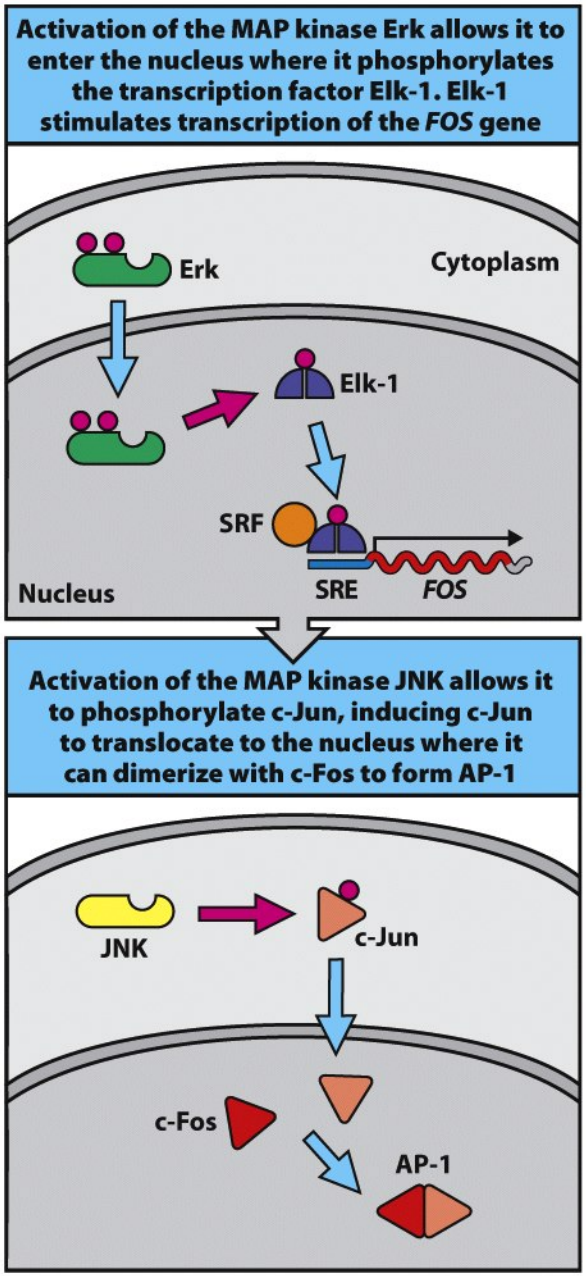


Figure 6-20 Immunobiology, 7ed. (© Garland Science 2008)

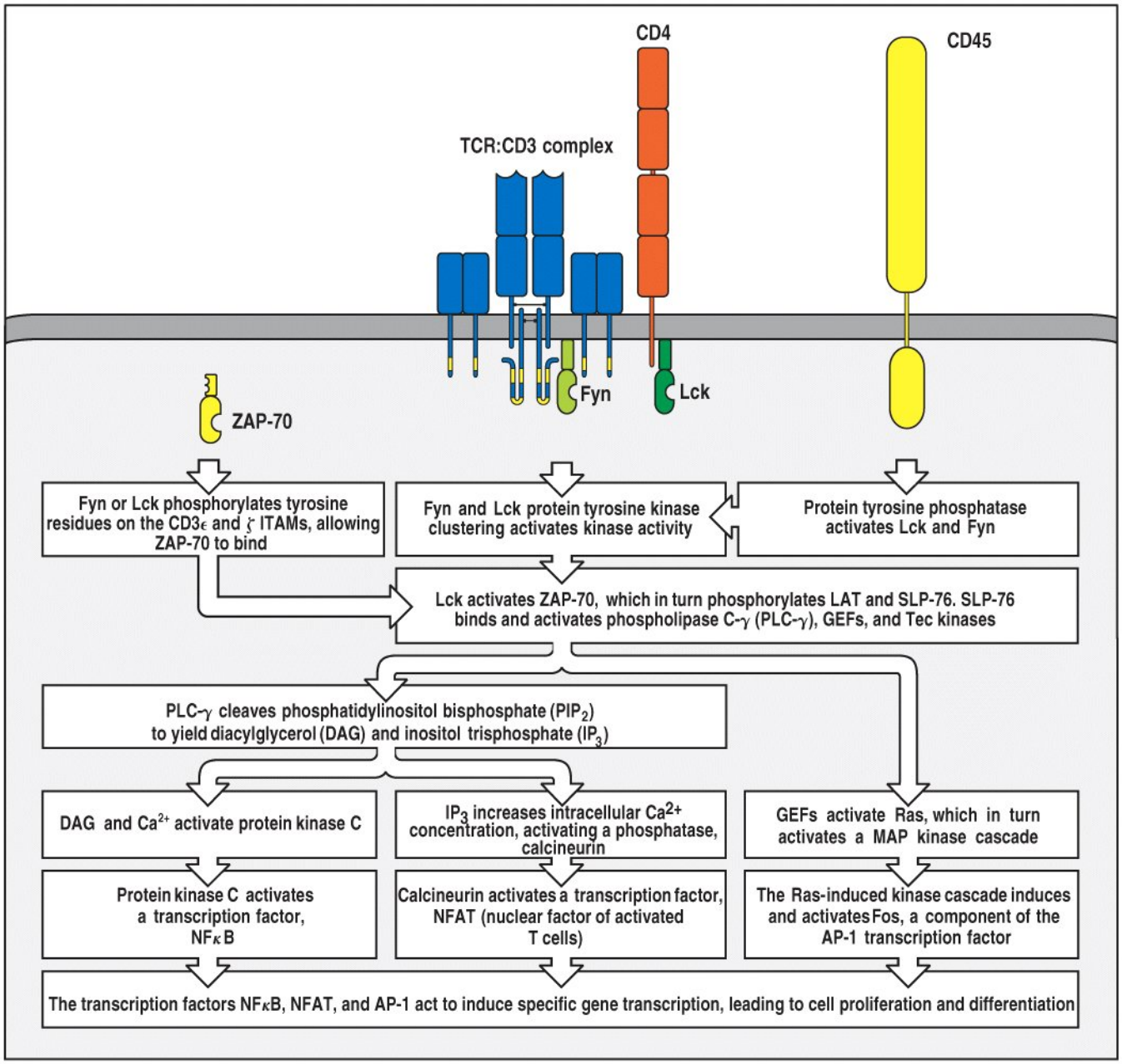


Figure 6-16 Immunobiology, 6/e. (© Garland Science 2005)

Das TCR Signal reicht nicht aus zur Aktivierung naiver T-Zellen

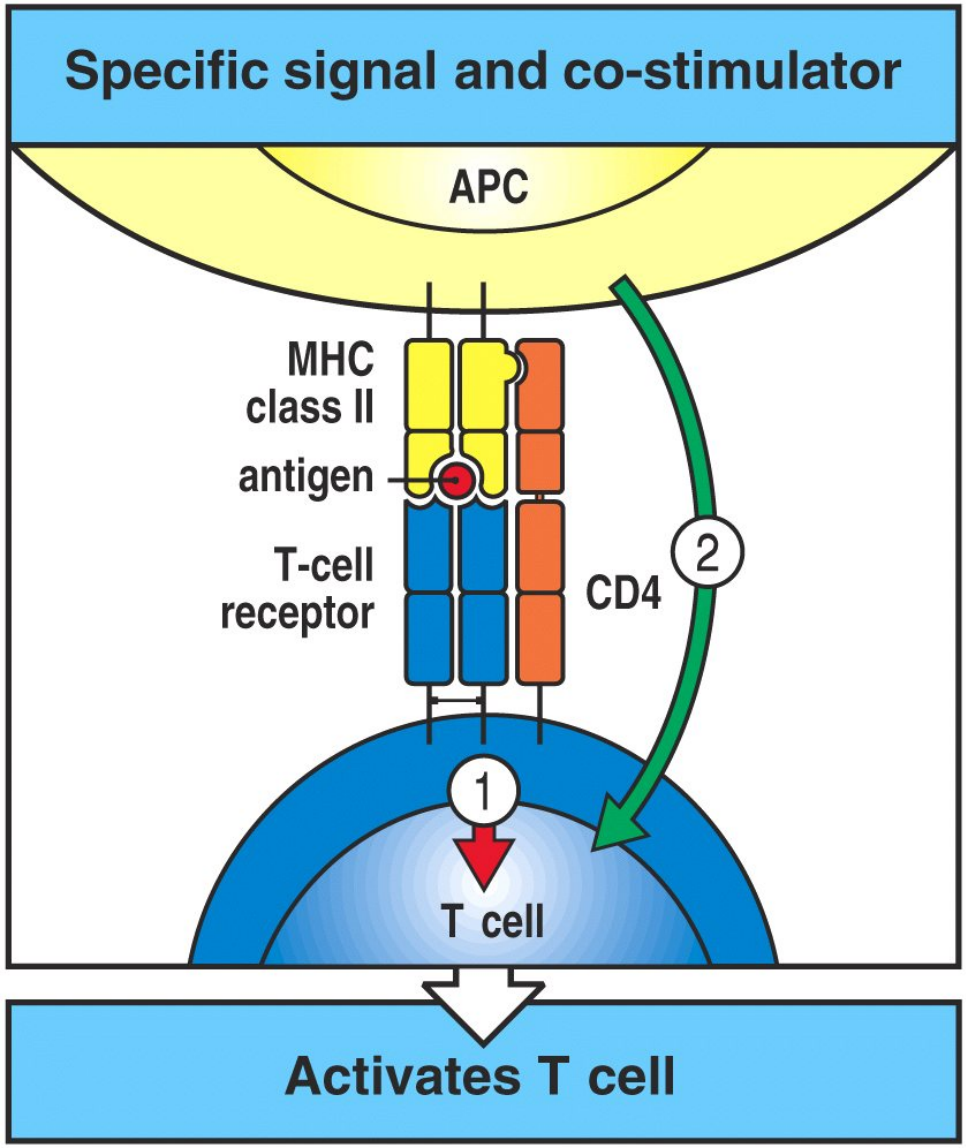


Figure 8-10 Immunobiology, 6/e. (© Garland Science 2005)

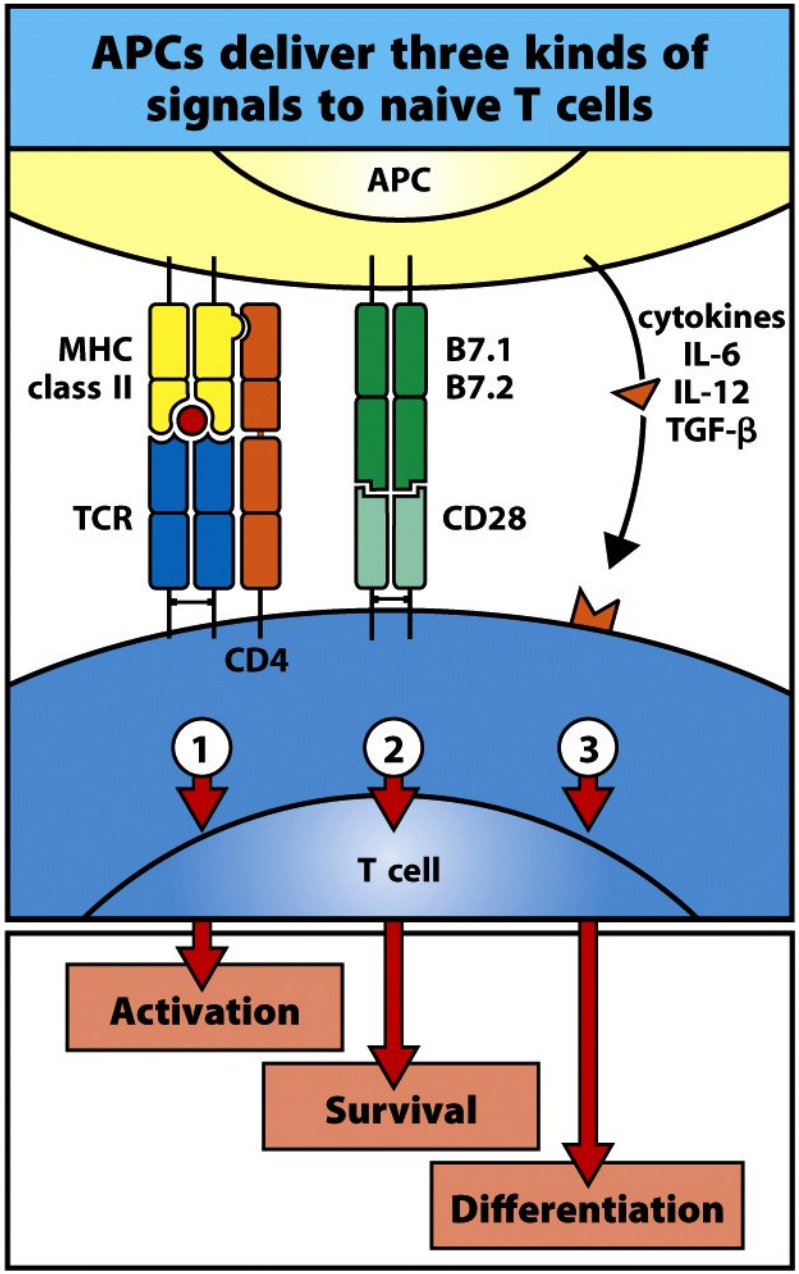


Figure 8-19 Immunobiology, 7ed. (© Garland Science 2008)

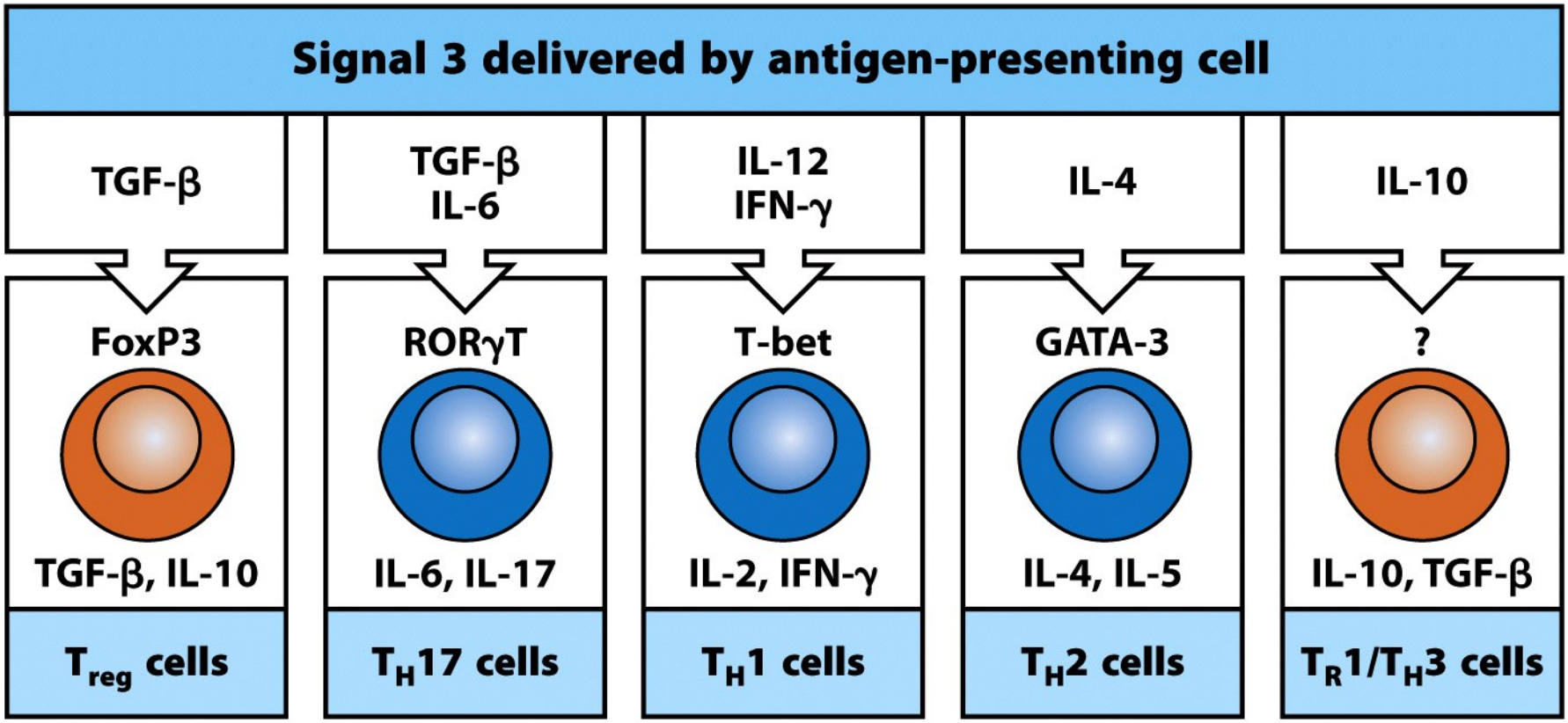


Figure 8-29 Immunobiology, 7ed. (© Garland Science 2008)

CD28 liefert das 2. Signal durch Bindung von CD80 und CD86 auf professionellen APC

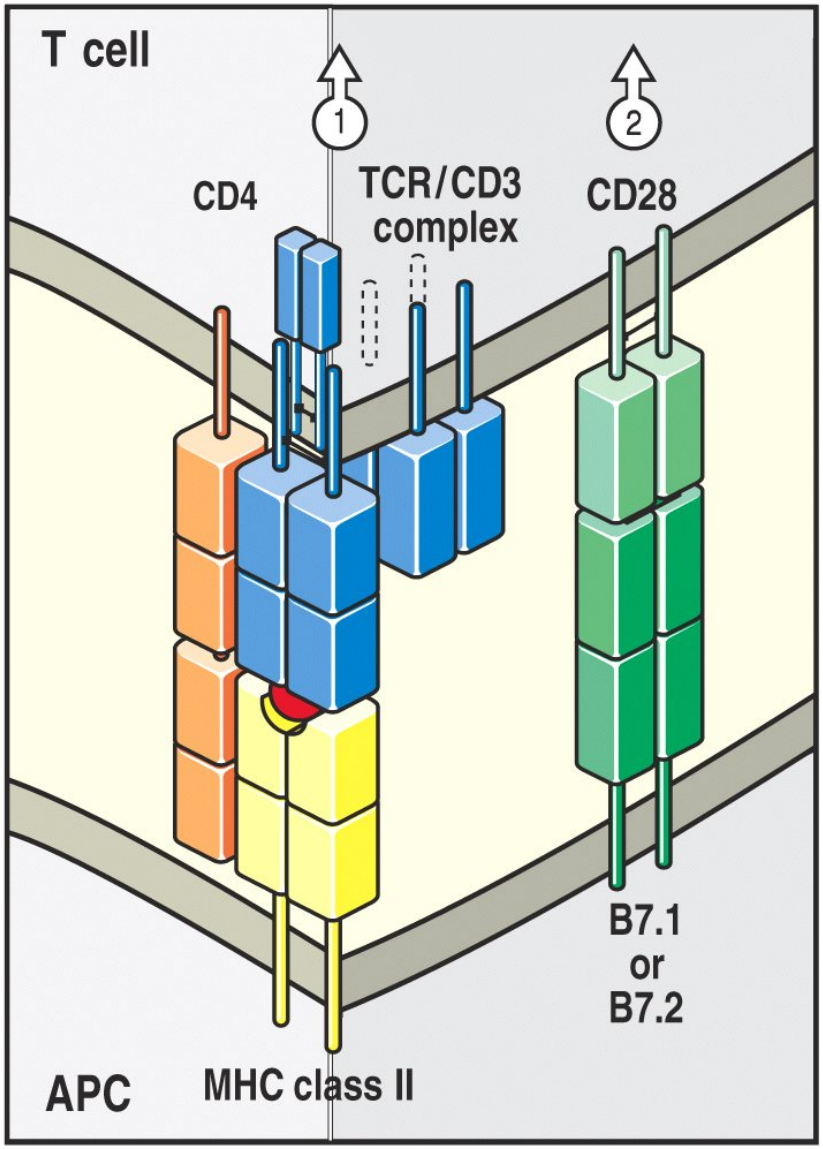


Figure 8-11 Immunobiology, 6/e. (© Garland Science 2005)

Signal 2 alleine: Kein Effekt auf T-Zelle
TCR Signal alleine: ANERGIE (funktionelle Inaktivierung)

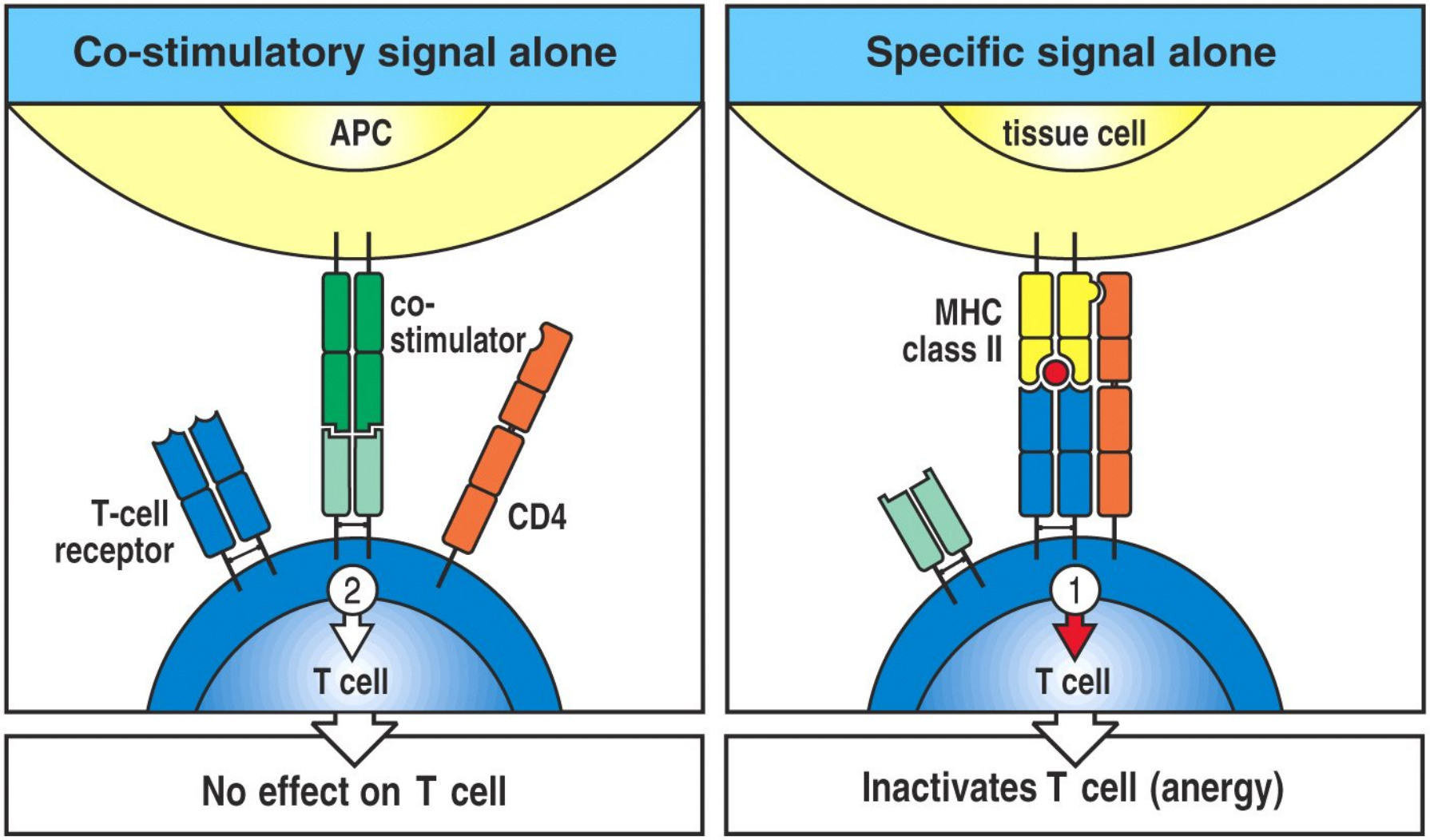


Figure 8-21 Immunobiology, 6/e. (© Garland Science 2005)

CD28 Signale verstärken TCR Signale quantitativ („Choke“-Funktion)

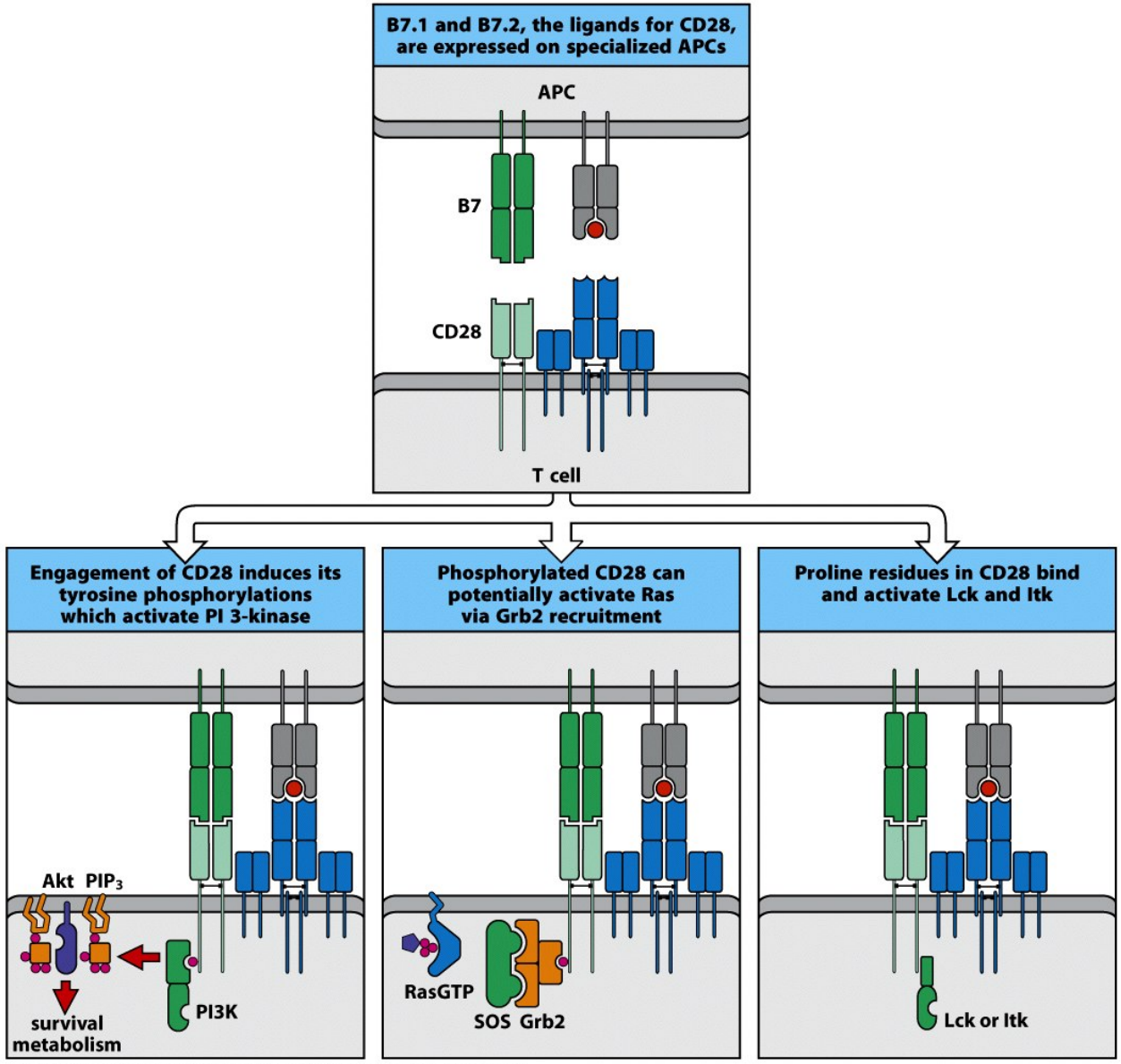


Figure 6-28 Immunobiology, 7ed. (© Garland Science 2008)

CTLA-4 (CD152) generiert hemmendes Signal in der T-Zelle („Cytotoxic T lymphocyte antigen 4“)

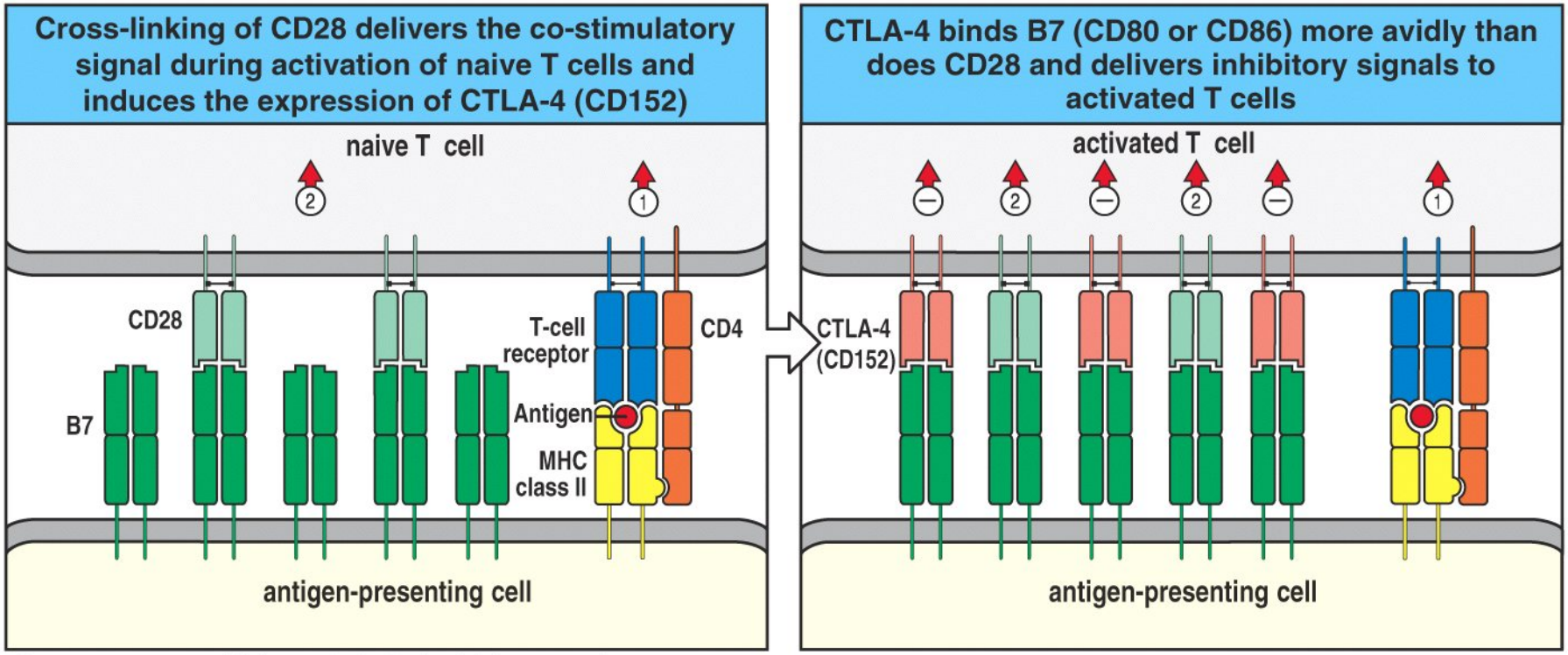
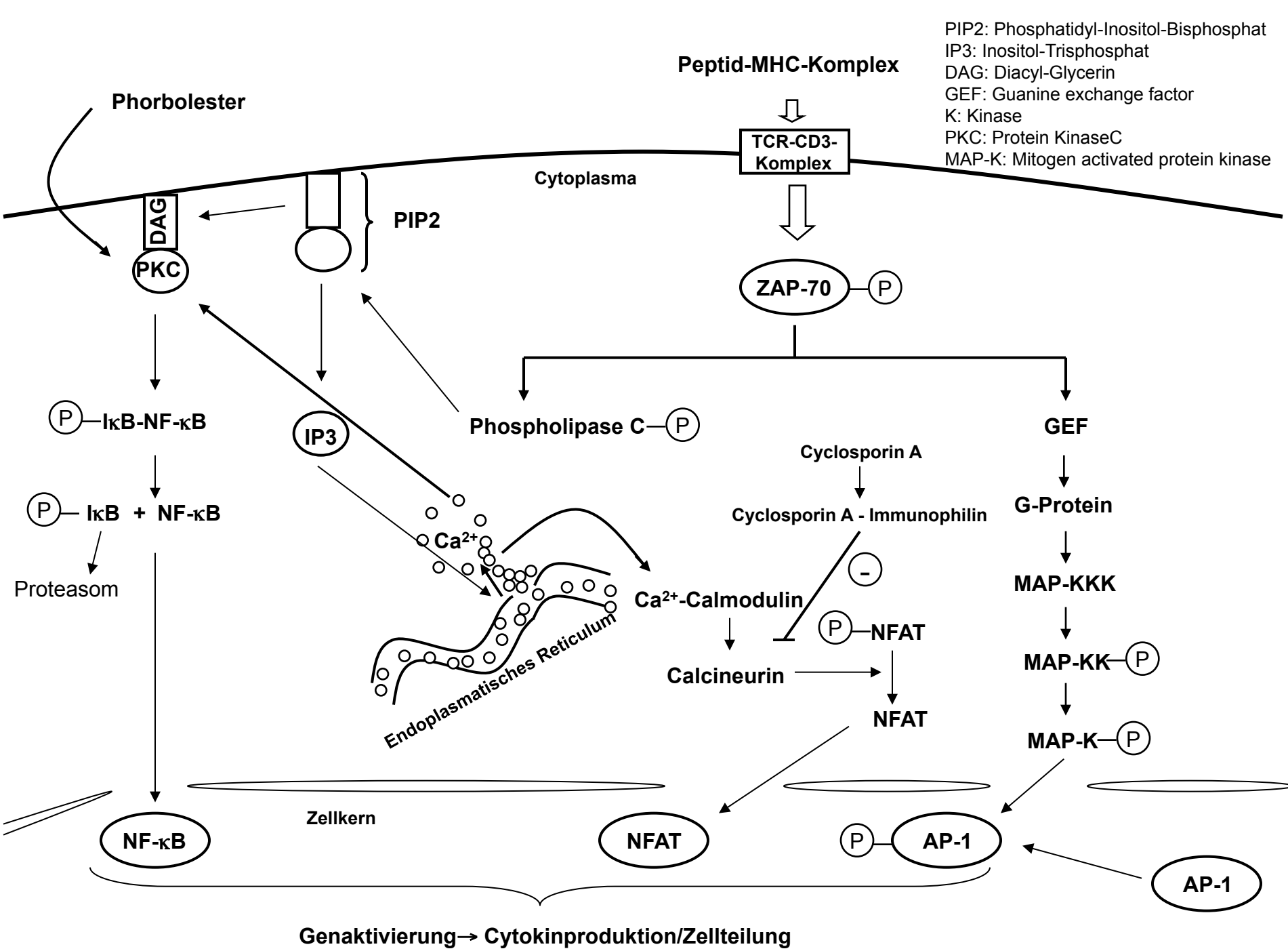


Figure 8-12 Immunobiology, 6/e. (© Garland Science 2005)


PIP2: Phosphatidyl-Inositol-Bisphosphat
IP3: Inositol-Trisphosphat
DAG: Diacyl-Glycerin
GEF: Guanine exchange factor
K: Kinase
PKC: Protein KinaseC
MAP-K: Mitogen activated protein kinase
Peptid-MHC-Komplex
Phorbolester
TCR-CD3-Komplex
Cytoplasma
DAG
PIP2
PKC
ZAP-70
P
IκB-NF-κB
IP3
Phospholipase C
GEF
Cyclosporin A
IκB + NF-κB
Ca2+
Cyclosporin A - Immunophilin
G-Protein
Proteasom
Ca2+-Calmodulin
MAP-KKK
NFAT
Calcineurin
MAP-KK
Endoplasmatisches Reticulum
NFAT
MAP-K
Zellkern
NF-κB
NFAT
AP-1
AP-1
Genaktivierung→ Cytokinproduktion/Zellteilung