Bruton’s tyrosine kinase phosphorylates scaffolding and RNA-binding protein G3BP1 to induce stress granule aggregation during host sensing of foreign ribonucleic acids

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The Ras-GTPase activating protein SH3 domain-binding protein 1 (G3BP1) plays a critical role in the formation of classical and antiviral stress granules in stressed and virus-infected eukaryotic cells, respectively. While G3BP1 is known to be phosphorylated at serine residues which could affect stress granule assembly, whether G3BP1 is phosphorylated at tyrosine residues and how this posttranslational modification might affect its functions is less clear. Here, we show using immunoprecipitation and immunoblotting studies with 4G10 antibody that G3BP1 is tyrosine-phosphorylated when cells are stimulated with the synthetic double-stranded RNA analog polyinosinic:polycytidylic acid to mimic viral infection. We further demonstrate via co-immunoprecipitation and inhibitor studies that Bruton’s tyrosine kinase (BTK) binds and phosphorylates G3BP1. The nuclear transport factor 2-like domain of G3BP1 was previously shown to be critical for its self-association to form stress granules. Our mass spectrometry, mutational and biochemical cross-linking analyses indicate that the tyrosine-40 residue in this domain is phosphorylated by BTK and critical for G3BP1 oligomerization. Furthermore, as visualized via confocal microscopy, pretreatment of cells with the BTK inhibitor LFM-A13 or genetic deletion of the btk gene or mutation of G3BP1-Y40 residue to alanine or phenylalanine all significantly attenuated the formation of antiviral stress granule aggregates upon polyinosinic:polycytidylic acid treatment. Taken together, our data indicate that BTK phosphorylation of G3BP1 induces G3BP1 oligomerization and facilitates the condensation of ribonucleoprotein complexes into macromolecular aggregates.

Stress granules (SGs) are nonmembranous, transiently assembled and cytoplasmic-localized subcellular aggregates of mRNA, RNA-binding proteins, and other structural, enzymatic, or signaling proteins (1). They are formed in eukaryotic cells in reaction to cellular insults such as heat, toxin, starvation or viral infections and played a role in stalling mRNA translation or facilitating their decay (2). Depending on the nature of the cellular stresses, the composition and morphology of the SG can vary (3) but certain protein components are critical to the formation of various classes of SG and one of these is the RAS-GTPase-activating protein (SH3 domain)-binding protein 1 (G3BP1).

G3BP1 belongs to a family of RNA-binding proteins that also include G3BP2a and its spliced variant G3BP2b (4). G3BP1 has been shown to bind various mRNA, miRNA, and viral RNA as well as various proteins and its overexpression is known to induce the formation of SG (5–8). In the quiescence state, G3BP1 exists primarily as a monomer in the cytoplasm and can also be found partially in the nucleus (9). Upon cell stress, G3BP1 forms oligomers to effect SG assembly in the cytosol (10).

Structurally, G3BP1 is composed of four distinct domains and starting from the N terminal, a nuclear transport factor 2 (NTF2)-like domain, an acidic and proline-rich region, an RNA recognition motif (RRM) and at the carboxyl terminal, arginine and glycine-rich boxes (RGG). The RRM of G3BP1 interacts with target RNA sequences of 2 to 8 nucleotide long and can also bind other proteins which may affect its RNA-binding specificity. On the other hand, the NTF2-like domain has been demonstrated to facilitate protein–protein interactions and can mediate the dimerization of G3BP1 (11, 12).

The role of G3BP1 in SG formation is known to be regulated by multiple posttranslational modifications (PTMs). For example, the de-methylation of arginine-447 in its RGG domain regulates SG assembly in response to oxidative stress induced by sodium arsenite (13). Similarly, the phosphorylation states of G3BP1 serine residues are known to affect SG assembly (10, 14). Aside from these mechanisms, it is not known if the role of G3BP1 in SG

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aggregation is regulated by tyrosine phosphorylation and if so, which tyrosine residue and kinase(s) are involved. This is particularly relevant since many protein–protein interactions are affected by tyrosine phosphorylation (15). In addition to PTM, the dimerization of G3BP1 mediated by its NTF2-like domain is also important for the induction of SG assembly (10, 16) and it is currently not known how this process is being regulated.

Viral infection is known to trigger the formation of a special class of SG known as the antiviral SG (avSG) that served as a platform for host innate immune response to foreign or pathogen-associated nucleic acids (17). We and others have shown that G3BP1 is a critical component of avSG (7, 18). Several viruses have evolved strategies to subvert or disrupt the formation of avSG by targeting G3BP1. For example, Chikungunya virus nonstructural protein nsP3 interacts with G3BP1 to inhibit avSG assembly and manipulates host cells to replicate viral RNA at the cytoplasmic foci (19). In addition, several viruses from the Picornaviridae family express proteases that could cleave G3BP1 and thus prevent the formation of avSG (20, 21).

Recently, we demonstrated that G3BP1 could bind the viral RNA sensor RIG-I in response to viral RNA or its synthetic agonist, polyinosinic:polycytidylic acid or p(I:C) (7). It is currently not known if the activation of these innate immune signaling pathways could induce any PTM, especially protein tyrosine phosphorylation, on G3BP1 and how this PTM is being brought about and its effect on avSG assembly.

In this study, we examined if G3BP1 is tyrosine-phosphorylated upon stimulation of cells with viral ribonucleic acid mimetic. We show that G3BP1 is tyrosine-phosphorylated upon p(I:C) stimulation. Furthermore, we show that Bruton’s tyrosine kinase (BTK) binds and phosphorylates G3BP1 at tyrosine-40 in its NTF2-like domain, and this PTM is critical for G3BP1 dimerization and the formation of avSG aggregates. Taken together, our work reveals how the sensing of viral RNA by innate immune receptors leads to the formation of avSG via tyrosine phosphorylation of G3BP1 by BTK.

Results

**G3BP1 is tyrosine phosphorylated upon p(I:C) stimulation**

While G3BP1 is known to be serine phosphorylated, and this process might affect stress granule formation (10, 14), whether G3BP1 is tyrosine phosphorylated and how it might affect its functions is not known. Since scaffolding proteins are frequently activated by tyrosine phosphorylation to dock other proteins (15), we first examined if G3BP1 can be tyrosine phosphorylated upon specific cellular stimulations. As G3BP1 is known to play a role in RIG-I signaling (7), we first treated mouse bone marrow–derived macrophages (BMDMs) with p(I:C) and found their endogenous G3BP1 to be tyrosine phosphorylated at 2 h (h) poststimulation (Fig. 1A). Similarly, we found endogenous G3BP1 to be tyrosine phosphorylated in p(I:C)-stimulated human HeLa cells (Fig. 1B). We also overexpressed HA-tagged G3BP1 in HEK293T cells and showed that the immunoprecipitated (IP) G3BP1 was tyrosine-phosphorylated at the 2 h timepoint after p(I:C) stimulation (Fig. 1C). Hence, G3BP1 undergoes tyrosine phosphorylation in various cells upon p(I:C) stimulation.

BTK has been shown to regulate various innate immune signaling pathways (22), some of which are involved in the sensing of foreign ribonucleic acids. For example, we had previously shown that BTK was involved in TLR3 and DDX41-cGAS pathways leading to type-I IFN production (23, 24). Hence, it is highly likely that BTK might be involved in G3BP1 phosphorylation. To examine if BTK is responsible for the tyrosine phosphorylation of G3BP1, we pretreated HEK293T cells harboring HA-tagged G3BP1 with various BTK inhibitors such as LFM-A13 and Terreic acid followed by p(I:C) stimulation and immunoprecipitation and immunoblotting of G3BP1 with the anti-phospho-tyrosine antibody 4G10 (Fig. 1D). We found that both LFM-A13 and terreic acid could inhibit G3BP1 tyrosine phosphorylation. On the other hand,
PP2 that targets Src did not inhibit p(I:C)-induced G3BP1 tyrosine phosphorylation (Fig. S1). Hence, BTK probably phosphorylates G3BP1.

Taken together, our data suggest that G3BP1 is tyrosine phosphorylated in cells upon their sensing of foreign ribonucleic acids, and this process probably involves BTK.

**BTK binds and phosphorylates G3BP1**

To ascertain a role for BTK in the phosphorylation of G3BP1, we cotransfected HeLa cells with HA-tagged G3BP1 and FLAG-tagged BTK to visualize their interaction via confocal microscopy and found increased colocalization of these two molecules after p(I:C) stimulation (Fig. 2A). We also examined if BTK and G3BP1 could be found together in the same complex biochemically. We stimulated COS7 cells with p(I:C) and found that BTK could coimmunoprecipitate (co-IP) with G3BP1 upon p(I:C) treatment (Fig. S2), suggesting that BTK and G3BP1 could interact endogenously in the same complex and that their association is induced upon p(I:C) stimulation.

Next, we overexpressed HA-tagged G3BP1 and FLAG-tagged BTK in HEK293T cells and found that BTK directly binds G3BP1 and that there was increased interaction between the two molecules upon p(I:C) stimulation (Fig. 2B). Furthermore, we showed by 4G10 antibody staining that G3BP1 was tyrosine-phosphorylated when it was co-IP with BTK in HEK293T cells overexpressing these constructs (Fig. 2C). We also pretreated HEK293T cells harboring these constructs with the BTK inhibitor LFM-A13 and demonstrated that this pre-treatment reduced BTK interaction with G3BP1 and at the same time attenuated the tyrosine phosphorylation of G3BP1 (Fig. 2D). Taken together, our results indicate that BTK colocalizes, binds, and tyrosine phosphorylates G3BP1 in p(I:C)-stimulated cells.

**Formation of p(I:C)-induced avSG requires BTK**

G3BP1 is a core scaffolding protein that facilitates the assembly of classical SG and avSG upon viral infections and foreign ribonucleic acid stimulations (7). Hence, we investigated if BTK can be found in and plays a role in avSG formation. As shown in Figure 3A, wildtype HeLa cells were able to form distinct avSG upon p(I:C) stimulation as visualized in confocal microscopy study using G3BP1 or TIA staining to mark the granules. Analysis using IMARIS software to define individual cell and to quantify avSG (granules greater than 1.24 μM) per cell indicated that p(I:C)-stimulation significantly increased the number of aggregates in wildtype HeLa cells (Fig. 3B). Interestingly, there was also increased co-association of BTK with G3BP1 or TIA in avSG upon p(I:C)-treatment as revealed by Pearson’s correlation coefficient analyses (Fig. 3C), suggesting that BTK can be found in avSG. Next, we employed CRISPR/Cas9 targeting to generate btk<sup>−/−</sup>, tlr3<sup>−/−</sup>, and rig<sup>−/−</sup> HeLa cells (Fig. S3) to study the contribution of BTK, TLR3, and RIG-I to the formation of avSG. Interestingly, in the absence of these proteins, the formation of avSG aggregates was significantly curtailed (Figs. 3, A and B and S4), suggesting that the formation of avSG requires BTK and the various ribonucleic acid sensors (TLR3 or RIG-I). It is interesting to note that the knockout of TLR3 or RIG-I did not yield an intermediate phenotype but significantly abrogated the formation of avSG aggregation.
BTK phosphorylation of G3BP1

BTK possesses Pleckstrin homology (PH), SH3/SH2, and kinase domains (23) and since it binds G3BP1, we sought to identify the motif that mediates this interaction. To accomplish this objective, we overexpressed HA-tagged full length and various truncated mutants of BTK (Fig. 4A) in HEK293T cells together with FLAG-tagged full-length G3BP1. Interestingly, through co-IP experiment, we found that an intact full-length BTK was required for the binding of G3BP1 (Fig. 4B).

Figure 3. G3BP1-mediated stress granule formation during p(I:C) stimulation is largely abrogated in the absence of BTK, TLR3, and RIG-I.
A, confocal microscopy analyses of G3BP1, BTK, and TIA colocalization in non-treated or p(I:C)-stimulated wildtype (WT), btk−/−, tlr3−/−, and rig-1−/− HeLa cells. Cells were stimulated with p(I:C) for 2 h and G3BP1 (red), BTK (green), and TIA (gray) were visualized using fluorochrome-conjugated antibodies. Width of white bar indicates 10 μM. B, quantification of G3BP1 containing granules per cell prior to and after p(I:C) stimulation in WT, btk−/−, tlr3−/−, and rig-1−/− HeLa cells. G3BP1 containing granules were quantified from 10 cells per treatment using IMARIS 9.7 software (Images shown here are representatives of images shown in Fig. S4). Confocal images from Figure 3A were used to render the 2D images of the G3BP1 granules (colored in red). Bar chart quantification of granules per cell + SD (lower figure). Width of white bar indicates 10 μM. C, Pearson’s correlation of BTK-G3BP1 (red) and BTK-TIA (green) correlation in WT cells in the absence or presence of p(I:C) stimulation. BTK, Bruton’s tyrosine kinase; G3BP1, RAS-GTPase-activating protein (SH3 domain)-binding protein 1; p(I:C), polyinosinic:polycytidylic acid.

G3BP1 tyrosine phosphorylation requires full-length and kinase activity of BTK

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BTK phosphorylation of G3BP1

To confirm that BTK directly phosphorylates G3BP1 and not act as a scaffold protein to recruit another kinase, we co-expressed HA-tagged kinase variants of BTK with FLAG-tagged G3BP1 in HEK293T cells and examined the phosphorylation status of G3BP1 (Fig. 4C). The E41K variant (BTK\textsuperscript{CA}) resulted in constitutive activation while the K430R variant (BTK\textsuperscript{KD}) abolished the kinase activity of BTK (23). Co-expression of BTK\textsuperscript{WT} led to the phosphorylation of G3BP1. The co-expression of BTK\textsuperscript{CA} resulted in enhanced G3BP1 tyrosine phosphorylation. On the other hand, co-expression of BTK\textsuperscript{KD} abolished G3BP1 tyrosine phosphorylation. Taken together, our data indicate that a full-length BTK is required to bind G3BP1 and that BTK directly phosphorylates G3BP1.

**BTK phosphorylates Y40 in the NTF2-like domain and enables G3BP1 dimerization**

G3BP1 comprises five well-characterized NTF2-like, acidic, PxxP, RRM, and RGG domains (7). To identify which G3BP1 protein domain mediates BTK interaction, we generated mutant constructs harboring various combinations of G3BP1 motifs (Fig. 5A) and co-expressed them with full-length BTK in HEK293T cells. As shown in Figure 5B, co-IP studies indicated that an intact full-length G3BP1 was required to bind BTK as the various truncations of G3BP1 resulted in the loss of BTK binding. This finding together with that in Figure 4 indicates that BTK–G3BP1 interaction is not mediated by any particular motif on individual protein but rather requires both proteins to be intact.

Next, we attempt to elucidate the tyrosine residue(s) within G3BP1 that BTK phosphorylates. Using the bioinformatics prediction software NetPhos 3.1, we identified two tyrosine sites within the NTF2-like domain of G3BP1 that could potentially be phosphorylated by BTK (Fig. 5F, above threshold score of 0.6). These residues were Y40 and Y125 (Fig. 5C, upper panel and Fig. S5). We proceeded to generate two G3BP1 mutants with tyrosine to alanine substitutions—Y40A and Y125A—to examine BTK phosphorylation of these residues (Fig. 5C, lower panel). We co-expressed wildtype and the two G3BP1 phospho-mutants together with FLAG-tagged BTK in HEK293T cells and found that BTK can still bind these mutants (Fig. 5D). However, the tyrosine phosphorylation of G3BP1 was significantly impaired when G3BP1\textsuperscript{Y40A} was co-expressed with BTK, suggesting that Y40 is the primary site whereby BTK phosphorylates G3BP1 (Fig. 5D).

G3BP1 was previously shown to form homodimer mediated via their NTF2-like domain (10, 16). Hence, we explored if Y40 could be involved in the formation of G3BP1 homodimers. We overexpressed wildtype G3BP1, tyrosine mutants G3BP1\textsuperscript{Y40A} and G3BP1\textsuperscript{Y125A}, and serine mutants known to affect SG formation, G3BP1\textsuperscript{S149E} and G3BP1\textsuperscript{S99P}, in HEK293T cells. Whole cell lysates were prepared and incubated with BS\textsubscript{3} cross-linker in increasing dosage. As shown in Figure 5E, G3BP1 homodimerization did not occur with G3BP1\textsuperscript{Y40A} and G3BP1\textsuperscript{S99P} variants but with wildtype G3BP1 and G3BP1\textsuperscript{Y125A} and G3BP1\textsuperscript{S149E} mutants. Taken together, the data indicate that BTK phosphorylates G3BP1 at Y40 and this residue regulates G3BP1 homodimerization.
G3BP1-Y40 is essential for the formation of SG

Since G3BP1 Y40 plays a critical role in G3BP1 dimerization, we examined if it plays a role in SG formation. Consistent with the biochemical data in Figure 5E, confocal microscopy examination of cells revealed the formation of discrete SG in HEK293T cells overexpressing either wildtype G3BP1 or G3BP1Y125A variant (Fig. 5F). BTK was also found to localize within these distinct granules. However, no such SG was seen in cells over-expressing G3BP1Y40A mutant. These data suggest that Y40 plays a role in SG condensation. Next, we generated two phospho-mimetic variants (Fig. 6A)—G3BP1Y40E to mimic constitutively phosphorylated and G3BP1Y40F to represent nonphosphorylated proteins. We chose to generate G3BP1Y40F variant as it represents a less disruptive modification compared to our previous G3BP1Y40A variant. We co-expressed HA-tagged G3BP1 wildtype and the two mutants together with FLAG-tagged BTK in HEK293T cells and found that BTK binding was enhanced with G3BP1Y40E and significantly reduced with G3BP1Y40F mutant (Fig. 6B). Confocal microscopy studies indicated that the overexpression of wildtype or G3BP1Y40E variant with BTK precipitated the formation of large and distinct SG aggregates (as indicated by the white arrows in Fig. 6C). However, the condensation of large SG was attenuated in cells co-expressing BTK and G3BP1Y40F. Although small granules can be seen, they generally lacked BTK co-localization. We also treated p(I:C)-stimulated LN-229 cells with the BTK inhibitor LFM-A13 and showed that the formation of SG was attenuated (Fig. S6). Collectively, the data thus far indicated that SG formation requires G3BP1 and BTK interaction and that the phosphorylation of G3BP1 Y40 residue is the molecular switch that governs SG clustering during cell’s sensing of foreign RNA.

Discussion

We show in this study that G3BP1 is tyrosine phosphorylated upon stimulation of cells with p(I:C) which activated the intracellular innate immune nucleic acid-sensing pathways. We also demonstrate that BTK is responsible for the phosphorylation of G3BP1. Our work further indicates that G3BP1 is phosphorylated at Y40 in the NTF2-like domain and that this residue is critical for G3BP1 dimerization and the formation of avSG aggregates. Treatment of cells with the BTK inhibitor LFM-A13 or deletion of the \( btk \) gene abrogated p(I:C) induced SG formation. Mutating G3BP1 Y40 to alanine or phenylalanine also abolished the ability of G3BP1 to induce the formation of SG aggregates. Hence, BTK and G3BP1 Y40 are essential for the condensation of avSG.
G3BP1 is characterized by five specific domains (7). Earlier work by Tourriere et al. had shown that the truncation of its NTF2-like and/or RGG domain significantly affected the formation of stress granules (10). Our current work is consistent with this earlier observation and further extended the finding by pinpointing the molecular mechanism involved, namely the phosphorylation of Y40 in the NTF2-like domain by BTK and the subsequent induction of G3BP1 dimerization leading to SG aggregation.

That Y40-mediated G3BP1 dimerization is important for SG clustering is also supported by two recent major publications which revealed that the physical crosslinking of G3BP1 drives RNA molecules into networked RNA/protein condensates (8) and that G3BP1, being the central node of this network, triggers liquid–liquid phase separation in response to an increase in intracellular RNA concentrations, and this process is critical for SG assembly (16). Incidentally, we had previously shown that G3BP1 could bind RNA via its RGG domain and that G3BP1 could further recruit via its RGG domain, RIG-I, which is a major sensor of viral RNA (7). These molecular interactions serve to further concentrate and condensate more RNA and proteins into a network that facilitates the formation of large avSG aggregates.

A recent article had revealed that the assembly of large SG also required ATP-driven reactions, but it was not known which kinase activity was involved (25). Interestingly, our current study implicates BTK to be an important component in the formation of avSG. BTK is involved in the signaling of various immune receptors such as the Toll-like receptors, C-type lectin receptors, B cell receptor, and various other intracellular innate immune receptors (23, 24). However, its role in avSG formation has not been documented. Here, we uncovered an important role for BTK in the phosphorylation of Y40 in the NTF2-like domain of G3BP1 that is critical for its dimerization and the clustering of avSG.

Taken together, our findings help to reveal a model for the formation of avSG in host foreign RNA sensing response. It directly illuminates how the sensing of viral ribonucleic acids by the various innate immune receptors such as RIG-I and TLR3 leads to the assembly of avSG. Upon virus infection, BTK is activated by the various immune receptors that recognize viral RNA. It subsequently phosphorylates G3BP1 at Y40 to induce its dimerization. G3BP1 also interacts with viral RNA and with intracellular RNA-sensing proteins such as RIG-I which helped to bind even more viral RNA, forming ribonucleoprotein complexes. It is interesting to note that the knock-out of either RIG-I or TLR3 significantly abrogate the formation of SG aggregates suggesting that the combined action of various RNA sensors might be necessary to "concentrate" enough RNA and ribonucleoprotein complexes. In turn, the dimerization of G3BP1 helps to condensate ribonucleo-protein complexes into large aggregates that segregate away viral RNA to prevent their translation, replication, or packaging into virions or facilitate their decay. Hence, it is not surprising that viruses have evolved strategies to cleave G3BP1 to prevent the formation and assembly of avSG.

**Experimental procedures**

**Mice**

C57BL/6 and btk<sup>−/−</sup> mice were purchased from Jackson Laboratory and bred in our animal housing facilities. All mice-
BTK phosphorylation of G3BP1

related experiments were performed in accordance to guidelines issued by A*STAR Biological Resource Centre Institutional Animal Care and Use Committee.

Isolation and differentiation of BMDMs

Bone marrow cells were extracted from the femurs of C57BL/6 and btk−/− mice. Red blood cells were lysed with Ammonium-Chloride-Potassium (ACK) lysis buffer, and the remaining cells were washed in PBS and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 20% L929 cell-conditioned media for 3 days. After 3 days, culture media was changed daily with fresh media for another 4 consecutive days before cells were ready for further experimentations.

Cell culture and reagents

COS-7, HEK293T, and LN-229 cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with heat-inactivated fetal bovine serum (Biowest) and Penicillin/Streptomycin (P/S) (Gibco). Cells were stimulated with pI:C (Invivogen), DMXAA (MedChem Express) or sodium arsenite (Sigma). BMDM, HEK293T, and LN-229 cells were pretreated with LFM-A13, terreic acid (MedChem Express), and PP2 (Milliopore) for an hour prior to stimulation with various ligands. Generation of CRISPR/Cas9-targeted HeLa cells performed as described (26). In brief, following primers: RIG-I (5’-CACGGGAAGTGTGGCAGCCTC-3’) and (5’-AAACATGAGCTGCACTTTAATTTCA-3’), TLR3 (5’-CACCGTGTCACCGTGACAGAAC-3’) and (5’-AACGTGTGGCAACGAAAGAACC-3’). Annealed primers were then ligated in pSB-CRISPR previously linearized using Esp3I (NEB) and purified. HeLa cells were co-transfected with pCMV-T7-SB100 and pSB-CRISPR (at 1:2 ratio) using Lipofectamine 3000 (Thermo Fisher Scientific), and statistical significance was determined based on a p-value less than 0.05.

Data availability

The data that support the findings of this article are available from the corresponding author upon reasonable request.

supporting information—This article contains supporting information.

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Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.
Abbreviations—The abbreviations used are: avSG, antiviral SG; BTK, Bruton’s tyrosine kinase; G3BP1, Ras-GTPase-activating protein (SH3 domain)-binding protein 1; NTF2, nuclear transport factor 2; p(I:C), polyinosinic-polycytidylic acid; PTMs, post-translational modifications; RRM, RNA recognition motif; RGG, arginine and glycine-rich boxes; SG, stress granule.

References