Structural And Biochemical Characterization Of An Interferon-Inducible Gtpase, Human Guanylate Binding Protein 2 (GBP2)

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The GTPase superfamily of proteins has always been a topic of interest due to their diverse biological roles, ranging from vesicular transport to membrane fission and fusion. The interferon-inducible family of GTPases has gained attention in recent years. On being induced by interferons, these GTPases potently induce antimicrobial effector functions against vacuolar pathogens. Within this family is a superfamily of guanylate binding proteins (GBPs), which are regarded as central players in cell-autonomous immunity. Out of the seven members expressed in humans, GBP1, 2, and 5 have a CaaX domain at their C-terminus that can be prenylated. Recent studies on the most well-characterized member, GBP1, revealed its capability to bind to pathogen membrane and form higher-order structures, but the roles played by GBP2 and GBP5 in host defense remain poorly understood. In our study, we applied a combination of biochemical, structural, and biophysical tools to understand the mechanism governing the activation of GBP2. We used X-ray crystallography to capture the nucleotide-free structure of full-length GBP2 and the structure of the GTPase domain of GBP2 bound to GDP, which denotes the post hydrolysis step. Structure-guided mutagenesis validated the role of key residues in GTP binding and hydrolysis. We also established the relationship between nucleotide binding and the oligomeric status of GBP2. Characterization of the GTPase activity of full-length GBP2 and the GTPase domain revealed differences in their rate of hydrolysis leading to the hypothesis that the C-terminal helical domain regulates the GTP hydrolysis by the G domain. Our work highlights the structural and biochemical properties of GBP2 that will help us to understand the molecular basis for its roles in vivo.