INTRODUCTION

All cells depend on tightly regulated signalling pathways to appropriately respond to their environment. The basic model assumes that extracellular information is received by cell surface and/or intracellular receptors. These signals are subsequently relayed through the cytoplasm. Ultimately, the information may reach the cell nucleus, where it is translated into gene activation/inactivation. Consequently, changes in gene expression should result in the adequate cellular response to external stimuli.

Immune cells are particularly dependent on tightly controlled information transfer from the cell exterior. These cells actively seek and respond to a myriad of challenges in an unpredictable environment. In addition, they must constantly be ready to adapt to ever-changing conditions. Immune cells must respond appropriately, as the whole organism’s safety depends on their activity.

Activated receptors trigger a complex network of signalling events leading to the modulation of protein activity or gene transcription. This occurs through transient alteration in the signalling proteins involved, by conformational change or post-translational modification, typically resulting in the formation of multi-protein complexes. A group of proteins, which are integral parts of these signalling pathways, may have been outshone by their enzymatically active binding partners. These proteins do not perform enzymatic functions. Since their only activity is binding,
they have been designated *adaptor proteins*. In order to perform their functions, these molecules may contain conserved interaction domains and/or unique binding motifs. This allows adaptor molecules to bring together proteins and adapt signalling pathways.

In this review, we discuss how adaptor proteins can form transient molecular complexes in a reversible, dynamic and inducible manner using their structured domains and disordered regions. Further, we explore the intriguing functions and roles of adaptor proteins, with emphasis on intracellular signalling in immune cells. To point out their relevance at the level of the organism, we examine their role in cancer as well as in genetic and infectious diseases. Finally, we present challenges faced in elucidating roles of adaptor proteins, as illustrated by the T cell–specific adaptor (TSAd) protein encoded by the *SH2D2A* gene.

### 1.1 Definition of adaptor proteins

Defining adaptor proteins is challenging and multiple efforts have been made to distinguish it from molecules with anchoring, scaffolding or docking functions.3,4 These terms are used interchangeably, and there is no consensus in the field as to what should be considered an adaptor as distinguished from a scaffold, an anchor or a docking protein. Therefore, for the sake of simplification and also since their functions are overlapping, in the context of this review, we have chosen to refer to all proteins with adaptor, scaffolding, anchoring or docking functions as adaptor proteins.

The simplest definition of an adaptor protein is an intracellular protein, which facilitates signal transduction through interactions with other proteins. However, this definition includes practically *all* signalling proteins. Therefore, adaptor proteins can be defined through exclusion. To narrow down the scope of the review, we have made a list of exclusion criteria to filter out non-adaptor proteins.

Firstly, proteins with any extracellular interactions, including ligand-transmembrane receptor interactions, are excluded.

Secondly, as adaptor proteins do not possess enzymatic activity, enzymes involved in cellular signalling are excluded. For instance, a kinase containing two separate binding domains in addition to the enzymatic domain, is not an adaptor, while a pseudokinase, which has lost its enzymatic activity, can be considered an adaptor.5

Thirdly, DNA- or RNA-binding proteins, that is transcription and translation factors, are excluded. By all the other means, the latter could be considered adaptor molecules due to their multivalency and lack of enzymatic activity.

Fourthly, adaptor proteins, which engage in the formation of stable complexes, are excluded. To appropriately perform their roles, adaptors generally form low affinity interactions of transient or repetitive nature. Proteins forming stable complexes usually only have one well-defined function, in contrast to most adaptors, which are very flexible in both interactions and functions.

The importance of moderate binding affinities for adaptor function is illustrated by artificial SH2 domains binding with high affinity to pTyr, that is superbinder SH2 domains, which may perturb phosphotyrosine signalling pathways.6 Similarly, *Legionella* can release superbinder SH2 domains, of even higher affinity but low specificity,7 into macrophages. Such a phenomenon may be a virulence factor for *Legionella*, resulting in perturbed signalling in the infected cell.

The requirement of low affinity interactions excludes molecules such as the CD3 chains. These proteins are necessary for signal transduction from the T cell receptor (TCR)8 and lack extraacellular receptor function as well as enzymatic domains. Although CD3 chains as standalone molecules could be referred to as adaptor proteins, they only function as integral parts of receptors, which lack similar domains on their own. Lastly, a group of proteins known as adaptins is excluded. These proteins have a unique role in clathrin-mediated vesicular transport by forming stable and well-defined complexes.

Taken together, an adaptor molecule is an intracellular protein, which lacks enzymatic activity as well as DNA or RNA binding properties and can form dynamic multi-protein complexes by binding to two or more proteins at the same time. Adaptor molecules additionally share a number of specific features, which equip these proteins with abilities necessary for their function. While this review focuses on adaptor proteins as defined above, it is important to be aware that many proteins, under given circumstances, may fulfil adaptor molecule functions in addition to enzymatic or other functions.

### 1.2 Features of adaptor proteins

Adaptor proteins assist in positioning other molecules to maintain proper signalling within a pathway. In order to do so, adaptors need special features—that is binding domains, binding motifs and structural flexibility. Binding domains are usually conserved regions with defined structure and specificity. In contrast, binding motifs can be flexible short linear motifs defined by the protein sequence
A  Cytosolic tyrosine kinases with SH2 domains

B  Adaptors with SH2 domains
or specific pockets defined by the secondary and tertiary protein structure. Structural flexibility can be created by intrinsically disordered regions that lack a defined three-dimensional (3-D) structure, or by post-translational modifications, which can dynamically change the structure/properties of the molecule.

**Binding domains** define the molecular pathway in which the protein participates. For example, Src homology (SH) 2

| Domain | Full name | Canonical target | Examples of adaptors containing the domain |
|--------|-----------|------------------|-------------------------------------------|
| 14-3-3 | pSer/pThr | YWHA/B/E/G/H/Q/Z, SFN |
| ADF-H  | Actin-depolymerizing factor homology | Actin | CFL1, SH3P7 |
| ANK    | Ankyrin repeat | No consensus, recognize tertiary structures | SHANK1, SHANK3, ANKS4B, ANKH1 |
| ARM    | Armadillo repeat | No consensus, hydrophobic residues, recognize tertiary structure | KPNA1, KPNA6 |
| C1     | Phorbol esters or diacylglycerol | UNC13A, AKAP13 |
| C2     | Ca²⁺-mediated membrane phospholipids binding | TOLLIP, BAIAJ3, ESYT1 |
| CARD   | Caspase activation and recruitment domain | Homo-oligomerization | CRADD, CARD16, NOL3 |
| CUE    | Coupling of ubiquitin to ER degradation | Ubiquitin | TAB2, TOLLIP, CUEDC1 |
| DD     | Death domain | Homo-oligomerization | FADD, TRADD, MYD88 |
| DED    | Death-effector domain | Homo-oligomerization | FADD, PEA15 |
| EVH1/WH1 | Ena/VASP homology 1/WASP-Homology 1 | FPPPP, LPPPEP | ENAH, HOMER1 |
| F-BOX  | Skp1 protein | SKP2, BTRC, FBXW8, FBXO11 |
| FERM   | 4.1 protein, Ezrin, Radixin, Moesin | Integral membrane proteins | EZR, FRMD6 |
| GYF    | Glycine-tyrosine-phenylalanine | Proline-rich motifs | CD2BP2, GIGYF1 |
| LIM    | Lin-1, Isl-1, Mec-3 | LIM homo-oligomerization, Tyr-containing motifs | TES, PDLIM2, LASP1, PINCH |
| LRR    | Leucine-rich repeats | No consensus | CHADL, DCN |
| NZF    | Npl4 zinc finger | Ubiquitin (not exclusive) | NPL4, VPS36 |
| PB1    | Phox and Bem1 | Homodimerization | PARD6A, PARD6B, PARD6G, TFG |
| PDZ    | Post-synaptic density 95, PSD-85; discs large, Dlg; zonula occludens-1, ZO-1 | C-terminal motifs, homodimerization, lipids | SHANK1-3, TJP1-3, SLC9A3R2, PARD6G, PDLIM2 |
| PH     | Pleckstrin homology | Polyphosphoinositides | IRS1-4, DOK1, PLEK |
| PTB    | Phosphotyrosine binding | NPX(p)Y motif | SHC1-4, IRS-1-4, DOK1/2/3/6/7 |
| SAM    | Sterile Alpha Motif | Homo- and hetero- oligomerization | LCP2, SHANK1-3, SASH1 |
| SH2    | Src homology 2 | pTyr | Refer to Figure 1 |
| SH3    | Src homology 3 | PXXP | Refer to Figure 3 |
| SOCS box | Suppressor of cytokine signalling box | Elongin BC complex | SOCS1-7, CISH, ASB2 |
| SPRY   | SPIa/ryanodine receptor | Phosphatidylinositol 4,5-bisphosphate, homodimerization | RANBP10, SPSB1 |
| TIR    | Toll/Il-1 Receptor | Homodimerization | MYD88, TIRAP, TICAM1, TICAM2 |
| TPR    | Tetratricopeptide repeat | Amphipathic residues, EEVD | STIP1, BBS4 |
| UBA    | Ubiquitin-associated | Ubiquitin | VPS13D, UBXN7, NUB1 |
| UEV    | Ubiquitin E2 variant | Ubiquitin or P[T/S][AP] | TSG101, MMS2 |
| WD40   | Trp-Asp 40 | pSer/pThr | BTRC, SEC13, ARPC1A, FBXW11 |
| WW     | Trp-Trp | pSer/pThr, [A/P][P][A/P][Y] | DMD, PLEKHA7 |

aData and information on interaction domains and motifs are retrieved from the Pawson Lab (Domains – Map at http://pawsonlab.mshri.on.ca/), UniProt²¹² and SMART²¹³ databases. Domains included here are common examples found in adaptor proteins. The list is not comprehensive.
domains are an essential part of protein tyrosine phosphorylation pathways in most immune cells responses. The protein tyrosine phosphorylation cascade involves tyrosine kinases and phosphatases, which add or remove a phosphate group of hydroxyl-containing amino acid, respectively. The phosphotyrosine motifs generated by kinases can be recognition sites for the evolutionary conserved SH2 domains.

The SH2 domain endows a protein with the ability to 'read' the activity of tyrosine kinases by docking onto particular phosphotyrosine motifs on other proteins. The human genome encodes 111 proteins harbouring a total of 121 SH2 domains. Of these, 28 are non-receptor protein tyrosine kinases. A large proportion of these is expressed in immune-related cells and tissues as compared to other tissues (Figure 1A). As discussed later in this review, 49 of the SH2 domain–containing proteins are considered adaptor proteins. Several of these SH2 domain–containing adaptor molecules display preferential expression in tissues enriched in immune cells (Figure 1B). The preferential expression of both tyrosine kinases and SH2 domain–containing adaptor proteins in tissues enriched in immune cells highlights the importance of phosphotyrosine mediated signalling in cells of the immune system. However, not all adaptor proteins involved in intracellular signalling harbour SH2 domains. A keyword search in the Pfam database (https://pfam.xfam.org/) retrieves 443 entries for 'interaction domain' and 1886 entries for 'binding domain'. Many of them can be found in adaptor molecules. An overview of representative binding domains, which may also be present in adaptor proteins, is given in Table 1.

The wide array of binding domains allows adaptor proteins to play roles in a plethora of intracellular pathways, depending on which domains they harbour. While SH2 domains participate in tyrosine phosphorylation cascades, 14-3-3 domains can be involved in serine-threonine phosphorylation cascades and Toll/interleukin-1 receptor (TIR) domains are implicated in Toll-like receptor (TLR) signalling. Other examples of interaction domains include SH3 domains binding proline-rich motifs, pTyr binding (PTB) domains recognizing defined phosphopeptide motifs and pleckstrin homology (PH) domains binding specific polyphosphoinositides (Table 1).

In addition to these structured domains, adaptor proteins can also carry out their function through the unstructured or disordered regions that they possess. Binding motifs and structural flexibility are both consequences of intrinsically disordered regions, which are very often a significant part of an adaptor molecule's sequence (48%-63% on average, depending on the prediction algorithm). Intrinsic disorder regions are a part of a protein that lacks a stable 3-D structure as defined by currently available scientific methods. Two decades ago, it was found that disordered regions of most proteins evolve faster than the structured regions. However, a more recent study has observed that within the disordered regions there are linear motifs, recognized by other domains (SH2, SH3 or Ser/Thr kinases), that are surprisingly well conserved. Additionally, the number of proteins containing unstructured regions of more than 50 amino acids in length is much higher in eukaryotes than in bacteria or archaea. This suggests that there is an evolutionary advantage in developing proteins with higher flexibility. The flexibility provided by intrinsically disordered regions allows complex organisms to produce multifunctional proteins, such as adaptor proteins, whose role could differ within a signalling pathway or between various cells (so-called moonlighting). The obvious importance of intrinsically disordered regions makes it surprising that many studies disregard their significance for protein function.

There are a substantial number of features, which characterize disordered regions and define their unique functions in intracellular signalling. Just to name a few, intrinsically disordered regions can drive membrane curvature; form phase-separated compartments; contain adaptable short linear motifs, which can overlap or be post-translationally modified; serve as flexible linkers between domains; increase the hydrodynamic radius of the protein; dynamically change protein structure; perform moonlighting; and promote oligomerization. Most of these features of disordered regions are highly relevant for the function of adaptor proteins.

Short linear motifs (SLiMs) (or molecular recognition features (MoRFs)) are possibly the most important feature from the examples mentioned above. While the specificity of protein domains and hence their binding partners can to a certain degree be mapped, this has been much more difficult to achieve with SLiMs. It was therefore not before applying sophisticated search algorithms that scientists were able to understand the commonality of SLiMs in the proteome. However, even the best software cannot predict binding to novel targets, as all programmes are trained on existing knowledge.

Short linear motifs present in disordered regions are more accessible to binding domains as they are continually exposed. If a binding motif is hidden within a structured domain, the binding conformation will be much more restricted, as in the classic model of lock-and-key protein-protein interactions. Unstructured regions may bind to proteins with high specificity, but with low affinity, which makes them ideal signalling hubs—both specific and reversible. High specificity comes from the ability of disordered regions to complement the binding interface, while low affinity comes from the transient nature of the interaction. However, this perspective was recently challenged as it was speculated that disordered structures, due to their flexibility, actually combines low specificity with a high affinity. Even if the binding affinity seems low, the disordered structure can adapt itself to the target surface by increasing its own recognition surface and therefore, increasing the interaction stability and its affinity.
Not all adaptor molecules consist of large portions of intrinsically disordered regions with SLiMs. There are certain families of adaptor proteins that consist nearly entirely of a single binding domain. For example, members of the signalling lymphocytic activation molecule (SLAM)—associating protein (SAP) family, which includes SAP (SH2D1A) and EAT2 (SH2D1B), contain only one SH2 domain, and the 14-3-3 proteins (YWHA/B/E/G/H/Q/Z and SFN) contain only the 14-3-3 domain. However, even in these cases, flexibility may be a key property of the adaptor. Firstly, SAP family proteins contain intrinsically disordered tails, which represent 30% of the total protein sequence. Similarly, 14-3-3 domain contain a hidden disordered region propensity, which most likely provides the protein with means to interact with numerous interaction partners and perform moonlighting functions.43 Secondly, a single isolated domain is a quintessence of flexibility as no internal structural constraints stop the protein from forming interactions. SAP itself is considered to be a detachable SH2 domain of FYN kinase as it binds to the FYN SH3 domain and extends the enzyme’s pool of interaction partners.44 Another unique property of the SAP SH2 domain is that it is not restricted to conventional pTyr-containing motifs. Although with a lower affinity, the non-canonical SH2 domain can bind unphosphorylated peptides providing a source of flexibility to its binding partners.45

**Post-translational modifications** are an important part of any signalling pathway due to their (generally) inducible and reversible nature, and their predominant occurrence in disordered regions, and consequently in SLiMs.23,46,47 A certain degree of sequence flexibility may be necessary for post-translational modifications to occur.47 Post-translational moieties may change the structure of a protein sequence, including the binding properties and functionality of SLiMs.37 The most commonly described modifications are additions of small chemical groups to specific amino acids, such as phosphate (to serines, threonines or tyrosines), methyl (to arginines or lysines) or acetyl (to lysines). Among these, tyrosine phosphorylation is of particular interest to immunologists.

One of the major adaptor proteins in T cells, linker for activation of T cells (LAT), contains multiple phosphotyrosine sites that are necessary for a molecule’s ability to bind to different targets48 and thus regulate T cell activation. Another important adaptor protein, MYD88, involved in TLR signalling, becomes inhibited when acetylated on Lys265. Consequently, autocrine production of growth-promoting IL-6 in B cell lymphoma is suppressed.49 There are also other, more complex modifications, such as lipidations (palmitoylation and myristoylation), ubiquitination and SUMOylation. Palmitoylation of LAT is necessary for the translocation of the protein to the plasma membrane. LAT lacking palmitoylated cysteines does not perform its functions, that is absence of these cysteines disrupts T cell development and inhibits TCR activation.50 Ubiquitination is usually, but not exclusively, connected to protein degradation, which can also affect cellular signalling. Ubiquitination of both LAT and SLP-76 (LCP2)—another adaptor protein involved in T cell activation—tags these proteins for degradation. In both cases, the lack of the ubiquitination tag increases TCR signalling.51,52 SUMOylation seems to be more variable in function. It can promote interaction between the adaptor protein GRB2 and the SOS1 guanine nucleotide exchange factor, which leads to increased ERK activity, and subsequently increased cell motility.53 On the other hand, SUMOylation of the adaptor TAK1-binding protein 2 (TAB2), which is involved in IL-1 signalling, may decrease the adaptor’s activity, as shown by the AP-1 luciferase reporter assay.54

## 2 | ADAPTER PROTEINS CONTROL SIGNALLING CASCADES IN SPACE AND TIME

The unique combination of conserved protein-binding domains and intrinsically disordered structures containing SLiMs allows adaptor molecules to control intracellular signalling cascades in space and time. Adaptor proteins can control the localization of signalling molecules, which defines the pathway’s space. In parallel, they can control the sequence of enzymatic events, which defines the pathway’s timing. These processes are often highly interrelated.

In the following, we will discuss various aspects of the adaptor protein functions. For the sake of simplification, we have separated these into two main, but not mutually exclusive, categories: control of (i) space and (ii) time of cellular signalling (Figure 2).

### 2.1 | Space functions of adaptor proteins

#### 2.1.1 | Molecular hub

All adaptor proteins serve, to various extents, as scaffolds for the recruitment of other molecules involved in a given signalling pathway (Figure 2A). One of the best-described examples is LAT.55 Upon triggering of the TCR, tyrosine phosphorylation of CD3 by LCK recruits and allows activation of another tyrosine kinase ZAP70. LAT is a major phosphorylation target of ZAP70. Membrane-bound LAT phosphorylated on multiple tyrosines becomes a molecular hub, which can recruit other proteins and diverge the signalling pathway ([48], reviewed in [8]. Phosphorylation of LAT on Tyr191 and Tyr226 recruits Vav guanine nucleotide exchange factor 1 (VAV1), which starts GTPase signalling, and subsequently, activation of mitogen-activated protein (MAP) kinase pathway and cytoskeleton reorganization. LAT pTyr171 associates with phosphoinositide 3-kinase
(PI3K), which mediates the generation of phosphatidylinositol-3,4,5-triphosphate, and subsequently, activation of protein kinase B (AKT)–induced cell proliferation and survival. Another protein recruited by LAT (to pTyr132) is phospholipase C gamma (PLCγ), which catalyses the generation of inositol 1,4,5-trisphosphate and diacylglycerol from
phosphatidylinositol 4,5-bisphosphate. These molecules trigger the intracellular release of calcium ions and activate the Ras-family of small GTPases and protein kinase C (PKC). All these pathways initiate gene transcription necessary for T cell activation.48

However, transmembrane adaptor proteins,56 such as LAT, are not the only adaptors known for their vast number of interaction partners. The growth factor receptor-bound protein 2 (GRB2) family is also involved in phosphorysine signalling cascades.57 GRB2, the most studied protein from this family, is ubiquitously expressed in the body (Figure 1B), allowing GRB2 involvement in numerous growth factor receptor tyrosine kinase pathways: epidermal (EGFR), vascular endothelial (VEGFR), hepatocyte (HGFR), platelet-derived (PDGFR) and fibroblast (FGFR). GRB2 interacts with various proteins, including LAT in the TCR pathway. However, the most well-established interaction of GRB2 is with SOS1, a protein that directly activates the MAP kinase pathway, resulting in differential expression of genes involved in cell survival and proliferation. Not surprisingly, GRB2 knockout mice are embryonic lethal. Moreover, GRB2 is often implicated in cancer transformation (see Adaptor proteins in human disease—Predisposition to cancer).58

2.1.2 | Signalling segregation

The ability of adaptor proteins to create signalling clusters (Figure 2B) is again the best described in the case of LAT in T cell signalling.59 Triggered TCR signalling induces spatiotemporal segregation of molecules into concentric patterns. This phenomenon is called the immunological synapse. It is well characterized but lacks a unified model.60 The immunological synapse facilitates the direct secretion of cytokines and cytotoxic granules, but it is also orchestrating intracellular signalling.61 For T cells, it is generally accepted that the TCR locates to the centre of the immunological synapse and is surrounded by co-receptors (CD4/8, CD28, CD2, CTLA-4, PD-1). Adhesion receptors (LFA-1) and larger molecules (CD45) locate to the periphery of the synapse. The nature of the molecular exclusion is still debated. While most of the proposed segregation models focus on the surface receptors, segregation of signalling molecules exists also intracellularly. This could be secondary to the aggregation of TCRs, which may serve as an ignition point for intracellular signalling. However, alternative possibilities exist. It was recently found that LAT pre-assembles signalling clusters, which are actively transported via actin towards the centre of an immunological synapse.59,62 Moreover, such clusters resemble liquid-liquid phase-separated microdomains.62,63 A phase-separated microdomain is a membrane-less cellular compartment, which concentrates certain molecules (reviewed in Ref. [64]). GRB2, NCK1, SLP-76, N-WASP (WASL), GADS (GRAP2), SLP-65 (BLNK) and CIN85 (SH3KBP1) are among adaptor proteins known to be involved in such phenomena, not only in TCR signalling,21 but also in B cell activation,65 cell adhesion and receptor tyrosine kinase signalling.64 Segregation of signalling molecules generally facilitates signalling by providing the basis for specific protein recruitment, signal multiplication and increased probability of interactions/enzymatic reactions. However, data describing microdomains have to be taken with a reasonable caution, as it is still an emerging field, prone to bias and technical artefacts.66

Another important notion is that many adaptors facilitate membrane anchoring of protein clusters, which further supports signalling segregation (see Increasing probability of interaction below). For this purpose, they can contain transmembrane domains,56 lipidation sites67 or phosphoinoside-binding domains and motifs68 (such as pleckstrin homology domains3). Even though the presence of a transmembrane domain is sufficient for protein localization to the cell membrane, additional modifications may be required for specific locations. For example, LAT has to be palmitoylated on Cys26, which serves as a sorting signal, in order to be correctly translocated to the cell membrane.50

Membrane anchoring can also be tightly regulated. TIR domain-containing adaptor protein (TIRAP) involved in TLR signal transduction contains a phosphoinositide-binding motif for recruitment to the cell membrane. The motif can be phosphorylated on Thr28, which not only disrupts the membrane binding but also promotes TIRAP ubiquitination and subsequent degradation.69 This mechanism is proposed to prevent prolonged TLR2 and TLR4 signalling.70

Thus, adaptor proteins can segregate molecules within signalling cascades, through relocation to or creation of cellular compartments. This will facilitate interactions of specific proteins with their activated receptors.

2.1.3 | Specific protein recruitment

Adaptor proteins may be critical for the recruitment of specific enzymes (Figure 2C). They contain domains related to the recruitment of proteins involved in particular signalling pathways.7 Such interactions are often localization dependent. For example, Src kinase–associated phosphoprotein 1 (SKAP1) is indispensable to recruit and form a complex between Ras-related Protein Rap-1A (RAP1A) and Ras Association Domain Family Member 5 (RASSF5) in the plasma membrane.71 The RAP1A-RASSF5 complex is necessary to transduce the signal from the LFA-1 receptor in T cells. Similarly, the adaptor TIRAP mentioned above recruits another adaptor protein MYD88 to the plasma membrane. This initiates the signalling of TLRs through IRAK4 kinase.72
As such, it should not come as a surprise that many enzymes are reported to bind to multiple adaptor proteins. For example, LCK may bind to LAT,73 SH2B3,74 HSH2D,75 TSAAd,76 LIME177,78 and SHC179 in T cells. This phenomenon allows the repurposing of a single kinase to various signalling cascades by modifying its location or by increasing its pool of substrates.

However, the flexibility of adaptor proteins is not only necessary for repurposing enzymes in a single cell, but also in various tissues. As previously mentioned, moonlighting refers to proteins with multiple functions (in different signalling pathways). Again, SLiMs and disordered regions are features that play a significant role in moonlighting.32 There are several examples of adaptor molecules that have important functions in distinct tissues. For example, SH2B3 is necessary for negative regulation of myeloid cell proliferation,80 T cell signalling74 and endothelial progenitor cell-driven neovascularization.81 SLP-76 is involved in signalling cascades of both platelet aggregation and TCR.82 Due to its ubiquitous expression, it is not a surprise that 14-3-3 proteins are involved in a plethora of cellular functions, including a chaperone function.83

While adaptors can retain a specific protein at a specific location, they may also deplete the pool of certain proteins (so-called prozone effect94) to, for instance, downregulate a signalling cascade. Low concentration of adaptors may promote signalling by the formation of molecular complexes or priming of enzymes. However, at higher concentration, adaptors may form incomplete, non-functional complexes or deplete the pool of enzymes or substrates, and as a consequence inhibit signalling. Such a competitive mechanism is commonly observed for members of the SOCS, SLAP, GRB7 and SH2B adaptor protein families, which can target components of tyrosine kinase pathways.85 One example is the Src-like adaptor (SLA), which can compete with SRC kinase for the interaction with PDGFR.86

### 2.1.4 Increasing probability of interaction

The hydrodynamic radius is the effective radius of the protein, which is the protein itself and all of the solvent molecules attracted by it. Proteins with large hydrodynamic radii make the largest contribution to membrane crowding.26 Both theoretical considerations27 and experimental data26 show that intrinsically disordered proteins have larger hydrodynamic radii than globular proteins. As many adaptor proteins contain a significant portion of intrinsically disordered regions, they are more predisposed to crowd the cellular space. Consequently, their capture radius is larger and a smaller amount of adaptor proteins is necessary to reach probabilities of interactions similar to structured proteins27 (so-called fly-casting mechanism28).

Signalling modules are typically triggered in the plasma membrane, which reduces the interaction space into two dimensions.88 Membrane anchoring and clustering at the cell membrane can induce favourable conformation and orientation of interacting proteins. For example, the presence of SLP-76 in membrane-bound microclusters is required for Tyr775 and Tyr783 phosphorylation of PLCγ by IL-2-inducible T cell kinase (ITK), which is an essential event for T cell activation89 (Figure 2D). SLP-76 is not only mediating the interaction necessary for enzymatic activity, but it is also stabilizing the catalytically active form of ITK (see Stabilizing the conformation of signalling nodes below).

Adaptor proteins often interact with other adaptors90 and promote oligomerization,33-35,63 which further increases their interaction radius. Such molecular clusters can be pre-existing, enhancing the probability of the interaction with activated receptors/enzymes.91 Moreover, due to intrinsically disordered regions, adaptor proteins can form fuzzy complexes.30 This occurs when the adaptors dynamically change their conformation within the complex and actively adjust to new/modified binding partners. However, one of the most important phenomena that enhances the probability of interactions is spatial confinement, which allows repetitive re-binding.92

### 2.2 Time functions of adaptor proteins

#### 2.2.1 Transducing the signal

A major function of adaptor proteins is to participate in cellular signalling (Figure 2E). Most signalling cascades lead to activation and translocation of transcription factors to the nucleus. The resulting altered gene expression may display a graded (analogue) response, exemplified in T cells by expression of CD69 or CD25, which correlates with the amount of specific transcription factor translocated to the nucleus.93 However, other genes display a binary (digital) response to transcription factors, for example, IL-2 and IFN-γ that are either expressed or not.94 Binary responses are a major part of the cellular decision-making process (see Signal multiplication below). When contributing to signalling cascades, adaptor proteins may not only provide graded signalling but also enable digital responses (with discrete, binary outcomes), sustained, transient or even oscillatory signalling.95

The most important role of adaptor molecules in cellular signalling is to ensure a correct order of events (specificity of signalling).82,96-98 They achieve this through their binding domains of relatively high specificity3 and SLiMs. The flexibility37 and the presence of overlapping SLiMs14 greatly enhance the functionality of the protein within the signalling pathway by providing it with fuzzy dynamics of interactions, or by allowing it to participate in distinct signalling...
pathways. SLiMs can also define the signalling speed. The phosphorylation rate of Tyr132 in LAT is governed by the preceding Gly131. Substituting Gly131 for Asp or Glu can accelerate TCR signalling, which suggests that Gly131 provides a necessary proofreading step.99

In addition, the number of ways, in which signalling pathways can be steered through post-translational molecular switches present in SLiMs, is very large. The best-known mechanisms include binary switches, specificity switches, cumulative switches, avidity switches and sequential switches (all reviewed in Ref. [100]). All of these mechanisms can establish and control the order of events within the signalling pathway.

Binary switches can be as simple as the phosphorylation-mediated binding of multiple proteins to LAT,48 or phosphorylation-mediated inhibition of the ADAP SH3 domain binding to the phosphorylated interaction motif on SKAP1.101 Both examples are involved in proper regulation of T cell activation. However, binary switches can be also more complicated and can involve allosteric control. When phosphorylated on Tyr221, one isoform of CRK, an adaptor that links tyrosine kinases to small G proteins, may be blocked from interactions with other proteins,102 as this phosphosite is a ligand for CRK SH2 domain itself.

Specificity switches can affect the range of binding partners conferred by a given motif. Adaptor protein DISC1, which is involved in neurogenesis, switches between two different binding partners depending on the phosphorylation status of its Ser710.103 If Ser710 is phosphorylated, DISC1 recruits BBS1 to the centrosome and stimulates cell migration. If Ser710 is not phosphorylated, DISC1 binds GSKβ, which activates the Wnt signalling pathway and subsequently, stimulates proliferation. Similarly, the location of some adaptor proteins defines their pool of interaction partners. The location within the cell can be controlled by post-translational molecular switches in the form of lipidation, for example, to correctly localize the adaptor to the plasma membrane.50

Cumulative switches work as positive or negative rheostats. For example, ADAP contains at least three phosphoryrosines recognized by SLP-76 SH2 domains, which are critical for proper microcluster formation.104 However, any two of these phosphosites can partially reconstitute protein oligomerization. This suggests that ADAP phosphorylation behaves like a positive rheostat of signalling microclusters formation in the T cells. The avidity switch can be exemplified with the interaction of adaptor protein TKS4 (SH3PXD2B) with SRC kinase. SRC binds with both its SH2 and SH3 domains to specific sites on TKS4.105 The double interaction gives synergistic enhancement of binding strength, which can lead to prolonged signalling from the EGF receptor. Sequential switches depend on the subsequent order of post-translational modifications. One example of a sequential switch was described above, where ubiquitination and degradation of TIRAP depend on the preceding phosphorylation of Thr28.69

The sequence of events is a crucial point of all signalling cascades and any mistake could bring disastrous effects, even to the whole organism. Adaptor proteins may prevent unspecific or inadequate molecular events from happening through functional misfolding.106 The disordered regions enable a protein to escape unspecific (non-native) interactions by dynamically sequestering recognition motifs through structural changes of the surrounding sequence.

### 2.2.2 Signal multiplication

While the sequence of events in the signalling cascade is of high importance, the strength (multiplication) of the signal is equally relevant for any signalling pathway (Figure 2F). Computational modelling indicates that adaptor molecules can regulate signal duration.107 As described above, adaptor proteins are excellent molecular hubs55,57,108,109 that may strengthen and stabilize the signal by recruiting multiple proteins. However, a more potent way of increasing the signal is through oligomerization,21,33-35,62 which may lead to the creation of phase-separated microdomains.21,62 It is a simple, but extremely efficient method of multiplying the function of a molecular hub.

Adaptor proteins can also promote a phenomenon called processive phosphorylation,110 which facilitates phosphorylation of multiple sites by a kinase in one single encounter with a substrate. For instance, GRB2 may promote the processive phosphorylation of LAT by ZAP70.111 Another example is LCK, which although not classified as an adaptor protein, may function as an adaptor by bridging ZAP70 to LAT, thus promoting processive phosphorylation of LAT by ZAP70.73

Signal multiplication via adaptor proteins is also an important part of the cellular decision-making process. Computational modelling shows that adaptor proteins involved in phosphorylation pathways improve their determinism (bistability).112 Moreover, proper organization of signalling nanoclusters allows digitizing the external stimuli to increase the fidelity of a system.113 Again, this is exemplified by LAT, which has been pinpointed as one of the elements of digitization in TCR signalling.114 The phenomenon might depend on a precise phosphorylation rate of specific tyrosines present in the LAT molecule.99 Finally, computational simulations have shown that adaptor molecules not only may amplify a stimulus but may also attenuate it, depending on the context115 and adaptor concentration.84

### 2.2.3 Joining signalling pathways

The necessity of activation through multiple distinct receptors is best exemplified by TCR co-signalling116 (Figure 2G).
The need for fine-tuning of the T cell response via co-receptors is clearly illustrated through the development of the CAR T cell technology. The first generation of CART cells poorly imitated the behavior of naturally activated T cells and was later improved by inclusion in the CAR construct of two T cell co-receptor intracellular domains.117 This observation supports the notion that coordination of proper immune cell activation, where several signalling pathways need to be activated simultaneously, requires a large number of adaptor molecules.116 This applies to the adaptors FYB1 and SKAP1, which translate the TCR pathway into an inside-out signal, through integrin-mediated adhesion.118 Another example is the adaptor GRB2 which is necessary for T cell co-receptor CD28 to elicit IL-2 production,119 an important part of proper activation of T cells. However, it seems that merging of the TCR and CD28 signalling pathways occurs at the level of transcription initiation, as both pathways depend on different adaptor proteins to activate NF-κB transcription factor, LAT/ADAP and GRB2/VAV1, respectively.120

Activation of NK cells also depends on the integration of signals coming from a variety of co-stimulatory receptors. A combination of certain receptors can provide synergistic signalling. For example, NKG2D (KLRK1) and 2B4 (CD244) signalling pathways converge at the phosphorylation of SLP-76.121 The former predominantly induces the phosphorylation of SLP-76 on Tyr113, whereas the latter induces phosphorylation of Tyr113. Phosphorylation of both sites is necessary to overcome VAV1 inhibition by CBL-C E3 ubiquitin-protein ligase (CBL-C) and subsequent activation of NK cells. This phenomenon is ligand/receptor-dependent because antibody-mediated activation of FcγRIII (CD16) phosphorylates SLP-76 on both tyrosines simultaneously.

2.2.4 | Stabilizing the conformation of signalling nodes

Adaptor proteins can control enzymatic activity through allosteric control5 (Figure 2H). In particular, this pertains to pseudokinases, which can be perceived as adaptor proteins due to their loss of enzymatic activity.122 For instance, in the ERK pathway, KSR pseudokinase can activate RAF kinase through its binding.123 Other adaptors can also influence the enzymatic activity of their binding partners, such as phosphorylated PAG1, which increases the activity of tyrosine kinase CSK by 6-fold, significantly affecting TCR activation.124 Similarly, SLP-76 binding of ITK stabilizes the kinase active state, allowing ITK to perform its functions.89 Allosteric modulation can also be understood as conformational changes, which promotes differential binding preferences as a result of interaction with an adaptor protein. Such a case was described for the interaction between LAT and GRB2, where binding to one phosphotyrosine is increased upon phosphorylation of another tyrosine.111 In addition, adaptor proteins provide a boosting platform for enzymatic reactions. This is termed substrate (metabolic) channelling and refers to a rapid transfer of a substrate between two subsequent enzymes.125 A similar phenomenon was reported between protein kinase C and its adaptor AKAP7, but it was instead referred to as the scaffold state switching model.126 The authors additionally confirmed the adaptor’s ability to insulate the enzyme from both substrate competition and ATP competition, but not activation competition. Lastly, an important aspect of the interplay between adaptor molecules and the corresponding enzymes is the protection of moieties. An example of this is when an SH2 domain is bound to a phosphorylated tyrosine, thus protecting it from phosphatases.127

In conclusion, adaptor proteins play a crucial role in fine-tuning signalling pathways. As such, it would be expected that in the absence of a given adaptor molecule, physiological processes will be perturbed resulting in human disease. Considering the importance of cellular signalling mechanisms for the flexibility and robustness of immune reactions, it is likely that genes encoding adaptor molecules may contribute to various disease phenotypes. To obtain a rough overview of diseases where adaptor molecules have been implicated, we performed a search for associated disease phenotypes on DisGeNET.128,129

3 | ADAPTOR PROTEINS IN HUMAN DISEASE

Genetic diseases are often associated with mutations or deletions in genes encoding proteins harbouring enzymatic activity. However, adaptor proteins, which interact with these enzymatically active proteins, may have important roles in a wide array of biological and physiological processes, given the possible functions they may accommodate, as discussed above.

In the following, we will explore the extent of how adaptor proteins have been implicated in genetic diseases. To this end, we took advantage of the DisGeNET repository, a collection of genes involved in human diseases, which are deposited in several publically available databases. This comprehensive platform integrates data from expert-curated repositories with text-mined data, GWAS catalogues as well as animal disease models. As such, the data range from large scale genome-wide studies to analysis of monogenic diseases.

As adaptor molecules are heterogeneous and do not have one common feature that is applicable across all proteins, it is difficult to search for adaptor proteins in a given database. Therefore, to get insight into associated human diseases, we decided to focus on adaptor proteins that contain either SH2 and/or SH3 domains as the target genes. To assess the
scope of genes implicated in disease, we performed a similar search for the SH2 domain–containing kinases. This search revealed on average twice as many diseases associated with SH2 domain–containing kinases than the number of diseases associated with SH2 domain–containing adaptor proteins. This may be explained by the more apparent phenotypes observed in kinase mutants as well as by the higher research attention they receive. Despite this, the search in DisGeNET revealed genes encoding adaptor molecules that are implicated in human genetic diseases. An overview of

| Gene       | Alternative names | SH2b | SH3b | Associated disease/phenotype                                      | Reference |
|------------|-------------------|------|------|------------------------------------------------------------------|-----------|
| BCAR1      | CKRAS, CAS, CASS1, p130Cas | 1    |      | Breast cancer                                                    | [132,133] |
| BLNK       | SLP-65, BASH      | 1    |      | Impaired B cell development, childhood pre-B ALL, Agammaglobulinemia | [148]     |
| CRK family proteins |                | 1/2d |      | Lung cancer, breast cancer, gastric cancer, glioblastoma         | Reviewed in [135] |
| CRKL       |                   | 2    |      | DiGeorge syndrome                                               | [214,215] |
| FYB1       | ADAP, FYB, SLAP130| 2    |      | Thrombocytopenia 3                                              | [216]     |
| GRAP2      | GADS, GRB2l, GRID | 2    |      | Peripheral T cell lymphoma                                       | [137,217] |
| GRB2       | ASH               | 2    |      | Various cancers                                                  | Reviewed in [58] |
| LCP2       | SLP-76            | 1    |      | Platelet function and signalling                                 | Reviewed in [82] |
| MAPK8IP1   | JIP1, IB1, PRKM8IP| 1    |      | Diabetes mellitus                                                | [154]     |
| MIA        | CD-RAP (in mouse) | 1    |      | Malignant melanoma, gastric cancer                               | [219,220] |
| NCK1       | NCK               | 3    |      | Colorectal cancer, multiple cancer associations                  | [161,162] |
| NCK2       | GRB4              | 3    |      | Primary melanoma                                                 | [163]     |
| NEDD9      | CasL, HEF1, CASS2 | 1    |      | Melanoma/neoplasm metastasis and other malignancies              | Reviewed in [134] |
| NPHP1      | NPH1              | 1    |      | Familial juvenile nephronophthisis (NPH)                         | [221,222] |
|            |                   |      |      | Joubert syndrome 4                                               | [155]     |
| PEX13      |                   | 1    |      | Peroxisome biogenesis disorders (Zellweger Syndrome)             | [223]     |
| SH2B1      | KIAA1299, SH2B    | 1    |      | Various cancers                                                  | Reviewed in [131] |
|            |                   |      |      | Obesity                                                          | [224]     |
| SH2B3      | LNK               | 1    |      | Essential thrombocytahemia, Erythrocytosis                       | [80,149,150] |
|            |                   |      |      | Coronary artery disease                                          | [225]     |
| SH2D1A     | SAP, DSHP         | 1    |      | X-linked Lymphoproliferative Disease B cell lymphoma              | [142-144] |
| SH2D2A     | TSAD, LAD, VRAP, SCAP | 1    |      | Multiple sclerosis                                               | [151,152] |
|            |                   |      |      | (see TSAd in human disease for more)                              |           |
| SH3BP2     | 3BP2, RES4-23     | 1    |      | Cherubism                                                       | [226]     |
| SH3PX2D2B  | TKS4, FAD49, KIAA1295 | 4    |      | Frank-Ter Haar syndrome                                         | [156]     |
| SH3TC1     |                   | 1    |      | Colorectal cancer                                                | [227]     |
| SH3TC2     | KIAA1985, PP12494 | 2    |      | Charcot-Marie-Tooth disease                                      | [228]     |
| SHANK2     | CORTBP1, KIAA1022, PROSAP1 | 1    |      | Intellectual disability (Autism)                                 | [229]     |
| SOCS1/SOCS3| SS11, TIP3/CIS3, SS13 | 1    |      | Various cancers                                                  | Reviewed in [230] |

*This is not a complete list of adaptor proteins associated with disease, but a selected overview of the most notable gene-disease associations.

bThe column indicates the number of the specified domain.

The number of SH3 domains varies depending on CRK protein isoform.
selected genes associated with human diseases is shown in Table 2.

3.1 | Predisposition to cancer

DisGeNET classifies the associated diseases based on its nature into 'Disease Types': Diseases, Groups and Phenotype. We noticed that the search results are mainly of the type Diseases; that is, they are associated with specific diseases (e.g., Diabetes mellitus) (Figure 3A). To get a better understanding of the type of diseases that the genes are associated with, we further grouped results in the type Disease based on their annotated MeSH code and/or semantic type (Box 1). This revealed that a majority of the SH2- and SH3-encoding genes are primarily associated with neoplasms (Figure 3B). This is possibly a skewed result, due to the vast amount of research available in the field of oncology. Several of these studies are GWAS studies that have been a powerful tool in searching for cancer targets. On the other hand, adaptor proteins are frequently implicated in signalling pathways that maintain normal cell growth and proliferation, including SH2B1, BCAR1, NEDD9 and several proteins in the CRK
family (Table 2). Therefore, it is perhaps not surprising that many adaptor molecules are reported to be involved in predisposition to cancer.

Of particular immunological interest are adaptor proteins implicated in several leukaemias. The GRB2 family proteins, GRB2 and GADS, have been shown to mediate oncogenic fusion protein BCR-ABL-driven myeloid and lymphoid leukaemia, respectively. The SH2 domain of GRB2 interacts with BCR-ABL, which recruits another adaptor protein GAB2. This complex subsequently activates the Ras signalling pathway and drives cellular transformation. Similarly, the SH2 domain of GADS binds BCR-ABL and creates a molecular hub, including SLP-76, which subsequently activates signalling pathways downstream of BCR-ABL. Furthermore, CRK family proteins are suggested as mediators of BCR-ABL-dependent leukemogenesis. CRKL and, to some extent, CRK have been shown to bind to BCR-ABL in transformed granulocytes and myeloid precursor cells (reviewed in Ref. [135]). Together, these events exemplify how adaptors may be exploited in transformed cells to promote advantageous protein complex formation (Figure 2A), specific protein recruitment (Figure 2C) and signal transduction (Figure 2E), as discussed earlier.

Several cancer treatments aim at targeting proteins of the JAK-STAT pathway, in particular inhibiting kinases or other enzymatically active proteins that drive cancer transformation. However, some of these treatments are over time met with resistance. In efforts to circumvent this problem, several groups have started to develop inhibitors targeting adaptor proteins instead, for instance by down-regulating BCAR1 in the tumour. Similarly, both the SH2 domains of GRB2 and NCK1, which are implicated in several cancers, have been suggested as targets for cancer therapies.

### 3.2 Monogenic disorders

In contrast to cancer, which is multifactorial, there are also distinct diseases caused by defined mutations in adaptor proteins. Several SH2 domain–containing adaptor proteins are associated with disease phenotypes, as previously reviewed. A prominent example of an adaptor molecule involved in disease is SAP, encoded by the gene SH2D1A. This molecule contains a single SH2 domain and is mainly associated with infectious diseases and diseases of the hematopoietic system (Figure 3B). In 1998, three groups simultaneously identified loss-of-function mutations in SH2D1A, which were associated with X-linked lymphoproliferative disease (XLP) triggered by infection with Epstein Barr virus. These mutations range from large deletions to point mutations and are mostly found within the SH2 domain and primarily cause structural mutations. Normally, SAP associates with SLAM and competes with the phosphatase SHP-2 for the same binding site, thereby limiting the recruitment of SHP-2 to the receptor. Furthermore, SAP bridges SLAM with FYN, a kinase that can enhance phosphorylation of SLAM receptors. In individuals harbouring mutations in SH2D1A, SLAM receptor signalling in T cells is consequently abrogated which causes the symptoms observed in XLP. Further, patients who have recovered

---

**Box 1 Identification of adaptor proteins associated with disease in the DisGeNET database**

DisGeNET is a comprehensive platform collecting gene-disease associations from several publically available databases. To identify adaptor proteins associated with disease in this database, we first identified all human proteins that contain an SH2 and/or an SH3 domain in UniProt (data retrieved on 16 April 2020). Of these, proteins with enzymatic activity such as kinases or guanine exchange factors, including those that have DNA- or RNA-binding activity or are involved in the endocytic pathway, were excluded from further analysis. All remaining genes were considered adaptor molecules, as defined within the scope of this review. Diseases associated with this list of genes were extracted from DisGeNET. Using the classification annotated by DisGeNET, the proteins were then grouped by Phenotype, Group and Disease, based on the nature of the associated disease as defined by DisGeNET. Within the Disease group, we further grouped the genes based on their MeSH codes and semantic type into one of the following groups: Neoplasms, Infections, Haematopoietic system, Nervous, Cardiovascular and Other. Several diseases in DisGeNET are assigned multiple MeSH codes, given the multifactorial nature of the disease. Genes associated with such diseases were placed into the group that was the initial cause of disease and not its consequence. For instance, for T cell lymphomas, which is assigned the following MeSH codes: Hemic and Lymphatic Diseases; Immune System Diseases; Neoplasms, were placed under Neoplasms, as this is the source cause of disease. The MeSH codes in our defined groups are divided as follows: Neoplasms (C04), Infections (C01, C02, C03), Haematopoietic system (C15, C20), Nervous (C10), Cardiovascular (C14) and Other (remaining MeSH codes).
from symptomatic XLP or asymptomatic individuals with malfunctioning SAP have been shown to develop B cell lymphomas. The underlying mechanisms are still unclear; however, recent data suggest that SAP is crucial for CD8+ T cell–mediated immune surveillance of transformed B cells. In the absence of SAP, signalling through the SLAM receptor 2B4 is disrupted in T cells and proper activation of naïve CD8+ T cells is abolished. Together, this illustrates how SAP may be involved in signal attenuation as well as signal multiplication, as discussed earlier (Figure 2C and 2F).

Other examples of SH2-encoding genes associated with immune dysfunction are BLNK, SH2B3 and SH2D2A. This could be explained by a relatively higher expression of these adaptor proteins in hematopoietic cells (Figure 1B). Similarly, mutations in SH3 domain-encoding genes are associated with specific diseases including MAPK8IP1 in diabetes mellitus, NPHP1 in Joubert syndrome and SH3PXD2B in Frank-Ter Haar Syndrome. These latter examples are diseases of specific tissues, possibly owing to their functional importance in given cells. However, it may also reflect the diverse function adaptor proteins play in that particular tissue as well as across tissues, illustrating the concept of moonlighting.

Taken together, the search on DisGeNET for human diseases reveals the vast number and diversity of types of diseases which adaptor proteins are implicated in. Not only are they involved in multifactorial diseases such as cancers, but deletions or point mutations of the adaptor molecules alone can cause severe disease and developmental defects.

### Table 3

| Gene knockout | Phenotype | References |
|---------------|-----------|------------|
| BCAR1         | Embryonic lethality, systemic congestion, growth retardation | [231] |
| BLNK          | Impaired B cell development, high incidence of pre-B cell lymphoma | [232,233] |
| CRKII         | Normal | [165] |
| CRKL (Crkol in mice) | Similar phenotype to DiGeorge Syndrome | [234] |
| FYB1 (Fyb/Slap) | Impaired T cell proliferation and responses | [235,236] |
| GRAP2 (GADS)  | Defective T cell development and some developmental defects | [237] |
| GRB2          | Impaired embryogenesis, limited malignant transformation | [238] |
| LCP2          | Foetal haemorrhage, perinatal mortality, dysregulation in separating lymphatic and blood vessels, block in T cell development | [239,240] |
| MAPK8IP1 (JIP) | Required for early embryogenesis, however viable in another study and no developmental defects and no signs of diabetes | [241,242] |
| MIA (CD-RAP in mice) | Ultrastructural Cartilage Abnormalities | [243] |
| NCK1/NCK2     | Functionally redundant and viable when either Nck adaptor is knocked out, however when both KO they are embryonic lethality | [164] |
| NEDD9 (CasL)  | Reduced lymphocytes in secondary lymphoid organs, loss of marginal zone B cells | [244] |
| NPHP1         | Male infertility | [245] |
| PEX13         | Similar phenotype to human Zellweger Syndrome | [246] |
| SH2B1         | Slight growth retardation and infertility, dysregulated glucose metabolism | [247,248] |
| SH2B3 (LNK)   | Dysregulated B cell production | [249] |
| SH2D1A (SAP)  | XLP-like phenotype when challenged with infectious agent | [250,251] |
| SH2D2A (TSAd) | Mild changes in T cell activation | [171] |
| SH3BP (3BP2)  | Normal TCR responses, impaired BCR responses | [252] |
| SH3PXD2B (TXS4) | Skeletal, eye and cardiac abnormalities, retarded growth | [253] |
| SH3TC2        | Progressive peripheral neuropathy | [254] |
| SHANK2 (ProSAP1/SHANK2) | Autistic-like behaviour, hyperactive | [255] |
| SOCS1         | Stunted growth, impaired survival | [256] |

*This list includes knockout mouse models of the genes listed in Table 1.
*Gene/protein names in brackets correspond to the name used in the reference.
*Refer to the groups who first generated the gene-targeted mice and reported its associated phenotype.
3.3  Diseases associated with other adaptor proteins

SH2 and SH3 domain–containing proteins make up only a fraction of all adaptor proteins present in the human genome. And so, the diseases mentioned here illustrate just the tip of the iceberg in terms of the imperative role adaptors play in human health. For example, KSR1 has been implicated in malignancies, and increased expression of GAB2 is associated with mammary carcinogenesis. Furthermore, the scaffolding protein SANS has been implicated in Usher syndrome.

Additionally, it remains important to bear in mind that disease-causing mutations associated with adaptor protein function may also occur in binding partners, which have lost their ability to bind to the adaptor protein. An example of this is in Noonan syndrome, where a mutation of RAF1 abrogates its interaction with 14-3-3 proteins, and consequently abolishes the autoinhibition of RAF1, otherwise promoted by 14-3-3 binding. This lack of negative regulation results in developmental deformities observed in Noonan syndrome. As such, given their regulatory function, when exploring drug targets it is relevant to identify and consider all potential interaction partners of adaptor proteins.

3.4  Mouse models

Groups interested in identifying the genetic cause of diseases mentioned above begin by searching for mutations by positional cloning (or more recently by whole genome sequencing) in families where more than one member has the disease. If a candidate gene is identified, the next questions are whether mutations also occur in the same gene in non-related patients and whether a disease mechanism can be identified in knockout (KO) animals where the gene of interest is deleted. While there are some exceptions, most of these KO models develop disease phenotypes similar to those observed in patients (Table 3).

Examples of exceptions are NCK1 and NCK2, which have been implicated in several types of cancer. In mice where either NCK1 or NCK2 are disrupted, the proteins seem to be functionally redundant and the mice are viable. However, when generating double KOs of these proteins, loss of NCK results in embryonic death. Similarly, CRK seems to harbour a redundant function. CRK KO mice are phenotypically normal and without embryonic abnormalities. This lack of phenotype is in stark contrast to the wide array of functions ascribed to CRK, including regulation of cellular processes from cell proliferation, migration, adhesion to apoptosis.

Examples where adaptor molecules appear to be redundant highlight several important issues. As mentioned above, the effect of mutations in genes encoding adaptor proteins may only be observed in specific tissues. Thus, creating a ubiquitous KO may not accurately recapitulate the disease phenotype as seen in humans. As such, it is important to consider conditional KOs, with a deletion of the gene in specific tissues. Although adaptor proteins initially seem to have redundant functions, they may also harbour multifunctional characteristics, which could explain the apparent lack of phenotype in KO animals.

The concept of multifunctional proteins was first termed as moonlighting proteins in the late 1990s, and it is still very much relevant today. As discussed earlier, given their flexibility and multi-functionality, adaptors are prodigious in moonlighting. This may explain the diverse range of diseases including several types of cancer that many adaptor proteins are involved in. Keeping this in mind, the function of an adaptor protein may be revealed only under defined conditions, for instance during an infection with a particular microbe (eg SH2D1A). However, with an improved standard of living, health care and reduced exposure to triggering pathogens, this may limit the prevalence of disease phenotypes caused by mutations in genes encoding adaptor molecules. Thus, it may be that a pool of genetic variants exists that are disease-causing in the context of specific pathogens and that have yet to be identified. For instance, it is likely that in the current pandemic, the new coronavirus, SARS-CoV-2 may trigger specific COVID-19 disease phenotypes in the context of specific genotypes. This is particularly interesting since entire populations are thought to be susceptible to the virus.

And while a large fraction of the population has already been infected, the resulting disease is highly variable both across different ages but also between seemingly healthy individuals of the same age and sex. Since the pathogenesis of SARS-CoV-2-induced disease is to a large extent dependent on a perturbed immune response against the virus, examining whether underlying polymorphisms in adaptor protein-encoding genes is associated with COVID-19 susceptibility could be highly relevant.

The SH2D2A gene encodes the T cell–specific adaptor protein (TSAd), an example of an adaptor, whose function is still a matter of ongoing discussion. This gene harbours a number of expression quantitative trait loci (eQTL) (GTEx database); however, it has only been suggested to be associated with a limited number of diseases (Figure 3B). The functional role of this adaptor, which is preferentially expressed in hematopoietic cells (Figure 1B), will be discussed in the last section of this review.

4  TSAD—AN ADAPTOR WITH AN AMBIGUOUS ROLE IN CELLULAR SIGNALLING

As we described in the previous sections, adaptor proteins fulfil a vast number of functions in cellular signalling.
However, their exact physiological role is often hard to define due to the absence of related diseases and mild or lack of phenotype in animal KO models. One example is the adaptor protein TSAd encoded by the \( \text{SH2D2A} \) gene. TSAd has two defined structural features: an SH2 domain and a long disordered C-terminal proline-rich tail containing four phosphotyrosines (Figure 4A), which are both highly conserved across species (Figure 4B). It has been found to interact with LCK, RLK and ITK as well as VEGF receptor 2 (VEGFR2), thus its alternate designations LCK adaptor protein (LAD),\(^{170}\) RLK/ITK-binding protein (RIBP)\(^{171}\) and VEGF receptor–associated protein (VRAP).\(^{172}\) Two decades after it was first identified in 1998,\(^{173}\) its role in cellular signalling is still only partially understood.

In the previous sections, we have presented a number of reasons highlighting why adaptor proteins may be understudied. Several of these reasons apply to TSAd including: (i) expression across various tissues, (ii) moonlighting functions, (iii) mild phenotype of \( \text{SH2D2A} \) KO mice, (iv) weak association with human disease, (v) multiple and distinct binding partners and (vi) poorly understood disordered regions, which constitute the majority of the protein.

In the following, we will present current knowledge about TSAd focusing on reasons why TSAd’s role in cellular signalling has been challenging to define.

### 4.1 Regulation of TSAd expression

Five alternative splicing variants of TSAd exist. Especially interesting is variant 5, which omits exon 7 and, as a result, does not contain the proline-rich region and the tyrosines.\(^{174}\)
This is consistent with the observation that alternatively spliced protein variants are enriched in unstructured regions,\textsuperscript{175} which is another way to increase intracellular signalling complexity.

As indicated in Figure 1B, TSAd (\textit{SH2D2A}) is predominantly expressed in lymphocytes (specifically in T cells\textsuperscript{173,176} and NK cells\textsuperscript{177}), endothelial\textsuperscript{172} and epithelial cells.\textsuperscript{178} TSAd expression is regulated by TCR\textsuperscript{173,176} and cAMP signalling,\textsuperscript{179} where the latter strongly induces TSAd mRNA expression in primary T cells. Additionally, protein kinase A (cAMP-dependent protein kinase) activity is required for TCR-dependent induction of TSAd expression.\textsuperscript{180} Another potent activator of TSAd expression is a combination of phorbol myristate acetate and ionomycin (which bypasses immunoreceptor tyrosine-based activation motif phosphorylation), both in T cells and NK cells.\textsuperscript{181}

### 4.2 TSAd in human disease

There are 474 eQTLs associated with \textit{SH2D2A}, most of which are single nucleotide polymorphisms (SNPs) (as listed in the GTEx database). A polymorphism in the promoter region of \textit{SH2D2A}, resulting in a shorter promoter sequence, has been associated with increased susceptibility to multiple sclerosis,\textsuperscript{151} juvenile rheumatoid arthritis,\textsuperscript{182} chronic inflammatory demyelinating polyradiculoneuropathy\textsuperscript{153} and Sjögren’s syndrome.\textsuperscript{183}

Further, T cells homozygous for shorter variants of the promoter region displayed lower levels of TSAd upon TCR stimulation.\textsuperscript{151} A non-synonymous SNP resulting in asparagine substitution at amino acid position 52 in TSAd increased susceptibility to multiple sclerosis\textsuperscript{152} and ovarian cancer.\textsuperscript{184}

Furthermore, asparagine in position 52 of TSAd was associated with increased transcriptional activity and promoted TSAd interaction with LCK as measured by the yeast \( \beta \)-galactosidase reporter assay.\textsuperscript{184} While these reports indicate that variation in TSAd expression may influence disease susceptibility, it is unclear how specifically this may affect the risk of disease.

### 4.3 Phenotypes of TSAd KO mice

The \textit{SH2D2A}-deficient mice were generated on the 129 genetic background and backcrossed to C57BL/6.\textsuperscript{171} The initial report of \textit{SH2D2A}-deficient mice having a mild autoimmune phenotype\textsuperscript{185} has not been confirmed by our group.\textsuperscript{186} It is possible that this discrepancy in observations is due to differences in the extent of genetic backcrossing, as many hybrid strains between 129 and C57BL/6 mice develop autoimmunity spontaneously.\textsuperscript{187} However, a number of phenotypic characteristics have been revealed when the \textit{SH2D2A}-deficient mice were challenged in various models. Tumour growth in TSAd KO mice is slower, possibly due to reduced angiogenesis.\textsuperscript{188}

Vessels in TSAd KO mice do not respond to VEGF with increased vascular permeability, and VEGF stimulation does not disrupt VE-cadherin junctions in endothelial cells lacking TSAd.\textsuperscript{189} Additionally, TSAd KO mice are more resistant to myeloma development than TSAd wild-type mice in a myeloma-specific TCR-transgenic model.\textsuperscript{186} Upon viral challenge, TSAd-deficient mice displayed reduced clearance of murine cytomegalovirus in the spleen.\textsuperscript{181} Murine \textit{SH2D2A}\textsuperscript{−/−} CD4\textsuperscript{+} T cells exhibited impaired polarization in the immunological synapse of multiple molecules involved in TCR signalling.\textsuperscript{190}

Moreover, TSAd KO mice displayed accelerated rejection of heart transplants in an MHC class II-mismatched model, and resistance to graft-prolonging therapy of costimulatory blockade in the fully mismatched model.\textsuperscript{191} As such, the TSAd KO phenotype is still not fully explored.

### 4.4 A unique role for TSAd in VEGFR signalling

TSAd has been reported to control the opening of adherens junctions of the endothelium and, consequently, vascular permeabilization.\textsuperscript{188,189,192} Upon VEGF binding to VEGFR2, the receptor dimerizes and initiates downstream signalling, which engages tyrosine protein kinases. The SH2 domain of TSAd recognizes pTyr951 (Tyr949 in mice) in the cytoplasmic part of VEGFR2. Through this interaction, TSAd bridges VEGFR2 with SRC kinase, which can bind to prolines in the TSAd unstructured region. As a result of membrane translocation, SRC kinase phosphorylates the VE-cadherin’s intracellular domain, which leads to the opening of adherens junctions.\textsuperscript{189} VEGFR2 Y949F mutant mice have significantly reduced vascular permeabilization, which blocks molecular extravasation, oedema and metastatic cancer.\textsuperscript{192}

### 4.5 TSAd in TCR signalling

While TSAd has been studied much more extensively in TCR signalling, there is no consensus, so far, on its role in T cells. However, the interaction of TSAd with LCK has been best characterized. LCK can bind with its SH3 domain to the proline-rich region of TSAd\textsuperscript{174} and with its SH2 domain to TSAd phosphotyrosines (Tyr280, Tyr290 and Tyr192,\textsuperscript{196} but downregulates phosphorylation of LAT, SLP-76, PLC\(\gamma\), ZAP70 and CD3\(\gamma\).\textsuperscript{176,197} Our group has also shown a connection between LCK pTyr192 and TSAd pTyr290. Phosphorylation of the LCK SH2 domain on Tyr305 (Tyr293 in mice) disrupts VE-cadherin junctions.\textsuperscript{193} The SH2 domain of TSAd recognizes pTyr290 peptides.\textsuperscript{196}
4.6 | Other interaction partners of TSAd

In addition to LCK, SRC and VEGFR, well-established interaction partners for TSAd also include ITK, which binds to the proline-rich region of TSAd via its SH3 domain.195,198 In mice, TSAd has been shown to become phosphorylated upon PDGFR activation in bronchial epithelial cells.199 This leads to the association of TSAd with PDGFR and GRB2. Furthermore, TSAd binds to MAP3K2 and co-localizes with it in the immunological synapse.200 Upon EGFR stimulation, the TSAd-MAP3K2 interaction results in the activation of MAPK7 and JNK, most likely by facilitating their phosphorylation by SRC.201,202 Upon stimulation with CXCL12 and CCL5 chemokines, TSAd brings together the β-subunit of G protein–coupled chemokine receptor, LCK and ZAP70, which is necessary for the activation of the latter molecule.203 The SH2 domain of NCK1 can bind to TSAd pTyr280 and pTyr305, while its SH3 domains can bind to the TSAd proline-rich region.204 This binding facilitates NCK1 interaction with LCK and SLP-76, which potentially promotes actin polymerization in T cells. Finally, TSAd is suggested to interact with DSCAM and DSCAML1 in neurons, recognizing their phosphotyrosine motifs, although the physiological relevance is unknown.205

The main structural feature of TSAd is its SH2 domain. It is thus of particular interest to identify interaction partners to the TSAd SH2 domain in T cells. As previously mentioned, the TSAd SH2 domain can bind pTyr951 on VEGFR2.189 Otherwise, it has been reported to interact with SMAD2 and SMAD3, which are involved in the TGF-β receptor (TGFBR) signal transduction pathway.206 Additionally, the TSAd SH2 domain and the proline-rich region mediates binding to the laminin-binding receptor (RPSA), which promotes T cell migration.207 TSAd may also bind via its SH2 domain to CD6 and LAT,208 and Valosin-containing protein (VCP).209

4.7 | TSAd—open questions in immune cell signalling

While TSAd may interact with cytosolic kinases in T cells and NK cells, TSAd’s role in immune receptor signalling is still not well defined. The recent report that TSAd is critical for graft rejection has revealed a novel and potent phenotype.191 However, its significance in the context of signalling in T cells still awaits deciphering.

Most of the TSAd protein sequence (75%) consists of an intrinsically disordered structure. To what extent this sequence has functional relevance is not well characterized. Mice expressing the TSAd SH2 domain in the absence of the disordered regions had T cells with reduced TCR-dependent production of IL-2, proliferation, migration and inflammatory responses.210

Parts of the disordered region in TSAd are conserved between species193 (Figure 4B). Predicting binding sites and motifs hidden within disordered regions remains difficult, as it is based on comparison with pre-existing knowledge.39 Identification of short sequence motifs that are conserved between species will help identify regions of interest within the unstructured regions. This may allow testing of single mutations of conserved amino acids, which may pinpoint their importance or even function. Such mutations can be further tested using genome editing technology such as CRISPR/Cas9,211 which mediates deciphering roles of adaptor proteins in signalling pathways in relevant cell lines.

5 | CONCLUSION

As described in this review, a straightforward explanation of the role of adaptor proteins in cellular signalling is unattainable. This is partly due to the multitude of different functions that these molecules may fulfil. While many adaptor molecules have been characterized in great detail, others are still understudied, and a larger focus on this intriguing group of proteins is required. Adaptors may be perceived as redundant parts of signalling pathways since their absence often does not result in striking phenotypes. However, increasing evidence points towards adaptors being imperative cellular components, by providing intracellular signalling networks with essential flexibility and fine-tuning in a dynamic manner. This is particularly true for cells of the immune system, which have evolved to depend on adaptor proteins in their constant effort to respond adequately to external challenges and, ultimately, keep the organism alive.

ACKNOWLEDGMENTS

Part of the text in this review was also included in PB’s doctoral thesis: Borowicz P Regulation of T cell activation by two conserved phosphotyrosines in Lck and its adapter protein TSAd. Oslo: Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, 2020. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health (US), and by NCI, NHGRI, NHLBI, NIDA, NIMH and NINDS.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

PB, HC and AS conceived the review, did literature search and wrote the manuscript. AH and HC performed database searches. All authors read and approved the final version of the manuscript.
REFERENCES

1. Hawiger J, Zienkiewicz J. Decoding inflammation, its causes, genomic responses, and emerging countermeasures. Scand J Immunol. 2019;90:e12812.

2. Bretscher PA. The history of the two-signal model of lymphocyte activation: A personal perspective. Scand J Immunol. 2019;89:e12762.

3. Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science. 1997;19(278):2075-2080.

4. Buday L, Tompa P. Functional classification of scaffold proteins and related molecules. Febs J. 2010;277:4348-4355.

5. Langeberg LK, Scott JD. Signalling scaffolds and local organization of cellular behaviour. Nat Rev Mol Cell Biol. 2015;16:232-244.

6. Kaneko T, Huang H, Cao X, et al. Superbinder SH2 domains act as antagonists of cell signaling. Sci Signal. 2012;5:ra68.

7. Kaneko T, Stogios PJ, Ruan X, et al. Identification and characterization of a large family of superbinding bacterial SH2 domains. Nat Commun. 2018;3(19):4549.

8. Gaud G, Lesourne R, Love PE. Regulatory mechanisms in T cell receptor signalling. Nat Rev Immunol. 2018;18:485-497.

9. Lim WA, Pawson T. Phosphotyrosine signaling: evolving a new cellular communication system. Cell. 2010;3(142):661-667.

10. Liu BA, Shah E, Jablonowski K, Stergachis A, Engelmann B, Nash PD. The SH2 domain-containing proteins in 21 species establish the provenance and scope of phosphotyrosine signaling in eukaryotes. Sci Signal. 2011;4:ra83.

11. Baláz A, Csizmok V, Buday L, et al. High levels of structural disorder in scaffold proteins as exemplified by a novel neuronal protein, CASK-interactive protein1. FEBS J. 2009;276:3744-3756.

12. Dunker AK, Lawson JD, Brown CJ, et al. Intrinsically disordered protein. J Mol Graph Model. 2001;19:26-59.

13. Brown CJ, Takayama S, Campen AM, et al. Evolutionary rate heterogeneity in proteins with long disordered regions. J Mol Evol. 2002;55:104-110.

14. Ren S, Uversky VN, Chen Z, Dunker AK, Obradovic Z. Short Linear Motifs recognized by SH2, SH3 and Ser/Thr Kinase domain are capable of encoding the spectrum of specificity of the SH2 domain. J Biol Chem. 2006;281:8209-8216.

15. Gonfloni S, Williams JC, Hattula K, Weijland A, Wierenga RK, Superti-Furga G. The role of the linker between the SH2 domain and catalytic domain in the regulation and function of Src. Embo J. 1997;15(16):7261-7271.

16. Kaltsoyanni A, Alves J, Urbaneke C, Knorr R, Ungewickell EJ. Structural disorder of the endocytic proteins AP180 and epsin 1. J Biol Chem. 2002;277:8209-8216.

17. Hofmann H, Soranno A, Borgia A, Galt K, Nettels D, Schuler B. Polymer scaling laws of unfolded and intrinsically disordered proteins quantified with single-molecule spectroscopy. Proc Natl Acad Sci USA. 2012;2(109):16155-16160.

18. Shoemaker BA, Portman JJ, Wolynes PG. Speeding molecular recognition by using the folding funnel: The fly-casting mechanism. Proc Natl Acad Sci USA. 2000;97(16):8868-8873.

19. Bah A, Vernon RM, Siddiqui Z, et al. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. Nature. 2015;5(519):106-U240.

20. Tompa P, Fuxreiter M. Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions. Trends Biochem Sci. 2008;33:2-8.

21. Berlow RB, Dyson HJ, Wright PE. Expanding the paradigm: intrinsically disordered proteins and allosteric regulation. J Mol Biol. 2018;3(430):2309-2320.

22. Tompa P, Szasz C, Buday L. Structural disorder throws new light on moonlighting. Trends Biochem Sci. 2005;30:484-489.

23. Houtman JCD, Yamaguchi H, Barda-Saad M, et al. Oligomerization of signaling complexes by the multipoint binding of GRB2 to both LAT and SOS1. Nat Struct Mol Biol. 2006;13:798-805.

24. Motshwene PG, Moncrieffe MC, Grossmann JG, et al. An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. J Biol Chem. 2009;284:25404-25411.

25. van Geersdaele LK, Stead MA, Harrison CM, et al. Structural basis of high-order oligomerization of the cullin-3 adaptor SPOP. Acta Crystallogr D Biol Crystallogr. 2013;69:1677-1684.

26. Cortese MS, Uversky VN, Dunker AK. Intrinsic disorder in scaffold proteins: getting more from less. Prog Biophys Mol Biol. 2008;98:85-106.

27. Van Roey K, Uyar B, Weatheritt RJ, et al. Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. Chem Rev. 2014;9(114):6733-6778.

28. Liu H, Huang H, Voss C, et al. Surface loops in a single SH2 domain are capable of encoding the spectrum of specificity of the SH2 family. Mol Cell Proteomics. 2019;18:372-382.

29. Edwards RJ, Palopoli N. Computational prediction of short linear motifs from protein sequences. Zhou P, Huang J, Computational...
Peptidology. Methods in Molecular Biology 1268. New York: Humana Press; 2015:89-141.

40. Dunker AK, Cortese MS, Romero P, Lakoucheva LM, Uversky VN. Flexible nets. The roles of intrinsic disorder in protein interaction networks. FEBS J 2005;272:5129-5148.

41. Ivarsson Y, Jemth P. Affinity and specificity of motif-based protein-protein interactions. Curr Opin Struct Biol. 2019;54:26-33.

42. Plewczynski D, Tkacz A, Wyrwicz LS, Rychlewski L. AutoMotif server: prediction of single residue post-translational modifications in proteins. Bioinformatics. 2005;15(21):2525-2527.

43. Sugase K, Dyson HJ, Wright PE. Mechanism of coupled folding and binding of an intrinsically disordered protein. Nature. 2007;21(447):1021-1025.

44. Sluchanko NN, Bustos DM. Intrinsic disorder associated with 14-3-3 proteins and their partners. Prog Mol Biol Trans Sci. 2019;166:19-61.

45. Latour S, Veillette A. The SAP family of adaptors in immune regulation. Semin Immunol. 2004;16:409-419.

46. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

47. Wang X, Li JP, Chiu LL, et al. Attenuation of T cell receptor signaling via the GRB2 family of adaptor proteins. Soboloff J, Kappes DJ. Signaling Mechanisms Regulating T Cell Diversity and Function. Boca Raton (FL): CRC Press/Taylor & Francis; 2018:147-176.

48. Wang X, Li JP, Chiu LL, et al. Attenuation of T cell receptor signaling via the GRB2 family of adaptor proteins. Soboloff J, Kappes DJ. Signaling Mechanisms Regulating T Cell Diversity and Function. Boca Raton (FL): CRC Press/Taylor & Francis; 2018:147-176.

49. New M, Sheikh S, Bekheet M, et al. TLR adaptor protein MYD88 promotes T cell receptor signal transduction. Science. 2016;29(352):595-599.

50. Kagan JC, Medzhitov R. Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling. Annu Rev Biophys. 2019;6(48):465-494.

51. Dustin ML. The immunological synapse. Cancer Immunol Res. 2014;2(1023-1033).

52. Case LB, Ditlev JA, Rosen MK. Regulation of Transmembrane Signaling by Phase Separation. J Immunol. 2010;12(11):848.

53. Wang X, Li JP, Chiu LL, et al. Attenuation of T cell receptor signaling via the GRB2 family of adaptor proteins. Soboloff J, Kappes DJ. Signaling Mechanisms Regulating T Cell Diversity and Function. Boca Raton (FL): CRC Press/Taylor & Francis; 2018:147-176.

54. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

55. Wang X, Li JP, Chiu LL, et al. Attenuation of T cell receptor signaling via the GRB2 family of adaptor proteins. Soboloff J, Kappes DJ. Signaling Mechanisms Regulating T Cell Diversity and Function. Boca Raton (FL): CRC Press/Taylor & Francis; 2018:147-176.

56. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

57. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

58. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

59. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.

60. Wang X, Jiang J, Lu Y, Shi G, Liu R, Cao Y. TAB2, an important upstream adaptor of interleukin-1 signaling pathway, is subject to SUMOylation. Mol Cell Biochem. 2014;385:69-77.
22 of 26
BOROWICZ et al.

77. Brdickova N, Brdicka T, Angelisova P, et al. LIME: a new membrane Raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. *J Exp Med*. 2003;198(1):1453-1462.

78. Hur EM, Son M, Lee OH, et al. LIME, a novel transmembrane adaptor protein, associates with p56lck and mediates T cell activation. *J Exp Med*. 2003;198(1):1463-1473.

79. Fukushima A, Hatanaka Y, Chang JW, Takamatsu M, Singh N, Iwashima M. Lck couples Shc to TCR signaling. *Cell Signal*. 2006;18:1182-1189.

80. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood*. 2010;12(116):988-992.

81. Kwon SM, Suzuki T, Kawamoto A, et al. Pivotal role of Link adaptor protein in endothelial progenitor cell biology for vascular regeneration. *Circ Res*. 2009;24(104):969-977.

82. Judd BA, Koretzky GA. The role of the adapter molecule SLP-76 in platelet function. *Oncoogene*. 2001;1(20):6291-6299.

83. Sluchanko NN, Gusev NB. Moonlighting chaperone-like activity of the universal regulatory 14-3-3 proteins. *FEBS J*. 2017;284:1279-1295.

84. Levchenko A, Bruck J, Sternberg PW. Scaffold proteins may biophysically affect the levels of mitogen-activated protein kinase signaling and reduce its threshold properties. *Proc Natl Acad Sci USA*. 2000;23(97):5818-5823.

85. Naudin C, Chevalier C, Roché S. The role of small adaptor proteins in the control of oncogetic signaling driven by tyrosine kinases in human cancer. *Oncoarget*. 2016;8(7):11033-11055.

86. Roche S, Alonso G, Kazlauskas A, Dixit VM, Courtenide SA, Pandey A. Src-like adaptor protein (Slap) is a negative regulator of mitogenesis. *Curr Biol*. 1998;27(8):975-978.

87. Scheve CS, Gonzales PA, Momín N, Stachowiak JC. Steric pressure between membrane-bound proteins opposes lipid phase separation. *J Am Chem Soc*. 2013;13(5):1185-1188.

88. Cebeau M, Spitalet M, Serge A, Magee AJ. Signalling complexes and clusters: functional advantages and methodological hurdles. *J Cell Sci*. 2010;1(123):309-320.

89. Bogen Y, Ainey C, Beach D, Yablonski D. SLP-76 mediates and maintains activation of the Tec family kinase ITK via the T cell antigen receptor-induced association between SLP-76 and ITK. *Proc Natl Acad Sci USA*. 2007;17(104):6638-6643.

90. Bisson N, James DA, Ivosev G, et al. Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor. *Nat Biotechnol*. 2011;29:653-1138.

91. Bray D. Signaling complexes: biophysical constraints on intracellular communication. *Annu Rev Biophys Biomol Struct*. 1998;27:59-75.

92. Mittag T, Ornicky S, Choy WY, et al. Dynamic equilibrium engagement of a polyvalent ligand with a single-site receptor. *Proc Natl Acad Sci USA*. 2008;18(105):17772-17777.

93. Fuhrmann F, Lischke T, Gross F, et al. Adequate immune response ensured by binary IL-2 and graded CD25 expression in a murine transfer model. *Elife*. 2016;30:5.

94. Fiering S, Northrop JP, Nolan GP, Mattila PS, Crabtree GR, Herzenberg LA. Single cell assay of a transcription factor reveals a threshold in transcription activated by signals emanating from the T-cell antigen receptor. *Genes Dev*. 1990;4:1823-1834.

95. Shaw AS, Filbert EL. Scaffold proteins and immune-cell signalling. *Nat Rev Immunol*. 2009;9:47-56.

96. Pan CQ, Sudol M, Sheetz M, Low BC. Modularity and functional plasticity of scaffold proteins as p(l)acemakers in cell signaling. *Cell Signal*. 2012;24:2143-2165.

97. Flynn DC. Adaptor proteins. *Oncogene*. 2001;1(20):6270-6272.

98. Yamamoto M, Takeda K, Akira S. TIR domain-containing adaptors define the specificity of TLR signaling. *Mol Immunol*. 2004;40:861-868.

99. Lo WL, Shah NH, Rubin SA, et al. Slow phosphorylation of a tyrosine residue in LAT optimizes T cell ligand discrimination. *Nat Immunol*. 2019;20:1481-1493.

100. Van Roey K, Gibson TJ, Davey NE. Motif switches: decision-making in cell regulation. *Curr Opin Struct Biol*. 2012;22:378-385.

101. Duke-Cohan JS, Kang H, Liu H, Rudd CE. Regulation and function of SKAP-55 non-canonical motif binding to the SH3c domain of adhesion and degranulation-promoting adaptor protein. *J Biol Chem*. 2006;12(281):13743-13750.

102. Kobashigawa Y, Sakai M, Naito M, et al. Structural basis for the transforming activity of human cancer-related signaling adaptor protein CRK. *Nat Struct Mol Biol*. 2007;14:503-510.

103. Ishizuka K, Kamiya A, Oh EC, et al. DISC1-dependent switch from progenitor proliferation to migration in the developing cortex. *Nature*. 2011;5(473):92-96.

104. Coussens NP, Hayashi R, Brown PH, et al. Multipoint binding of the SLP-76 SH2 domain to ADAP is critical for oligomerization of SLP-76 signaling complexes in stimulated T cells. *Mol Cell Biol*. 2013;33:4140-4151.

105. Dülk M, Szeder B, Glatz G, et al. EGF regulates the interaction of Tks4 with Src through its SH2 and SH3 domains. *Biochemistry*. 2018;17(57):4186-4196.

106. Uversky VN. Intrinsically disordered proteins may escape unwanted interactions via functional misfolding. *BBa-Proteins Proteome*. 2011;1814:693-712.

107. Locasale JW, Chakraborty AK. Regulation of signal duration and the statistical dynamics of kinase activation by scaffold proteins. *PLoS Comput Biol*. 2008;27(4):e1000099.

108. Fang KA, Wilson J, Russo A, et al. Intersectin (ITSN) family of scaffolds function as molecular hubs in protein interaction networks. *PLoS One*. 2012;27:7.

109. Di Stefano P, Leal MPC, Tornillo G, et al. The adaptor proteins p140CAP and p130CAS as molecular hubs in cell migration and invasion of cancer cells. *Am J Cancer Res*. 2011;1:663.

110. Patwardhan P, Miller WT. Processive phosphorylation: mechanism and biological importance. *Cell Signal*. 2007;19:2218-2226.

111. Huang WYC, Ditlev JA, Chiang HK, Rosen MK, Groves JT. AllostERIC modulation of Grb2 recruitment to the intrinsically disordered scaffold protein, LAT, by remote site phosphorylation. *J Am Chem Soc*. 2017;13(13):18009-18015.

112. Chan C, Liu XF, Wang LM, Bardwell L, Nie Q, Enciso G. Protein scaffolds can enhance the bistability of multisite phosphorylation systems. *PLoS Comput Biol*. 2012;8(6):e1002531.

113. Khodolenko BN, Hancock JP, Kolch W. Signalling ballet in space and time. *Nat Rev Mol Cell Biol*. 2010;11:414-426.

114. Huang WY, Yan Q, Lin WC, et al. Phosphotyrosine-mediated LAT assembly on membranes drives kinase bifurcation in recruitment dynamics of the Ras activator SOS. *Proc Natl Acad Sci USA*. 2016;19(113):8218-8223.

115. Locasale JW, Shaw AS, Chakraborty AK. Scaffold proteins confer diverse regulatory properties to protein kinase cascades. *Proc Natl Acad Sci USA*. 2007;14(104):13307-13312.
116. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and coinhibition. *Nat Rev Immunol.* 2013;13:227-242.

117. Stoiber S, Cadilha BL, Bembearek MR, Lesch S, Endres S, Kobold S. Limitations in the design of chimeric antigen receptors for cancer therapy. *Cells.* 2019;17(8):472.

118. Wang H, Rudd CE. SKAP-55, SKAP-55-related and ADAP adapters modulate integrin-mediated immune-cell adhesion. *Trends Cell Biol.* 2008;18:486-493.

119. Kim HH, Tharayil M, Rudd CE. Growth factor receptor-bound protein 2 SH2/SH3 domain binding to CD28 and its role in co-signaling. *J Biol Chem.* 1998;272(29):301-306.

120. Thaker YR, Schneider H, Rudd CE. TCR and CD28 activate the transduction factor NF-xB in T-cells via distinct adaptor signaling complexes. *Immunol Lett.* 2015;163:113-119.

121. Kim HS, Long EO. Complementary phosphorylation sites in the adaptor protein SLP-76 promote synergistic activation of natural killer cells. *Sci Signal.* 2012;5(232):ta9.

122. Rajakulendran T, Sichiari F. Allosteric protein kinase regulation by pseudokinases: insights from STRAD. *Sci Signal.* 2010;3:pe8.

123. Rajakulendran T, Sahmi M, Lefrancois M, Sichiari F, Therrien M. A dimerization-dependent mechanism drives RAF catalytic activation. *Nature.* 2009;24(461):542-545.

124. Takeuchi S, Takayama Y, Ogawa A, Tamura K, Okada M. Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl-terminal Src Kinase, Csk. *J Biol Chem.* 2000;275(22):29183-29186.

125. Jadwin JA, Curran TG, Lafontaine AT, White FM, Mayer BJ. Scaffold state switching amplifies, accelerates, and insulates protein kinase activities on scaffolds. *ACS Nano.* 2013;7:8658-8665.

126. Greenwald EC, Redden JM, Dodge-Kafka KL, Sauermann JJ. Scaffold state switching amplifies, accelerates, and insulates protein kinase C signaling. *J Biol Chem.* 2014;289(24):2353-2360.

127. Jadwin JA, Curran TG, Lafontaine AT, White FM, Mayer BJ. Src homology 2 domains enhance tyrosine phosphorylation in vivo by protecting binding sites in their target proteins from dephosphorylation. *J Biol Chem.* 2018;12:29183-29186.

128. Bauer-Mehren A, Rautschka M, Sanz F, Furlong LL. DisGeNET: a Cytoscape plugin to visualize, integrate, search and analyze gene-disease networks. *Bioinformatics.* 2010;15:2924-2926.

129. Pinero J, Bravo A, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res.* 2017;45(4):D833-D839.

130. Liang B, Ding H, Huang L, Luo H, Zhu X. GWAS in cancer: progress and challenges. *Mol Genet Genom.* 2020;295(3):537-561.

131. Cheng Y, Duan C, Zhang C. New perspective on SH2B1: An accelerator of cancer progression. *Biomed Pharmacother.* 2020;121:109651.

132. Brinkman A, van der Flier S, Kok EM, Dorssers LC. BCR1, a human homologue of the adapter protein p130Cas, and anti-estrogen resistance in breast cancer cells. *J Natl Cancer Inst.* 2000;19(92):112-120.

133. van der Flier S, Brinkman A, Look MP, et al. Bacr1/p130Cas protein and primary breast cancer: prognosis and response to tamoxifen treatment. *J Natl Cancer Inst.* 2000;19(92):120-127.

134. Shagisultanova E, Gaponova AV, Gabbasov R, Nicolas E, Golemis EA. Preclinical and clinical studies of the NEDD9 scaffold protein in cancer and other diseases. *Gene.* 2015;1(567):1-11.

135. Sriram G, Birge RB. Emerging roles for crk in human cancer. *Genes Cancer.* 2010;1(1):1132-1139.

136. Sattler M, Mohi MG, Pride YB, et al. Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell.* 2002;1:479-492.

137. Gillis LC, Berry DM, Minden MD, McClage CJ, Barber DL. Gads (Grb2-related adaptor downstream of Shc) is required for BCR-ABL-mediated lymphoid leukemia. *Leukemia.* 2013;27:1666-1676.

138. Makino Y, Hamamura K, Takei Y, et al. A therapeutic trial of human melanomas with combined small interfering RNAs targeting adaptor molecules p130Cas and paxillin activated under expression of ganglioside GD3. *Biochim Biophys Acta.* 2016;1860:1753-1763.

139. Chaki SP, Barhoumi R, Rivera GM. Nck adapter proteins promote podosome biogenesis facilitating extracellular matrix degradation and cancer invasion. *Cancer Med.* 2019;8:7385-7398.

140. Moriachi P, Robertson FM, Klostergaard J, McMurray JS. Targeting SH2 domains in breast cancer. *Future Med Chem.* 2019;6:1909-1926.

141. Liu BA, Jablonowski K, Raina M, Arcé M, Paukun T, Nash PD. The human and mouse complement of SH2 domain proteins-establishing the boundaries of phosphotyrosine signaling. *Mol Cell.* 2006;23(22):851-868.

142. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet.* 1998;20:129-135.

143. Nichols KE, Harkin DP, Levitz S, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci USA.* 1998;10(95):13765-13770.

144. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature.* 1998;1(395):462-469.

145. Lappalainen I, Thusberg J, Shen B, Vihinen M. Genome wide analysis of pathogenic SH2 domain mutations. *Proteins.* 2008;72:779-792.

146. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol.* 2014;34:772-779.

147. Huang YH, Tsai K, Tan SY, et al. 2B4-SAP signaling is required for the priming of naive CD8(+) T cells by antigen-expressing B cells and B lymphoma cells. *Oncoimmunology.* 2017;6:e1267094.

148. Minegishi Y, Rohrer J, Coustan-Smith E, et al. An essential role for BLNK in human B cell development. *Science.* 1999;3(286):537-561.

149. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. *Leukemia.* 2016;30(10):1713-1718.

150. Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutations in blast-phase myeloproliferative neoplasms and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia.* 2014;18:486-493.

151. Dai KZ, Harbo HF, Celsus EG, et al. The T cell regulator gene SH2D2A contributes to the genetic susceptibility of multiple sclerosis. *Genes Immun.* 2001;2(2):263-268.

152. Lorentzen AR, Smestad C, Lie BA, et al. The SH2D2A gene and primary erythrocytosis. *Leukemia.* 2010;16(363):1189-1190.

153. D'Souza SM, D'Souza SP, D'Souza FM, et al. Preclinical and clinical studies of the NEDD9 scaffold protein in cancer and other diseases. *Genes Cancer.* 2010;1(567):11-11.

154. Notturno F, Pace M, De Angelis MV, Caporale CM, Giovannini A, Uncini A. Susceptibility to chronic inflammatory demyelinating neuropathies.
polyradiculoneuropathy is associated to polymorphic GA repeat in the SH2D2A gene. *J Neuroimmunol.* 2008;15(197):124-127.

154. Waebler G, Delplanque J, Bonny C, et al. The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. *Nat Genet.* 2000;24:291-295.

155. Parissi MA, Bennett CL, Eckert ML, et al. The NPH1 gene deletion associated with juvenile nephronophthisis is present in a subset of individuals with Joubert syndrome. *Am J Hum Genet.* 2004;75:82-91.

156. ter Haar B, Hamel B, Hendriks J, de Jager J. Melnick-Needles syndrome: indication for an autosomal recessive form. *Am J Med Genet.* 1982;13:469-477.

157. Zhang H, Koo CY, Stebbing J, Giamas G. The dual function of KSR1: a pseudokinase and beyond. *Biochem Soc Trans.* 2013;41:1078-1082.

158. Bentires-Alj M, Gil SG, Chan R, et al. A role for the scaffolding adapter Gab2 in breast cancer. *Nat Med.* 2006;12:114-121.

159. Weil D, El-Amraoui A, Masmoudi S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet.* 2003;12(1):463-471.

160. Pandit B, Sarkozy A, Pennacchio LA, et al. Gain-of-function RAF1 mutations cause Noonan and LEPoard syndromes with hypertrophic cardiomyopathy. *Nat Genet.* 2007;39:1007-1012.

161. Law PJ, Sud A, Mitchell JS, et al. Genome-wide association study identifies NCK1 as a novel protein tyrosine phosphatase gene with altered expression in breast cancer. *J Exp Med.* 2012;2(209):1363-1377.

162. Zhang F, Lu YX, Chen Q, et al. Identification of NCK1 as a novel Src homology (SH)2 domain and putative SH3 and phosphotyrosine binding sites. *J Biol Chem.* 1998;273:4539-4546.

163. Granum S, Sundvold-Gjerstad V, Dai KZ, et al. Structure function analysis of SH2D2A isoforms expressed in T cells reveals a crucial role for the proline rich region encoded by SH2D2A exon 7. *BMC Immunol.* 2006;13(7):15.

164. Buljan M, Chalancon G, Dunker AK, et al. Alternative splicing of intrinsically disordered regions and rewiring of protein interactions. *Curr Opin Struct Biol.* 2013;23:443-450.

165. Zhang H, Koo CY, Stebbing J, Giamas G. The dual function of KSR1: a pseudokinase and beyond. *Biochem Soc Trans.* 2013;41:1078-1082.

166. Bentires-Alj M, Gil SG, Chan R, et al. A role for the scaffolding adapter Gab2 in breast cancer. *Nat Med.* 2006;12:114-121.

167. Weil D, El-Amraoui A, Masmoudi S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet.* 2003;12(1):463-471.

168. Pandit B, Sarkozy A, Pennacchio LA, et al. Gain-of-function RAF1 mutations cause Noonan and LEPoard syndromes with hypertrophic cardiomyopathy. *Nat Genet.* 2007;39:1007-1012.

169. Law PJ, Sud A, Mitchell JS, et al. Genome-wide association study identifies NCK1 as a novel protein tyrosine phosphatase gene with altered expression in breast cancer. *J Exp Med.* 2012;2(209):1363-1377.

170. Wu LW, Mayo LD, Dunbar JD, et al. VRAP is an adpoter protein that binds KDR, a receptor for vascular endothelial cell growth factor. *J Biol Chem.* 2000;3(275):6059-6062.

171. Spurkland A, Brinchmann JE, Markussen G, et al. Molecular cloning of a T cell-specific adaptor protein (TSAd) containing an Src homology (SH) 2 domain and putative SH3 and phosphotyrosine binding sites. *J Biol Chem.* 1998;273:4539-4546.

172. Granum S, Sundvold-Gjerstad V, Dai KZ, et al. Structure function analysis of SH2D2A isoforms expressed in T cells reveals a crucial role for the proline rich region encoded by SH2D2A exon 7. *BMC Immunol.* 2006;13(7):15.

173. Buljan M, Chalancon G, Dunker AK, et al. Alternative splicing of intrinsically disordered regions and rewiring of protein interactions. *Curr Opin Struct Biol.* 2013;23:443-450.

174. Zhang H, Koo CY, Stebbing J, Giamas G. The dual function of KSR1: a pseudokinase and beyond. *Biochem Soc Trans.* 2013;41:1078-1082.

175. Bentires-Alj M, Gil SG, Chan R, et al. A role for the scaffolding adapter Gab2 in breast cancer. *Nat Med.* 2006;12:114-121.

176. Weil D, El-Amraoui A, Masmoudi S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet.* 2003;12(1):463-471.

177. Pandit B, Sarkozy A, Pennacchio LA, et al. Gain-of-function RAF1 mutations cause Noonan and LEPoard syndromes with hypertrophic cardiomyopathy. *Nat Genet.* 2007;39:1007-1012.

178. Law PJ, Sud A, Mitchell JS, et al. Genome-wide association study identifies NCK1 as a novel protein tyrosine phosphatase gene with altered expression in breast cancer. *J Exp Med.* 2012;2(209):1363-1377.
190. Abrahamsen G, Sundvold-Gjerstad V, Habtamu M, Bogen B, Spurkland A. Polarity of CD4+ T cells towards the antigen presenting cell is regulated by the Lck adapter TSAd. *Sci Rep.* 2018;8(6):1-13.

191. Wedel J, Stack MP, Seto T, et al. T cell-specific adaptor protein regulates mitochondrial function and CD4(+) T regulatory cell activity in vivo following transplantation. *J Immunol.* 2019;15(203):2328-2338.

192. Li X, Padhan N, Sjostrom EO, et al. VEGFR2 pY949 signalling regulates adherens junction integrity and metastatic spread. *Nat Commun.* 2016;23(7):11017.

193. Granum S, Andersen TCB, Sorlie M, et al. Modulation of Lck function through multisite docking to T cell-specific adapter protein. *J Biol Chem.* 2008;28(3):21909-21919.

194. Kapoor-Kaushik N, Hinde E, Compeer EB, et al. Distinct mechanisms regulate Lck spatial organization in activated T cells. *Front Immunol.* 2016;7:83.

195. Berge T, Sundvold-Gjerstad V, Granum S, et al. T cell specific adapter protein (TSAd) interacts with Tec kinase ITK to promote CXCL12 induced migration of human and murine T cells. *PLoS One.* 2010;18(5):e9761.

196. Granum S, Sundvold-Gjerstad V, Gopalakrishnan RP, et al. The kinase Itk and the adapter TSAd change the specificity of the kinase Lck in T cells by promoting the phosphorylation of Tyr192. *Sci Signal.* 2014;7(355):ra118.

197. Marti F, Garcia GG, Lapinski PE, MacGregor JN, King PD. Essential role of the T cell-specific adapter protein in the activation of LCK in peripheral T cells. *J Exp Med.* 2006;20(203):281-287.

198. Andersen TCB, Kristiansen PE, Huszenicza Z, et al. The SH3 domain proteins of the protein kinases ITK and LCK compete for adjacent sites on T cell-specific adapter protein. *J Biol Chem.* 2019;18(294):15480-15494.

199. Park D, Choi YB, Han MK, Kim UH, Shin J, Yun Y. Adaptor protein Lad relays PDGF signal to Grb2 in lung cells: a tissue-specific PDGF signal transduction. *Biochem Biophys Res Commun.* 2001;8(284):275-281.

200. Sun W, Kesavan K, Schafer BC, et al. MEK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem.* 2001;16(276):5093-5100.

201. Yao Z, Yoon S, Kalie E, Raviv Z, Seger R. Calcium regulation of EGF-induced ERK5 activation: role of Lad1-MEKK2 interaction. *PLoS One.* 2010;7(5):e12627.

202. Sun W, Wei X, Kesavan K, et al. MEK kinase 2 and the adaptor protein Lad regulate extracellular signal-regulated kinase 5 activation by epidermal growth factor via Src. *Mol Cell Biol.* 2003;23:2298-2308.

203. Park D, Park I, Lee D, Choi YB, Lee H, Yun Y. The adaptor protein Lad associates with the G protein beta subunit and mediates chemokine-dependent T-cell migration. *Blood.* 2007;15(109):5122-5128.

204. Hem CD, Sundvold-Gjerstad V, Granum S, et al. T cell specific adaptor protein (TSAd) promotes interaction of Nck with Lck and SLP-76 in T cells. *Cell Commun Signal.* 2015;11(13):31.

205. Sachse SM, Lieve S, Ribeiro LF, et al. Nuclear import of the DSCAM-cytoplasmic domain drives signaling capable of inhibiting synapse formation. *Embo J.* 2019;15:38.

206. Richard KC, Bertolosei GE, Dunfield LD, McMaster CR, Nachtigal MW. TSAd interacts with Smad2 and Smad3. *Biochem Biophys Res Commun.* 2006;18(347):266-272.

207. Park E, Choi Y, Ahn E, Park I, Yun Y. The adaptor protein LAD/TSAd mediates laminin-dependent T cell migration via association with the 67 kDa laminin binding protein. *Exp Mol Med.* 2009;31(41):728-736.

208. Hem CD, Ekornhol M, Granum S, Sundvold-Gjerstad V, Spurkland A. CD6 and linker of activated T cells are potential interaction partners for T cell-specific adaptor protein. *Scand J Immunol.* 2017;85:104-112.

209. Marti F, King PD. The p95–100 kDa ligand of the T cell-specific adaptor (TSAd) protein Src-homology-2 (SH2) domain implicated in TSAd nuclear import is p97 Valosin-containing protein (VCP). *Immunol Lett.* 2005;15(97):235-243.

210. Choi Y, Park E, Ahn E, Park I, Yun Y. The effector functions of mature T lymphocytes are impaired in transgenic mice expressing the SH2 domain of TSAd/Lad. *Mol Cells.* 2009;28:183-188.

211. Borowicz P, Chan H, Medina D, Gumpelmair S, Kjelstrup H, Spurkland A. A simple and efficient workflow for generation of knock-in mutations in Jurkat T cells using CRISPR/Cas9. *Scand J Immunol.* 2020;91:e12862.

212. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.* 2019;8(47):D506-D515.

213. Letunic I, Bork P. 20 years of the SMART protein domain annotation resource. *Nucleic Acids Res.* 2018;46(24):D493-D496.

214. Lopez-Rivera E, Liu YP, Verbitsky M, et al. Genetic drivers of kidney defects in the DiGeorge syndrome. *N Engl J Med.* 2017;23(376):742-754.

215. Racedo SE, McDonald-McGinn DM, Chung JH, et al. Mouse and human CRKL is dosage sensitive for cardiac outflow tract formation. *Am J Hum Genet.* 2015;5(96):235-244.

216. Levin C, Koren A, Pretorius E, et al. Deleterious mutation in the FYB gene is associated with congenital autosomal recessive small-platelet thrombocytopenia. *J Thromb Haemost.* 2015;13:1285-1292.

217. Agostinelli C, Rizvi H, Paterson J, et al. Intracellular TCR-signaling pathway: novel markers for lymphoma diagnosis and potential therapeutic targets. *Am J Surg Pathol.* 2014;38:1349-1359.

218. Sun J, Wan C, Jia P, et al. Application of systems biology approach identifies and validates GRB2 as a risk gene for schizophrenia in the Irish Case Control Study of Schizophrenia (ICCSS) sample. *Schizophr Res.* 2011;125:201-208.

219. Bosserhoff AK, Lederer M, Kaufmann M, et al. MLB, novel serum marker for progression of malignant melanoma. *Anticancer Res.* 1999;19:2691-2693.

220. Aung PP, Oue N, Mitani Y, et al. Systematic search for gastric cancer-specific genes based on SAGE data: melanoma inhibitory activity and matrix metalloproteinase-10 are novel prognostic factors in patients with gastric cancer. *Oncogene.* 2006;20(25):2546-2557.

221. Hildebrandt F, Otto E, Rensing C, et al. Essential role of the T cell-specific adapter protein in the activation of LCK in peripheral T cells. *J Exp Med.* 2006;20(203):281-287.

222. Andersen TCB, Kristiansen PE, Huszenicza Z, et al. The SH3 domains of the protein kinases ITK and LCK compete for adjacent sites on T cell-specific adapter protein. *J Biol Chem.* 2019;18(294):15480-15494.

223. Shimozawa N, Suzuki Y, Zhang Z, et al. Nonsense and temperature-sensitive mutations in PEX13 are the cause of complementation group H of peroxisome biogenesis disorders. *Hum Mol Genet.* 1999;8:1077-1083.
224. Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet*. 2009;41:18-24.

225. Consortium CAD, Deloukas P, Kanoni S, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45:25-33.

226. Ueki Y, Tiziani V, Santanna C, et al. Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. *Nat Genet*. 2001;28:125-126.

227. Yi JM, Dhir M, Van Neste L, et al. Genomic and epigenomic integration identifies a prognostic signature in colon cancer. *Clin Cancer Res*. 2011;17(15):3585-3595.

228. Senderek J, Bergmann C, Stendel C, et al. Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. *Am J Hum Genet*. 2003;73:1106-1119.

229. Berkel S, Marshall CR, Weiss B, et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet*. 2010;42:489-491.

230. Inagaki-Ohara K, Kondo T, Ito M, Yoshimura A. SOCS, inflammation, and cancer. *Jakstat*. 2013;1(2):e24053.

231. Honda H, Oda H, Nakamoto T, et al. Cardiovascular anomaly, impaired actin bundling and resistance to Src-induced transformation in mice lacking p130Cas. *Nat Genet*. 1998;19:361-365.

232. Flemming A, Brummer T, Reth M, Jumaa H. The adaptor protein SLP-65 acts as a tumor suppressor that limits pre-B cell expansion. *Nat Immunol*. 2003;4:38-43.

233. Pappu R, Cheng AM, Li B, et al. Requirement for B cell linker protein (BLNK) in B cell development. *Science*. 1999;286:1949-1954.

234. Griffiths EK, Krawczyk C, Kong YY, et al. Positive regulation of T cell activation and integrin adhesion by the adapter Fyb/Slap. *Science*. 2001;293(5531):2260-2263.

235. Peterson EJ, Woods ML, Dmowski SA, et al. Coupling of the TCR to integrin activation by Slap-130/Fyb. *Science*. 2001;293(5531):2263-2265.

236. Yoder J, Pham C, Iizuka YM, et al. Requirement for the SLP-76 adaptor GADS in T cell development. *Science*. 2001;291:1987-1991.

237. Cheng AM, Saxton TM, Sakai R, et al. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. *Cell*. 1998;91(5):793-803.

238. Clements JL, Lee JR, Gross B, et al. Fetal hemorrhage and platelet dysfunction in SLP-76-deficient mice. *J Clin Invest*. 1999;103:19-25.

239. Pivniouk V, Tsitsikov E, Swinton P, Rathbun G, Alt FW, Geha RS. Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *Cell*. 1998;94(4):229-238.

240. Thompson NA, Haefliger JA, Senn A, et al. Islet-brain1/JNK-interacting protein-1 is required for early embryogenesis in mice. *J Biol Chem*. 2001;276(74):27745-27748.

241. Whitmarsh AJ, Kuan CY, Kennedy NJ, et al. Requirement of the JIP1 scaffold protein for stress-induced JNK activation. *Genes Dev*. 2001;15(15):2421-2432.

242. Moser M, Bosserhoff AK, Hunziker EB, Sandell L, Fassler R, Buettner R. Ultrastructural cartilage abnormalities in MIA/CD-RAP-deficient mice. *Mol Cell Biol*. 2002;22:1438-1445.

243. Seo S, Asai T, Saito T, et al. Crk-associated substrate lymphocyte type is required for lymphocyte trafficking and marginal zone B cell maintenance. *J Immunol*. 2005;175(3):3492-3501.

244. Jiang ST, Chiu YY, Wang E, et al. Targeted disruption of Nphp1 causes male infertility due to defects in the later steps of sperm morphogenesis in mice. *Hum Mol Genet*. 2008;17(17):3368-3379.

245. Maxwell M, Bjorkman J, Nguyen T, et al. Pex13 inactivation in the mouse disrupts peroxisome biogenesis and leads to a Zellweger syndrome phenotype. *Mol Cell Biol*. 2003;23:5947-5957.

246. Ohhtsuka S, Takaki S, Iseki M, et al. SH2-B is required for both male and female reproduction. *Mol Reprod Dev*. 2002;66:3066-3077.

247. Duan C, Yang H, White MF, Rui L. Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol Cell Biol*. 2004;24:7435-7443.

248. Takaki S, Sauer K, Iritani BM, et al. Control of B cell production by the adaptor protein Ink. Definition Of a conserved family of signal-modulating proteins. *Immunity*. 2000;13:599-609.

249. Wu C, Nguyen KB, Pien GC, et al. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat Immunol*. 2001;2:410-414.

250. Czar MJ, Kersh EN, Mijares LA, et al. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. *Proc Natl Acad Sci USA*. 2001;98(9):7449-7454.

251. de la Fuente MA, Kumar L, Lu B, Geha RS. 3BP2 deficiency impairs the response of B cells, but not T cells, to antigen receptor ligation. *Mol Cell Biol*. 2006;26:5214-5225.

252. Iqbal Z, Cezudo-Martin P, de Brouwer A, et al. Disruption of the podosome adaptor protein TKS4 (SH3PXD2B) causes the skeletal dysplasia, eye, and cardiac abnormalities of Frank-Ter Haar Syndrome. *Am J Hum Genet*. 2010;86(2):254-261.

253. Arnaud E, Zenker J, de Preux Charles AS, et al. SH3TC2/KIAA1985 protein is required for proper myelination and the integrity of the node of Ranvier in the peripheral nervous system. *Proc Natl Acad Sci USA*. 2009;106(106):17528-17533.

254. Schmeisser MJ, Ey E, Wegener S, et al. Autistic-like behaviours and synaptic modulators of immune cell signalling. *Scand J Immunol*. 2009;70(5):293-298.

255. BOROWICZ et al.

256. Arnaud E, Zenker J, de Preux Charles AS, et al. SH3TC2/KIAA1985 protein is required for proper myelination and the integrity of the node of Ranvier in the peripheral nervous system. *Proc Natl Acad Sci USA*. 2009;106(106):17528-17533.

257. Scand J Immunol. 2009;70(5):293-298.

How to cite this article: Borowicz P, Chan H, Hauge A, Spurkland A. Adaptor proteins: Flexible and dynamic modulators of immune cell signalling. *Scand J Immunol*. 2020;92:e12951. [https://doi.org/10.1111/sji.12951]