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Regulation of innate immune functions by guanylate-binding proteins

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ABSTRACT

Guanylate-binding proteins (GBP) are a family of dynamin-related large GTPases which are expressed in response to interferons and other pro-inflammatory cytokines. GBPs mediate a broad spectrum of innate immune functions against intracellular pathogens ranging from viruses to bacteria and protozoa. Several binding partners for individual GBPs have been identified and several different mechanisms of action have been proposed depending on the organisms, the cell type and the pathogen used. Many of these anti-pathogenic functions of GBPs involve the recruitment to and the subsequent destruction of pathogen containing vacuolar compartments, the assembly of large oligomeric innate immune complexes such as the inflammasome, or the induction of autophagy. Furthermore, GBPs often cooperate with immunity-related GTPases (IRGs), another family of dynamin-related GTPases, to exert their anti-pathogenic function, but since most IRGs have been lost in the evolution of higher primates, the anti-pathogenic function of human GBPs seems to be IRG-independent. GBPs and IRGs share biochemical and structural properties with the other members of the dynamin superfamily such as low nucleotide affinity and a high intrinsic GTPase activity which can be further enhanced by oligomerisation. Furthermore, GBPs and IRGs can interact with lipid membranes. In the case of three human and murine GBP isoforms this interaction is mediated by C-terminal isoprenylation. Based on cell biological studies, and in analogy to the function of other dynamins in membrane scission events, it has been postulated that both GBPs and IRGs might actively disrupt the outer membrane of pathogen-containing vacuole leading to the detection and destruction of the pathogen by the cytosolic innate immune system of the host. Recent evidence, however, indicates that GBPs might rather function by mediating membrane tethering events similar to the dynamin-related atlastin and mitofusin proteins, which mediate fusion of the ER and mitochondria, respectively. The aim of this review is to highlight the current knowledge on the function of GBPs in innate immunity and to combine it with the recent progress in the biochemical characterisation of this protein family.

1. Introduction

Interferons are cytokines released upon viral or microbial infections to induce the expression of several hundred antivirally or antimicrobially active genes (MacMicking, 2012; Platanias, 2005; Boehm et al., 1998). Of these, four families of GTPases are among the most strongly induced genes: the guanylate-binding proteins (GBP), the immunity-related GTPases (IRG), the Mx-proteins and the very large inducible GTPases (VLIG) (Li et al., 2009; MacMicking, 2012; Meunier and Broz, 2016; Pilla-Moffett et al., 2016).

The guanylate-binding proteins were identified due to their strong induction by type II interferons (IFN-γ) and their ability to interact with guanine nucleotides (Cheng et al., 1983). The characterisation of the GBP promotor was crucial for the elucidation of interferon-gamma signalling (Darnell et al., 1994; Lew et al., 1991; Platanias, 2005) long before the cellular functions of GBPs were identified. Cloning of the first human and murine GBPs showed an unusual conservation of the canonical GTP-binding motifs. While humans express seven GBPs, the murine genome codes for 11 genes (Kresse et al., 2008; Olszewski et al., 2006). In both, humans and mice, three GBPs carry a C-terminal isoprenylation motif, the CaaX-box (Cheng et al., 1991). While the sequence homology between dynamin and the antiviral Mx GTPases had been identified around that same time (Obar et al., 1990), the GBPs were only included in the dynamin superfamily when the biochemical and structural similarities became apparent and when other dynamin-related protein families were identified (Ghosh et al., 2006; Praefcke et al., 1999; Praefcke and McMahon, 2004; Prakash et al., 2000a; Schwemmle and Staeheli, 1994)(Fig. 1). The common feature of dynamin GTPases include a high intrinsic GTPase activity, which is further stimulated by oligomerisation, and the interaction with biological membranes. Activation of the GTPases on membranes induces a change in membrane conformation resulting in either fission or fusion (Daumke

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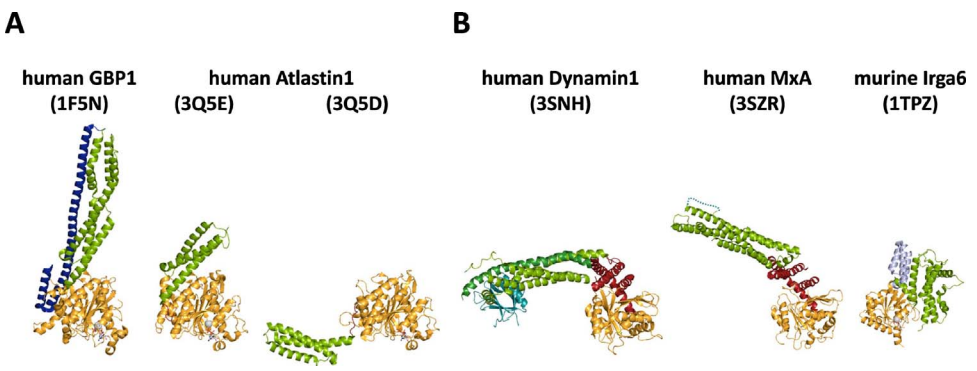

Fig. 1. Structural comparison of mammalian dynamin superfamily proteins. A: Structural comparison of hGBP1 and two conformations of atlastin1. The large GTPase (LG)-domains are shown in orange, the middle domains in green and the C-terminal α12/13-domain in hGBP1 in blue. Note that the structures of atlastin only contain the N-terminal part of the protein up to the first transmembrane region. B: Structural comparison of dynamin1, MxA and Irga6. The LG-domains are shown in orange, the bundle signal element in red and the stalk in green. The membrane binding PH-domain of dynamin1 and the L4-region of MxA are shown in viridian green and the N-terminal helical region of Irga6 in blue-grey, respectively. Note that the structure of dynamin1 does not contain the C-terminal proline-rich domain.

and Praefcke, 2016). The closest homologues of the GBPs within the dynamin superfamily are the atlastin proteins. Atlastins are conserved from yeast to man and regulate dynamics of the endoplasmic reticulum (ER) by tethering and fusion of ER tubules (Hu and Rapoport, 2016; McNew et al., 2013).

The first publication of an innate immune function of GBPs reported an antiviral effect against vesicular stomatitis virus (VSV) and encephaloymyocarditis virus (EMCV) (Anderson et al., 1999). The antiviral effect was not very prominent compared to the resistance mediated by e.g. human MxA protein against influenza virus, which may be due to the incomplete depletion of hGBP1 and −2 by the used antisense RNA. A similar effect was found for murine GBP2 in a later study (Carter et al., 2005). The first publications showing an anti-pathogenic activity of GBPs against protozoa (Degrandi et al., 2013; Yamamoto et al., 2012) and bacteria (Tietzel et al., 2009) appeared a decade late. Since then is has become clear that GBPs are important for the defence against a broad array of intracellular pathogens (Meunier and Broz, 2016; Pilla-Moffett et al., 2016).

The immunity-related GTPases (IRGs), were initially identified in mice due to their strong induction by IFN-γ starting with IRGM1 under the name LRG-47 (Gilly and Wall, 1992) followed by five other isoforms (Boehm et al., 1998; Carlow et al., 1995; Lafuse et al., 1995; Sorace et al., 1995; Taylor et al., 1996). Later it became apparent that the genes coding for this protein family, which was referred to as p47 GTPases to distinguish them from the previously identified p65/67 GBPs, is evolving fast leading to more than 20 murine isoforms depending on the mouse strain (Lilue et al., 2013). In contrast, IRGs are almost completely lost in humans (Bekpen et al., 2005; Bekpen et al., 2009). Biochemically, the best studied IRG protein is Irga6 (Ghosh et al., 2004; Pawlowski et al., 2011; Schulte et al., 2016; Uthaiah et al., 2003) which displays a GTP-dependent oligomerisation and a dimerisation induced stimulation of the GTPase activity. In cells Irga6 can be N-terminally myristoylated (Martens et al., 2004; Papic et al., 2008). Despite their later identification, the biological function of IRGs was more intensively studies than the GBPs. The first report of an anti-pathogenic activity of an IRG identified the apicomplexan parasite *Toxoplasma gondii* as a target pathogen (Taylor et al., 2000). The study of murine IRG knock-out strains strongly accelerated the identification of additional resistance phenotypes. Cell biological studies showed that a subgroup of the IRGs, the so called GMS-proteins, serve as negative regulators of the other IRG family members. (Hunn et al., 2008). In the absence of pathogens these GMS-proteins reside on different endomembrane compartment and protect them from the attack by the other IRGs, the so-called GKS proteins. Certain pathogens (e.g. *T. gondii*, *Chlamydia* and *E. cuniculi*), enter the host cell by non-phagocytic mechanisms. During the active invasion by *T. gondii*, most transmembrane proteins of the host are excluded from the membrane of the forming PV (Mordue et al., 1999). The GKS proteins identify such PVs as non-self structures by the absence of GMS-proteins and are recruited to them resulting in the disruption of the PV and the destruction of the parasite (Haldar et al., 2013; Khaminets et al., 2010; Zhao et al., 2009). The current model for the mechanism of IRGs is based on analogy to other dynamin-related proteins and on the careful observation of the sequential order of pathogen entry, IRG recruitment to the PV membrane (PVM), shape change and finally permeabilisation of the PV membrane. A direct disruption of the PVM by IRG proteins, however, has so far not been formally proven (Zhao et al., 2009). Over time it became clear that the deletion of individual regulatory GMS proteins can disrupt this mutual regulatory network. In the case of Irgm1-/- mice, the mislocalisation of GKS-proteins leads to a loss of lysosomal acidity and autophagic processing resulting in IFN-γ-induced lymphopenia (Feng et al., 2008; King et al., 2011; Maric-Biresev et al., 2016; Traver et al., 2011). For some pathogens this effect has been interpreted as loss of resistance (Feng et al., 2008), but against others, such as *Chlamydia*, Irgm1 does mediate cell autonomous resistance (Coers et al., 2011). In mice the recruitment of IRGs and GBPs to PVs are linked to proteins from the autophagy pathway (Al-Zeer et al., 2009; Haldar et al., 2014; Khaminets et al., 2010) (Fig. 2). After relocalisation to the PV of *T. gondii* and C. trachomatis, the GKS IRGs mediate recruitment of ubiquitin E3 ligases such as TRAF6 to the membrane. The ubiquitylation of proteins on the PV, probably including the IRGs (Traver et al., 2011), creates binding sites for the ubiquitin-binding protein p62/sequestosome (SQSTM1), which is itself a binding partner of mGBP2 (Haldar et al., 2015) and mGBP1 (Kim et al., 2011). The recruitment of GBPs to the *T. gondii* PV in murine cells requires GTP-binding but is independent of GTP-hydrolysis and of isoprenylation (Kravets et al., 2012; Virreira Winter et al., 2011). Accordingly, pathogen strains which express virulence factors that disable the IRG system (Behnke et al., 2012; Fentress et al., 2010; Fleckenstein et al., 2012; Steinfeldt et al., 2010) also indirectly inactivate the GBPs and genetic deletions of GMS IRGs resulting in mislocalisation of GKS IRGs also affect GBPs (Traver et al., 2011). In human cells, IRGM is the only IRG isoform with an immune function (Bekpen et al., 2009; Chauhan et al., 2016). The protein is truncated compared to canonical IRG proteins and its expression if not inducible by interferons. IRGM is involved in the regulation of autophagy and polymorphisms in the gene are linked to Crohn's disease and the susceptibility to tuberculosis. So far, a connection to the function of human GBPs has not been shown.

2. Structural and biochemical framework of GBPs

The domain architecture of GBPs consists of three parts (Fig. 1). The N-terminal large GTPase (LG) domain harbours the GTP-binding site which can be identified by three GTP-binding motifs: the phosphate binding P-loop, the DxxG motif and the RD-motif, which deviates from the N/TKxD motif in other GTP-binding proteins (Cheng et al., 1991; Praefcke et al., 1999). Upon GTP-binding the GBPs dimerise, which stimulates their enzymatic activity (Ghosh et al., 2006; Praefcke et al., 2004; Prakash et al., 2000a; Prakash et al., 2000b; Wehner et al., 2012; Wehner and Herrmann, 2010). The LG-domain is followed by a helical