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Emerging themes in IFN- γ -induced macrophage immunity by the p47 and p65 GTPase Families

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Abstract

Vertebrates have evolved complex immune specificity repertoires beyond the primordial components found in lower multi-cellular organisms to combat microbial infections. The type II interferon (IFN- γ) pathway represents one such system, bridging innate and acquired immunity and providing host protection in a cell-autonomous manner. Recent large-scale transcriptome analyses of IFN- γ -dependent gene expression in effector cells such as macrophages have highlighted the prominence of two families of GTPases - p47 IRGs and p65 GBPs – that are now beginning to emerge as major determinants of antimicrobial resistance. Here we discuss the recent clarification of known family members, their cellular biochemistry and host defense functions as a means to understanding the complex innate immune response engendered in higher vertebrates such as humans and mice.

Keywords

macrophage; IFN- γ ; p47 IRG; GBP; GTPase; phagosome; autophagy

Introduction

Of the soluble stimuli sensed by mammalian macrophages to trigger antimicrobial activation, few match the effectiveness of interferon-gamma (IFN- γ) (Nathan et al., 1983). IFN- γ is over 100,000-fold more potent in aiding the oxidative burst of human mononuclear phagocytes, for example, than other macrophage-activating cytokines like tumor necrosis factor (TNF- α) or type I IFNs (IFN- α , IFN- β) (Nathan et al., 1984). This outcome is even more remarkable given the relative paucity of IFN- γ receptors on the cell surface - ~4,000–12,000 - well below the number found for most plasma membrane proteins (Pace et al., 1983; Finbloom et al., 1985).

In addition, IFN- γ priming heightens the macrophage response to microbial products such as lipopolysaccharide (LPS) that signal via Toll-like receptors (TLRs) (Schroder et al., 2006) while basal MyD88 activity in turn augments IFN- γ -induced gene expression and mRNA stability (Shi et al., 2003; Sun and Ding, 2006). Thus, IFN- γ can act singly or in synergy with host-derived cytokines or pathogen-derived products to restrict intracellular infections, a nexus

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that makes it one of the most critical of all vertebrate immune pathways enlisted in this fight (Schroder et al., 2006).

The ability of IFN- γ to endow macrophages with the capacity to kill ingested micro-organisms stems largely from the complex transcriptional programs it elicits within these cells (Ehrt et al., 2001). Here, over 1,000 genes may be engaged. Within this group are mRNA transcripts that encode host proteins with long-recognized antimicrobial activity, notably inducible nitric oxide synthase (iNOS/NOS2), phagocyte oxidase and natural resistance associated macrophage protein-1 (NRAMP1) (MacMicking et al., 1997; Nathan and Shiloh, 2000; Skamene et al., 1998).

In addition to these more established mechanisms, two new families of GTPases have emerged that provide cell-autonomous resistance against a variety of bacterial, eukaryotic and viral pathogens. On the basis of molecular mass and guanosine nucleotide-binding activity, these have been labeled the p47 immunity-related GTPases (p47 IRGs) and p65 guanylate-binding proteins (p65 GBPs), respectively (MacMicking, 2004; Taylor et al., 2004; MacMicking, 2005; Vestal, 2005; Martens and Howard, 2006). Members of both groups were initially isolated in piecemeal cloning efforts that spanned decades (see MacMicking, 2004; Vestal, 2005; Martens and Howard, 2006). More recently, however, the application of bioinformatics has begun to usher in a new era of understanding the rich genetic diversity and powerful biological functions of these novel host defense factors.

Genes and genomes

Refined annotations suggest 23,000–26,000 genes in humans and 28,000–30,000 genes in mice (Venter et al., 2001; Waterston et al., 2002); ~5–10% are thought to subsume immune-related activities (Venter et al., 2001). A particularly striking feature of the latter category is the large number of multigene families or even superfamilies present (Venter et al., 2001). One common explanation for such preponderance is that strong evolutionary pressure leads to immune gene expansion in the face of microbial diversity (Sabeti et al., 2006). Both the p47 IRG and p65 GBP families may be good examples of this, especially in mice, while in humans alternative splicing appears to be the preferred mechanism for generating additional protein isoforms (MacMicking, 2004; Vestal, 2005; Martens and Howard, 2006; MacMicking et al., unpublished).

In silico analyses posit as many as 23 p47 *Irg* and 6 p65 *Gbp* loci in the euchromatic mouse genome (Bekpen et al., 2005). Humans, in contrast, appear to possess 2 *IRG* and 7 *GBP* genes (Bekpen et al., 2005) (Fig. 1). Numerical disparity between the mouse and human IRG families can be explained in part by duplicative expansion of a common *Irga* ancestor on mouse chromosome 18 and the existence of multiply-spliced transcripts for human *IRGs* (eg. *IRGM*) (Bekpen et al., 2005; MacMicking et al., unpublished). In fact the sizeable mouse *Irg* family appears anomalous among vertebrates: rats, for example, possess only 7 *Irg* genes, with dogs, zebrafish and pufferfish harboring 9, 11 and 2 members, respectively (Bekpen et al., 2005; MacMicking et al., unpublished). Reexamination of the current mouse genome also suggests that the total number of original *Irg* entries may be an over-estimate, while in the case of the *Gbps*, more members have arisen (MacMicking et al., unpublished).

For the p47 IRG family, pseudogenic ascriptions are now predicted for *Irgal* (LOC546714) and *Irga8* (LOC667597) while an additional *Irg* gene, *Irg11*, lies between *Irgb7* and *ligp1/Irga6* genes on chromosome 18 (see Fig. 2). Moreover, re-assembly of mouse chromosome 11 indicates that the *Irgb3/4* and *Irgb5/6* regions are probably not duplicated as first thought (Bekpen et al., 2005) (Fig. 2). Previous analysis also divided a number of GTPase bidomain proteins into smaller single domain *Irg* genes (Bekpen et al., 2005). Newer EST deposits, however, do not support this partition. Instead larger single domain proteins or even entire in-

frame ORFs with tandem GTPase domains (e.g. AK145236) appears more likely. These larger Irg proteins could conceivably function as dimers or co-operatively regulate the activities of monomers within an oligomeric complex, biochemical behavior reminiscent to that already seen for Iigp1/Irga6 (Uthaiah et al., 2003). As such we have considered Irgb1/Irgb2, Irgb3/Irgb5 and Irgb8/Irgb9 groupings as bidomain or bigger single domain p47 Irg GTPases and thus refer to them here as Large-Irg proteins 1–3 (*Lirg1-3*) with a predicted M_r of ~95 kDa (Fig. 2).

For the GBP family, several additional mouse orthologs appear within pre-existing clusters on chromosome 5 (Fig. 2). Hereafter these have been termed *Gbps-7* through *-12* (Figs. 1, 2). On chromosome 3, an additional gene 3' to *Gbp2* appears intact and has been called *Gbp13* (EG621605) (Figs. 1, 2). Phylogenetic representation of the p65 GBP G-domains indicates that as a family they are more interrelated than are the p47 IRGs (Fig. 1). Moreover, interspecies comparison between mouse and human GBPs suggests a relatively recent evolution of these highly homologous genes as compared to the p47 IRGs (Olszewski et al., 2006). Reasons for this divergence are unknown but could include specific pathogen-host range dependencies influencing one family or host species more than the other (Frank, 2002). Alternatively, a large number of disparate murine *Irg* genes may be needed if they also assume non-immune activities. To date such activities have not been found (see MacMicking, 2004; Taylor et al., 2004; Martens and Howard, 2006) but it remains an issue for investigation.

Stretches and structures

At the primary sequence level, both the p47 IRGs and p65 GBPs are defined by a single stretch of 200–300 amino acids encompassing the guanosine nucleotide-binding or G-domain (Fig. 3A and B). p47 IRGs share ~40–80% similarity within this domain while GBPs exhibit ~55–80% similarity across the corresponding 280 residue stretch. Interfamily relatedness, however, is only ~20–25% over the first 4 motifs (G1-G4) of the G-domain, a difference that may help account for their disparate nucleotide-binding specificities.

Prototypic G-domains exemplified by Ras and the heterotrimeric G-proteins act as molecular switches with distinct conformations in their GTP- and GDP-bound states (Vetter and Wittinghofer, 2001). Proteins are often active in their GTP-bound form and can be inactivated by specific GTPase activating proteins (GAPs) that accelerate the conversion of GTP to GDP. Attendant conformational changes in the G-domain are then transmitted to proximal domains or interacting effector proteins, bringing about GTPase-dependent regulation of output function (s). The p47 IRGs hydrolyze GTP to GDP while certain GBPs (hGBP1 and hGBP2) convert GDP further to GMP (Schwemmle and Staeheli, 1994; Neun et al., 1996; Taylor et al., 1996; Carlow et al., 1998; Han et al., 1998; Uthaiah et al., 1999; Tiwari and MacMicking, unpublished). Two-step hydrolysis is unusual for a G-domain and insights into the reaction mechanism have been obtained from crystal structures of transition-state analog-bound hGBP1 (Prakash et al., 2000a; Prakash et al., 2000b) and its isolated catalytic region (Ghosh et al., 2006) (Fig. 3A and B). An ability to bind GMP is a unique feature of the GBPs compared with other GTPases, although the physiological relevance of this nucleotide preference is still unclear.

Several evolutionarily conserved sequence motifs - labeled G1 to G5 - are involved in binding the phosphate moieties (G1,G2,G3) or purine ring (G4,G5) of GTP, co-ordinating Mg^{2+} at the active site (G1,G2,G3) and regulating effector functions (G2 and G3) (Sprang, 1997; Vetter and Wittinghofer, 2001). Each in turn is considered below.

The G1-motif, also called the glycine-rich loop, P-loop or the Walker-A motif, corresponds to $GxxGx^K/M_S$ and $GXXRxKS$ in the p47 IRGs and p65 GBPs, respectively (Fig. 3C). Mutation of the last G1 lysine or serine residue leads to profound loss of GTPase activity as noted for

hGBP1 (Lys₅₁, Ser₅₂) (Praefcke et al., 2004). This is because Lys₅₁ binds the β/γ -phosphate of GTP whereas in its absence a polar bond with Thr₉₈ (G3) is formed. Other G1 residues also contribute to activity: hGBP1 Arg₄₈ is required for transactivation and cooperative GTPase activity of the homodimeric structure (Ghosh et al., 2006). This mechanism of activation is analogous to that brought about by arginine-fingers in GAPs (Vetter and Wittinghofer, 2001; Scheffzek and Ahmadian, 2005). The crystal structure of Iigp1/Irga6 also highlights the requirement for Lys₇₈ in binding the γ -phosphate and Mg²⁺ (Ghosh et al., 2004). Interestingly, 3 mouse *Irg* proteins (Lrg-47/Irgm1, Gtpi/Irgm2, Igtp/Irgm3) and 1 putative human ortholog (IRGM) possess methionine instead of lysine at this position although any detrimental effects of this substitution on enzyme activity have not been found (Taylor et al., 1996; Tiwari and MacMicking, unpublished).

A role for the G2 loop in folding back on the active site nucleotide to form a phosphate cap is seen for GBPs but not p47 IRGs (see Fig. 3B; Prakash et al., 2000b). The invariant threonine normally involved in binding the γ -phosphate and Mg²⁺ as part of a switch-I region is missing in IRGs. Instead, residues within the G3 motif (Iigp1/Irga6 Asp₁₂₆ for example) may take on the role of co-ordinating Mg²⁺ (Ghosh et al., 2004). This residue is conserved in hGBP1 (Thr₇₅; switch-I) and its mutation leads to near-inactivation of the enzyme (Praefcke et al., 2004). Another critical catalytic residue adjacent to the G3 motif (Gln₆₀ in Ras) is absent in both p47s and GBPs, pointing to a divergent reaction mechanism in these GTPases (Praefcke et al., 2004).

Lastly, the G4 region in p47 IRGs and p65 GBPs can be represented as Y_{/D}FFVWTxxD with non-canonical substitutions outside the classical N_{/K}TxD motif giving rise to different substrate-binding capacities (Fig. 3C). In Iigp1/Irga6, G4 Lys₁₈₄ and Asp₁₈₆ make specific contacts with the nucleotide (Ghosh et al., 2004). For hGBP1, additional conserved residues in the G4 motif termed the arginine-aspartate ('RD') pair also contribute to GTP binding (Fig. 3B). A loose G5 motif (SA^{K/L}) corresponding to Ser₂₃₁-Lys₂₃₃ is found in Iigp1/Irga6 (Ghosh et al., 2004) but is absent in the GBPs. The latter instead employ a unique guanine cap composed of two short helices to allow for tight substrate binding (Fig. 3) (Ghosh et al., 2006).

These differences in the GTP-binding pocket of GBPs lead to a significant change in the active site nucleotide N-glycosidic bond angle ($\sim -75^\circ$) compared to that in Ras ($\sim -112^\circ$) (Ghosh et al., 2006). Moreover, the torsion angle at the C4'-C5' bond in the ribose moiety undergoes a major change to the extent that the α -phosphate in the GMP/AlF₃-bound structure is in a position occupied by β -phosphate in the GDP/AlF₄⁻ bound structure (Ghosh et al., 2006). The implications of this conformational change are twofold: first, flexibility brought about in the nucleotide may allow GBPs to convert GTP to GMP, and second, GDP hydrolysis involves the same reaction mechanism as the hydrolysis of GTP (Ghosh et al., 2006).

Apart from the G-domain, motifs for post-translational lipid modifications are noted for several members of both the p47 IRG and p65 GBP families. Here a C-terminal CaaX motif targets human and mouse GBPs1, 2 and 5 for isoprenylation (Vestal, 2005) and an N-terminal myristoylation site (MGxxxS) is predicted in 11 mouse *Irga* subfamily proteins (Bekpen et al., 2005). At least in the case for Iigp1/Irga6, mutation of the second N-terminal glycine residue leads to diminished Golgi membrane binding (Martens et al., 2004).

Regulation and regulators

The p47 IRGs and p65 GBPs are among the most abundant genes activated upon exposure of cells to IFN- γ . For the p47 IRGs, Lrg-47/Irgm1 and Iigp1/Irga6 can also respond directly to IFN- α/β (Sorace et al., 1995; Zerrahn et al., 2002), while these two members plus Igtp/Irgm3 and Iigp1/Irga6 are solicited by indirect signaling with lipopolysaccharide (LPS) (Zerrahn et

al., 2002; Lapaque et al., 2006). To date most p47 proteins are not induced by other inflammatory cytokines such as interleukin (IL)-1 α , IL-1 β , IL-2, IL-4, IL-6 and TNF- α (see MacMicking, 2004; Martens and Howard, 2006). STAT1-dependent induction of p47 members after IFN stimulation is based on the identification of GAS sites (γ IFN-activated sites; GAS, Fig. 4) in their promoters and is supported by studies from STAT1^{-/-} (Collazo et al., 2002; Gavrilescu et al., 2004; Lieberman et al., 2004) and phosphorylation-deficient STAT^{S727A} mice (Varinou et al., 2003).

Many p47 IRG promoters also contain the interferon stimulated response elements (ISRE) bound by the heterotrimeric STAT-1/STAT-2/IRF-9 (interferon-regulatory factor-9) transcription factor complex (called interferon stimulated gene factor (ISGF)-3 (Honda and Taniguchi, 2006; Fig. 4). To date only the mouse *Irg-47/Irgd* promoter ISRE and YY1 motifs have been verified biochemically to recruit this transcription factor complex (Gilly et al., 1996). The ISRE is indistinguishable from the binding site for the IRF-1 transcription factor (Honda and Taniguchi, 2006), and in the absence of IRF-1 expression, several p47 IRGs are compromised (Boehm et al., 1998). ISGF3 binding may also require phosphorylation of STAT1 via an I κ B kinase- ϵ (IKK- ϵ) signaling cascade as seen for *Irg-47/Irgd*, *Ilgp1/Irga6* and *Gtp1/Irgm2* in *Ikkbe*^{-/-} fibroblasts (tenOever et al., 2007). For the recently-cloned human IRGs no evidence exists regarding immunologic induction and the canonical transcription factor binding sites outlined above appear absent within 1–2kb upstream of the start site (Belkpen et al., 2005; MacMicking et al, unpublished). Thus the human and mouse p47 IRGs diverge considerably in terms of their induction.

Unlike the p47 IRGs that are primarily immediate-early genes, expression of the p65 GBPs require *de novo* protein synthesis and secondary transcription factors such as IRF-1 that recognize ISREs in their promoters (Fig. 4). As compared to IFN- γ , type I interferons are stronger inducers of the ISGF-3 complex, which also bind ISREs; thus human p65 GBPs can be induced by IFN α/β (Colonno and Pang, 1982) although the response to this cytokine is much weaker in mouse macrophages (Boehm et al., 1998; Kim and MacMicking, unpublished). Induction of murine GBPs by IFN- γ can be augmented by TNF- α , IL-1 or LPS (Nguyen et al., 2002) and in the case of hGBP1, TNF- α and IL-1 β were shown to act through the NF- κ B binding site in its promoter (Naschberger et al., 2004).

Early studies using luciferase reporters in fibroblasts and differentiated embryonic stem cells from IRF-1^{-/-} mice demonstrated the requirement of IRF-1 for *Gbp2* induction (Lew et al., 1991; Briken et al., 1995). More recent molecular efforts have dissected the contributions of STAT1 and IRF-1 in the transcriptional induction of GBPs. Here STAT1 dimers are responsible for binding and recruiting accessory transcription co-activators such as the histone acetyl transferase, CREB-binding protein (Varinou et al., 2003), histone deacetylase-1, a presumed helicase from the mini chromosome maintenance (MCM) complex (Snyder et al., 2005), and BRG1, an ATPase of the key SWI/SNF complex (Pattenden et al., 2002; Ni et al., 2005). These studies allude to the possibility that STAT1 binds the *Gbp2* promoter in a BRG1-dependent manner and by recruiting HAT prepares the chromatin for subsequent IRF-1-driven transcription.

LPS-dependent induction of p47 IRG proteins is primarily via TLR4 and is MyD88-independent, enlisting the Toll/IL-1 receptor (TIR)-domain-containing adaptor inducing IFN- β (TRIF), IKK- ϵ and TANK-binding kinases (TBK1), and lastly, IRF3 (Doyle et al., 2002; Shi et al., 2003; Hemmi et al., 2004; Weighardt et al., 2004). Such induction is also more responsive to classical LPS agonists that carry a bisphosphorylate pyranose moiety than non-classical lipid A species belonging to pathogens such as *L. pneumophila* RC1 or *B. melitensis* (Lapaque et al., 2006). Other bacterial cell wall products – lipoarabinomannans from *Mycobacterium* spp. or lipoteichoic acid from Gram-positive bacteria – also display stimulatory p47 IRG activity,

most likely as an indirect result of type I IFN secretion (MacMicking et al., 2003; McCaffrey et al., 2004).

Residence and residents

IFN- γ -inducible GTPases reside within lipid microdomains or cytoskeletal scaffolds that may help partition signaling complexes involved in intracellular traffic. Here they could also recruit binding partners to fulfill their antimicrobial functions. Targeting to membrane-bound compartments enlists isoprenylation for hGBP1, mGbp1 and mGbp2, and myristoylation for Iigp1/Irga6 (Nantais et al., 1996; MacMicking, 2004; Martens et al., 2004; Modiano et al., 2005; Vestal, 2005). A reliance on post-translational modification presumably stems from the fact that neither p47 IRGs nor p65 GBPs contain predicted transmembrane regions, especially given that membrane-association is emerging as the rule for most members of both GTPase families.

Organellar membranes to which p47 IRGs have been found to localize include Igtp/Irgm3 to the endoplasmic reticulum (ER) (Taylor et al., 1997), Iigp1/Irga6 to the ER and Golgi (Zerrahn et al., 2002; Martens et al., 2004), and Lrg-47/Irm1 to the ER, *cis*-Golgi, phagocytic cups and plasma membrane (MacMicking et al., 2003; Martens et al., 2004). For the GBPs, hGBP1 resides in the ER and Golgi (Modiano et al., 2005) and mGBP2 on unidentified intracellular vesicles (Vestal et al., 2000). Additionally, several mouse GBPs localize to distinct subpopulations of intracellular vesicles not unlike autophagosomes (Kim and MacMicking, unpublished).

Members of the p47 IRG family can translocate to specific pathogen compartments from their resting locations as first noted for Lrg-47/Irm1 which travels via a brefeldin A-sensitive pathway to the *M. tuberculosis* phagosome (MacMicking et al., 2003). Here it is thought to facilitate the cytokine-stimulated phagosome maturation cascade which may include recruitment of the autophagic machinery (MacMicking et al., 2003; Gutierrez et al., 2004). Later studies have also observed Lrg-47/Irm1 on nascent *Listeria monocytogenes* phagosomes within 10–15 min after uptake (Matsuzawa and MacMicking, unpublished; see Fig. 5) and the presence of Igtp/Irgm3, Gtpi/Irgm2, Iigp1/Irga6 and Tgtp/Irgb6 on *Toxoplasma gondii* parasitophorous vacuoles (Martens et al., 2005; Martens and Howard, 2006).

Steady-state organellar residence and phagosome recruitment relies on a bidomain structure for p47 IRGs. An isolated G-domain of Lrg-47/Irm1 and Iigp1/Irga6 can localize to the plasma membrane, unlike the full-length protein that is retained in the ER/Golgi (Martens et al., 2004). The region responsible for Lrg-47/Irm1 retention lies in a C-terminal amphipathic helical domain that is shared with Gtpi/Irgm2 and Igtp/Irgm3 and can confer correct localization on GFP chimeras (Martens et al., 2004). Amino acid substitutions which destroy amphipathicity of this helix prevent the LRG-47/Irm1 α K fragment or holoenzyme from targeting the *cis*-Golgi (Martens et al., 2004). These same mutations interfere with binding of Lrg-47/Irm1 to specific phosphoinositols generated on internal membranes, providing an additional reason for failure to localize properly (Tiwari and MacMicking, unpublished). In contrast, the Iigp1/Irga6 α K does not seem to contain membrane-targeting sequences; instead, an N-terminal myristoylation site when mutated leads to complete partitioning into the aqueous phase (Martens et al., 2004).

Golgi/ER localization of Lrg-47/Irm1, Iigp1/Irga6 and Igtp/Irgm3 appears independent of GTP hydrolysis as seen from proteins with null mutations in the G1 motif (Taylor et al., 1997; Martens et al., 2004). Lrg-47/Irm1 relocation to the plasma membrane during phagocytosis and to mycobacterial phagosomes, however, may require GTP binding, as does targeting of Iigp1/Irga6 to *T. gondii* vacuoles (Martens et al., 2004; Martens and Howard,

2006; MacMicking, unpublished). Remarkably, none of the aforementioned trafficking events appear to enlist IFN- γ -induced accessory factors.

Compared with the p47 GTPases, p65 GBPs differ in that hGBP1 requires GTP binding, other IFN- γ -stimulated proteins and isoprenyl modification for Golgi residence (Modiano et al., 2005). Moreover, oligomerization status of the native, GTPase- and farnesylation-mutant proteins affects localization of hGBP1 in these cells. For mGbp2, the need for enzyme activity in organelle targeting is less clear. Here a presumed inactive (S52N) mutant localizes to punctuate vesicles in a manner similar to that of the parent protein (Gorbacheva et al., 2002). Additionally, our own studies in murine macrophages suggest that several other GBPs localize to distinct intracellular vesicles and are likely to be membrane bound despite lacking canonical isoprenylation or palmitoylation/myristoylation motifs in their primary sequence (Fig. 5; Kim and MacMicking, unpublished).

Identifying binding partners or interactions with resident proteins will undoubtedly provide some insight into the sub-cellular behavior of these GTPases. Yeast two-hybrid efforts have retrieved the Golgi protein hook3 as an interacting partner for Iigp1/Irga6 and the fatty-acid binding protein ADRP for Irg-47/Irgd (Kaiser et al., 2004; Yamaguchi et al., 2006). Hook3 interaction with Iigp1/Irga6 required the latter to be in a GTP-bound conformation as demonstrated by the G1 motif S83N mutant. Hook3 itself is a novel microtubule binding protein with four coiled-coiled domains, the fourth of which associates with Iigp1/Irga6, and treatment of cells with the microtubule-disrupting agent nocodazole abolished *in vivo* binding (Kaiser et al., 2004). The functional consequences of this interaction have not been explored further; however, it is known that the *Salmonella* type III secretion system effector, SpiC, targets hook3 to prevent phagolysosome fusion (Shotland et al., 2003). Thus Iigp1/Irga6 may be part the host cellular machinery needed to control trafficking or remodeling of this organelle.

C-terminal binding of Irg-47/Irgd to ADRP is suggestive of a role for p47 IRGs in intracellular lipolysis although little overlap within cells was noted for these two proteins (Yamaguchi et al., 2006). Involvement of the Irg-47/Irgd C-terminus may also argue against a common mechanism shared by other p47 IRGs since this region is the least conserved across family members. Further experiments are therefore needed to establish the relevance of this partner for p47 IRG-mediated antimicrobial activity. At the time of writing, no binding partners for members of the p65 GBP family have been isolated.

Microbes and macrophages

A single vertebrate species can serve as host to an estimated 1,400 phylogenetically distinct pathogens (Mascie-Taylor and Karim, 2003). Pathogen discrimination via TLRs and nucleotide-oligomerization domain-leucine rich repeat (NOD-LRR) proteins help reduce this complexity, yet recognition alone does not guarantee microbial clearance. This is because many of the effector proteins needed for killing are invoked by a different set of signals. Such is the case in *M. tuberculosis*-infected macrophages (Shi et al., 2003). Here, TLR stimulation itself has no direct effect on bacterial viability although it can enhance IFN- γ -induced activation of genes that subsequently curtail growth (Shi et al., 2003). Thus PRRs are often required to work in tandem with IFN- γ -responsive proteins to limit infection at the level of the individual cell (Schroder et al., 2006).

Both the p47 IRGs and p65 GBPs fall into this latter category; their over-expression confers cell autonomous protection while their removal renders IFN- γ -activated macrophages more permissive for microbial replication. Initial evidence for a role of p47 IRGs in macrophage-mediated killing came from studies of the classic facultative intracellular pathogen, *Mycobacterium tuberculosis* (MacMicking et al., 2003). Here, genetic removal of Irg-47/Irgm1 led to cell-autonomous defects at the level of the activated macrophage which have been

corroborated using siRNAs against the murine gene and possibly a related human orthologue, IRGM, although the latter needs independent confirmation (Singh et al., 2006).

Other pathogens targeted by the Lrg-47/Irgm1 pathway within macrophages include the protozoan parasites *Toxoplasma gondii* (Butcher et al., 2005) and *Trypanosoma cruzi* (Santiago et al., 2005). Additional members of the p47 IRG family operate to curtail *T. gondii* growth including Igtp/Irgm3 and Iigp1/Irga6 (Halonen et al., 2001; Bernstein-Hanley et al., 2006; Ling et al., 2006) while both p47 IRGs help restrict *Chlamydia trachomatis* replication in epithelia and embryonic fibroblasts, respectively (Nelson et al., 2005; Bernstein-Hanley et al., 2006). For the latter bacterium, a third p47 IRG gene - *Irgb10* – was recently mapped to the susceptibility locus *Ptrq-3* and can complement resistance *in trans* to C3H/HeJ susceptible host cells (Bernstein-Hanley et al., 2006). Thus members of the p47 IRG family may act in concert to provide effective host defense.

Defects in cell-autonomous p47 IRG activity are also manifested at the level of the whole organism. Lrg-47/Irgm1^{-/-} mice, for example, fail to control *M. tuberculosis*, *M. avium*, *L. monocytogenes*, *S. typhimurium*, *T. gondii*, *T. cruzi*, and *L. major* infections; Igtp/Irgm3^{-/-} mice are vulnerable to *T. gondii* and *C. trachomatis* while Irg-47/Irgd and possibly Iigp1/Irga6 are needed for resistance to *T. gondii* as well (reviewed in MacMicking, 2004; Taylor et al., 2004; Martens and Howard, 2006). Interestingly, none of these deficiencies led to compromised anti-viral activity, although MCMV and Ebola have been the only viruses examined to date (Taylor et al., 2000; Collazo et al., 2001). Earlier over-expression studies had implicated a role for Tgtp/Irgb6 against VSV (Carlow et al., 1998) and Igtp/Irgm3 against coxsackievirus B3 (Zhang et al., 2003) but it was unclear whether these effects were due to direct antiviral activity or enhanced non-cytopathic cell survival.

On the basis of the above susceptibility studies it is clear that p47 IRGs can respond to pathogens inhabiting a range of membrane-bound compartments -phagosomes, parasitophorous vacuoles and inclusion bodies. An emerging consensus is therefore one in which the activities of these small GTPases impact the biogenesis or remodeling of the pathogen-containing niche. Studies from several groups have documented the presence of endogenous p47 IRGs on such organelles: Lrg-47/Irgm1 to *M. tuberculosis* and *L. monocytogenes* phagosomes (MacMicking et al., 2003; Matsuzawa and MacMicking, unpublished; see Fig. 5), Gtpi/Irgm2, Igtp/Irgm1, Iigp1/Irga6 and Tgtp/Irgb6 to *T. gondii* parasitophorous vacuoles (Butcher et al., 2005; Martens et al., 2005; Ling et al., 2006) and Iigp1/Irga6 to *C. trachomatis* inclusion bodies (Nelson et al., 2005). Where tested, p47 IRG recruitment correlated with host protection.

Once relocated to the site of microbial replication, p47 IRGs may help facilitate fusion with lysosomes by soliciting components of the autophagic machinery (MacMicking et al., 2003; Gutierrez et al., 2004; Ling et al., 2006; Singh et al., 2006), disrupt the vacuolar membrane (Martens et al., 2005; Ling et al., 2006) or target lipid membrane intermediates (Nelson et al., 2005). Each could reflect contingent steps in a series of events, for example, membrane disruption invoking autophagic engulfment prior to eventual fusion with lysosomes. That the p47 IRGs participate in this cascade specifically during infection is reinforced by recent experiments on mannose receptor-mediated latex bead internalization; here, general phagocytosis did not require Lrg-47/Irgm1, Igtp/Irgm3 or Irg-47/Irgd (Yates et al., 2007).

Precise modes of p47 IRG action could also be dictated by the specific pathogen encountered or the presence of additional p47 IRGs in the vicinity. Antimicrobial activity requires GTP hydrolysis, at least in the case of Iigp1/Irga6 and Lrg-47/Irgm1, suggesting they are likely to engage other proteins or even members of the same family to bring about these changes (Martens et al., 2005; Martens and Howard, 2006; Choi and MacMicking, unpublished).

Because the few p47 IRG-interacting partners isolated so far span several trafficking pathways, it is conceivable that a given p47 GTPase fulfills more than one task, from postER/Golgi cargo transport to assembly of pre-fusion pore complexes on the nascent phagosomal membrane.

An absence of binding partners for the p65 GBPs and a penchant for self-assembly implies a different mechanism may be employed for this family. The antimicrobial profile of p65 GBPs is also less well characterized, to date having been restricted to viruses. In ectopic over-expression studies, hGBP1 inhibited VSV, hepatitis C and encephalomyocarditis viruses by up to 50% (Anderson et al., 1999; Carter et al., 2005). hGBP1 siRNAs have also yielded an ~60% contribution to the control of hepatitis C virus (Itsui et al., 2006). Importantly, neither cell viability nor signal transduction cascades targeting ISRE, GAS, AP-1 or NF- κ B transcription factor binding sites were affected by siRNA treatment.

mGbp2 also exerted a similar effect against encephalomyocarditis virus that appeared dissociated from its GTPase activity (Carter et al., 2005). This latter finding is surprising in light of biochemical evidence that demonstrates self-stimulated GTPase activity arising from nucleotide-dependent dimeric and tetrameric assembly (Kunzelmann et al., 2005). Perhaps the p65 GBPs do not act as mechanoenzymes in a manner analogous to the dynamin superfamily to inhibit microbial growth. Alternative explanations for their antiviral activity, however, remain to be proffered.

Summary

Vertebrates are endowed with at least two classes of IFN- γ -induced GTPases -p47 IRGs and p65 GBPs - that combat intracellular infections as part of the cell-autonomous response during innate host defense. Pathogen specificity is exemplified by p47 IRGs that are effective against vacuolarized bacteria and protozoa, whereas the p65 GBPs can also act against cytosolic rhabdoviruses and flaviviruses. Control of microbial replication at the level of the infected cell may involve GTPase-assisted fusogenic complex formation on the phagosomal membrane or viral assembly site, or regulation of the vesicular cargo that traffics to and from that compartment. A major challenge for the future, therefore, will be a complete molecular description of the trafficking events governed by these GTPases, a task made more complex by the recent discoveries of yet more family members within the activated macrophage.

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References

- Anderson SL, Carton JM, Lou J, Xing L, Rubin BY. Interferon-induced guanylate binding protein-1 (GBP-1) mediates an antiviral effect against vesicular stomatitis virus and encephalomyocarditis virus. *Virology* 1999;256:8–14. [PubMed: 10087221]
- Bekpen C, Hunn JP, Rohde C, Parvanova I, Guethlein L, Dunn DM, Glowalla E, Leptin M, Howard JC. The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage. *Genome Biol* 2005;6:R92. [PubMed: 16277747]
- Bernstein-Hanley I, Coers J, Balsara ZR, Taylor GA, Starnbach MN, Dietrich WF. The p47 GTPases Igtg and Irgb10 map to the Chlamydia trachomatis susceptibility locus Ctrq-3 and mediate cellular resistance in mice. *Proc Natl Acad Sci USA* 2006;103:14092–14097. [PubMed: 16959883]
- Boehm U, Guethlein L, Klamp T, Ozbek K, Schaub A, Futterer A, Pfeffer K, Howard JC. Two families of GTPases dominate the complex cellular response to IFN-gamma. *J Immunol* 1998;161:6715–6723. [PubMed: 9862701]

- Briken V, Ruffner H, Schultz U, Schwarz A, Reis LF, Strehlow I, Decker T, Staeheli P. Interferon regulatory factor 1 is required for mouse Gbp gene activation by gamma interferon. *Mol Cell Biol* 1995;15:975–982. [PubMed: 7823961]
- Butcher BA, Greene RI, Henry SC, Annecharico KL, Weinberg JB, Denkers EY, Sher A, Taylor GA. p47 GTPases regulate *Toxoplasma gondii* survival in activated macrophages. *Infect Immun* 2005;73:3278–3286. [PubMed: 15908352]
- Carlow DA, Teh SJ, Teh HS. Specific antiviral activity demonstrated by TGTP, a member of a new family of interferon-induced GTPases. *J Immunol* 1998;161:2348–2355. [PubMed: 9725230]
- Carter CC, Gorbacheva VY, Vestal DJ. Inhibition of VSV and EMCV replication by the interferon-induced GTPase, mGBP-2: differential requirement for wild-type GTP binding domain. *Arch Virol* 2005;150:1213–1220. [PubMed: 15717119]
- Chekmenov DS, Haid C, Kel AE. P-Match: transcription factor binding site search by combining patterns and weight matrices. *Nucl Acids Res* 2005;33:W432–W437. [PubMed: 15980505]
- Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tessarollo L, Woude GF, Sher A, Taylor GA. Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. *J Exp Med* 2001;194:181–188. [PubMed: 11457893]
- Collazo CM, Yap GS, Hieny S, Caspar P, Feng CG, Taylor GA, Sher A. The function of gamma interferon-inducible GTP-binding protein IGTP in host resistance to *Toxoplasma gondii* is Stat1 dependent and requires expression in both hematopoietic and nonhematopoietic cellular compartments. *Infect Immun* 2002;70:6933–6939. [PubMed: 12438372]
- Colonna RJ, Pang RH. Induction of unique mRNAs by human interferons. *J Biol Chem* 1982;257:9234–9237. [PubMed: 6179932]
- Do CB, Mahabhashyam MS, Brudno M, Batzoglu S. ProbCons: Probabilistic consistency-based multiple sequence alignment. *Genome Res* 2005;15:330–340. [PubMed: 15687296]
- Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T, Rao G, Sun R, Haberland M, Modlin R, Cheng G. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. *Immunity* 2002;17:251–263. [PubMed: 12354379]
- Ehrt S, Schnappinger D, Bekiranov S, Drenkow J, Shi S, Gingeras TR, Gaasterland T, Schoolnik G, Nathan C. Reprogramming of the macrophage transcriptome in response to interferon-gamma and *Mycobacterium tuberculosis*: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. *J Exp Med* 2001;194:1123–1140. [PubMed: 11602641]
- Finbloom DS, Hoover DL, Wahl LM. The characteristics of binding of human recombinant interferon-gamma to its receptor on human monocytes and human monocyte-like cell lines. *J Immunol* 1985;135:300–305. [PubMed: 3158703]
- Frank, SA. Immunology and evolution of infectious disease. Princeton University Press; Princeton, N.J: 2002.
- Gavrilescu LC, Butcher BA, Del Rio L, Taylor GA, Denkers EY. STAT1 is essential for antimicrobial effector function but dispensable for gamma interferon production during *Toxoplasma gondii* infection. *Infect Immun* 2004;72:1257–1264. [PubMed: 14977926]
- Ghosh A, Uthaiiah R, Howard J, Herrmann C, Wolf E. Crystal structure of IIGP1: a paradigm for interferon-inducible p47 resistance GTPases. *Mol Cell* 2004;15:727–739. [PubMed: 15350217]
- Ghosh A, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C. How guanylate-binding proteins achieve assembly-stimulated processive cleavage of GTP to GMP. *Nature* 2006;440:101–104. [PubMed: 16511497]
- Gilly M, Damore MA, Wall R. A promoter ISRE and dual 5' YY1 motifs control IFN-gamma induction of the IRG-47 G-protein gene. *Gene* 1996;179:237–244. [PubMed: 8972906]
- Gorbacheva VY, Lindner D, Sen GC, Vestal DJ. The interferon (IFN)-induced GTPase, mGBP-2. Role in IFN-gamma-induced murine fibroblast proliferation. *J Biol Chem* 2002;277:6080–6087. [PubMed: 11726669]
- Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 2004;119:753–766. [PubMed: 15607973]

- Halonen SK, Taylor GA, Weiss LM. Gamma interferon-induced inhibition of *Toxoplasma gondii* in astrocytes is mediated by IGTP. *Infect Immun* 2001;69:5573–5576. [PubMed: 11500431]
- Han BH, Park DJ, Lim RW, Im JH, Kim HD. Cloning, expression, and characterization of a novel guanylate-binding protein, GBP3 in murine erythroid progenitor cells. *Biochim Biophys Acta* 1998;19:373–386. [PubMed: 9659399]
- Hemmi H, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H, Kawai T, Hoshino K, Takeda K, Akira S. The roles of two IkappaB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J Exp Med* 2004;199:1641–1650. [PubMed: 15210742]
- Henikoff S, Henikoff JG. Automated assembly of protein blocks for database searching. *Nucl Acids Res* 1991;19:6565–6572. [PubMed: 1754394]
- Honda K, Taniguchi T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol* 2006;6:644–658. [PubMed: 16932750]
- Itsui Y, Sakamoto N, Kurosaki M, Kanazawa N, Tanabe Y, Koyama T, Takeda Y, Nakagawa M, Kakinuma S, Sekine Y, Maekawa S, Enomoto N, Watanabe M. Expressional screening of interferon-stimulated genes for antiviral activity against hepatitis C virus replication. *J Viral Hepat* 2006;13:690–700. [PubMed: 16970601]
- Kaiser F, Kaufmann SH, Zerrahn J. IIGP, a member of the IFN inducible and microbial defense mediating 47 kDa GTPase family, interacts with the microtubule binding protein hook3. *J Cell Sci* 2004;117:1747–1756. [PubMed: 15075236]
- Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 2004;5:150–163. [PubMed: 15260895]
- Kunzelmann S, Praefcke GJ, Herrmann C. Nucleotide binding and self-stimulated GTPase activity of human guanylate-binding protein 1 (hGBP1). *Methods Enzymol* 2005;404:512–527. [PubMed: 16413296]
- Lapaque N, Takeuchi O, Corrales F, Akira S, Moriyon I, Howard JC, Gorvel JP. Differential inductions of TNF-alpha and IGTP, IIGP by structurally diverse classic and non-classic lipopolysaccharides. *Cell Microbiol* 2006;8:401–413. [PubMed: 16469053]
- Lew DJ, Decker T, Strehlow I, Darnell JE. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. *Mol Cell Biol* 1991;11:182–191. [PubMed: 1898761]
- Lieberman LA, Banica M, Reiner SL, Hunter CA. STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis. *J Immunol* 2004;172:457–463. [PubMed: 14688355]
- Ling YM, Shaw MH, Ayala C, Coppens I, Taylor GA, Ferguson DJ, Yap GS. Vacuolar and plasma membrane stripping and autophagic elimination of *Toxoplasma gondii* in primed effector macrophages. *J Exp Med* 2006;203:2063–2071. [PubMed: 16940170]
- MacMicking JD. IFN-inducible GTPases and immunity to intracellular pathogens. *Trends Immunol* 2004;25:601–609. [PubMed: 15489189]
- MacMicking JD. Immune control of phagosomal bacteria by p47 GTPases. *Curr Opin Microbiol* 2005;8:74–82. [PubMed: 15694860]
- MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. *Annu Rev Immunol* 1997;15:323–350. [PubMed: 9143691]
- MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science* 2003;302:654–659. [PubMed: 14576437]
- Martens S, Howard J. The interferon-inducible GTPases. *Annu Rev Cell Dev Biol* 2006;22:559–589. [PubMed: 16824009]
- Martens S, Sabel K, Lange R, Uthaiiah R, Wolf E, Howard JC. Mechanisms regulating the positioning of mouse p47 resistance GTPases LRG-47 and IIGP1 on cellular membranes: retargeting to plasma membrane induced by phagocytosis. *J Immunol* 2004;173:2594–2606. [PubMed: 15294976]
- Martens S, Parvanova I, Zerrahn J, Griffiths G, Schell G, Reichmann G, Howard JC. Disruption of *Toxoplasma gondii* parasitophorous vacuoles by the mouse p47-resistance GTPases. *PLoS Pathog* 2005;1:e24. [PubMed: 16304607]
- Mascie-Taylor CG, Karim E. The burden of chronic disease. *Science* 2003;302:1921–1922. [PubMed: 14671291]

- McCaffrey RL, Fawcett P, O’Riordan M, Lee KD, Havell EA, Brown PO, Portnoy DA. A specific gene expression program triggered by Gram-positive bacteria in the cytosol. *Proc Natl Acad Sci USA* 2004;101:11386–11391. [PubMed: 15269347]
- Modiano N, Lu YE, Cresswell P. Golgi targeting of human guanylate-binding protein-1 requires nucleotide binding, isoprenylation, and an IFN-gamma-inducible cofactor. *Proc Natl Acad Sci USA* 2005;102:8680–8685. [PubMed: 15937107]
- Nantais DE, Schwemmle M, Stickney JT, Vestal DJ, Buss JE. Prenylation of an interferon-gamma-induced GTP-binding protein: the human guanylate binding protein, huGBP1. *J Leukoc Biol* 1996;60:423–431. [PubMed: 8830800]
- Naschberger E, Werner T, Vicente AB, Guenzi E, Topolt K, Leubert R, Lubeseder-Martellato C, Nelson PJ, Sturzl M. Nuclear factor-kappaB motif and interferon-alpha-stimulated response element cooperate in the activation of guanylate-binding protein-1 expression by inflammatory cytokines in endothelial cells. *Biochem J* 2004;379:409–420. [PubMed: 14741045]
- Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* 1983;158:670–689. [PubMed: 6411853]
- Nathan CF, Prendergast TJ, Wiebe ME, Stanley ER, Platzer E, Remold HG, Welte K, Rubin BY, Murray HW. Activation of human macrophages. Comparison of other cytokines with interferon-gamma. *J Exp Med* 1984;160:600–605. [PubMed: 6206183]
- Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates and the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci USA* 2000;97:8841–8848. [PubMed: 10922044]
- Nelson DE, Virok DP, Wood H, Roshick C, Johnson RM, Whitmire WM, Crane DD, Steele-Mortimer O, Kari L, McClarty G, Caldwell HD. Chlamydial IFN-gamma immune evasion is linked to host infection tropism. *Proc Natl Acad Sci USA* 2005;102:10658–10663. [PubMed: 16020528]
- Neun R, Richter MF, Staeheli P, Schwemmle M. GTPase properties of the interferon-induced human guanylate-binding protein 2. *FEBS Lett* 1996;390:69–72. [PubMed: 8706832]
- Nguyen TT, Hu Y, Widney DP, Mar RA, Smith JB. Murine GBP-5, a new member of the murine guanylate-binding protein family, is coordinately regulated with other GBPs in vivo and in vitro. *J Interferon Cytokine Res* 2002;22:899–909. [PubMed: 12396730]
- Ni Z, Karaskov E, Yu T, Callaghan SM, Der S, Park DS, Xu Z, Pattenden SG, Bremner R. Apical role for BRG1 in cytokine-induced promoter assembly. *Proc Natl Acad Sci USA* 2005;102:14611–14616. [PubMed: 16195385]
- Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 2000;302:205–217. [PubMed: 10964570]
- Olszewski MA, Gray J, Vestal DJ. In silico genomic analysis of the human and murine guanylate-binding protein (GBP) gene clusters. *J Interferon Cytokine Res* 2006;26:328–352. [PubMed: 16689661]
- Pace JL, Russell SW, Schreiber RD, Altman A, Katz DH. Macrophage activation: priming activity from a T-cell hybridoma is attributable to interferon-gamma. *Proc Natl Acad Sci USA* 1983;80:3782–3786. [PubMed: 6407020]
- Pattenden SG, Klose R, Karaskov E, Bremner R. Interferon-gamma-induced chromatin remodeling at the CIITA locus is BRG1 dependent. *EMBO J* 2002;21:1978–1986. [PubMed: 11953317]
- Praefcke GJ, Kloep S, Benscheid U, Lilie H, Prakash B, Herrmann C. Identification of residues in the human guanylate-binding protein 1 critical for nucleotide binding and cooperative GTP hydrolysis. *J Mol Biol* 2004;344:257–269. [PubMed: 15504415]
- Prakash B, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C. Structure of human guanylate-binding protein 1 representing a unique class of GTP-binding proteins. *Nature* 2000a;403:567–571. [PubMed: 10676968]
- Prakash B, Renault L, Praefcke GJ, Herrmann C, Wittinghofer A. Triphosphate structure of guanylate-binding protein 1 and implications for nucleotide binding and GTPase mechanism. *EMBO J* 2000b;19:4555–4564. [PubMed: 10970849]
- Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Vavilys P, Shamovsky O, Palma A, Mikkelsen TS, Altshuler D, Lander ES. Positive natural selection in the human lineage. *Science* 2006;312:1614–1620. [PubMed: 16778047]

- Santiago HC, Feng CG, Bafica A, Roffe E, Arantes RM, Cheever A, Taylor G, Vieira LQ, Aliberti J, Gazzinelli RT, Sher A. Mice deficient in LRG-47 display enhanced susceptibility to *Trypanosoma cruzi* infection associated with defective hemopoiesis and intracellular control of parasite growth. *J Immunol* 2005;175:8165–8172. [PubMed: 16339555]
- Scheffzek K, Ahmadian MR. GTPase activating proteins: structural and functional insights 18 years after discovery. *Cell Mol Life Sci* 2005;62:3014–3038. [PubMed: 16314935]
- Schneider TD, Stephens RM. Sequence logos: a new way to display consensus sequences. *Nucl Acids Res* 1990;18:6097–6100. [PubMed: 2172928]
- Schroder K, Sweet MJ, Hume DA. Signal integration between IFN γ and TLR signalling pathways in macrophages. *Immunobiology* 2006;211:511–524. [PubMed: 16920490]
- Schwemmle M, Staeheli P. The interferon-induced 67-kDa guanylate-binding protein (hGBP1) is a GTPase that converts GTP to GMP. *J Biol Chem* 1994;269:11299–11305. [PubMed: 7512561]
- Shi S, Nathan C, Schnappinger D, Drenkow J, Fuortes M, Block E, Ding A, Gingeras TR, Schoolnik G, Akira S, Takeda K, Ehrt S. MyD88 primes macrophages for full-scale activation by interferon- γ yet mediates few responses to *Mycobacterium tuberculosis*. *J Exp Med* 2003;198:987–997. [PubMed: 14517275]
- Shotland Y, Kramer H, Groisman EA. The Salmonella SpiC protein targets the mammalian Hook3 protein function to alter cellular trafficking. *Mol Microbiol* 2003;49:1565–1576. [PubMed: 12950921]
- Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006;313:1438–1441. [PubMed: 16888103]
- Skamene E, Schurr E, Gros P. Infection genomics: Nramp1 as a major determinant for natural resistance to intracellular infections. *Ann Rev Med* 1998;49:275–287. [PubMed: 9509263]
- Snyder M, He W, Zhang JJ. The DNA replication factor MCM5 is essential for Stat1-mediated transcriptional activation. *Proc Natl Acad Sci USA* 2005;102:14539–14444. [PubMed: 16199513]
- Sorace JM, Johnson RJ, Howard DL, Drysdale BE. Identification of an endotoxin and IFN-inducible cDNA: possible identification of a novel protein family. *J Leukoc Biol* 1995;58:477–484. [PubMed: 7561525]
- Sprang SR. G proteins, effectors and GAPs: structure and mechanism. *Curr Opin Struct Biol* 1997;7:849–856. [PubMed: 9434906]
- Sun D, Ding A. MyD88-mediated stabilization of interferon- γ -induced cytokine and chemokine mRNA. *Nat Immunol* 2006;7:375–381. [PubMed: 16491077]
- Taylor GA, Jeffers M, Largaespada DA, Jenkins NA, Copeland NG, Woude GF. Identification of a novel GTPase, the inducibly expressed GTPase, that accumulates in response to interferon γ . *J Biol Chem* 1996;271:20399–20405. [PubMed: 8702776]
- Taylor GA, Stauber R, Rulong S, Hudson E, Pei V, Pavlakis GN, Resau JH, Vande Woude GF. The inducibly expressed GTPase localizes to the endoplasmic reticulum, independently of GTP binding. *J Biol Chem* 1997;272:10639–10645. [PubMed: 9099712]
- Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, Taylor LS, Eagleson B, Secrest L, Southon EA, Reid SW, Tessarollo L, Bray M, McVicar DW, Komschlies KL, Young HA, Biron CA, Sher A, Vande Woude GF. Pathogen-specific loss of host resistance in mice lacking the IFN- γ -inducible gene IGTP. *Proc Natl Acad Sci USA* 2000;97:751–755. [PubMed: 10639151]
- Taylor GA, Feng CG, Sher A. p47 GTPases: regulators of immunity to intracellular pathogens. *Nat Rev Immunol* 2004;4:100–109. [PubMed: 15040583]
- tenOever BR, Ng SL, Chua MA, McWhirter SM, Garcia-Sastre A, Maniatis T. Multiple functions of the IKK-related kinase IKK ϵ in interferon-mediated antiviral activity. *Science* 2007;315:1274–1278. [PubMed: 17332413]
- Uthiaiah RC, Praefcke GJ, Howard JC, Herrmann C. IIGP1, an interferon- γ -inducible 47-kDa GTPase of the mouse, showing cooperative enzymatic activity and GTP-dependent multimerization. *J Biol Chem* 2003;278:29336–29343. [PubMed: 12732635]
- Varinou L, Ramsauer K, Karaghiosoff M, Kolbe T, Pfeffer K, Muller M, Decker T. Phosphorylation of the Stat1 transactivation domain is required for full-fledged IFN- γ -dependent innate immunity. *Immunity* 2003;19:793–802. [PubMed: 14670297]
- Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD,

Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, et al. The sequence of the human genome. *Science* 2001;291:1304–1351. [PubMed: 11181995]

Vestal DJ. The guanylate-binding proteins (GBPs): proinflammatory cytokine-induced members of the dynamin superfamily with unique GTPase activity. *J Interferon Cytokine Res* 2005;25:435–443. [PubMed: 16108726]

Vestal DJ, Gorbacheva VY, Sen GC. Different subcellular localizations for the related interferon-induced GTPases, MuGBP-1 and MuGBP-2: implications for different functions? *J Interferon Cytokine Res* 2000;20:991–1000. [PubMed: 11096456]

Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. *Science* 2001;294:1299–1304. [PubMed: 11701921]

Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, Antonarakis SE, Attwood J, Baertsch R, Bailey J, Barlow K, Beck S, Berry E, Birren B, Bloom T, Bork P, Botcherby M, Bray N, Brent MR, Brown DG, Brown SD, Bult C, Burton J, Butler J, Campbell RD, Carninci P, Cawley S, Chiaromonte F, Chinwalla AT, Church DM, Clamp M, Clee C, Collins FS, Cook LL, Copley RR, Coulson A, Couronne O, Cuff J, Curwen V, Cutts T, Daly M, David R, Davies J, Delehaunty KD, Deri J, Dermitzakis ET, Dewey C, Dickens NJ, Diekhans M, Dodge S, Dubchak I, Dunn DM, Eddy SR, Elnitski L, Emes RD, Eswara P, Eyraas E, Felsenfeld A, Fewell GA, Flicek P, Foley K, Frankel WN, Fulton LA, Fulton RS, Furey TS, Gage D, Gibbs RA, Glusman G, Gnerre S, Goldman N, Goodstadt L, Grafham D, Graves TA, Green ED, Gregory S, Guigo R, Guyer M, Hardison RC, Haussler D, Hayashizaki Y, Hillier LW, Hinrichs A, Hlavina W, Holzer T, Hsu F, Hua A, Hubbard T, Hunt A, Jackson I, Jaffe DB, Johnson LS, Jones M, Jones TA, Joy A, Kamal M, Karlsson EK, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002;420:520–562. [PubMed: 12466850]

Weighardt H, Jusek G, Mages J, Lang R, Hoebe K, Beutler B, Holzmann B. Identification of a TLR4- and TRIF-dependent activation program of dendritic cells. *Eur J Immunol* 2004;34:558–564. [PubMed: 14768061]

Yamaguchi T, Omatsu N, Omukae A, Osumi T. Analysis of interaction partners for perilipin and ADRP on lipid droplets. *Mol Cell Biochem* 2006;284:167–173. [PubMed: 16532261]

Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. *Traffic* 2007;8:241–250. [PubMed: 17319801]

Zerrahn J, Schaible UE, Brinkmann V, Guehlich U, Kaufmann SH. The IFN-inducible Golgi- and endoplasmic reticulum-associated 47-kDa GTPase IIGP is transiently expressed during listeriosis. *J Immunol* 2002;168:3428–3436. [PubMed: 11907101]

Zhang HM, Yuan J, Cheung P, Luo H, Yanagawa B, Chau D, Stephan-Tozy N, Wong BW, Zhang J, Wilson JE, McManus BM, Yang D. Overexpression of interferon-gamma-inducible GTPase inhibits coxsackievirus B3-induced apoptosis through the activation of the phosphatidylinositol 3-kinase/Akt pathway and inhibition of viral replication. *J Biol Chem* 2003;278:33011–33019. [PubMed: 12819192]

Fig. 1.

Phylogenetic analyses of p47 IRGs and p65 GBPs. G-domains of p47 IRGs, p65 GBPs and other representative GTPases were first aligned separately using ProbCons (Do et al., 2005) followed by two step profiles using TCoRe (Notredame et al., 2000) which generated the best alignment of this divergent group of proteins. The neighbor-joining tree shown was based on this alignment using the Molecular Evolutionary Genetics Analysis package (version 3.1; Kumar et al., 2004). The p47 IRGs separate into distinct sub-families, particularly the N- and C-terminal GTPase domains (indicated as G1 and G2 respectively) of L-Irg1-3 that form two sub-clusters. Human IRGM lies nearest to the cluster containing mouse Lrg-47/Irgm1, Gtpi/Irgm2 and Igtp/Irgm3. Note the groupings within the GBP family indicating a closer relation between genes in this family. Scale bar designates number of substitutions per site.

A. Human genome

B. Mouse genome

Fig. 2.

Genomic organization of human and mouse p47 IRGs and p65 GBPs. (A) Schematic representation of the human genomic regions containing p47 *IRG* and p65 *GBP* genes on chromosomes 1, 5 and 19. Arrows represent 5'–3' direction of mRNA transcripts and boxes represent the canonical Iigp1/Irga6-like GTPase domain (Pfam accession PF05049). Small green boxes are presumed GBP pseudogenes. Red boxes represent genes unrelated to the GBP family. (B) Schematic representation of the mouse genomic regions containing multiple p47 *Irg* and p65 *Gbp* genes on chromosomes 3, 5, 7, 11 and 18. Three longer genes (labeled *L-Irg1-3*) may contain two tandem GTPase domains previously treated as individual proteins (Bekpen et al., 2005). The N-terminal GTPase domain of *L-Irg1* is identical to *Irgb5*, a genomic duplication that has been pointed out earlier (Bekpen et al., 2005). Blue boxes indicate presumed IRG pseudogenes (*Irgb7*, *Irga1*, *Irga5* and *Irga8*) and red boxes represent genes unrelated to the IRG family. *Irg11* is a new IRG gene upstream of *Iigp1/Irgb6*. For the p65 *Gbp* family, new genes have been labeled as *Gbp8-13*. The gene nomenclature endorsed by HUGO is shown on the top, with NCBI GeneID numbers below.

Fig. 3.

Structures and sequence relationship within the p47 IRGs and p65 GBPs. (A) A computer rendering of full-length dimeric Lig1/Irg6 (pdb code 1tq2) crystallized in the presence of phosphoaminophosphonic acid-guanylate ester (GNP; shown in red stick representation). Subunits in the dimer are colored in shades of blue and green. For the sake of clarity, N- and C-terminal helical domains and the G-domain are shaded and marked as indicated for the protomer on the left. The axis relates the protomers by 2-fold non-crystallographic symmetry. The α K region responsible for membrane targeting in Lrg47/Irgm1 is indicated. (B) A model of monomeric full-length hGBP1 (pdb code 1f5n) shaded to represent its subdomains. The elongated nature of hGBP1 versus Lig1/Irg6 is apparent. The active site nucleotide (GNP; red stick model) was imported from the hGBP1 G-domain crystal structure (pdb code 2bc9). (C) Conserved motifs from an alignment of G-domains of p47 IRGs and p65 GBPs (see legend to Fig. 1) were identified using BLOCKS (Henikoff and Henikoff, 1991), and LOGO (Schneider and Stephens, 1990) representations of these are shown in the panel on top. The bottom panel shows a closer view of the G-domain of Lig1/Irg6 (1tq2) and hGBP1 (2bc9). Positions of the conserved GTPase motifs shown in the upper panel are depicted with the rest of the protein drawn as ribbons. The divergent switch-I region is shown in red and orange for the IRGs and GBPs, respectively. GBPs lack a G5 motif but instead have a 'guanine cap' that closes the nucleotide binding pocket as shown in blue.

Fig. 4. IFN-inducible transcription factor-binding sites within p47 IRG and p65 GBP promoters. Results from earlier studies of known genes are summarized schematically (Bekpen et al., 2005; Olszewski et al., 2006). Analyses of newly identified members were carried out using P-match (Chekmenev et al., 2005), and results for GAS (interferon-gamma activated site) or ISRE (interferon stimulated response elements) are shown.

Fig. 5.

Recruitment of endogenous Lrg-47/Irgm1 to the bacterial phagosome. Upper panel depicts single-channel and merged (overlay) confocal images of Lrg-47/Irgm1 on the mycobacterial phagosome (*M. bovis* BCG) in IFN- γ -activated RAW264.7 murine macrophages. Antigens were detected using goat anti-Lrg-47 peptide (A19) pAb coupled to Alexa-488 with rabbit anti-PPD pAb-linked Alexa 594. Lower panel demonstrates early recruitment (10–15 min post-uptake) of Lrg-47/Irgm1 to the *L. monocytogenes* vacuole in IFN- γ -activated mouse macrophages. Rabbit anti-*L. monocytogenes* (ATCC strain 43251) pAb was used for bacterial visualization. Original magnification, 100 $\times$. Courtesy of T. Matsuzawa.