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Authors: Sinead Flannery, Andrew G. Bowie

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The interleukin-1 receptor-associated kinases: critical regulators of innate immune signalling

Sinead Flannery and Andrew G. Bowie

School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland.

Corresponding author:

Andrew G. Bowie

School of Biochemistry and Immunology

Trinity College Dublin

Dublin 2

Ireland

Ph: 00 353 1 8962435

Fax: 00 353 1 6772400

Email: agbowie@tcd.ie

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Abstract

The interleukin-receptor-associated kinase (IRAK) family are involved in regulating Toll-like receptor (TLR) and interleukin-1 (IL-1) signalling pathways. TLRs are pattern recognition receptors of the innate immune response that are responsible for sensing pathogens and initiating immunity, while IL-1 is one of the key cytokines that mediates inflammation. As such, IL-1/TLR signalling pathways and the IRAK family are critical in anti-pathogen responses, inflammation and autoimmunity. The family comprises of four members, IRAK-1, IRAK-2, IRAK-M (IRAK-3) and IRAK-4, and has a role in both positive and negative regulation of signal transduction. While it was once thought that the family displayed some redundancy, each member of the family is emerging as a distinct and vital contributor to IL-1/TLR signalling mechanisms. Knockout mouse studies have explored the relative contribution of each of the IRAKs in IL-1/TLR signalling, while the recent generation of kinase-inactive knock-in IRAK-4 mice have revealed which of IRAK-4 functions require its kinase activity. IRAK-2, previously thought of as a pseudokinase, has recently been proposed to have kinase activity that is essential for TLR signalling. Not surprisingly given their critical role in IL-1/TLR signalling, the IRAK family members have been implicated in certain disease models including human immunodeficiencies. Thus the potential targeting of these essential protein kinases therapeutically is also discussed.

Abbreviations: ARE, Adenine and uridine Rich Elements; ATP, Adenosine Triphosphate; BAFF,B-cell activating factor belonging to the TNF family; BMDM, Bone Marrow Derived Macrophages; CpG, Cytidine-phosphate-guanosine; DD, Death Domain; dsDNA Double Stranded DNA; dsRNA, Double Stranded RNA; ID, Intermediary Domain; IFN, Interferon; IκB, Inhibitor of Kappa B; IKK, IκB kinase; IRAK, Interleukin Receptor Associated Kinase; IRF, Interferon Regulatory Factor; JNK, c-jun N terminal Kinase; KD, Kinase Dead; KO, Knockout; LMCV, Lymphocytic Choriomeningitis Virus; LPS, Lipopolysaccharide; LRR, Leucine Rich Repeats; LT, Lymphotoxin β receptor; MAL, MyD88-adaptor like; MEFs, Mouse Embryonic Fibroblasts; MyD88, myeloid differentiation primary response gene 88; NEMO, NFκB Essential Modifier; NIK, NFκB Inducing Kinase; PAMP, Pathogen Associated Molecular Patterns; PBMC, Peripheral Blood Mononuclear Cells; PRR, Pattern Recognition Receptor; Poly IC, Polyinosine-polycytidylic acid; RIG-I, Retinoic acid Inducible Gene 1; SAM, sterile α motif; SARM, Sterile α and ARMadillo motif containing protein; SINTBAD, Similar to NAP-1 TBK-1 adaptor; SLE, Systemic Lupus Erythematosus; ssRNA, Single Stranded RNA; TAB,TAK Binding Protein; TAK, TGF-β Activated Kinase; TANK, TRAF-associated NFκB activator; TBK-1, TANK binding kinase-1; TICAM, TIR-containing adaptor molecule; TIR, Toll/IL-1R; TIRAP, TIR-adaptor protein; TLR, Toll-Like-Receptor; TNF, Tumour Necrosis Factor; TRAF, TNFR-associated factor; TRAM, TRIF-Related Adaptor Molecule; TRIF, TIR domain containing adaptor protein inducing IFN-β; TTP, Tristetraprolin; UTR, Untranslated Region; VACV, Vaccinia Virus; VSV, Vesicular Stomatitis Virus

1. Introduction

The IRAK family are defined as intracellular kinases that play a significant role in the innate immune system as they participate in signalling networks of the innate axis of the immune response. These signalling networks are critical for the regulation of inflammation, the antiviral response, the subsequent activation of the adaptive immune response and the control of autoimmune and inflammatory disease. Innate immune signalling is activated upon detection of pathogens through pattern recognition receptors (PRRs) which recognise pathogen associated molecular patterns (PAMPs) (1). PAMPs are conserved motifs on microorganisms essential for their survival and distinguishable from host structures (2). One central group of PRRs are the Toll-like receptors (TLRs) which have been well characterised since their discovery in the late 1990's (3). TLRs are defined by having a Toll/IL-1_Receptor (TIR) domain located cytoplasmically and leucine rich repeats (LRRs) located extracellularly. The TLRs are expressed on a variety of cell types and differentially recognise distinct PAMPs (2). They can be broadly divided into two categories: TLRs that are located at the plasma membrane namely TLR1, TLR2 and TLR6 which recognise lipoproteins and TLR4 which recognises LPS, and TLRs located endosomally namely TLR3 (which recognises dsRNA), TLR7 (ssRNA) TLR8 (ssRNA) and TLR9 (CpG motifs in DNA) TLR4 is known to translocate to the endosome and signal from there also (4). TLR7, TLR8 and TLR9 form an evolutionary conserved sub-group within the TLR family. TLR7, 8 and 9 signal through similar signalling mechanisms although they are located on different cell types and are known to induce different cytokine responses (5-7). One major TLR-induced set of

responses is the activation of transcription factors leading to the induction of proinflammatory cytokines and type-I interferons (IFNs).

Engagement of TLRs by PAMPs causes receptor dimerisation leading to the recruitment of one or more of five TIR domain-containing adaptor proteins. They are myeloid differentiation primary-response gene 88 (MyD88), MyD88-adaptor-like (Mal), TIR-domain-containing adaptor inducing IFN-β (TRIF), TRIF-related adaptor molecule (TRAM) and sterile-α- and armadillo-motif-containing protein (SARM) (8-12). MyD88 is required for all TLR signalling pathways except for TLR3 and a TLR4/MyD88-independent pathway (13). IL-1R also signals through MyD88. As well as a TIR domain, MyD88 also contains a death domain (DD). Its death domain facilitates its interaction with IRAK proteins (14).

In terms of the four IRAK family members, human IRAK-1, IRAK-2 and IRAK-4 are ubiquitously expressed, whereas human IRAK-M is only detectable in monocytes and macrophages in an inducible manner (15-16). Structurally IRAK family members share similar domains (See Figure 1). They contain an N-terminal DD, a proST domain, a central conserved kinase domain and a C terminal domain (except for IRAK-4 which lacks a C terminal domain) (17, 18). The DD is vital for signalling since it interacts with other signalling molecules such as MyD88 and IRAK members that lack the death domain act in a dominant negative manner (14, 19). The proST region is rich in serines, prolines and threonines. IRAK-1 is reported to undergo hyperphosphorylation in this region (18). This

domain for IRAK-1 is said to contain two potential PEST sequences which may facilitate its degradation. IRAK-2 does not have these sequences and it is not degraded (20). The central kinase domain contains an activation loop which is important for kinase activity. Each IRAK kinase domain also contains an invariant lysine residue in its ATP binding site which is also critical for the catalytic activity (21). Recently the crystal structure of the kinase domain of IRAK-4 has been reported by two separate groups (22, 23). IRAK-4 contains characteristic structural features of both Ser/Thr and also tyrosine kinases. The IRAK family have a tyrosine gatekeeper residue at the centre of the ATP binding site (22). The gatekeeper residue refers to the residue upstream of the hinge that controls access to a pre-existing internal hydrophobic pocket at the back of the ATP-binding site (22). The tyrosine residue as a gatekeeper is exclusive to the IRAK family making them a unique family of kinases (23). The different IRAK proteins have different residues that undergo phosphorylation (see Figure 1). Lastly the C-terminal domain is important for interaction with TRAF6 (24). IRAK-1 contains three TRAF6 interaction motifs, IRAK-2 is reported to have two TRAF6 interaction motifs and IRAK-M contains one TRAF6 interaction motif (24).

The IRAK family contribute to multiple signalling pathways “downstream” of the TIR adaptors including, but not restricted to, activation of NFκB, MAP kinases and IFN regulatory factors (IRFs). For IL-1/TLR-induced NFκB activation, phosphorylation of the IRAKs results in the subsequent activation of TNF-receptor-associated factor 6 (TRAF6) E3 ligase activity and

polyubiquitination events essential for signalling (see Figure 2). TRAF6 then recruits to a TGF-β Activated Kinase-1 (TAK)/ TAK Binding Protein-2/3 (TAB2/3) complex leading to TAK-1 activation by phosphorylation (25). TAK-1 then activates the IκB kinase (IKK) complex which contains two catalytic subunits which can form homo or heterodimers (IKKα or IKKβ) and a regulatory subunit NFκB-essential modifier (NEMO), also known as IKKγ (26). The IKK complex phosphorylates IκB, an inhibitory subunit of NFκB, thus allowing an active NFκB dimer to translocate into the nucleus.

For MAP kinase activation, the IKK complex also phosphorylates p105 which is a negative regulator of serine/threonine kinase tumour progression locus 2 (Tpl2) (27). Thus upon phosphorylation and subsequent degradation of p105, Tpl2 is activated. Tpl2 then activates MKK1 and MKK2 leading to the phosphorylation of the extracellular signal-regulated kinases ERK-1 and ERK2 (27). p38 and JNK MAP kinases are also activated by TLR signalling, since TAK-1 activates MKK3/6 and MKK4/7 which in turn stimulates p38 and JNK respectively (28).

In addition certain TLRs, namely TLR3, TLR4, TLR7, TLR8 and TLR9 also activate the IRFs in response to viral PAMPs. A broad range of viral infections activate IRF3 and IRF7 while IRF5 activation is more restricted (29). In order to be activated, IRF3 and IRF7 are phosphorylated by two kinases: TBK-1 (TRAF family member associated NFκB activator (TANK)-binding kinase-1) and IKKε (30). These kinases are recruited upon activation of TLR3 and this pathway is thought to be IRAK-independent (see Figure 3).

While the NFκB pathway has NEMO as its scaffolding protein strong evidence suggests a role for three proteins, TANK, NAP-1 (NFκB-activating kinase-associated protein) and SINTBAD (similar to NAP-1 TBK-1 adaptor), as scaffolding proteins for the assembly of TBK-1 and IKKε kinase complexes (31). TLR7/8/9 are also known to activate IRF5 and IRF7 in a TBK-1/IKKε-independent, but IRAK-dependent manner (32). IRAK-1 has been shown to stimulate IRF5 ubiquitination via TRAF6 both in mouse and human cells (33). Through the use of IRAK-1 -/- mice, it has been demonstrated that IRAK-1 is important for IRF7 phosphorylation. Furthermore IKKα plays a role in this pathway through the activation of IRF7 (see Figure 3) (33, 34).

2. IRAK-1

IRAK-1 was the first member of the IRAK family to be discovered and was initially shown to have a role in IL-1 signalling (15). It is a protein of 712 aa in length giving it a molecular mass of ~85kDa. Human IRAK-1 is ubiquitously expressed while interestingly, murine IRAK-1 has a more restricted expression being primarily expressed in liver, kidneys and testis (15, 35). Human IRAK-1 has three splice variants (36). Since the TLRs share the TIR domain with the IL-1 receptor it was hypothesized that IRAK-1 might also participate in TLR-mediated signalling and many studies have now shown that various TLR pathways utilise IRAK-1. Many roles for IRAK-1 have been proposed including roles in NFκB activation, IRF activation and STAT3 activation (34, 37, 38).

2.1 IRAK-1 post-translational modification during IL-1R/TLR signal transduction

Upon ligand binding to the IL-1R or a TLR, MyD88 is rapidly recruited to the receptor via interaction of its TIR domain (40). IRAK-1 interacts with MyD88 through DD interactions (see Figure 2). Thr66 in the DD has been shown to be vital for the formation of homodimers of IRAK-1 but mutation of this residue did not prevent IRAK-1 interaction with IRAK-2 or IRAK-M but prevented activation of NFκB (41).

IRAK-1 has also been shown to undergo phosphorylation upon TLR stimulation and some residues on IRAK-1 have been suggested as phosphorylation targets for IRAK-4 (See Figure 1). The phosphorylation of IRAK-1 occurs in a number of steps: It has been shown, *in vitro*, that IRAK-1 is initially phosphorylated at Thr 209 (18). This is a critical residue in IRAK-1 as mutation of this residue completely disrupts its kinase activity (18). Phosphorylation of Thr 209 results in a conformational change in the kinase domain of IRAK-1 allowing subsequent phosphorylations to then take place including phosphorylation of Thr 387, a critical residue in the activation loop in the kinase domain of IRAK-1 (see Figure 1) (18). This residue has also been suggested to be a potential target for phosphorylation by IRAK-4. The final step of these sequential phosphorylations occurs in the ProST region of the protein (also previously referred to as the undetermined domain), which is subject to hyper-autophosphorylation. MyD88 only binds non phosphorylated IRAK-1 (42), thus upon phosphorylation, IRAK-1 is released from the receptor complex and binds to TRAF6, ultimately leading to NFκB activation. However, if and how IRAK-1 “activates” TRAF6 is still unclear. IRAK-1 is subject to other

modifications besides phosphorylation. It also undergoes ubiquitination and sumoylation (37, 43). After phosphoryation and activation of IRAK-1, it has been reported that IRAK-1 undergoes K48-linked polyubiquitination leading to its rapid degradation (42). Until recently it was assumed that IRAK-1 was only targeted by K48 ubiquitination, however recent studies have shown that IRAK-1 undergoes K63-linked polyubiquitination (43-45). K63-linked polyubiquitination of a protein is normally required for signal transduction rather than degradation and upon ubiquitination of IRAK-1 it can interact with NEMO (44). Mutation of the ubiquitination sites on IRAK-1 prevents NEMO binding and subsequent IL-1/TLR-induced NFκB activation. Thus IRAK-1 is ubiquitinated, although what protein ubiquitinates it is still uncertain. Separate groups have proposed TRAF6 and the pellino proteins as the E3 ligase for IRAK-1 ubiquitination (43-45). The pellino proteins are a family of E3 ubiquitin ligases that play an important role in IL-1/TLR signalling. One report has shown IRAK-1 undergoes ubiquitin editing and thus is subject to both K63- and K48- linked ubiquitination (46).

2.2 NFκB activation and IRAK-1 kinase activity

IRAK-1-deficient mice were used to examine the role of IRAK-1 in IL-1/TLR – induced activation of NFκB and MAPK pathways (47-49) (See Table 1). IRAK-1 -/- macrophages showed a partial decrease in LPS-induced IKKβ activation and IL-1- and LPS-induced NFκB DNA binding were also affected (47, 48). Further, IL-1-induced p38 and JNK activation were shown to be reduced in IRAK-1 -/- mouse primary embryonic fibroblast (EF) cells (49). Interestingly at low concentrations of IL-1, IκB degradation was affected in IRAK-1 -/-

fibroblasts, however at higher concentration IκB was completely degraded in wild type and IRAK-1 -/- cells (49). Furthermore, an effect on IL-1 and LPS induced cytokine production was only significant at lower concentrations of stimuli in IRAK-1 deficient cells. Therefore deletion of IRAK-1 attenuates, but does not eliminate, IL-1/TLR-induced NFκB, MAPK activation and gene induction (47-49). One particular study of interest showed that IRAK-1 was completely dispensable for TLR7/9 mediated NFκB and MAPK cytokine production in plasmacytoid dendritic cells (pDCs) (34). However this study revealed a novel role for IRAK-1 in IRF activation which will be discussed later (see Section 2.3)

Despite the fact that IRAK-1 is a kinase, the function/relevance of its kinase activity in the activation of NFκB by IL-1/TLRs is still uncertain. It has been shown that the kinase activity of IRAK-1 is not essential for NFκB activation as a kinase inactive mutant of IRAK-1 can still activate NFκB (50, 51). In contrast to this IRAK-1 has been shown to phosphorylate the pellino proteins upon overexpression in cells and also directly *in vitro* (43-45).

Overall, whether the kinase activity of IRAK-1 is directly required for NFκB is still uncertain and the generation of a knock-in mouse expressing catalytically inactive IRAK-1 will be required to resolve this issue. Given that IRAK-1 is an active kinase and that its kinase activity may not be essential for TLR-induced NFκB, IRAK-1 may play other roles in innate signalling where its kinase activity is critical.

2.3 IRAK-1 and IRF activation

This holds true for the role for IRAK-1 in IRF activation that has emerged in recent years. A study by Uematsu *et al.,* mentioned earlier, showed that TLR7- or TLR9-induced NFκB and MAPK activation was normal in IRAK-1 -/- pDCs (34). Strikingly, TLR7 or TLR9 induction of IFNα was completely abolished in these mice. Interestingly, in this study IRAK-1 was shown to directly phosphorylate IRF7 *in vitro* using human cells and the kinase activity of IRAK-1 was shown to be critical for IRF7 transcriptional activation. Thus this study was the first indication that IRAK-1 may have a more essential role in TLR-induced IRF activation than NFκB in certain contexts (34). In contrast to this it has also been proposed that IRAK-1 is a negative regulator of the IFN pathway since SHP-1 was shown to promote type I IFN induction by inhibiting IRAK-1 (52).

IRAK-1 was subsequently shown to be important for TLR7- and TLR8-induced IRF5 activation in both mouse and human cells (32,33). Convincingly the kinase activity of IRAK-1 was required for ubiquitination of IRF5 as using a kinase inactive mutant of IRAK-1 failed to induce the formation of polyubiquitinated IRF5 (33). It is thought that the explanation for this is that IRAK-1 regulates the TRAF6-mediated ubiquitination of IRF5 (See Figure 3) (33). A single nucleotide polymorphism in IRF5 has been revealed as a risk factor for systemic lupus erythematosus (SLE) and more recently it has also been shown that IRAK-1 has a crucial role in the development of this disease (53, 54). IRAK-1 has also been shown to interact with TRAF3 (55), which is a

key player in TLR-induced type I IFN production, thus highlighting further the role of IRAK-1 in the IRF axis of TLR signalling.

2.4 IRAK-1 and STAT activation

IRAK-1 has been shown to a have a novel role in TLR4-mediated STAT3-dependent IL-10 expression (38). Surprisingly IRAK-1 -/- mice that were stimulated with LPS in this study were shown to induce multiple NFκB-dependent genes normally. However the IRAK-1 -/- mice failed to induce any IL-10 message in comparison to wild type mice and LPS-induced IL-10 production was severely impaired in IRAK-1 -/- splenocytes (38). LPS-mediated IL-10 gene expression has been shown to be dependent on STAT3 and phosphoryation of STAT3 on a crucial serine residue was not observed in IRAK-1 -/- splenocytes. Further it was demonstrated that IRAK-1 and STAT3 localise together in the nucleus after stimulation. In addition, IRAK-1 interacts with the endogenous IL-10 promoter.

A role for IRAK-1 has also been revealed in STAT1 activation since IL-1-mediated phosphorylation of STAT1 requires IRAK-1 (56). Furthermore IRAK-1 and STAT1 have been shown to interact *in vivo* in human glioblastoma cells T98G (56).

3. IRAK-2

In 1997, a protein of 590 amino acids which shared sequence and functional similarity to IRAK-1 was discovered and this molecule was named as IRAK-2 (Figure 1) (19). Initial reports showed that IRAK-2 when overexpressed,

activated NFκB. This required the IRAK-2 DD since a truncated IRAK-2 (97-590) which lacks the DD failed to activate NFκB and moreover, acted in a dominant negative manner (19). Further evidence that IRAK-2 played a role in the TLR pathway emerged when it was shown that IRAK-2 interacted with adaptor molecules MyD88 and Mal and also with TRAF6 (8, 19)

3.1 Role of human IRAK-2 revealed by viral targeting

For 10 years after its discovery the exact function of IRAK-2 remained unclear. It was presumed to only have a redundant role with IRAK-1. However the importance of IRAK-2 in TLR-induced NFκB activation was discovered through studies with Vaccinia Virus (VACV). VACV is a member of the poxviridae family, which are large DNA viruses and the proteins they express have diverse ways of evading and subverting the innate immune system (57). A52 is one such protein shown to be important for virus virulence (57). It was found that A52 interacts with IRAK-2 and TRAF6, but not IRAK-1. Subsequently A52 was shown to inhibit all IL-1/TLR pathways to NFκB solely through its interaction with IRAK-2 (58). As the virus was specifically targeting IRAK-2 and not IRAK-1 to inhibit TLR-mediated NFκB signalling, this suggested a predominant role for IRAK-2 in NFκB activation.

Indeed the significance of IRAK-2 in TLR-mediated NFκB activation is now recognised. Knockdown of human IRAK-2 expression by siRNA impaired NFκB activation by TLR3, TLR4 and TLR8 (59). Of note, knockdown of IRAK-2 in human peripheral blood mononuclear cells (PBMCs) impaired LPS-induced IL8 production. However further studies in primary human cells will be required to fully understand the contribution of IRAK-2 to human TLR

signalling pathways. The role for IRAK-2 in the TLR3 pathway is particularly intriguing as no other IRAK family member has been shown to play a role in this pathway (60, 61). In support of this role for IRAK-2, it has been shown that endogenous IRAK-2 is recruited to the TLR3 receptor (59). Mechanistically how IRAK-2 functions is also still uncertain. It may act very proximal to the receptor complex for all TLRs as is the case with TLR3. However it has been shown that IRAK-2 can stimulate the formation of polyubiquitin chains associated with TRAF6 (see Figure 2) (59). It had always been assumed that IRAK-1 was the IRAK family member that triggered polyubiquitination of TRAF6, however overexpression of IRAK-2 induced polyubiquitination of TRAF6 while overexpression of IRAK-1 did not (59). This was shown to be independent of IRAK-1 as exogenous IRAK-2 could induce TRAF6 ubiquitination in cells deficient in IRAK-1 (59).

3.2 Function of murine IRAK-2 revealed by knockout mouse studies

While the importance of IRAK-2 was confirmed in multiple TLR pathways to NFκB in human cell lines, the relative importance of IRAK-2 in the murine system was still uncertain until very recently. In 2008 IRAK-2 knockout mice were generated (62). These mice were found to be highly resistant to LPS and CpG-induced septic shock (see Table1). Since previously it had been shown that the difference in mortality between wild type and IRAK-1 knockout mice was only subtle, this indicated a more critical role for IRAK-2 compared to IRAK-1 (48).

The role of IRAK-2 in TLR2 signalling to NFκB and MAPKs was examined using macrophages from IRAK-2 KO mice. This revealed that IRAK-2

functions redundantly with IRAK-1 in early signalling but is important for late and sustained NFκB and MAPK activation (62). Similar results were shown for TLR7 signalling to NFκB by a separate group using independently generated IRAK-2 -/- mice (63). Only when IRAK-2 -/- mice were crossed with IRAK-1 -/- mice was a dramatic impairment of NFκB activation by TLR2 observed

3.3 Differential expression of murine IRAK-2 splice variants in inbred versus wild-derived mice

Mouse and human IRAK-2 show 67% sequence identity (64). They share the same domain structure and are highly conserved in their death domains and kinase domains. Their C-terminal differs slightly as the C-terminal of murine-IRAK-2 is 35 amino acids longer than human IRAK-2 (see Fig 1). Interestingly one study has reported that overexpression of murine IRAK-2 cannot activate NFκB (64). Another distinction between human IRAK-2 and murine IRAK-2 is that while no evidence of splice variants exist for human IRAK-2, four splice variants are found in the murine form (IRAK-2a,- 2b, -2c and -2d) (65). Since overexpression of IRAK-2a and IRAK-2b activated NFκB whereas Irak-2c and IRAK-2d inhibited NFκB activation, IRAK-2c and IRAK-2d have been proposed as having a negative role on TLR signalling (65). Consistent with this, IRAK-2c completely lacks a DD which may prevent it from interacting with certain signalling molecules (64, 65).

Intriguingly, a recent study examining the innate immune response of wild-derived mice versus classical inbred strains revealed differential expression of IRAK-2 splice variants and suggested a more critical role for IRAK-2 in wild-

derived mice (66). A classical inbred strain of mice, C57BL/6J, expressed high levels of the inhibitory isoform of IRAK-2, IRAK-2c. This isoform inhibits the proinflammatory isoform IRAK-2a. This may explain the dominant role of IRAK-1, rather than IRAK-2, in early signalling events. However as time progresses the inhibitory isoform expression is decreased and IRAK-2a, which is no longer being inhibited, is able to function in a proinflammatory manner. Thus this isoform (in inbred mouse) is responsible for sustained NFκB activation (66). Wild-derived mice differ significantly from experimental models as the genetic diversity of wild derived mice has arisen in an evolutionary context and these mice display a higher degree of polymorphisms. In the wild-derived strain MOLF/Ei, a natural mutation occurs in the promoter of IRAK-2c. This results in IRAK-2c being expressed significantly less leading to less inhibition of IRAK-2a. Thus when IRAK-2a expression was suppressed by siRNA, IRAK-2a was found to be indispensable for early activation of NFκB and p38 MAPK. Thus the murine IRAK-2 from wild derived mice may behave in a similar manner to human IRAK-2 as the human IRAK-2 contains no splice variants and is shown to be important for human TLR signalling to NFκB (66).

3.4 Is IRAK-2 an active kinase?

It had been historically established that IRAK-1 and IRAK-4 were the only active kinases from the IRAK family based on the fact that an aspartate residue in the IRAK kinase domain is an asparagine residue in IRAK-2 and a serine in IRAK-M. However IRAK-2, like all other family members, contains a functional ATP-binding pocket with an invariant lysine residue in the protein

kinase subdomain (21). It has been recently proposed that this residue is sufficient for IRAK-2 to act as an active kinase (62). An *in vitro* kinase assay showed that IRAK-2 is phosphorylated upon stimulation with a TLR2 ligand (62). It is thought that IRAK-4 phosphorylates IRAK-2 and induces its kinase activity. It is of interest to note that overexpression of IRAK-1 and IRAK-4 kinase dead mutants can still activate NFκB whereas the kinase mutant of IRAK-2 fails to do so (62 and Joanna Szymak & Andrew Bowie, unpublished data). Thus this reveals a potential crucial role for the IRAK-2 kinase activity in TLR-mediated NFκB activation.

4. IRAK-M

The third family member to be discovered, IRAK-M, is a protein of 596 amino acids with a molecular mass of 68 kDa (67). While the other members of the IRAK family share a lot of similar features, IRAK-M is more unique. As mentioned previously, while expression of other human IRAK members are ubiquitous, expression of human IRAK-M is limited to monocytes and macrophages (67). Murine IRAK-M, which shares a 71% sequence similarity with its human counterpart, has been shown to be expressed in many cell types occurring most predominantly in the liver and thymus (16). Furthermore, given that IRAK-2 is no longer assumed to be a pseudo kinase, IRAK-M is now the only member of the family to lack kinase activity. Its most distinct feature is its function as a negative regulator of TLR signalling (68).

Initial reports on IRAK-M showed that upon overexpression it was able to activate NFκB (67). Furthermore when IRAK-M was expressed in cells lacking

IRAK-1 it was able to restore NFκB activation. Thus it was initially assumed that like IRAK-1 and IRAK-2, IRAK-M was a positive regulator of TLR-mediated NFκB signalling (67). However IRAK-M -/- mice showed an increased inflammatory response (but not increased susceptibility) to the bacterium *Salmonella typhimurium* (68). IRAK-M -/- mice were shown to have reduced survival upon influenza infection *in vivo* (69). Furthermore macrophages derived from IRAK-M knockout mice displayed enhanced activation of IL-1/TLR signalling, thus suggesting a negative role for this family member (68, 69) (see Table 1). It was initially proposed that IRAK-M may prevent IRAK-1 and/or IRAK-4 from dissociating from the MyD88 complex thus preventing TLR-mediated signalling to NFκB (68). A novel role for IRAK-M recently has recently been shown in specifically negatively regulating TLR2-induced p38, but not JNK or ERK, activation (70). The regulation of p38 by IRAK-M was found to be completely IRAK-1 independent but rather occured through IRAK-M-mediated stabilisation of the phosphatase MKP-1. MKP-1 had been previously shown to negatively regulate p38 phosphorylation (71). Thus in contrast to the proposal that IRAK-M may interact with and regulate IRAK-1 (68), this study suggests that IRAK-M does not contribute to IRAK-1 regulation and function.

IRAK-M was also shown to negatively regulate the alternative NFκB pathway in a TLR2-specific manner (72). The alternative or non canonical pathway of NFκB relies on the activation of NFκB-inducing kinase (NIK) and the subsequent phosphorylation of p100 through an IKKα-dependent mechanism. The alternative NFκB pathway is predominantly triggered by CD40, LT

(lymphotoxin β receptor) and the BAFF receptor (B-cell activating factor belonging to the TNF family) (73). However this study shows that upon stimulation of cells by the TLR2 agonist Pam_3Cysk_4 elevated levels of NIK protein and altered distribution of the NFκB subunit RelB were observed in IRAK-M -/- cells (72). This hinted at the possible regulation of the alternative NFκB pathway by IRAK-M. It appeared that IRAK-M did not regulate the TLR-2-induced classical NFκB pathway as p65/RelA phosphorylation and nuclear translocation were unchanged in wild-type and IRAK-M -/- cells (72). Thus it seems that the role for IRAK-M may be more vital in the alternative NFκB pathway, and for TLR2-induced p38 regulation, rather than in the classical NFκB pathway as originally thought.

5. IRAK-4

IRAK-4 is the most recent member of the IRAK family to be discovered (74). It was discovered through a database search as a human cDNA sequence that encodes a polypeptide sharing significant but previously unrecognised homolog with IRAK-1. It is the closest human homolog to the *Drosophila* Pelle protein. Pelle, the only IRAK in the fly, is involved in signalling downstream of the Toll-Dorsal pathway during embryonic development. The human IRAK-4 protein is 460 amino acids long and shares 87% similarity and 84% identity with murine-IRAK-4 (75).

5.1 Role of IRAK-4 in TLR signalling

The essential role of IRAK-4 was revealed through knockout studies. In contrast to IRAK-1 KO mice, mice depleted of IRAK-4 were shown to be

completely resistant to LPS-induced septic shock and lacked a cytokine response when challenged with various TLR ligands (75). Furthermore IL-1-induced NFκB, JNK and p38 activation were all severely defective in cells lacking IRAK-4. LPS-induced JNK activation was also inhibited and LPS-induced NFκB activation was delayed (75). These mice also failed to respond to lymphocytic choriomeningitis virus (LCMV). LCMV is dependent on IL-12 and IL-18, and IL-18 binds to a receptor that is homologous to the IL-1R (75). IRAK-4 plays a critical role proximal to the receptor and MyD88 and mediates NFκB activation through initially interacting with MyD88 (76). It has recently been described that the DDs of IRAK-4 and MyD88 form large oligomeric structures termed the Myddosome (77). The DDs were shown to assemble in a complex with two layers containing 7/8 MyD88 subunits and 4 IRAK-4 subunits altogether. This complex may allow for the recruitment of other molecules such as IRAK-2 or IRAK-M (77).

MyD88 acts as a scaffold protein for the interaction between IRAK-1 and IRAK-4 (77). IRAK-4 is presumed to phosphorylate IRAK-1, which leads to the autophosphorylation and activation of IRAK-1 itself. It has been shown that IRAK-4 also induces the degradation of IRAK-1 (78), thus acting in a negative feedback loop to regulate the MyD88-dependent pathway.

IRAK-4 has also been shown to have an important role in type I IFN induction by TLR7/8/9 (61). IRAK-4 was also shown to interact with IRAK-2 (79 and Sinead Keating & Andrew Bowie unpublished work) but unlike IRAK-2 appears to have no role in the TLR3 pathway. Overall, IRAK-4 is essential for

MyD88-dependent pathways but is dispensable for MyD88 independent signalling pathways, such as that used by TLR3 via TRIF (61).

5.2 Differential requirements for IRAK-4 kinase activity in signalling

While it was apparent from knockout studies that IRAK-4 has a vital role in IL-1/TLR signalling, whether the kinase activity of IRAK-4 was essential for its function was unknown. Initial studies using IRAK-4 deficient murine embryonic fibroblasts (MEFs) that were reconstituted with an IRAK-4 kinase inactive mutant showed that the kinase activity of IRAK-4 was required for the optimal induction of IL-1 induced NFκB, JNK activation and proinflammatory cytokines (80).Then with the generation of IRAK-4 kinase inactive knock-in mice it was shown that the kinase activity of IRAK-4 is required for its function (81, 82). *In vivo,* these mice were highly resistant to TLR-induced shock (81, 82). However macrophages from these mice revealed that the kinase activity of IRAK-4 was dispensable for the activation of IL-1-, TLR2-, TLR4- and TLR7-induced NFκB (81, 82). Interestingly in one particular study it was observed that TLR2-induced NFκB DNA binding still occurred in IRAK-4 knockout macrophages, indicating the existence of an IRAK-4 independent TLR2 pathway (81).

While IL-1/TLR-induced NFκB was not greatly affected in IRAK-4 kinase inactive knock-in mice, there was a dramatic impairment of IL-1/TLR induced cytokine and chemokine production (82-84).

Furthermore MAP kinase activation appears to have a higher dependency on IRAK-4 kinase activity than NFκB. IL-1-, TLR2-, TLR4- and TLR7-induced JNK activation was shown to be strongly dependent on IRAK-4 kinase activity (81-84). Whether the kinase activity of IRAK-4 is required for IL-1/TLR-induced p38 activation is still controversial (81-84). Two groups have shown that the kinase activity is required for IL-1/TLR-induced p38 activation in macrophages and fibroblasts (83,84), while others showed that IRAK-4 kinase activity is not required for TLR4/TLR7-induced p38 activation (82).

While studies from kinase inactive IRAK-4 knock-in mice have revealed that the kinase activity of IRAK-4 is required for certain functions in IL-1/TLR signalling, studies from human cells have shown that the kinase activity of IRAK-4 is generally redundant (85, 86). Using human IRAK-4 deficient fibroblasts reconstituted with a kinase inactive IRAK-4 mutant it was shown that the kinase inactive mutant was able to restore IL-1 induced NFκB, JNK activation and IL8 gene expression to a similar degree as the wild-type IRAK-4 (85). In addition a recent study which depleted human endothelial cells of IRAK-4 using IRAK-4 siRNA and reconstituted the cells with a kinase inactive IRAK-4 revealed that the kinase activity of IRAK-4 was not required for IL-1 induced IL-8 expression (86). Furthermore in this study, with the use of selective small kinase inhibitors, it was revealed that the kinase activity of IRAK-4 was redundant for the activation of IL-1-induced p38, JNK and IL-6 expression. Thus in the human system it appears that the kinase activity of IRAK-4 is not required, perhaps being redundant with IRAK-1 or IRAK-2.

5.3 IRAK-4 and human disease

IRAK-4 is one of the few IL-1/TLR signalling proteins to be implicated in human disease to date. A cohort of patients who had recurrent infections and a poor inflammatory response were discovered to have an inherited IRAK-4 deficiency (87-90). These patients were susceptible to extracellular pyogenic bacteria such as *Streptococcus pneumoniae*. While IRAK4 -/- mice are sensitive to a wide range of microorganisms patients with an IRAK-4 deficiency suffered from a narrow spectrum of infections (87). Furthermore for these patients, infections began early in life but decreased with age, presumably due to the help of the adaptive immune system (88). In contrast, the susceptibility of the immune system of IRAK-4 -/- mice does not decrease with age (88). This suggests a rather specific role for IRAK-4 in terms of which human pathogens the human immune system requires it for.

Even though IRAK-4 is required for all MyD88-dependent signalling pathways to NFκB and also for TLR7/8/9 signalling to IFNα, why IRAK-4-deficient patients were only susceptible to a narrow range of bacteria was unclear. However it has now been revealed that IRAK-4 is critical for human TLR7/8/9 responses to viruses, but that it is largely dispensable for the induction of IFNα/β and IFNλ in response to TLR3/TLR4. PBMCs that were deficient in IRAK-4 failed to respond to stimulation with TLR7/8 agonists, and no IFNα, IFNβ and IFN λ mRNA or protein was induced in these cells (61). In contrast, normal p38, JNK, IRF3 and NFκB responses to poly (I:C) and LPS were observed in

IRAK-4 deficient PBMCs and fibroblasts. The TLR3 and TLR4-MyD88 independent pathway have been shown to be IRAK-4 independent. This suggests that the MyD88-independent TRIF axis, together with the non-TLR PRRs, are sufficient for the recognition of most viruses by IRAK-4 deficient patients (61).

6. IRAKs and post-transcriptional regulation

Although much of the research into IL-1/TLR signalling focuses on transcriptional regulation many short-lived inflammatory mRNAs induced by TLRs are regulated at the post-transcriptional level. These mRNAs contain AU-rich elements (ARE) in their 3' untranslated (UTR) region. (91). ARE-binding proteins (ARE-BP), such as tristetraprolin (TTP), HuR and AUF1 bind to these ARE regions and positively and negatively regulate mRNA decay (91-94). The MAPK pathways mediate the regulation of these ARE-BPs (91). Some of the ARE-BPs regulate both mRNA decay and the translational pathway (95).

A common theme for the three positive IRAK family members, IRAK-1, IRAK-2 and IRAK-4 is that they have all been shown to have a role in post transcriptional regulation (63, 82, 96). IRAK-1 has been shown to be essential for IL-1-induced stabilisation of the mRNAs of two chemokines MIP2 and KC, via a signalling pathway that did not require TRAF6 (96). IRAK-2 has been shown to have a role in LPS-induced post-transcriptional and translational regulation of cytokine and chemokine expression (63). While it was shown, using IRAK-2 -/- macrophages that murine IRAK-2 has no role in TLR4-induced early or

sustained NFκB activation, TLR-4 induced cytokines and chemokine production was greatly affected since TLR4-induced stability of IL-6 and KC mRNA was shown to be regulated by IRAK-2 (63). It was found that the role of IRAK-2 in mRNA stability was ligand specific as there was no role for murine IRAK-2 in TLR7-induced post-transcriptional control (63). In addition to its role in post-transcriptional regulation, IRAK-2 was also shown to regulate LPS-induced TNFα mRNA translation. A contrasting report with IRAK-2 knockout mice showed no effect of IRAK-2 on mRNA stability for TLR-2 induced cytokines and chemokines tested thus suggesting a specific role for IRAK-2 in TLR4-mediated post-transcriptional regulation (62).

It was also reported that IRAK4 regulates mRNA stability of various cytokines (82). As with murine IRAK-2, the absence of murine IRAK-4 did not significantly reduce IL-1/TLR-induced NFκB activation, but it did have a significant effect on IL-1-, TLR4- and TLR7-mediated induction of cytokines and chemokines. In addition, the kinase activity of IRAK-4 has been shown to be essential for IL-1/TLR-induced mRNA stability (82). IRAKs themselves are regulated by post transcriptional mechanisms such as microRNAs (miRNAs). miRNAs are small non-coding RNAs that suppress gene expression through binding the 3-UTR of target mRNAs and mir146a has been shown to regulate IRAK-1 and IRAK-2 (97).

7. Future Perspectives

While it is apparent that the IRAK family is essential for IL-1/TLR signalling, the intricate details of how each family member is involved are still emerging. Additionally more and more studies are showing that the role for IRAKs in IL-1/TLR signalling is ligand- and cell type-dependent. This adds a further level of complexity to IL-1/TLR signalling and suggests that signalling pathways are more complex than initially imagined.

Moreover signalling these studies have revealed an emerging potential difference between mouse and human IRAKs. Increasing evidence suggests that the IRAK members function differently in mouse and human. Whether this is due to the experimental systems used rather than a clear species difference remains to be proven. When IRAK-4 is depleted in mice, this dramatically affects responses to a wide range of microorganisms. In contrast, humans with an IRAK-4 deficiency are only susceptible to a narrow range of bacteria. Similarly IRAK-2 in the murine system is thought to be somewhat redundant with IRAK-1 for early post-receptor signalling events, whereas there is increasing evidence for a potential significant role for IRAK-2 in these events in both human cell lines (59) and primary cells (Sinéad Flannery & Andrew Bowie unpublished data). These emerging species differences suggest caution is advised on extrapolating data from experimental murine studies to the human system.

As new research on the IRAK family emerges it is clear that each member plays a vital but non-redundant role in the IL-1/TLR signalling. Thus the IRAK family may be prospective candidates as therapeutic targets in disease (98). Drugs/inhibitors targeting individual IRAKs could be used specifically for a certain IL-1/TLR pathway in certain contexts, while not affecting the entire IL-1/TLR system. This would prevent a treated individual from being susceptible to a wide range of infections. For example inhibition of IRAK-2 would be predicted to block inflammation via NFκB-dependent genes, while leaving the TLR-IFN arm unaffected. Similarly blocking the kinase activity of IRAK-4 would block certain proinflammatory responses by multiple TLR pathways but would still allow the IFNα/β responses. Alternatively targeting the kinase activity of IRAK-1 may block TLR7/9 induction of IFN (an underlying trigger of some autoimmune diseases) while not affecting activation of NFκB.

Interestingly, is has also become clear that the role of the IRAK family is now not restricted to only the IL-1/TLR pathways. Studies now show a role for IRAK-4 in the adaptive immune response and a critical role in T-cell receptor signalling (99, 100). In addition it has been shown that IRAK-4 is required for Th17 differentiation (101). IRAK-1 and IRAK-2 have also been shown to participate in the RIG-1 antiviral pathway upon VSV infection (97). Thus, further ongoing investigations into the roles of the IRAK family members are likely to reveal further surprises, not only in the IL-1/TLR pathway but also in the broader immune response.

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References

1 Akira S, Uematsu S and Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 124, 783-801

2 Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010;19;140(6):805-20.

3 O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev. 2008;226:10-8. Review.

4 Kagan CJ, Su T, Horng T, Chow A, Akira S and Medzhitov, R. TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. Nat Immunol 2008. 9, 361-368

5 Wei T, Gong J Jamitzky F, Heckl WM, Stark RW and Rossle SC. Homolgy modelling of human Toll-like receptors TLR7, 8 and 9 lignad-binding domains. Protein Science 2009 18 1684-1691

6 Gibbard RJ, Morley PJ and Gay NJ. Conserved Features in the extracellular domain of human Toll-like Receptor 8 are essential for pH-dependent signaling. J Biol Chem 2006 281;37

7 Gorden KB, Gorski KS, Gibson SJ, Kedl RM, Kieper WC, Qiu X, Tomai MA, Alkan SS and Vasilakos JP. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. Journal of Immunology 2005 174: 1259-1268.

8 Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D,

Smith DE, Sims JE, Bird TA. and O'Neill LA. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature. 2001 413, 78-83

9 Horng T, Barton GM and Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol. 2001 2, 835-841

10 Oshiumi H, Matsumoto M, Funami K, Akazawa T and Seya T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol 9 2003. 4, 161-167

11 Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K and Akira S. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol. 2003 4, 1144-1150

12 Carty M, Goodbody R, Schroder M, Stack J, Moynagh PN. and Bowie AG. The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. Nat Immunol. 2006 7, 1074-1081

13 Kawai T, Takeuchi O, Fujita T, Inoue J, Mühlradt PF,Sato S, Hoshino K and Akira S. Lipopolysaccharide Stimulates the MyD88-Independent Pathway and Results in Activation of IFN-Regulatory Factor 3 and the Expression of a Subset of Lipopolysaccharide-Inducible Genes. The Journal of Immunology, 2001 167: 5887-5894

14 Neumann D, Kollewe C, Pich A, Cao P, Resch K, Martin MU. Threonine 66 in the death domain of IRAK-1 is critical for interaction with signaling molecules but is not a target site for autophosphorylation. J Leukoc Biol. 2008 Sep;84(3):807-13. Epub 2008 Jun 4.

15 Cao Z, Henzel WJ, and Gao X. IRAK: a kinase associated with the interleukin-1 receptor. Science 1996 271, 1128-1131

16 Rosati O, and Martin MU. Identification and characterisation of murine IRAK-M. Biochem. Biophys. Res. Commun 2003. 293, 1472-1478

17 Feinstein E, Kimchi A, Wallach D, Boldin M, Varfolomeev E. The death domain: a module shared by proteins with diverse cellular functions. Trends Biochem Sci. 1995;20(9):342-4.

18 Kollewe C, Mackensen AC., Neumann D, Knop J, Cao P, Li S, Wesche H and Martin MU. Sequential autophosphorylation steps in the interleukin-1 receptor-associated kinase-1 regulate its availability as an adapter in interleukin-1 signaling. J Biol Chem. 2004 279, 5227-5236

19 Muzio M, Ni J, Feng P and Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. Science. 1997 278, 1612-1615

20 Martin MU and Kollewe C. Interleukin-1 receptor associated kinase-1 (IRAK-1): A self regulatory adaptor molecule in the signalling cascade of the Toll/IL-1 receptor family. Signal Transduction 2001;1-2, 37-50.

21 Meylan E, Tschopp J. IRAK2 takes its place in TLR signaling. Nat Immunol. 2008;9(6):581-2

22 Wang Z, Liu J, Sudom A, Ayres M, Li S, Wesche H, Powers JP, Walker NP. Crystal structures of IRAK-4 kinase in complex with inhibitors: a serine/threonine kinase with tyrosine as a gatekeeper. Structure. 2006;14(12):1835-44.

23 Kuglstatter A, Villaseñor AG, Shaw D, Lee SW, Tsing S, Niu L, Song KW, Barnett JW, Browner MF. Cutting Edge: IL-1 receptor-associated kinase 4 structures reveal novel features and multiple conformations. J Immunol. 2007 1;1 78(5):2641-5.

24 Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, Wu H. Distinct molecular mechanism for initiating TRAF6 signalling. Nature. 2002 25;418(6896):443-7.

25 Wang C, Deng Li, Hong M, Akkaraju GR, Inoue J. and Chen ZJ. TAK-1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 2001 412, 346-351

26 Yi AK and Krieg AM. CpG DNA rescue from anti-IgM-induced WEHI-231 B lymphoma apoptosis via modulation of IκBα and IκBβ and sustained activation of nuclear factor-κB/c-Rel. J. Immunol. 1998;160,1240-1245

27 Beinke S, Robinson MJ, Hugunin M, and Ley SC. Lipopolysaccharide activation of the TPL-2/MEK extracellular signal-regulated kinase mitogen-activated protein kinase cascade is regulated by IκB kinase-induced proteolysis of NFκB p105. Mol. Cell. Biol 2004;9658-9667

28 Zhong J and Kryiakis JM. Dissection of a signalling pathway by which pathogen-associated molecular patterns recruit the JNK and p38 MAPKs and trigger cytokine release. J. Biol. Chem 2007;282, 24246-24254

29 Barnes BJ, Richards J, Mancl M, Hanash S, Beretta L, Pitha PM. Global and distinct targets of IRF-5 and IRF-7 during innate response to viral infection. J Biol Chem. 2004; 22;279(43):45194-207.

30 Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao S and Maniatis T. IKKepsilon and TBK-1 are essential components of the IRF3 pathway. Nat Immunol. 2003; 4:491

31 Chau TL, Gioia R, Gatot JS, Patrascu F, Carpentier I, Chapelle JP, O'Neill L, Beyaert R, Piette J, Chariot A. Are the IKKs and IKK-related kinases

TBK1 and IKK-epsilon similarly activated? Trends Biochem Sci. 2008; 33(4):171-80.

32 Schoenemeyer, A, Barnes BJ, Mancl ME, Latz E, Goutagny N, Pitha PM, Fitzgerald KA and Golenbock DT. The interferon regulatory factor, IRF5, is a central mediator of toll-like receptor 7 signaling. J Biol Chem. 2005; 280, 17005-17012

33 Balkhi MY, Fitzgerald KA, Pitha PM. Functional regulation of MyD88-activated interferon regulatory factor 5 by K63-linked polyubiquitination. Mol Cell Biol. 2008;28(24):7296-308.

34 Uematsu S, Sato S, Yamamoto M, Hirotani T, Kato H, Takeshita F, Matsuda M, Coban C, Ishii KJ, Kawai T, Takeuchi O and Akira S. Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-{alpha} induction. J Exp Med. 2005; 201, 915-923

35 Trofimova M, Sprenkle AB, Green M, Sturgill TW, Goebl MG, Harrington MA. Developmental and tissue-specific expression of mouse pelle-like protein kinase. J Biol Chem 1996; 26;271(30):17609-12.

36 Su J, Richter K, Zhang C, Gu Q, Li L. Differential regulation of interleukin-1 receptor associated kinase 1 (IRAK1) splice variants. Mol Immunol. 2007; 44(5):900-5.

37 Conze DB, Wu CJ, Thomas JA, Landstrom A and Ashwell JD. Lys63-linked polyubiquitination of IRAK-1 is required for interleukin-1 receptor- and toll-like receptor-mediated NF-kappaB activation. Mol Cell Biol. 2008; 28, 3538-3547

38 Huang Y, Li T, Sane DC, Li L. IRAK1 serves as a novel regulator essential for lipopolysaccharide-induced interleukin-10 gene expression. J Biol Chem. 2004; 3;279(49):51697-703.

39 Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity. 1997;7(6):837-47.

40 Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer JL, Di Marco F, French L, Tschopp J. MyD88, an adapter protein involved in interleukin-1 signaling. J Biol Chem. 1998 15;273(20):12203-9.

41 Ross K, Yang L, Dower S, Volpe F, Guesdon F. Identification of threonine 66 as a functionally critical residue of the interleukin-1 receptor-associated kinase. J Biol Chem. 2002 4;277(40):37414-21.

42 Yamin TT. and Miller DK. The interleukin-1 receptor-associated kinase is degraded by proteasomes following its phosphorylation. J Biol Chem. 1997 272, 21540-21547

43 Conze DB, Wu CJ, Thomas JA, Landstrom A and Ashwell JD. Lys63-linked polyubiquitination of IRAK-1 is required for interleukin-1 receptor- and toll-like receptor-mediated NF-kappaB activation. Mol Cell Biol. 2008 28, 3538-3547

44 Windheim M, Stafford M, Peggie M and Cohen P. Interleukin-1 (IL-1) induces the Lys63-linked polyubiquitination of IL-1 receptor-associated kinase 1 to facilitate NEMO binding and the activation of IkappaBalpha kinase. Mol Cell Biol. 2008; 28, 1783-1791

45 Ordureau A, Smith H, Windheim M, Peggie M, Carrick E, Morrice N and Cohen P. The IRAK-catalysed activation of the E3 ligase function of

Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. Biochem J. 2008 409, 43-52

46 Newton K, Matsumoto ML, Wertz IE, Kirkpatrick DS, Lill JR, Tan J, Dugger D, Gordon N, Sidhu SS, Fellouse FA, Komuves L, French DM, Ferrando RE, Lam C, Compaan D, Yu C, Bosanac I, Hymowitz SG, Kelley RF and Dixit VM. Ubiquitin Chain Editing Revealed by Polyubiquitin Linkage-Specific Antibodies. Cell 2008 134 668-678

47 Thomas JA, Allen JL, Tsen M, Dubnicoff T, Danao J, Liao XC, Cao Z, Wasserman SA. Impaired cytokine signaling in mice lacking the IL-1 receptor-associated kinase.. J Immunol. 1999 15;163(2):978-84.

48 Swantek JL, Tsen MF, Cobb MH, Thomas JA. IL-1 receptor-associated kinase modulates host responsiveness to endotoxin. J Immunol. 2000 15;164(8):4301-6

49 Kanakaraj P, Schafer PH, Cavender DE, Wu Y, Ngo K, Grealish PF, Wadsworth SA, Peterson PA, Siekierka JJ, Harris CA, Fung-Leung WP. Interleukin (IL)-1 receptor-associated kinase (IRAK) requirement for optimal induction of multiple IL-1 signaling pathways and IL-6 production. J Exp Med. 1998 15;187(12):2073-9.

50 Li X, Commane M, Burns C, Vithalani K, Cao Z, Stark GR. Mutant cells that do not respond to interleukin-1 (IL-1) reveal a novel role for IL-1 receptor-associated kinase. Mol Cell Biol. 1999;19(7):4643-52.

51 Maschera B, Ray K, Burns K, Volpe F. Overexpression of an enzymically inactive interleukin-1-receptor-associated kinase activates nuclear factor-kappaB. Biochem J. 1999 15;339 (Pt 2):227-31.

52 An H, Hou J, Zhou J, Zhao W, Xu H, Zheng Y, Yu Y, Liu S, Cao X.

Phosphatase SHP-1 promotes TLR- and RIG-I-activated production of type I interferon by inhibiting the kinase IRAK1. Nat Immunol. 2008;9(5):542-50.

53 Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, Bauer JW, Ortmann WA, Koeuth T, Gonzalez Escribano MF, Pons-Estel B, Petri M, Daly M, Gregersen PK, Martin J, Altshuler D, Behrens TW and Alarcon-Riquelme ME. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nat Genet 2006. 38, 550-555

54 Jacob CO, Zhu J, Armstrong DL, Yan M, Han J, Zhou XJ, Thomas JA, Reiff A, Myones BL, Ojwang JO, Kaufman KM, Klein-Gitelman M, McCurdy D, Wagner-Weiner L, Silverman E, Ziegler J, Kelly JA, Merrill JT, Harley JB, Ramsey-Goldman R, Vila LM, Bae SC, Vyse TJ, Gilkeson GS, Gaffney PM, Moser KL, Langefeld CD, Zidovetzki R, Mohan C. Identification of IRAK1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus. Proc Natl Acad Sci U S A. 2009 14;106(15):6256-61.

55 Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, Perry A, Cheng G. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. Nature. 2006 12;439(7073):208-11.

56 Nguyen H, Chatterjee-Kishore M, Jiang Z, Qing Y, Ramana CV, Bayes J, Commane M, Li X, Stark GR. IRAK-dependent phosphorylation of Stat1 on serine 727 in response to interleukin-1 and effects on gene expression. J Interferon Cytokine Res.2003;23(4):183-92.

57 Harte MT, Haga IR, Maloney G, Gray P, Reading PC, Bartlett NW, Smith GL, Bowie A, O'Neill LA. The poxvirus protein A52R targets Toll-like

receptor signaling complexes to suppress host defense. J Exp Med. 2003 3;197(3):343-51.

58 Maloney G, Schroder M and Bowie AG. Vaccinia virus protein A52R activates p38 mitogen-activated protein kinase and potentiates lipopolysaccharide-induced interleukin-10. J Biol Chem. 2005 280, 30838-30844

59 Keating SE, Maloney GM, Moran EM and Bowie AG. IRAK-2 participates in multiple toll-like receptor signaling pathways to NFkappaB via activation of TRAF6 ubiquitination. J Biol Chem. 2007 282, 33435-33443

60 Jiang Z, Zamanian-Daryoush M, Nie H, Silva AM, Williams BR and Li X. Poly(I-C)-induced Toll-like receptor 3 (TLR3)-mediated activation of NFkappa B and MAP kinase is through an interleukin-1 receptor-associated kinase (IRAK)-independent pathway employing the signaling components TLR3-TRAF6-TAK1-TAB2-PKR. J Biol Chem. 2003 278, 16713-16719

61 Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, Picard C, von Bernuth, H, Senechal B, Plancoulaine S, Al-Hajjar S, Al-Ghonaium A, Marodi L, Davidson D, Speert D, Roifman C, Garty BZ, Ozinsky A, Barrat FJ, Coffman RL, Miller RL, Li X, Lebon P, Rodriguez-Gallego C, Chapel H, Geissmann F, Jouanguy E and Casanova JL. Human TLR-7-, -8-, and -9-mediated induction of IFN-alpha/beta and -lambda Is IRAK-4 dependent and redundant for protective immunity to viruses. Immunity. 2005 23, 465-478

62 Kawagoe T, Sato S, Matsushita K, Kato H, Matsui K, Kumagai Y, Saitoh T, Kawai T, Takeuchi O and Akira S. Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. Nat Immunol. 2008 9, 684-691

63 Wan Y, Xiao H, Affolter J, Kim TW, Bulek K, Chaudhuri S, Carlson D, Hamilton T, Mazumder B, Stark GR, Thomas J and Li X. IL-1 receptor-associated kinase 2 is critical for LPS-mediated post-transcriptional control. J Biol Chem 2009; J Biol. Chem 17;284 (16) 10367-75

64 Rosati O, Martin MU. Identification and characterization of murine IRAK-2. Biochem Biophys Res Commun. 2002 13;297(1):52-8.

65 Hardy MP and O'Neill LA. The murine IRAK2 gene encodes four alternatively spliced isoforms, two of which are inhibitory. J Biol Chem. 2004 279, 27699-27708

66 Conner JR, Smirnova II, Poltorak A. A mutation in Irak2c identifies IRAK-2 as a central component of the TLR regulatory network of wild-derived mice. J Exp Med. 2009 6;206(7):1615-31.

67 Wesche H, Gao X, Li X, Kirschning CJ, Stark GR, Cao Z. IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. J Biol Chem. 1999 2;274(27):19403-10.

68 Kobayashi K, Hernandez LD, Galán JE, Janeway CA Jr, Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. Cell. 2002 26;110(2):191-202.

69 Seki M, Kohno S, Newstead MW, Zeng X, Bhan U, Lukacs NW, Kunkel SL and Standiford TJ. Critical role of IL-1 receptor-associated kinase-M in regulating chemokine-dependent deleterious inflammation in murine influenza pneumonia. Journal of Immunology 2010. 184: 1410-1418.

70 Su J, Xie Q, Wilson I, Li L. Differential regulation and role of interleukin-1 receptor associated kinase-M in innate immunity signaling. Cell Signal. 2007;19(7):1596-601.

71 Hammer M, Mages J, Dietrich H, Servatius A, Howells N, Cato AC, Lang R. Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. J Exp Med. 2006 23;203(1):15-20.

72 Su J, Zhang T, Tyson J, Li L. The interleukin-1 receptor-associated kinase M selectively inhibits the alternative, instead of the classical NFkappaB pathway. J Innate Immun. 2009 ;1(2):164-74.

73 Pomerantz, JL and Baltimore D. Two pathways to NF-kappaB. Mol Cell. 2002 10, 693-695

74 Li S, Strelow A, Fontana EJ and Wesche H. IRAK-4: A novel member of the IRAK family with the properties of an IRAK kinase. Proc. Natl. Acad. Sci. U.S.A 2002 99, 5567-5572

75 Suzuki S, Suzuki GS, Duncan DG, Millar T, Wada C, Mirtsos H, Takada A, Wakeham A, Itie S, Li, J. M., Pennnger H, Wesche PS, Ohashi TW, Mak W and Yeh C. Severe Impairement of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature 2002 416,750-756

76 Burns K, Janssens S, Brissoni B, Olivos N, Beyaert R, Tschopp J. Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. J Exp Med. 2003 20;197(2):263-8.

77 Motshwene PG, Moncrieffe MC, Grossmann JG, Kao C, Ayaluru M, Sandercock AM, Robinson CV, Latz E, Gay NJ. An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. J Biol Chem. 2009 11;284(37):25404-11.

78 Kubo-Murai M, Hazeki K, Nigorikawa K, Omoto T, Inoue N, Hazeki OJ Biochem. IRAK-4-dependent degradation of IRAK-1 is a negative feedback signal for TLR-mediated NF-kappaB activation. 2008;143(3):295-302.

79 De Nardo D, Nguyen T, Hamilton JA and Scholz GM. Down-regulation of IRAK-4 is a component of LPS- and CpG DNA-induced tolerance in macrophages. Cell Signal. 2009 21, 246-252

80 Lye E, Mirtsos C, Suzuki N, Suzuki S, Yeh WC. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. J Biol Chem. 2004 24;279(39):40653-8.

81 Kawagoe T, Sato S, Jung A, Yamamoto M, Matsui K, Kato H, Uematsu S, Takeuchi O, Akira S. Essential role of IRAK-4 protein and its kinase activity in Toll-like receptor-mediated immune responses but not in TCR signaling. J Exp Med. 2007 14;204(5):1013-24.

82 Kim TW, Staschke K, Bulek K, Yao J, Peters K, Oh KH, Vandenburg Y, Xiao H, Qian W, Hamilton T, Min B, Sen G, Gilmour R, Li X. A critical role for IRAK4 kinase activity in Toll-like receptor-mediated innate immunity. J Exp Med. 2007 14;204(5):1025-36.

83 Koziczak-Holbro M, Joyce C, Gluck A, Kinzel B, Muller M, Tschopp C, Mathison JC, Davis CN and Gram H.IRAK-4 kinase activity is required for interleukin-1 (IL-1) receptor- and toll-like receptor 7-mediated signaling and gene expression. J Biol Chem. 2007 282, 13552-13560

84 Koziczak-Holbro M, Glück A, Tschopp C, Mathison JC, Gram H. IRAK-4 kinase activity-dependent and -independent regulation of lipopolysaccharide-inducible genes. Eur J Immunol. 2008;38(3):788-96.

85 Qin J, Jiang Z, Qian Y, Casanova JL, and Li X. IRAK-4 kinase activity is redundant for interleukin-1 (IL-1) receptor-associated kinase phosphorylation and Il-1 responsiveness. J. Biol Chem. 2004 279; 40653-40658)

86 Song KW, Talamas FX, Suttmann RT, Olson PS, Barnett JW, Lee SW, Thompson KD, Jin S, Hekmat-Nejad M, Cai TZ, Manning AM, Hill RJ, Wong BR. The kinase activities of interleukin-1 receptor associated kinase (IRAK)-1 and 4 are redundant in the control of inflammatory cytokine expression in human cells. Mol Immunol. 2009;46(7):1458-66.

87 Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, Soudais C, Dupuis S, Feinberg J, Fieschi C, Elbim C, Hitchcock R, Lammas D, Davies G, Al-Ghonaium A, Al-Rayes H, Al-Jumaah S, Al-Hajjar S, Al-Mohsen IZ, Frayha HH, Rucker R, Hawn TR, Aderem A, Tufenkeji H, Haraguchi S, Day NK, Good RA, Gougerot-Pocidalo MA, Ozinsky A and Casanova JL. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science. 2003 299, 2076-2079

88 Ku CL, von Bernuth H, Picard C, Zhang SY, Chang HH, Yang K, Chrabieh M, Issekutz AC, Cunningham CK, Gallin J, Holland SM, Roifman C, Ehl S, Smart J, Tang M, Barrat FJ, Levy O, McDonald D, Day-Good NK, Miller R, Takada H, Hara T, Al-Hajjar S, Al-Ghonaium A, Speert D, Sanlaville D, Li X, Geissmann F, Vivier E, Marodi L, Garty BZ, Chapel H, Rodriguez-Gallego C, Bossuyt X, Abel L, Puel A and Casanova JL. Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. J Exp Med. 2007 204, 2407-2422

89 Puel A, Picard C, Ku CL, Smahi A, Casanova JL. Inherited disorders of NF-kappaB-mediated immunity in man. Curr Opin Immunol. 2004;16(1):34-41

90 Medvedev AE, Lentschat A, Kuhns DB, Blanco JC, Salkowski C, Zhang S, Arditi M, Gallin JI, Vogel SN. Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. J Exp Med. 2003 18;198(4):521-31.

91 Anderson P. Post-transcriptional control of cytokine production. Nat Immunol.2008 9, 353-359

92 Carbello E, Lai WS. and Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-α production by tristetraprolin. Science 1998 281, 1001-1005

93 Fan XC and Steitz JA. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. EMBO J. 1998 17, 3448-3460

94 Lu JY, Sadri N and Schneider RJ. Endotoxic shock in AUF1 knockout mice mediating by failure to degrade proinflammatory cytokine mRNAs Genes Dev 2006; 20 3174-3184.

95 Anderson P. Post-transcriptional regulons coordinate the initiation and resolution of inflammation Nat Rev Immunol. 2010;10(1):24-35.

96 Hartupee J, Li X and Hamilton T. Interleukin 1alpha-induced NFkappaB activation and chemokine mRNA stabilization diverge at IRAK1. J Biol Chem. 2008 283, 15689-15693

97 Hou J, Wang P, Lin L, Liu X, Ma F, An H, Wang Z and Cao X. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. J Immunol. 2009 1;183(3):2150-8.

98 Cohen P. Targeting protein kinases for the development of anti-inflammatory drugs. Current Opinion in Cell Biology 2009 21: 317-324

99 Suzuki N, Suzuki S, Millar DG, Unno M, Hara H, Calzascia T, Yamasaki S, Yokosuka T, Chen N, Elford AR, Suzuki J, Takeuki A, Mirtsos C, Bouchard D, Ohashi PS, Yeh W and Saito T. A critical role for the innate immune signalling molecule IRAK-4 in T-cell activation. Science 2006; 311 1927-1932

100 Suzuki N and Saito T. IRAK-4- a shared NFκB activator in innate and acquired immunity. Trends in Immunology 2006 Vol 27;(12) 566-72

101 Staschke K, Dong S, Saha J, Zhao J, Brooks N, Hepburn D, Xia J, Gulen M, Kang Z, Altuntas CZ, Tuohy VK, Gilmour R, Li X and Na S. IRAK-4 Kinase Activity Is Required for Th17 Differentiation and Th17-Mediated Disease. J. Immunol 2009 183: 568-577

Figure Legends

Figure 1. Functional domains of the human interleukin receptor associated kinases (IRAKs). Each member has a death domain, a proST domain, a conserved kinase domain, and C terminal domain. The only exception is IRAK-4 which lacks a C-terminal domain. For human **IRAK-1,** the death domain contains a critical residue at Thr66 which is important for signalling. The proST domain has been shown to be vital for autophosphorylation. The kinase domain contains Thr209 and Thr387, located in the activation loop, which are potential phosphorylation sites for IRAK-4. The invariant lysine residue in the ATP binding pocket of IRAK-1 is located at K239 and there is a critical aspartate residue at D340, both of which are critical for IRAK kinase function. The tyrosine gatekeeper is located at Y288. The C-terminus contains three TRAF6 binding motifs (E544, E587 and E707). The kinase domain of human **IRAK-2** contains an invariant lysine residue in the ATP binding pocket at K237, which is said to be important for its kinase activity. The tyrosine gatekeeper is located at Y286. Its C-terminal contains two TRAF6 binding motifs (E528 and E559). E528 is critical for IRAK-2 function. The invariant lysine residue of **IRAK-M** is located at K192. The tyrosine gatekeeper is located at Y242. IRAK-M also has a TRAF6 binding motif in its C-terminus (E480). Lastly **IRAK-4** contains a death domain, a proST region and a kinase domain. The invariant lysine residue of IRAK-4 is located at K213. There is a critical aspartate residue at D311 which is essential for IRAK-4 kinase function. Other important residues in IRAK-4 kinase domain include T342, T345, T346. The tyrosine gatekeeper is located at residue Y262.

Figure 2. The role of IRAKs in IL-1/TLR—induced MyD88-dependent activation of NFκB and MAPK.

1) Stimulation of IL-1/TLR, by TLR ligand or IL-1, results in the recruitment of TIR adaptors to the plasma membrane. IRAK4 interacts with MyD88 through death domain interactions. 2) IRAK-4 is thought to phosphorylate both IRAK-1 and IRAK-2 which induces their autophosphorylation activity. 3) Hyperphosphorylated IRAK-1 (and possibly IRAK-2) are released from the receptor complex and subsequently associate with TRAF6. 4) IRAK-1 phosphorylates Pellino which can ubiquitinate IRAK-1. IRAK-1 and Pellino form a complex with TRAF6. 5) NEMO binds to ubquitinated IRAK-1. 6) IRAK-2 induces the polyubiquitination of TRAF6. The polyubiquitination of TRAF6 results in the recruitment of the TAK-1/TAB2 complex and activation of TAK-1. 7) TAK-1 activates the IKK complex. 8) The IKK complex phosphorylates IκBα which allows p65/p50 to translocate in the nucleus.9) For MAP kinase activation, TAK-1 activates MKK3/6 and MKK4/7 for p38 and JNK activation respectively. 10) The IKK complex phosphorylates the inhibitory protein p105. Upon phosphorylation and degradation of p105, tpl2 is activated and subsequently activates MKK1/MKK2. MKK1/MKK2 activate ERK1 and ERK2. (Dashed arows indicate less defined pathways).

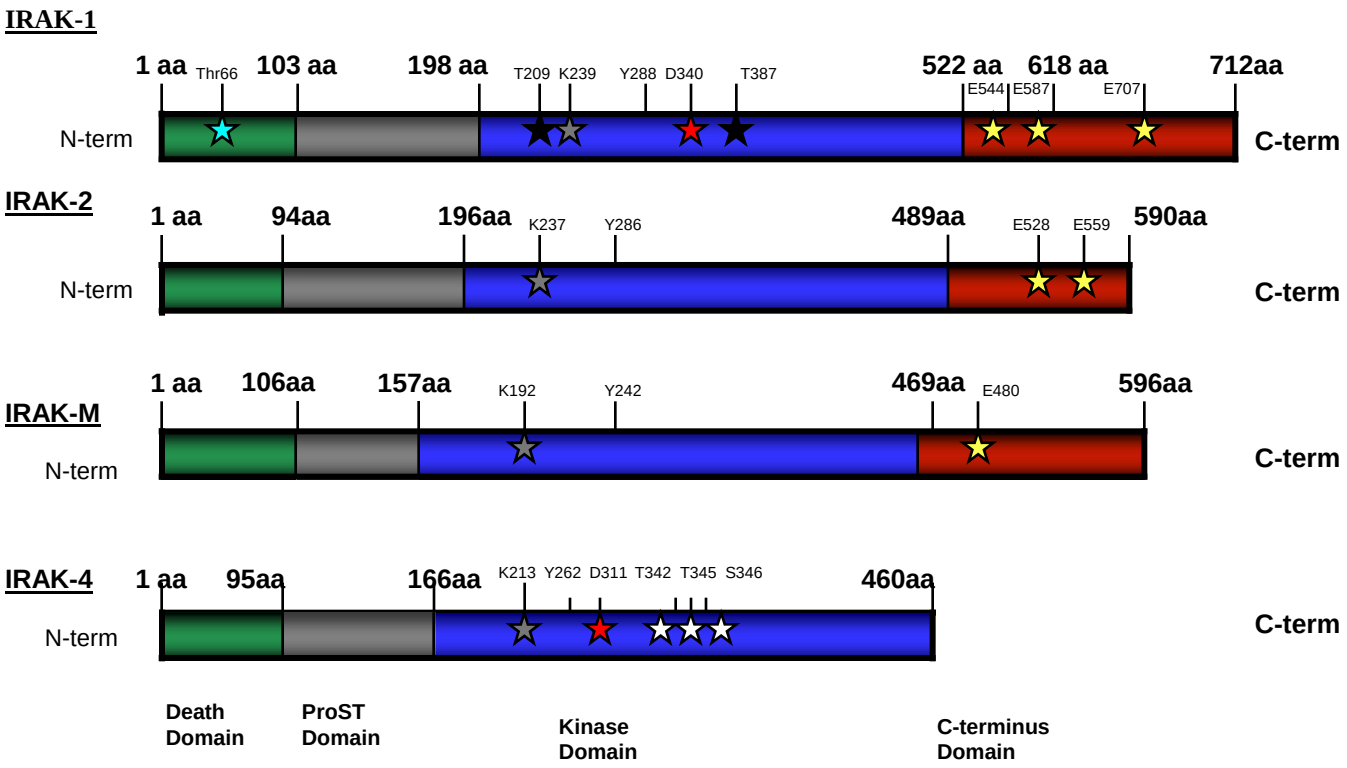
Figure 3. IRAK-dependent and IRAK-independent pathways in TLR-induced IRF activation.

1) Recognition of ds RNA (by TLR3), ssRNA (by TLR7/8) or CpG motifs (by TLR9) in the endosomes of cells results initially in the dimerisation of the TLRs. 2) The adaptor molecules TRIF (for TLR3) or MyD88 (for TLR7/8/9) are recruited to the receptor. The TLRs, TRIF and MyD88 signal via their TIR domains. 3) IRAK-1 and IRAK-4 have been shown to play an important role in the TLR7, 8, 9- MyD88-dependent pathway to IFN but is not thought to have a role in TLR3 activation of IFNs. IRAK-2 is not thought to be involved in the TLR7/8-induced IFN pathway but may have a role in TLR7/8-induced NFκB. 4) IRAK-1 has been shown to stimulate the ubiquitination of IRF5 via TRAF6 both in mouse and human cells. 5) IRAK-1 has also been shown to interact with TRAF3 which plays an important role in IFN activation. 6) IRAK-1 is vital for IRF7 phosphorylation and its kinase activity is vital for IRF7 transcriptional regulation both in mice and human cells. 7) IKKα which was thought to mainly function in NFκB activation has also been shown to play a part in TLR7/9-induced IRF7 activation. 8) For TLR3, activation of the IRFs occurs via TRAF3 and the kinases TBK-1 and IKKε which phosphorylate IRF3 and IRF7. 9) Upon activation, the IRFs translocate into the nucleus and induce the transcription of type I IFN (IFNα and IFNβ). (Dashed arrows indicate less defined pathways. TLR9 omitted from picture but is thought to signal similarly to TLR7/8).

Table 1: Knockout studies showing the effect of absence of IRAKs on IL-1/TLR signalling.

	Phenotype *in vivo*	Effect on NFκB activation	Effect on MAPK	Effect on IRF activation	Effect on mRNA stability
IRAK-1	Partially resistant to LPS-induced septic shock (47)	Partial impairment for IL-1/TLR 4 (47-49)	Impairment for IL-1 /TLR4 (47-49) No impairment of TLR2-induced p38 (70) Dramatic impairment of TLR2-induced ERK and JNK (70)	Effect on TLR7/9-induced IFN (34)	Required for IL-1-induced mRNA stabilisation (96)
IRAK-2	Mice completely resistant to LPS and CpG-induced septic shock (62)	Impairment of late TLR2/TLR7-activation(62) No impairment for TLR4 (63)	Impairment of late TLR2-activation (62)	N/D	Required for TLR4-induced mRNA stability (63).
IRAK-M	Reduced survival upon viral infection (69)	Enhanced activation of TLR4 and TLR9 (68)	Enhanced activation TLR4 and TLR9 (68) Enhanced TLR2-induced p38 (70) No effect on TLR2-induced ERK and JNK (70)	N/D	N/D
IRAK-4	Mice completely resistant to LPS and CpG-induced septic shock (75)	Impairment of IL-1 and MyD88-dependent TLR (61,75) No effect on TLR3 (61)	Impairment of TLR4 induced JNK (75) Impairment of IL-1 induced p38 (75)	Effect on TLR7/8/9-induced IFN (61)	Required for TLR4 and TLR7-induced mRNA stability (82)

Figure 1-3



☆	Important for dimerisation
☆	Important for kinase activity
★	Invariant lysine residue
★	Phosphorylated by IRAK-4
★	Critical asparate residue
★	TRAF6 binding motif

Figure 1

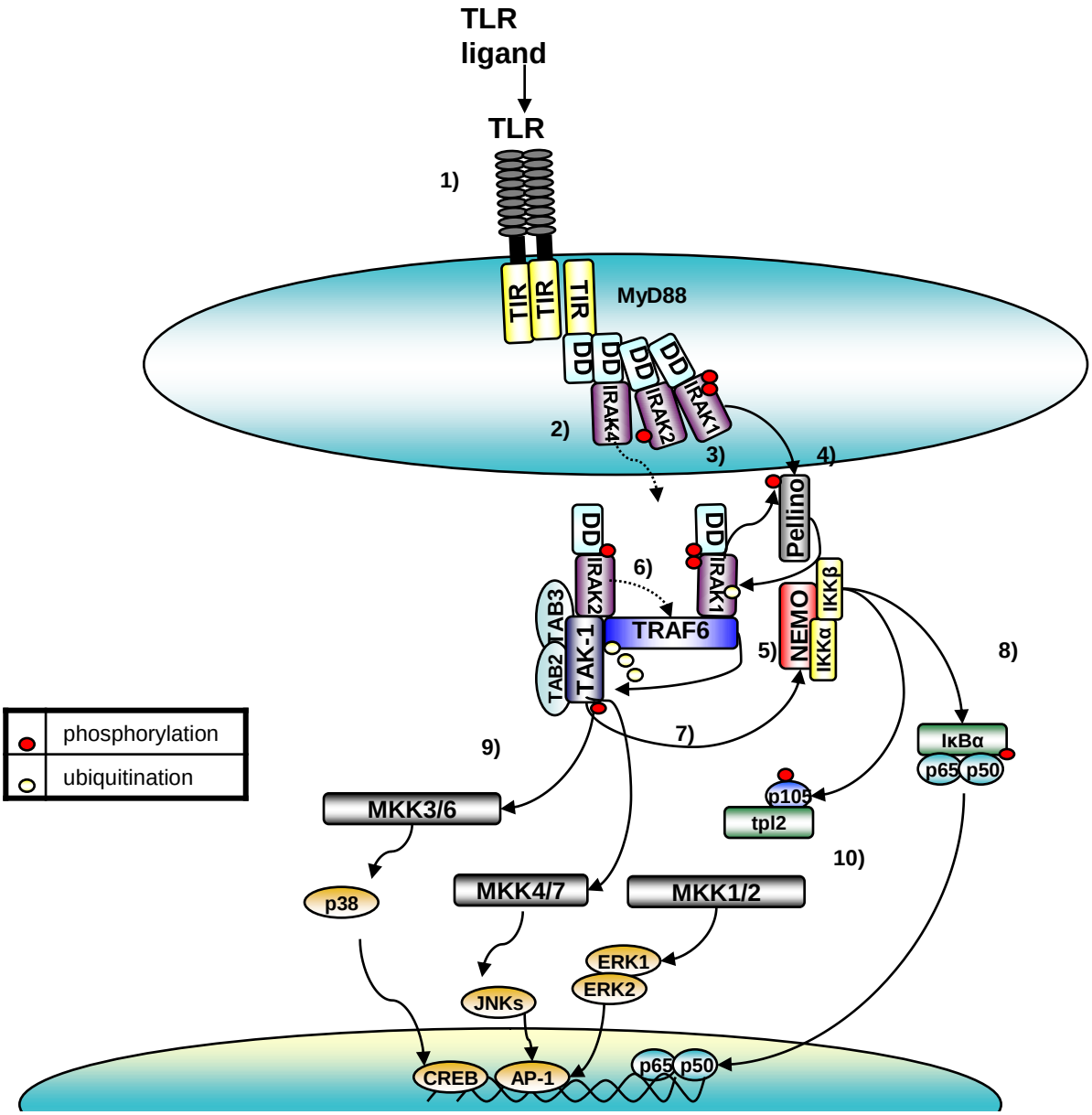
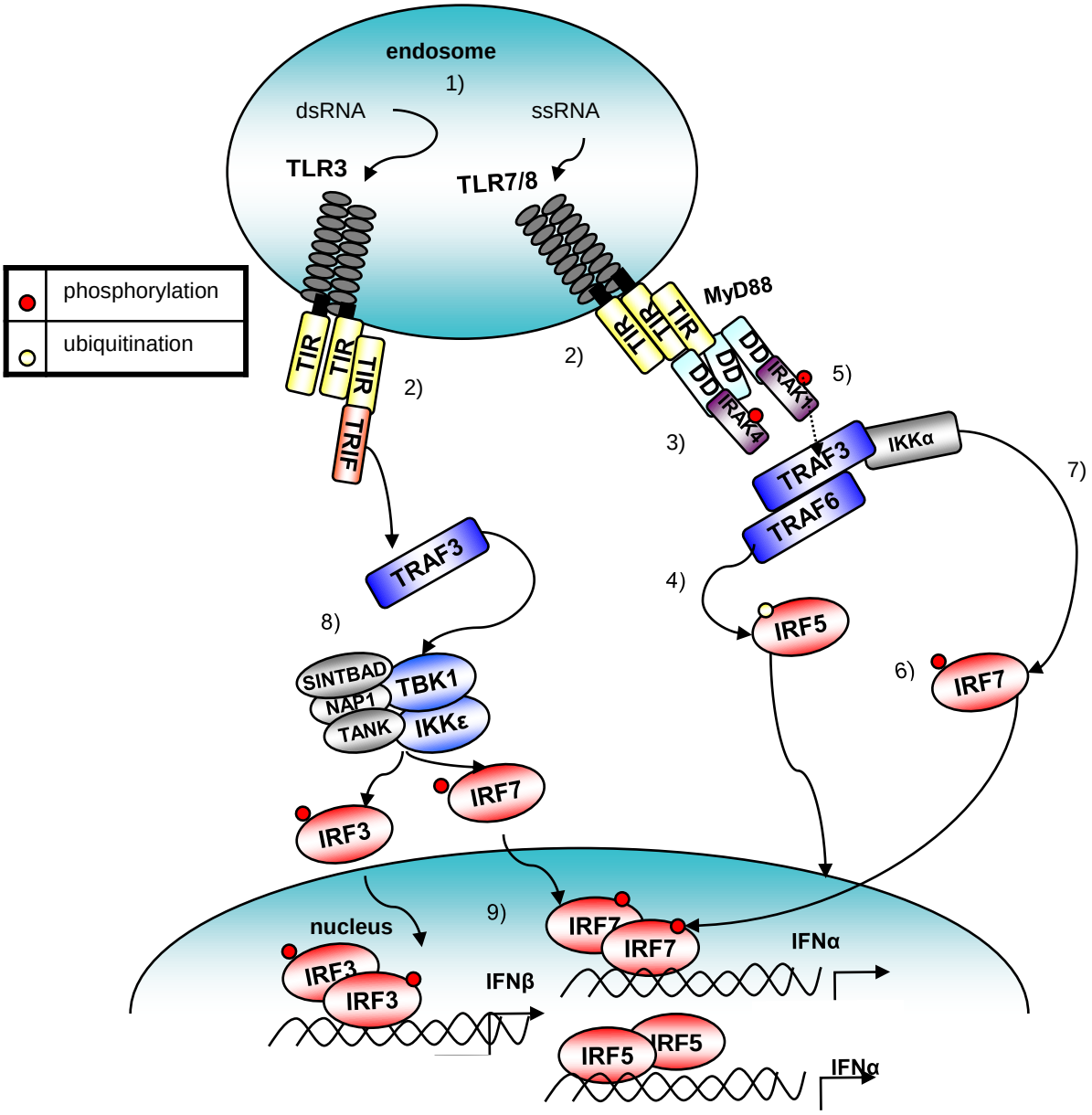

TLR
ligand
TLR
1)
TIR
TIR
TIR
MyD88
DD
DD
DD
DD
IRAK4
IRAK2
IRAK1
2)
3)
4)
Pellino
DD
IRAK2
6)
DD
IRAK1
TAB3
TAB2
TAK-1
TRAF6
NEMO
IKKβ
IKKα
5)
8)
phosphorylation
ubiquitination
7)
9)
IκBα
p65
p50
p105
tpl2
MKK3/6
10)
p38
MKK4/7
MKK1/2
ERK1
ERK2
JNKs
CREB
AP-1
p65
p50

Figure 2


endosome
1)
dsRNA
ssRNA
TLR3
TLR7/8
phosphorylation
ubiquitination
TIR
TRIF
2)
MyD88
DD
IRAK1
IRAK4
5)
3)
TRAF3
IKKα
TRAF6
7)
4)
IRF5
6)
IRF7
TRAF3
8)
SINTBAD
NAP1
TANK
TBK1
IKKε
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IRF3
nucleus
9)
IRF7
IRF7
IFNα
IRF3
IRF3
IFNβ
IRF5
IRF5
IFNα

Figure 3

*Graphical Abstract