

## Preface

IMMUNORECEPTOR SIGNALING AS A TOPIC OF RESEARCH has a history of about 30 years. Though the effects of plant lectins on lymphocyte activation were studied back in the 1960s, the modern era began once the lymphocyte antigen receptors were isolated and characterized. After a number of false starts, the T-cell antigen receptor (TCR) was finally identified and its multi-subunit character described in the 1980s. Though surface immunoglobulin was well known to be the recognition component of the B-cell antigen receptor (BCR), the signaling components of this receptor as well as the family of Fc receptors were also identified during this period.

Since then and with the involvement of increasing numbers of researchers, the field has expanded and has flourished due to the power of modern multidisciplinary research. Cellular immunology approaches demonstrated how receptor engagement affects lymphocyte activation, cytokine production, and elaboration of cell-surface molecules. Biochemical approaches not only resulted in isolation of the receptors but also an increasing list of downstream signaling molecules. The latter include protein tyrosine and serine kinases as well as a growing list of their substrates, all central to immunoreceptor signaling. The analysis of complexes of signaling molecules and the protein–protein interactions that make up these complexes remains an active area of research.

The molecular biology revolution that affected all of biomedical research over the same timespan also helped to transform immunoreceptor signaling research. Modern molecular biological tools allowed for the identification and characterization of the receptors and downstream signaling molecules. Genetic approaches such as the generation of mutants and their expression then allowed for the role of these molecules to be defined in cells and in engineered mice. Recently, the application of cell biologic and modern imaging techniques has triggered a new wave of discoveries. The application of these methods led to the discovery of synapses formed during cell–cell contact that are involved in signaling. Microscopic studies now track interactions of single signaling molecules within cells in real time. With the increasing acquisition of large quantities of data, computational approaches are being used to help understand the complexity of these signaling pathways.

Our hope here is to familiarize the reader with the latest discoveries in this field. We also hope that they convey a sense of the excitement about the incredible progress achieved over the last 20 years. The chapters cover a wide range of topics and, in aggregate, our hope is that they provide a comprehensive sense of the current state of the field.

We begin with discussion of TCR and BCR structure and function. Downstream of both of these receptors and receptors of the innate immune system, discussed next, are the protein tyrosine kinases (PTKs) of the Syk family. We continue with a chapter reviewing one of these PTKs, the central TCR-coupled kinase, ZAP-70, followed by chapters focusing on two of the proximal substrates of this kinase, LAT and SLP-76. Both chapters cover the topic of protein–protein interactions and signaling complexes. Members of the Tec family of PTKs, which are also activated following immunoreceptor engagement and are in turn responsible for activation of a variety of signaling enzymes, are the subject of the next chapter.

Engagement of antigen or immunoglobulin receptors alone does not result in full cellular activation. A number of additional receptors are required and this topic is reviewed in chapters on CD28 co-stimulation and the SLAM and SAP families of molecules. The activation of immunoreceptors leads to the activation of serine kinases and changes in lipid metabolism and ultimately in activation of transcription factors such as NF- $\kappa$ B, as well as to changes in the cytoskeleton; chapters on these

topics follow. We conclude with two chapters with a more global perspective. The first describes formation of the immune synapse of activated lymphocytes. Many of the molecules reviewed in the preceding chapters localize to this structure. The final chapter brings a systems approach to the study of the immense complexity of immunoreceptor signaling.

We hope that this collection brings together many of the major topics and approaches in this field. Perhaps this overview will aid in stimulating the next many years of fruitful research. We very much appreciate the efforts of several individuals at Cold Spring Harbor Laboratory Press. Richard Sever initiated the project and provided enthusiastic support for it. Kaaren Kockenmeister supervised production of the chapters and Joan Ebert guided and prodded editors and authors alike. Finally, we are grateful to our colleagues whose work has led the field and whose authorship is responsible for *any* success of this volume.

LARRY SAMELSON

ANDREY SHAW

# Index

- A**
- Actin  
  meshwork in T-cell receptor function  
  scaffolding, 238–241  
  translocation, 241–243  
  triggering of activation, 234–238
- ADAP, 95, 114, 117–118, 122
- AGC kinase, T-cell function, 189
- Akt  
  CD28 signaling, 152, 158–159  
  PIP<sub>3</sub> regulation, 201–202  
  T-cell function, 187–189, 192
- All, 21
- AMPK, 189–190
- Antigen-presenting cell (APC), B-cells, 28
- AP-1, 154
- APC, *See* Antigen-presenting cell
- Arp2/3, 95
- B**
- B7-1, 150
- B7-2, 150
- Bam32, 47
- B-cell receptor (BCR)  
  activation imaging on bilayers, 28–29  
  antigen presentation, 28  
  cytoskeleton in B-cell activation  
    CD19 in B-cell spreading and activation, 47  
    cell contraction and immunological synapse formation, 48–49  
    microcluster formation after antigen stimulation, 44–46  
  overview, 39–40  
  prospects for study, 49  
  receptor distribution regulation in resting cell, 40–42  
  spreading propagation of microsignalosomes, 46–47  
  T-cell activation homology, 42–44
- signaling  
  affinity and isotype influences on initiation, 32–34  
  mIg ectodomain, 30–32, 35  
  oligomerization  
    consequences of spontaneous, chronic oligomerization, 35–36  
    multivalent antigens in crosslinking, 29–30  
    translation into signaling, 34–35  
    prospects for study, 36–37
- BCL10  
  lymphocyte activation, 223–225  
  lymphoma dysregulation, 227–228  
  structure, 219
- Bcl-X<sub>L</sub>, 158–159
- BCR, *See* B-cell receptor
- Bimp3, *See* CARMA1
- Blnk, 46
- BRSK, 189
- Btk, 46, 64
- BTLA, 151
- C**
- Calcineurin, 137
- Calcium/calmodulin-dependent protein kinase kinase (CAMKK), T-cell function, 186
- CAMKK, *See* Calcium/calmodulin-dependent protein kinase
- CARD, 155
- CARD9, 56, 58
- CARD11, *See* CARMA1
- CARMA1  
  CD28 signaling, 155  
  domains and function, 219–220  
  lymphoma dysregulation, 227–228  
  phosphorylation, 220–223  
  structure, 218–219
- Casein kinase-1 (CK1), T-cell function, 188
- c-CbL, 93, 96
- CBM proteins, *See* BCL10; CARMA1; MALT1
- CCR2, 63
- CCR5, 63
- CCR7, 192
- CD1d, 3
- CD2, 42
- CD3  
  LAT complex studies, 97–98  
  T-cell development role, 20–21  
  T-cell receptor complex assembly  
    conservation of membrane-based receptor complex assembly, 9  
    functional ramifications, 9–10  
    mechanisms, 7–9

- CD3 (*continued*)  
 stoichiometry, 6–7  
 structural basis for intramembrane assembly, 9
- CD14, 64
- CD19  
 B-cell spreading and activation role, 47  
 T-cell function, 28–29
- CD21, 47
- CD22, 179
- CD28  
 adaptor proteins in signaling pathway initiation, 152–153  
 costimulation overview, 149–151  
 differentiation of T cells, 159–160  
 disease studies, 160  
 immunological synapse, 157–158  
 interleukin-2 production regulation  
 posttranscriptional regulation, 156–157  
 transcriptional regulation, 153–156  
 motif-specific protein–protein interactions, 151–152  
 survival enhancement of T cells, 158–159
- CD40, 63
- CD43, 97
- CD44, 63
- CD45, 42–44, 62, 97, 237, 271
- CD80, *See* B7-1
- CD81, 47
- CD84, *See* SLAM receptors
- CD86, *See* B7-2
- CD148, 44, 62
- CD152, *See* CTLA-4
- Cdc42, 46, 115, 153, 238
- Central supramolecular activating complex (cSMAC), 235, 240, 243, 248, 252–254
- Chronic lymphocytic leukemia (CLL), ZAP-70 defects, 79–80
- CLL, *See* Chronic lymphocytic leukemia
- Cofilin, 46
- CRACC, *See* SLAM receptors
- CrkL, 77
- Csk, 130
- cSMAC, *See* Central supramolecular activating complex
- CTLA-4, 150
- CXCR1, 63
- CXCR4, 63
- D**
- DAG, *See* Diacylglycerol
- DAP10, 9
- DAP12, 9, 60, 62, 64
- Dendritic cell, *See* Innate immune cell signaling
- DGK, 201
- Diacylglycerol (DAG)  
 kinases, 204–205  
 metabolism, 203  
 microtubule-organizing center effects, 250  
 prospects for study, 208–209  
 Ras and protein kinase C activation control, 203–204
- Diffuse large B-cell lymphoma (DLBCL), 36, 228
- DLBCL, *See* Diffuse large B-cell lymphoma
- DOCK8, 47
- Dok-1, 95
- Dok-2, 95
- E**
- EAT2  
 deficiency phenotypes, 178–180  
 knockout mice, 176–177  
 properties, 174–175  
 prospects for study, 180–181  
 signaling mechanisms, 177–178  
 SLAM receptor interactions, 174  
 switch-of-function mechanisms, 180
- ERK, *See* Mitogen-activated protein kinase
- ERT  
 deficiency phenotypes, 178–180  
 knockout mice, 176–177  
 properties, 174–175  
 prospects for study, 180–181  
 signaling mechanisms, 177–178  
 SLAM receptor interactions, 174  
 switch-of-function mechanisms, 180
- F**
- FAK, *See* Focal adhesion kinase
- FcγR, signaling in platelets and neutrophils, 118–119
- Fgr, *See* Src-family kinases
- Fluorescence recovery after photobleaching (FRAP), LAT dynamics studies, 99
- Focal adhesion kinase (FAK)  
 Src-family kinases in signaling, 64–65, 14-3-3, 190–192
- FoxO, 192
- FRAP, *See* Fluorescence recovery after photobleaching
- Fyn, 18–19
- G**
- GAD5, 155
- GPCR, *See* G protein-coupled receptor
- G protein-coupled receptor (GPCR), Src-family kinases in signaling, 63
- Granulocyte, *See* Innate immune cell signaling
- Grap, 89, 93
- Grb2, LAT interactions, 18, 89, 93–97
- GSK3, T-cell function, 188

## H

Hck, *See* Src-family kinases  
HDAC, *See* Histone deacetylase  
Histone deacetylase (HDAC), 191–192  
HPK1, 220, 222

## I

ICAM-1, 238, 240, 249, 252–253  
ICOS, 150–151  
I $\kappa$ B kinase (IKK), 155, 218, 223, 225–227  
IKK, *See* I $\kappa$ B kinase  
IL-2, *See* Interleukin-2  
IL-6, *See* Interleukin-6  
IL-17A, *See* Interleukin-17A  
Immunological synapse (IS)  
    B-cell contraction formation, 48–49  
    CD28 signaling, 157–158  
    central and peripheral supramolecular activating complex function, 253–254  
    cessation mechanisms, 249–250  
    computational modeling, 255–256  
    cytolytic killing function, 256  
    functional overview, 248–249  
    lymphocyte polarity  
        antigen recognition, 250–251  
        cell migration, 250  
        Par proteins, 251  
    microclusters, 252–253  
    stable contacts for T-cell activation, 253  
Inflammasome, Syk function, 65–66  
Innate immune cell signaling  
    activating pathways  
        CARD9 and receptor diversity, 56, 58  
        classic immunoreceptor pathways, 56–57  
    inhibitory pathways  
        classical inhibitory pathways, 58–60  
        indirect down-modulation of pathway crosstalk, 61  
    ITAMs, 60–61  
    prospects for study, 66  
Src-family kinases  
    focal adhesion kinase/Pyk2 signaling, 64–65  
    G protein-coupled receptor signaling, 63  
    immunoreceptor pathways, 62  
    interleukin-6 signaling, 63  
    membrane-bound receptor signaling, 64  
    overview, 61–62  
    selectin signaling, 63–64  
    Tec kinase signaling, 64  
    TRAF6 signaling complex, 63  
Syk  
    inflammasome, 65–66  
    ITAM pathways, 65  
    overview, 65

Integrins, signaling in platelets and neutrophils, 119  
Interleukin-2 (IL-2), CD28 in production regulation  
    postranscriptional regulation, 156–157  
    transcriptional regulation, 153–156  
Interleukin-6 (IL-6), Src-family kinases in signaling, 63  
Interleukin-17A (IL-17A), Itk role in T<sub>H</sub>17 cell production, 136–137  
IP<sub>3</sub>  
    metabolism, 203, 208  
    T-cell function, 205  
IP<sub>4</sub>  
    metabolism, 206, 208  
    T-cell function, 206–208  
IRAK, 218  
IS, *See* Immunological synapse  
Isotype switching, B-cell receptor signaling, 32–34  
ITAM  
    computer modeling of T-cell activation, 263–264  
    innate immune cell signaling  
        activating pathways, 56–58  
        inhibitory pathways, 59–61  
        Src-family kinases, 62  
        Syk, 65  
    lipid binding, 10–11  
    membrane release mechanisms, 12  
    structure of membrane-bound ITAM, 11  
T-cell receptor signaling  
    developmental functions, 20–22  
    distribution, 16–17  
    function of individual receptor chains and ITAMs, 18–20  
    initiation, 17–18  
    overview, 15  
    prospects for study, 22–23  
    tolerance role, 22  
ZAP-70 recruitment, 71–72, 74–76  
Itk  
    activation downstream of T-cell receptor, 128–129  
    disease defects, 141–142  
    PLC $\gamma$ 1 activation, 129, 131–132  
    regulation  
        downregulation, 131  
        multimerization, 132–133  
        upregulation, 130–131  
    substrate recognition mechanism, 131–132  
T-cell function  
    CD8<sup>+</sup> T-cell signaling, 137–138  
    development functions, 138–141  
    differentiation and effector functions, 134  
    interleukin-17A production in T<sub>H</sub>17 cells, 136–137  
    knockout mouse studies, 133–134  
    SLP-76 interactions, 115–116, 129–130

- I $\kappa$ k (*continued*)  
 T<sub>H</sub>1 differentiation and effector function,  
 135–136  
 T<sub>H</sub>2 differentiation and cytokine production,  
 134–135  
 Tec kinase domain structure, 127–128  
 Itpk, 200, 207–208
- J**  
 JNK, 153
- K**  
 KLF2, 192
- L**  
 LAT, *See* Linker for activation of T-cells  
 Lck, 76–77, 81, 157, 237–238, 240  
 Leu13, 47  
 LFA-1, 28, 48, 236, 241, 247–249, 252–253  
 Linker for activation of T cells (LAT)  
 computer modeling of T-cell activation, 264–265  
 functional overview, 72, 76, 89  
 gene cloning, 89–90  
 internalization and ubiquitylation, 101  
 mouse studies of function  
 knock-in mutants of cysteine and phosphotyrosine sites, 102–103  
 knockout mice, 101–102  
 LAT-independent signaling, 103–104  
 palmitoylation and membrane localization, 90–91  
 structure, 89–90  
 T-cell signaling  
 cooperativity  
 associated proteins, 95–96  
 binding studies, 96  
 oligomerization induction, 96–97  
 Grb2 interactions, 89, 93–97  
 imaging of complexes  
 assembly mechanism studies, 99–101  
 dynamics studies, 101  
 microclusters, 97–99  
 inhibitory actions, 95  
 overview, 91–92  
 phosphorylation, 92  
 PLC $\gamma$ 1 binding, 92–96  
 SLP-76 regulation, 89, 94–95  
 LKB1, 190  
 Ly-9, *See* SLAM receptors  
 Lyn, *See* Src-family kinases
- M**  
 Mac-1, 60  
 Macrophage, *See* Innate immune cell signaling
- Major histocompatibility complex (MHC), T-cell  
 receptor structure in restriction  
 mechanism, 3–6
- MALT1  
 lymphocyte activation, 225–226  
 lymphoma dysregulation, 227–228  
 nuclear factor- $\kappa$ B activation, 226–227  
 prospects for study, 228  
 structure, 219
- MAPK, *See* Mitogen-activated protein kinase
- MARK, 189
- Mast cell, *See* Innate immune cell signaling
- MEKK1, 153
- MELK, 189
- MHC, *See* Major histocompatibility complex
- Microtubule-organizing center (MTOC),  
 250–251
- Mitogen-activated protein kinase (MAPK)  
 computer modeling of T-cell activation, 266–268,  
 270  
 T-cell function, 187–188, 190, 192–193, 238
- Mlg, *See* B-cell receptor
- MTOC, *See* Microtubule-organizing center
- MyD88, 61, 218
- N**  
 Nck, 94, 112–114  
 Neutrophil, SLP-76 signaling, 118–122  
 NF- $\kappa$ B, *See* Nuclear factor- $\kappa$ B
- NFAT  
 computer modeling of T-cell activation,  
 267–268  
 T-cell function, 77, 137, 154, 188–190
- Nkt1*, 173
- NLRP3, 65
- NTB-A, *See* SLAM receptors
- NUAK, 189
- Nuclear factor- $\kappa$ B (NF- $\kappa$ B)  
 CBM proteins in lymphocyte activation, *See* BCL10;  
 CARMA1; MALT1  
 CD28 signaling, 155, 158  
 transcriptional activation, 217
- O**  
 Oct-1, 154–155  
 ORAI, 205
- P**  
 p21- $\zeta$ , 19  
 p23- $\zeta$ , 19  
 p85, PI3K, 18–19, 89, 94, 151–152  
 Par proteins, polarity, and immunological synapse  
 function, 251  
 PD-1, 150

PDK1, 152, 159, 188–189, 192, 201  
Peripheral supramolecular activating complex  
    (pSMAC), 250, 252–253  
Phosphatidylinositol 3-kinase (PI3K)  
    deficiency and T-cell effects, 199  
    functional overview, 197  
    p85, 18–19, 89, 94, 151–152  
Phospholipase C $\gamma$  (PLC $\gamma$ )  
    knockout mice, 200  
    PLC $\gamma$ 1  
        Itk activation, 129, 131–132  
        LAT binding, 92–96, 98, 203–205  
        SLP-76 binding, 116  
    PLC $\gamma$ 2 in B-cell activation, 46  
    structure, 203  
    T-cell function, 186, 203  
PI3K, *See* Phosphatidylinositol 3-kinase  
Pin1, 132  
PIP<sub>2</sub>  
    metabolism, 195–197, 203  
    prospects for study, 208–209  
    protein domain binding, 196–197  
PIP<sub>3</sub>  
    Akt regulation, 201–202  
    metabolism, 195–197  
    PH domain binding, 196–198, 201  
    prospects for study, 208–209  
    Tec kinases as effectors, 202–203  
PKB, *See* Akt  
PKC, *See* Protein kinase C  
PKD, *See* Protein kinase D  
Platelet, SLP-76 signaling, 118–122  
PLC $\gamma$ , *See* Phospholipase C $\gamma$   
Protein kinase B, *See* Akt  
Protein kinase C (PKC)  
    DAG in activation control, 203–204  
    PKC $\theta$ , 155, 157–159, 253  
Protein kinase D (PKD), T-cell function, 186  
PSGL-1, 63  
pSMAC, *See* Peripheral supramolecular activating  
    complex  
PTEN, 192, 200, 208  
Pyk2, Src-family kinases in signaling, 64–65

## Q

QIK, 189

## R

Rac, 238  
Ras, DAG in activation control, 203–204  
Ras-GAP, 18–19  
RIP-1, 218  
Rlk, 134, 138, 140  
RSK, 189

## S

SAP  
    deficiency phenotypes, 178–180  
    knockout mice, 176–177  
    properties, 174–175  
    prospects for study, 180–181  
    signaling mechanisms, 177–178  
    SLAM receptor interactions, 174  
    switch-of-function mechanisms, 180  
    X-linked lymphoproliferative disease mutations,  
        175–176  
SCID, *See* Severe combined immunodeficiency  
Selectin, Src-family kinases in signaling, 63–64  
Severe combined immunodeficiency (SCID), ZAP-70  
    defects, 80–82  
SGK, 189  
Shb, 95  
Shc, 18–19  
SHIP-1, 95, 180, 200, 208  
SHIP-2, 200, 208  
SHP-1, 19, 180, 268–269, 272  
SHP-2, 180  
SIK, 189  
SLAM receptors  
    functions  
        antibody stimulation studies, 171–172  
        ectopic expression studies, 172  
        genetic linkage analysis, 172–173  
        overview, 169–170  
        transgenic mouse studies, 173–174  
    properties, 170–171  
    prospects for study, 180–181  
    SAP family interactions. *See* EAT2; ERT; SAP  
    switch-of-function without SAP adaptors,  
        179–180  
    types, 170  
SLP-76  
    domains and T-cell signaling  
        amino terminus and SAM domain, 114–117  
        function modeling following immunoreceptor  
            engagement, 112–114  
        P1 domain, 117  
        SH2 domain, 117–118  
    homologs, 111  
    Itk interactions, 115–116, 129–130  
    LAT regulation, 72, 89, 94–96  
    miscellaneous functions, 122  
    neutrophil signaling, 118–122  
    platelet signaling, 118–122  
SMAC, *See* Supramolecular activation cluster  
Sos1, 96  
Src-family kinases  
    innate immune cell signaling  
        focal adhesion kinase/Pyk2 signaling,  
            64–65  
    G protein-coupled receptor signaling, 63

- Src-family kinases (*continued*)
- immunoreceptor pathways, 62
  - interleukin-6 signaling, 63
  - membrane-bound receptor signaling, 64
  - selectin signaling, 63–64
  - Tec kinase signaling, 64
  - TRAF6 signaling complex, 63
- Lyn, 29
- overview, 61–62
- Supramolecular activation cluster (SMAC), 48
- Syk
- functional overview, 65, 77–79
  - innate immune cell signaling
    - inflammasome, 65–66
    - ITAM pathways, 65
  - structure, 77–78
  - T-cell development role, 77–79
- T**
- T-cell receptor (TCR)
- actin meshwork function
    - scaffolding, 238–241
    - translocation, 241–243
    - triggering, 234–238
  - antigen-driven intrinsic event regulation, 35
  - CD3 complex assembly
    - conservation of membrane-based receptor complex assembly, 9
    - functional ramifications, 9–10
    - mechanisms, 7–9
    - stoichiometry, 6–7
    - structural basis for intramembrane assembly, 9
  - computer modeling of activation
    - antagonism and synergism studies, 268–269
    - overview, 261–262
    - prospects, 272–273
    - quantitative data and modeling approaches, 262–265
    - reconciliation of robustness and variability of activation, 271–272
    - spatial regulation studies, 270–271
    - specificity, sensitivity, and speed of signaling, 264, 266–268
    - stochasticity, 270
    - tunability of ligand response, 269–270
  - ITAM
    - lipid binding, 10–11
    - membrane release mechanisms, 12
    - signaling
      - developmental functions, 20–22
      - distribution, 16–17
      - function of individual receptor chains and ITAMs, 18–20
      - initiation, 17–18
      - overview, 15
      - prospects for study, 22–23
      - tolerance role, 22
      - structure of membrane-bound ITAM, 11
      - ligand binding by extracellular domains, 1–3
      - major histocompatibility complex restriction mechanism, 3–6
      - overview of signaling, 233, 235
- TCR, *See* T-cell receptor
- Tec kinase family, *See also* Itk; Rlk
  - innate immune cell signaling, 64
  - PIP<sub>3</sub> effector activity, 202–203
- TIRFM, *See* Total internal reflection microscopy
- TLRs, *See* Toll-like receptors
- Tolerance, ITAM-mediated signal amplification, 22
- Toll-like receptors (TLRs), Src-family kinases, and Syk in innate signaling, 61–62
- Total internal reflection microscopy (TIRFM), B-cell receptor activation studies, 41–42, 44–45
- TRAF2, 218
- TRAF6, 63, 225
- TRTEM-2, 60
- V**
- Vav, 46, 76, 94
- Vav1, 94, 112–113, 115, 117, 238–240
- VLA-4, 48, 243
- W**
- WASP, 77, 94–95, 114, 152
- X**
- XIAP, 176
- X-linked agammaglobulinemia, 141
- X-linked lymphoproliferative disease (XLP), SAP mutations, 175–176
- XLP, *See* X-linked lymphoproliferative disease
- Z**
- ZAP-70
- disease studies
    - chronic lymphocytic leukemia, 79–80
    - hypomorphic mutant mice, 82–83
    - severe combined immunodeficiency, 80–82
  - functional overview, 17, 19, 71–72
  - ITAM recruitment, 71–72, 74–76
  - regulation
    - negative regulation, 77
    - positive regulation, 76–77
    - recruitment to T-cell receptor, 75–76
  - structure, 72–75, 84
  - T-cell development role, 77–79
  - therapeutic targeting, 83–84