Immunol Res (2012) 52:176–181
DOI 10.1007/s12026-011-8256-4

Evolution of immunity: no development without risk

Alexander B. Sigalov

Published online: 18 November 2011

Abstract Signal transduction by cell surface receptors in the context of heterogeneous and variable cellular environments plays a pivotal role in regulating many biological processes, including development, activation, and homeostasis of the immune system. In some receptors, extracellular ligand-binding and intracellular signaling domains are located on the same protein chain (single-chain receptors), while in the so-called multichain immune recognition receptors (MIRRs), recognition and signaling functions are separated between different protein chains. Why did nature separate recognition and signaling functions for MIRRs, thereby increasing the risk of malfunction and potential attack by pathogens? The risk is real: in order to escape the immune response, viruses are able to disrupt functional coupling between recognition and signaling aspects of MIRR machinery. Intrinsic disorder of intracellular signal-generating regions of MIRRs adds further intrigue to the story. Why did nature select protein disorder for MIRRs to translate recognition of distinct antigens into appropriate activation signals that would induce specific functional outcomes? Here, I suggest that nature takes the risks associated with intrareceptor separation of functions as well as with the chaos and indeterminacy of protein disorder in exchange for providing diversity and variability of signal transduction. Not only does this phenomenon serve as the molecular basis for the development and evolution of the immune and other complex biological systems, but it fits closely to Darwinian evolutionary biology.

Keywords Cell receptors · Signal transduction · Protein intrinsic disorder · Single-chain receptors · Multichain immune recognition receptors · MIRR · Innate immunity · Adaptive immunity · Evolution of the immune response · SCHOOL concept

A. B. Sigalov (✉)
SignaBlok, Inc., P.O. Box 4064, Shrewsbury, MA 01545, USA
e-mail: Sigalov@signablok.com

Introduction

Cell surface receptors mediate cells' communication with each other and with their environment by inducing a response specific to individual stimuli. Depending on whether extracellular ligand-binding and intracellular signaling (effector) domains are located on the same or separate protein chains, cell receptors can be divided into two main structural families: single-chain and multichain receptors (SRs and MRs) (Fig. 1) [1, 2]. Most MRs are immune receptors. For this reason, they are commonly referred to as multichain immune recognition receptors (MIRRs) [1, 2]. The signature feature of MIRR signal-generating chains is the presence of one or more copies of the immunoreceptor tyrosine-based activation motif (ITAM) regions [3] or the YxxM motif [4] in their cytoplasmic domains. Upon receptor triggering, tyrosine residues of the ITAM/YxxM regions are phosphorylated in an early and obligatory event in the signaling cascade.

Ligand binding outside the cell is mediated by well-structured protein domains in both receptor families (Fig. 1). In contrast, while intracellular signaling domains of SRs are also well-structured, those of MIRRs lack a well-defined three-dimensional structure under physiological conditions (Fig. 1) [5–7], i.e., represent intrinsically disordered regions (IDRs) [8]. These immune signaling-related IDRs exhibit several unusual and previously unreported biophysical phenomena [5, 6, 9–11] that open new perspectives on the molecular mechanisms of receptor signaling with numerous applications in biology and medicine [12, 13].

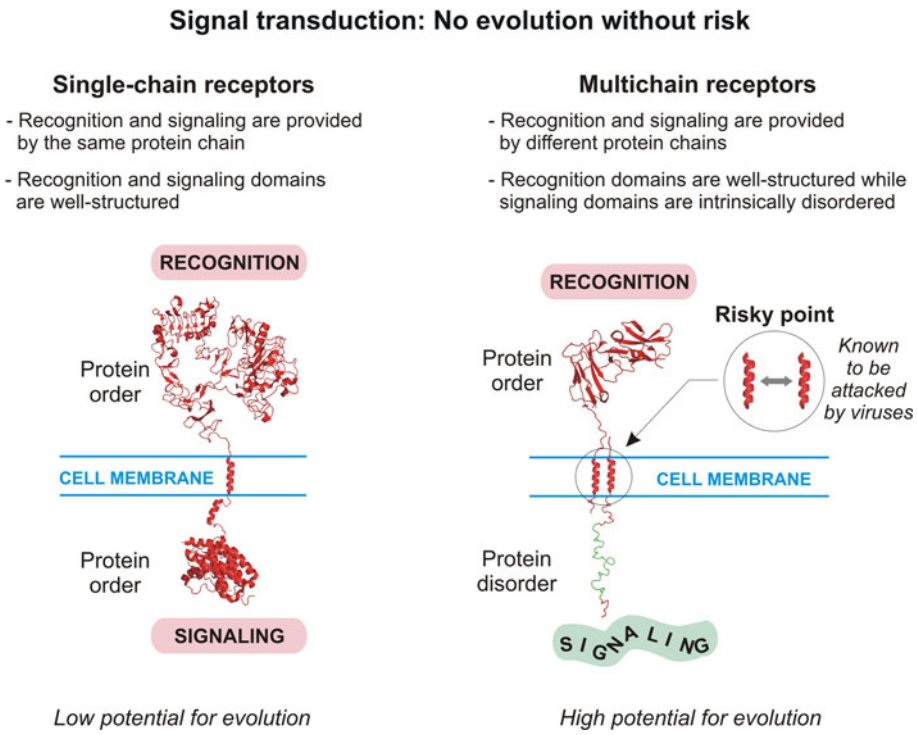

Fig. 1 Protein order and disorder in cell surface receptors. Images were created using PyMol (http://www.pymol.org) from Protein Data Bank entries 1NQL and 3GOP for the EGFR extra- and intracellular (juxtamembrane and kinase) domains, respectively (shown as an example of structure of a single-chain receptor), and entry 1UCT for the FcαRI extracellular domain (shown as an example of structure of a multichain receptor recognition subunit). For illustrative purposes, the cytoplasmic domain of a multichain receptor-associated signaling subunit is shown as a monomer and using arbitrary idealized structural elements to represent the ensemble of unfolded conformations of an IDR. The immunoreceptor tyrosine-based activation motif (ITAM) of multichain receptors is depicted in *green*. Transmembrane interactions between recognition and signaling subunits of multichain receptor are shown by a *gray* arrow. Adapted with permission from [14]. *EGFR*, epidermal growth factor receptor, *FcαRI*, Fc receptor I for IgA

Together, these observations raise some important questions [14], but here, I will focus on two that are of particular interest. Answering these questions can uncover the general principles of signal transduction that underlie the development and evolution of the immune system within the paradigm of Darwinian evolutionary biology.

Why did nature separate recognition and signaling functions for MIRRs, thereby increasing the risk of malfunction and potential attack by pathogens?

Within the modular assembly of MIRR, communication between recognition and signaling protein modules is provided mostly by non-covalent transmembrane interactions (Fig. 1). Thus, as biochemical processes that can be influenced and controlled [15–19], these interactions represent a potential point of attack by pathogens. Examples are viruses that, in order to escape the immune response, can disrupt functional coupling between recognition and signaling aspects of MIRR machinery [20, 21].

What is obtained in exchange for this risk? Among the benefits that can accrue from the modular assembly of MIRRs with separated recognition and signaling functions is the capability to diversify and vary signal transduction [14]. This capability in turn provides the mechanistic basis for the diversity and variability of the immune response. According to Charles Darwin, diversity and variability are at the very core of evolutionary processes [22]. One can conclude that nature takes the risks associated with intra-receptor separation of functions in exchange for high potential for the evolution of signal transduction (Fig. 1), which is of great importance in the development of the immune system in general.

Why did nature select the chaos of protein disorder for MIRRs to provide efficient and highly specific signaling?

The central premise of modern structural biology assumes that a protein's structure determines its function. For many decades, this theory has provided molecular interpretations and explanations for numerous biological processes, from enzymatic catalysis to signal transduction. However, in the past fifteen or so years, this classical structure–function

paradigm has been challenged by the emergence of intrinsically disordered proteins (IDPs), proteins that lack a well-defined three-dimensional structure under physiological conditions [23–25].

As recently revealed [5–7], separation of recognition and signaling functions in MIRRs is accompanied by substantial increase in protein disorder in intracellular signaling domains (Fig. 1). *What is obtained in exchange for the risk of chaos and indeterminacy of protein disorder?* First, protein intrinsic disorder both enables and enhances the features most important for MIRR-mediated signaling: high-specificity low-affinity interactions, the multiple binding of one protein to many partners, and the multiple binding of many proteins to one partner [26, 27]. Second, a novel model of signal transduction, the Signaling Chain HOmoOLigomerization (SCHOOL) model, suggests that upon antigen binding, the chaos of protein disorder of the ITAM-containing signaling regions is under specific and tight control of ligand-promoted cytoplasmic homooligomerization [2, 12]. Within the model, formation of competent signaling homooligomers in a cytoplasmic milieu is the necessary and sufficient event to trigger MIRRs and to induce cell activation, representing a functional link between protein intrinsic disorder and oligomericity [2, 12]. Such a condition assumes that signal diversification and variability may be achieved through different patterns of MIRR signaling subunit oligomerization [12] in combination with distinct activation signals provided by different MIRR signaling modules [28–32] and/or different ITAMs located on the same signaling module [33].

Thus, protein disorder contributes to the diversity and variability of signal transduction without compromising efficiency and specificity of signaling. The higher the number of different signaling subunits in the MIRR complex, the higher the extent of this contribution [14]. Here, I suggest that, similarly to intrareceptor separation of functions, immune signaling-related protein disorder has evolutionary and developmental benefits that outweigh attendant disadvantages, which are largely compensated by increasing the host defense.

Signal transduction in the context of the innate and adaptive immune response

The primary role of the immune system is to protect the host from a variety of invading pathogens. Thus, the immune system must be able to discriminate between foreign (i.e., "non-self") and the host's own (i.e., "self") molecules and to destroy "non-self."

The immune system can be divided into the innate and adaptive arms that have been classically represented as two separate systems with distinct properties. In this context, there exists two types of immune receptors: the innate receptors, the germ line-encoded receptors that detect a limited set of conserved antigens; and the adaptive receptors, the somatically generated antigen receptors of the T and B cells (T cell receptor, TCR and B cell receptor, BCR, respectively) (Table 1). While the innate system responds to antigens with fast kinetics and lacks memory capabilities, the adaptive system reacts with relatively delayed kinetics and possesses effective recall responses [34, 35]. Collectively, the innate and adaptive immune systems work cooperatively to defend against infection, pathogenic proliferation, and disease. Interestingly, recent advances reveal that not all immune cells can be strictly assigned to either the innate or the adaptive immune system, which revises our perspective on the immune system as an organizational continuum, rather than a dichotomy [34].

Table 1 The characteristics of the innate and adaptive receptors

Receptors	
Innate	Adaptive
Germ line-encoded	Encoded in multiple gene segments
Non-clonal distribution	Clonal distribution
Do not require gene rearrangement	Require gene rearrangement
Trigger immediate response	Trigger delayed response
Broad specificity: recognize pathogen-associated molecular patterns	Narrow specificity: recognize a particular epitope

In an evolutionary context, the adaptive immune response evolved long after the innate mechanisms of self-defense and provided significant added value to the innate immune system in promoting survival [36–38]. It should be noted here that immune receptors not only recognize potentially lethal pathogen invaders outside the cell but also translate this information into intracellular signaling pathways, initiating a protective response against these pathogens. Hence, it is equally important to understand potential evolutionary mechanisms of both aspects of receptor machinery: recognition and signaling.

In a structural context, the adaptive receptors are MIRRs with two (BCR) and four (TCR) signaling chains (Fig. 2). In contrast, the innate receptors represent SRs (e.g., toll-like receptors, TLRs) or MIRRs that contain one signaling chain (e.g., triggering receptors expressed on myeloid cells, TREMs, or natural killer cell receptors, NK receptors). Consideration of the distinct rather than redundant functions for the ITAM modules, including those located on different signaling chains [28–32, 39] and those located on the same chain (e.g., 3 different ITAMs on the ζ chain) [33] provides a molecular explanation for significantly higher diversity and variability of the adaptive response as

compared to those of the innate response. Interestingly, the extent of diversity and variability of NKp30-mediated signal transduction in this context should be intermediate between of those for the innate (e.g., TLRs) and the adaptive receptors (Fig. 2), which is consistent with the idea that NK cells represent an "evolutionary bridge" between innate and adaptive immunity [40].

Conclusions and remarks

As Charles Darwin pointed out in his *Origin of Species* [22], "In the struggle for survival, the fittest win out at the expense of their rivals because they succeed in adapting themselves best to their environment." In such an ongoing struggle, development and evolution of the self-defense mechanisms have become the key for survival of the diverse species in fiercely competitive interactions between them.

Since a major function of the immune system is to distinguish "self" from "non-self" and to protect the host against invading pathogens, the need for self-defense no doubt led to the evolutionary refinement of intricate immune systems, including the adaptive immune system. In the context of signal transduction, this process was accompanied by two remarkable structural features: intra-receptor separation of recognition and signaling functions, and protein disorder of signal-generating intracellular regions. They both contribute significantly to the diversity and variability of the immune response, adapting organisms to a varying environment [14].

Separation of functions suggests a modular assembly with distinct functional units and is one of the properties of controllable systems [41]. From an evolutionary standpoint [41], it represents the tendency toward the complication of structural organization, or, in other words, the tendency to restrict freedom and can be considered as an ascending limb of evolution. In contrast, the tendency to disordering (increasing freedom) can represent a descending limb of evolution. The latter aspect of evolution is known to contribute substantially to the increase in biodiversity, adaptation to the environment, and natural selection. One can see that incarnation of both sides of evolution is observed in the adaptive receptors.

On the other hand, upon antigen binding, protein disorder of signal-generating subunits of adaptive receptors is under specific and tight control of ligand-promoted homooligomerization [2, 5, 12]. Oligomerization per se is the simplest example of ordering because the formation of oligomers restricts the rotational and translational degrees of freedom of monomers. However, unusual features of homooligomerization of immune signaling-related IDPs (e.g., specific, reversible, and rapid binding without folding) [5, 11] minimize the restrictions of freedom of monomers upon oligomerization: molecules remain disordered and are in fast equilibrium between free and bound states. Thus, in the context of signal transduction, the controlled chaos and indeterminacy of protein disorder combines the advantages of ordering and disordering in order to provide specificity, sensitivity, diversity, and variability of signaling. This reveals an interesting functional mechanism of the convergence of two opposite tendencies of evolution.

In the struggle for the existence, in order to establish a successful infection, replicate, and persist in the host, viruses have evolved numerous strategies to counter and

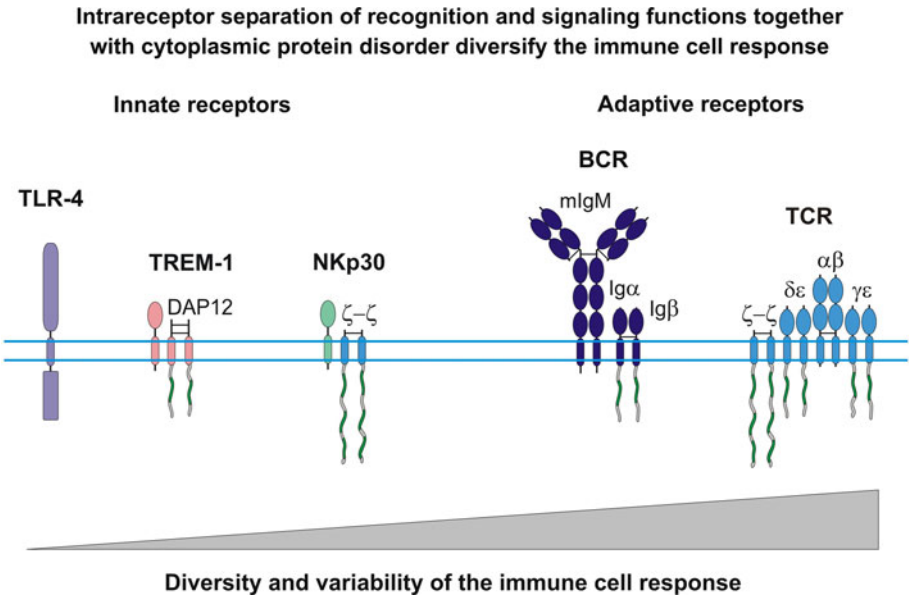

Fig. 2 Diversity and variability of the immune response. The immunoreceptor tyrosine-based activation motif (ITAM) is shown in *green*. *Curved lines* depict intrinsic disorder of the cytoplasmic domains of MIRR signaling subunits. Abbreviations: BCR, B cell receptor; DAP-12, DNAX adapter protein of 12 kD; Ig, immunoglobulin; NK, natural killer cell; TCR, T cell receptor; TLR-4, toll-like receptor; TREM-1, triggering receptor expressed on myeloid cells

evade host antiviral immune responses as well as to exploit them for productive viral replication. Several different viruses that are pathogenic for humans uniformly target members of the MIRR family, including innate and adaptive receptors. It is intriguing that these viruses use either a modular assembly of MIRRs to disrupt receptor-mediated signaling or cytoplasmic protein disorder of MIRRs to surprisingly augment cell activation as required for self-preservation [20, 21, 42]. This example of the coevolution of viruses and their hosts confirms that there is no evolution and development without risks. However, although we cannot avoid these risks, they can be compensated for by the benefits of learning from nature how to target the immune system for therapeutic purposes [13]. Thus, it is of fundamental and clinical interest to further evaluate the molecular mechanisms of evolution and development of the immune system.

Acknowledgments I would like to thank Marcia Ross for her generous editorial assistance.

References

1. Keegan AD, Paul WE. Multichain immune recognition receptors: similarities in structure and signaling pathways. Immunol Today. 1992;13:63–8.
2. Sigalov AB. Multichain immune recognition receptor signaling: different players, same game? Trends Immunol. 2004;25:583–9.
3. Reth M. Antigen receptor tail clue. Nature. 1989;338:383–4.
4. Wu J, Cherwinski H, Spies T, Phillips JH, Lanier LL. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. J Exp Med. 2000;192:1059–68.
5. Sigalov A, Aivazian D, Stern L. Homooligomerization of the cytoplasmic domain of the T cell receptor zeta chain and of other proteins containing the immunoreceptor tyrosine-based activation motif. Biochemistry. 2004;43:2049–61.
6. Sigalov AB, Aivazian DA, Uversky VN, Stern LJ. Lipid-binding activity of intrinsically unstructured cytoplasmic domains of multichain immune recognition receptor signaling subunits. Biochemistry. 2006;45:15731–9.
7. Sigalov AB, Uversky VN. Differential occurrence of protein intrinsic disorder in the cytoplasmic signaling domains of cell receptors. Self Nonself. 2011;2:55–72.
8. Uversky VN. What does it mean to be natively unfolded? Eur J Biochem. 2002;269:2–12.
9. Sigalov AB, Hendricks GM. Membrane binding mode of intrinsically disordered cytoplasmic domains of T cell receptor signaling subunits depends on lipid composition. Biochem Biophys Res Commun. 2009;389:388–93.
10. Sigalov AB, Kim WM, Saline M, Stern LJ. The intrinsically disordered cytoplasmic domain of the T cell receptor zeta chain binds to the nef protein of simian immunodeficiency virus without a disorder-to-order transition. Biochemistry. 2008;47:12942–4.
11. Sigalov AB, Zhuravleva AV, Orekhov VY. Binding of intrinsically disordered proteins is not necessarily accompanied by a structural transition to a folded form. Biochimie. 2007;89:419–21.
12. Sigalov AB. The SCHOOL of nature. I. Transmembrane signaling. Self Nonself. 2010;1:4–39.
13. Sigalov AB. New therapeutic strategies targeting transmembrane signal transduction in the immune system. Cell Adh Migr. 2010;4:255–67.
14. Sigalov AB. Cells diversify transmembrane signaling through the controlled chaos of protein disorder. Self Nonself. 2011;2:75–9.
15. Archakov AI, et al. Protein-protein interactions as a target for drugs in proteomics. Proteomics. 2003;3:380–91.
16. Pagliaro L, et al. Emerging classes of protein–protein interaction inhibitors and new tools for their development. Curr Opin Chem Biol. 2004;8:442–9.
17. Arkin MR, Wells JA. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. Nat Rev Drug Discov. 2004;3:301–17.
18. Berg T. Modulation of protein–protein interactions with small organic molecules. Angew Chem Int Ed Engl. 2003;42:2462–81.
19. Fletcher S, Hamilton AD. Protein-protein interaction inhibitors: small molecules from screening techniques. Curr Top Med Chem. 2007;7:922–7.
20. Sigalov AB. Novel mechanistic insights into viral modulation of immune receptor signaling. PLoS Pathog. 2009;5:e1000404.
21. Cohen T, Cohen SJ, Antonovsky N, Cohen IR, Shai Y. HIV-1 gp41 and TCRalpha trans-membrane domains share a motif exploited by the HIV virus to modulate T-cell proliferation. PLoS Pathog. 2010;6:e1001085.
22. Darwin CR. On the origin of species by means of natural selection or The preservation of favoured races in the struggle for life. London: John Murray; 1861.
23. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. Nat Rev Mol Cell Biol. 2005;6:197–208.
24. Iakoucheva LM, Brown CJ, Lawson JD, Obradovic Z, Dunker AK. Intrinsic disorder in cell-signaling and cancer-associated proteins. J Mol Biol. 2002;323:573–84.
25. Minezaki Y, Homma K, Nishikawa K. Intrinsically disordered regions of human plasma membrane proteins preferentially occur in the cytoplasmic segment. J Mol Biol. 2007;368:902–13.
26. Sickmeier M, et al. DisProt: the database of disordered proteins. Nucleic Acids Res. 2007;35:D786–93.
27. Patil A, Nakamura H. Disordered domains and high surface charge confer hubs with the ability to interact with multiple proteins in interaction networks. FEBS Lett. 2006;580:2041–5.
28. Pitcher LA, van Oers NS. T-cell receptor signal transmission: who gives an ITAM? Trends Immunol. 2003;24:554–60.
29. Pike KA, Baig E, Ratcliffe MJ. The avian B-cell receptor complex: distinct roles of Igalpha and Igbeta in B-cell development. Immunol Rev. 2004;197:10–25.
30. Storch B, Meixlsperger S, Jumaa H. The Ig-alpha ITAM is required for efficient differentiation but not proliferation of pre-B cells. Eur J Immunol. 2007;37:252–60.
31. Sanchez-Mejorada G, Rosales C. Signal transduction by immunoglobulin Fc receptors. J Leukoc Biol. 1998;63:521–33.
32. Jensen WA, Pleiman CM, Beaufils P, Wegener AM, Malissen B, Cambier JC. Qualitatively distinct signaling through T cell antigen receptor subunits. Eur J Immunol. 1997;27:707–16.
33. Chae WJ, et al. Qualitatively differential regulation of T cell activation and apoptosis by T cell receptor zeta chain ITAMs and their tyrosine residues. Int Immunol. 2004;16:1225–36.
34. Borghesi L, Milcarek C. Innate versus adaptive immunity: a paradigm past its prime? Cancer Res. 2007;67:3989–93.
35. Janeway C. Immunobiology: the immune system in health and disease. New York: Garland Science; 2005. p. 823.
36. Kimbrell DA, Beutler B. The evolution and genetics of innate immunity. Nat Rev Genet. 2001;2:256–67.
37. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell. 2006;124:815–22.
38. Litman GW, Cooper MD. Why study the evolution of immunity? Nat Immunol. 2007;8:547–8.

39. Roifman CM. CD3 delta immunodeficiency. Curr Opin Allergy Clin Immunol. 2004;4:479–84.
40. Sun JC, Lanier LL. Natural killer cells remember: an evolutionary bridge between innate and adaptive immunity? Eur J Immunol. 2009;39:2059–64.
41. Galimov EM. Phenomenon of life: between equilibrium and nonlinearity. Orig Life Evol Biosph. 2004;34:599–613.
42. Sigalov AB. Interaction between HIV gp41 fusion peptide and T cell receptor: putting the puzzle pieces back together. Faseb J. 2007; 21:1633–1634; author reply 1635.