Histone arginine demethylase JMJD6 is linked to stress granule assembly through demethylation of the stress granule–nucleating protein G3BP1

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Stress granules (SGs) are membrane-less organelles that are condensates of stalled translation initiation complexes and mRNAs. SG formation is a cytoprotective response to environmental stress and results from protein interactions involving regions of low amino acid complexity and poorly defined post-translational modifications of SG components. Many RNA-binding proteins are methylated, and we previously demonstrated that the potent SG–nucleating protein G3BP1 is methylated by protein arginine methyltransferase 1 and 5 (PRMT1 and PRMT5). G3BP1 methylation represses SG formation and is reversible. Here we functionally link JMJD6 (Jumonji C domain–containing protein 6) to G3BP1 demethylation. Our findings reveal that JMJD6 is a novel SG component that interacts with G3BP1 complexes, and its expression reduces G3BP1 monomethylation and asymmetric dimethylation at three Arg residues. Knockdown of JMJD6 repressed SG formation and G3BP1 demethylation, but SG formation and G3BP1 demethylation were rescued with catalytically active but not mutant JMJD6. These results suggest that JMJD6 functions directly or indirectly as an arginine demethylase of G3BP1 that promotes SG formation.

Stress granules (SGs) are cytoplasmic mRNP complexes that rapidly formed in response to unfavorable environments in eukaryotic cells. Stalled translation initiation complexes containing translation initiation factors, mRNA, 40S ribosome subunits, and key RNA-binding proteins such as G3BP1, Tia1, HuR, TDP43, FUS, and FRMP concentrate in SGs (1–6). Stress granules are thought to function as short-term repositories for mRNA to prevent mRNA degradation (7), as platforms for innate immune activation of double-stranded RNA-dependent protein kinase PKR (8, 9), and function as mediators of signaling cascades (10–13). SG formation typically follows translation inhibition caused by stress-induced eIF2α phosphorylation (1, 2, 14, 15), disruption of eIF4A and eIF4G function with small molecule inhibitors or virus infection (16) (17), and stress-induced tRNA cleavage (18–21) via an eIF2α-independent pathway.

Some of these RNA-binding proteins have been characterized as SG–nucleating proteins, particularly G3BP1 and Tia1. SG–nucleating proteins can induce SG formation when overexpressed, independent of exogenous stressors (3, 22, 23). Current models of SG structure involve stable cores enriched for G3BP1 surrounded by a dynamic shell that rapidly undergoes assembly and disassembly (24). Consistent with this model, knock-out of G3BP1 and its isoform G3BP2 deplete SG formation (25, 26), thus the classification of G3BP1 as a SG–nucleating protein. G3BP1 is also targeted by many viruses for proteolytic cleavage or sequestration to block SG formation during establishment of successful infections (27, 28).

Two models for SG formation exist. The first model posits that untranslated mRNPs promote liquid–liquid phase separation (LLPS), forming liquid droplets that are held together by weak interactions involving intrinsically disordered regions (IDR) of interacting proteins (29). Core formation then results from higher-affinity interactions that form during a structural transition at the center of the droplet. The second model proposes that stalled 48S preinitiation complexes can serve as a seed for SG condensation (30, 31), which attracts nucleating RNA-binding proteins such as G3BP1 already bound to 40S ribosomes (26), thus increasing their local concentration and favoring LLPS, liquid droplet formation, and nucleation of SGs.

SG assembly is highly dependent on post-translational modification (PTM) of IDRs of key SG–associated RNA-binding proteins (3, 32–37). Many RNA-binding proteins, including G3BP1, are highly enriched with IDR, compared with the rest of the human proteome (38).

Although many PTMs on IDR of SG–nucleating proteins have been proposed to influence SG assembly (3, 32–34, 36, 37), only phosphorylation of G3BP1 at Ser-149 by CKII (3, 39) and arginine methylation of the RGG domain of G3BP1 (25) have been shown to modulate SG assembly. We recently showed that G3BP1 is differentially methylated on Arg-435, Arg-447, and Arg-458. Demethylation of G3BP1 in response to stress (40) (41), as well as its interaction with mRNAs (42), supports a role for arginine methylation in SG formation.
Arg-460 by protein arginine methyltransferase 1 (PRMT1) and PRMT5. Arsenite stress quickly and reversibly decreased asymmetric methylation on G3BP1 promoting SG assembly (25). However, the arginine demethylase responsible for this activity was not identified.

JMJD6 (Jumonji C (JmjC) domain-containing protein 6) was the first arginine demethylase described, capable of demethylating arginine residues on histone H3R2 and histone H4R3 (40). The demethylation activity is driven by the JmjC domain that requires Fe(II) and 2-oxoglutarate to hydroxylate methyl groups following deamination producing formaldehyde (41).

Recently, studies from other groups validated demethylation activity of JMJD6 via biochemical and cell-based assays, confirmed by mass spectrometry (42–46). JMJD6 demethylase activity has also been reported on non-histone proteins, including estrogen receptor ERα (43), RNA helicase A (44), HSP70 (45), PAX3 paired box 3) (47), and TNF receptor-associated Factor 6 (46), indicating that JMJD6 also demethylates non-histone substrates.

Our previous work demonstrated rapid demethylation of G3BP1 following arsenite stress. The emerging role of JMJD6 as a demethylase prompted us to investigate JMJD6 activity on G3BP1. Here we show that JMJD6 is a new component of SGs that directly interacts with G3BP1. Expression of JMJD6 specifically reduced the levels of ω-Nε,Nε-asymmetric dimethylarginine (ADMA) modifications on proteins, including on G3BP1, and promoted SG assembly during arsenite stress. Furthermore, shRNA silencing of JMJD6 or chemical inhibition with a pan-JmjC domain inhibitor decreased SG formation during stress. Mass spectrometry confirmed that G3BP1 was demethylated at Arg-435, Arg-447, and Arg-460 by overexpression of JMJD6. Lastly, rescue experiments showed that expression of wild-type JMJD6, but not a catalytically inactive mutant, caused demethylation of G3BP1 and promoted SG assembly during arsenite stress. Collectively, these data indicate that catalytic activity of JMJD6 results in G3BP1 demethylation that triggers SG assembly during oxidative stress and strongly suggest methylated G3BP1 is a substrate of JMJD6.

Results

Arginine demethylase JMJD6 associated with stress granule complexes

G3BP1 is a dominant SG–nucleating protein regulated by phosphorylation and arginine methylation. Recently, the kinase responsible for G3BP1 phosphorylation was identified (39), but the demethylase(s) that governs SG condensation has not been resolved. G3BP1 is arginine-methylated, and JMJD6 was reported to demethylate methylarginine substrates, suggesting that JMJD6 might catalyze G3BP1 demethylation (40, 43–46). To investigate whether JMJD6 is involved in the cellular stress granule response, we visualized the distribution of endogenous JMJD6 in U2OS and HeLa cells during arsenite stress. In unstressed cells, immunofluorescence analysis (IFA) indicated that JMJD6 was most strongly localized in the nucleus (Fig. 1a, top panels), which agreed with previous studies from other groups (48, 49). However, we found much of the cytoplasmic fraction of JMJD6 concentrated in cytoplasmic punctate foci after 30 min of arsenite treatment, which colocalized with the SG marker Tia1 in HeLa cells after arsenite stress. The cells were treated with arsenite 30 min to induce SGs, and cells were counterstained against the SG marker Tia1 (red) and DAPI for nuclei (blue), b; SGs formed by thapsigargin-induced (4 h) ER stress or heat shock (60 min) in U2OS cells were visualized by IFA showing endogenous JMJD6 (green) and G3BP1 (red). Yellow squares indicate regions depicted in vignettes. c; pulldown of endogenous G3BP1 in unstressed (UT) and arsenite-treated (AS) HeLa cells. JMJD6 was detected by Western blot analysis. IgG was antibody control for pulldown assay. The quantification of JMJD6 intensity is shown relative to untreated condition and normalized versus G3BP1 levels in the pulldown. The results are representative of three independent experiments that were conducted, with 100 cells counted in each. *, p < 0.05 versus untreated control. Original magnification was 63×. IB, immunoblot; IP, immunoprecipitation.

Expression of catalytically active JMJD6 promotes SG formation

To understand the role of JMJD6 in stress responses, we used methods to manipulate JMJD6 activity to examine methylation levels in cells and how it impacts SG assembly. Overexpression of GFP-JMJD6 in U2OS cells resulted in 30% reduction of global ADMA signal in cells compared with a GFP-transfected control (Fig. 2b, left panel). Symmetric dimethyl arginine (SDMA) is another common arginine methylation pattern on proteins, but global SDMA signals are unaffected by JMJD6 overexpression.
indicating that JMJD6 bears a substrate preference for ADMA-modified proteins. To further confirm the alteration of ADMA levels in cells correlated with enzymatic activity of JMJD6, we generated dual point mutations H187A/D189A in the catalytic domain of JMJD6, which has been shown to abolish enzymatic activity of JMJD6 (40, 50). U2OS cells expressing JMJD6M showed no alteration of ADMA levels in cells (Fig. 2b, left panel), suggesting that the reduction of ADMA levels of proteins correlated with enzymatic activity of JMJD6. Next, we examined SG abundance in response to expression of JMJD6. In untreated cells, we did not see spontaneous SG formation in cells expressing GFP, GFP-JMJD6, or GFP-JMJD6M (Fig. 2c, top panels). There was an approximately 1.5-fold increase in SG formation in cells that expressed GFP-JMJD6 during arsenite stress compared with GFP transfected cells (Fig. 2c, bottom panels, and d), whereas cells expressing GFP-JMJD6M were modestly repressed in SG formation (0.9-fold in HeLa, and 0.5-fold in U2OS) (Fig. 2d). Immunoblot analysis indicated that JMJD6 or JMJD6M expression did not alter G3BP1 levels in cells (Fig. 2b). Taken together, these data indicate that expression of JMJD6 modulates ADMA signals on proteins and promotes SG assembly.  

Figure 2. Overexpression of JMJD6 promotes SG formation. U2OS cells were transfected with GFP (C), GFP-JMJD6 (J6), or GFP-catalytic domain mutant JMJD6 (J6M) for 24 h before analysis. a and b, antibodies specific for GFP (a), asymmetric (ADMA) methyl modification (b, left panel), and symmetric methyl modification (SDMA) (b, right panel) were used in Western blot analysis. Arrows in a indicate GFP or GFP-tagged transgene. c, SG formed in GFP, GFP-JMJD6, and GFP-JMJD6 transfected U2OS cells after treatment with arsenite (60 min). Cells were counterstained with SG markers G3BP1 in gray and eIF3B in red. Yellow arrows point to transfected cells. d, quantification of average SGS/cell in HeLa and U2OS cells expressing either GFP (white bar), GFP-JMJD6 (gray bars), or GFP-JMJD6M (black bars). *, p < 0.05 versus untreated (UT) control; #, p < 0.05; ##, p < 0.01 versus GFP-JMJD6 transfected arsenite (Ars) cells. The results shown in all panels were performed three times, and those in b were performed five times. For IFA, 100 cells were counted in each experimental replicate. Original magnification was 63×.
We observed a 1.5-fold increase in ADMA levels on total proteins after 24 h of NOG treatment in HeLa cells (Fig. 3d, left panel) but no change in SDMA levels (Fig. 3d, right panel). Treatment with NOG did not induce spontaneous SGs in untreated cells (Fig. 3, e, top panels, and f), whereas NOG treatment resulted in ~3-fold and 2-fold less arsenite-induced SGs in HeLa and U2OS cells, respectively (Fig. 3, e, bottom panels, and f). Collectively, we observed a defect in SG assembly when enzymatic activity of JMJD6 was impaired either by knockdown or with an inhibitor, suggesting that JMJD6 is an important protein that promotes SG assembly.

To further determine whether JMJD6 modulates SGs through G3BP1 we tested SG formation in JMJD6-silenced G3BP1 KO cells that were rescued with either GFP or GFP-G3BP1 constructs. In agreement with our previous study and those from other groups (25, 26), ablation of G3BP1 (Fig. 4a) strongly inhibited SG formation in untreated and arsenite-treated cells, and further, SGs were not rescued by JMJD6 KD in the G3BP1-null condition (Fig. 4b). Restoration of G3BP1 expression by transfecting GFP-G3BP1 (Fig. 4a) effectively rescued SG assembly in untreated and arsenite-stressed shScramble cells (Fig. 4b). However, this expression of GFP-G3BP1 was ~2-fold less effective in restoring SGs in G3BP1KO cells where JMJD6 was also knocked down (Fig. 4, c, top panels, and b). In these cells, JMJD6 knockdown also resulted in a ~2-fold reduction of SG assembly during arsenite stress (Fig. 4, c, bottom panels, and b), suggesting a link between JMJD6 and G3BP1 in promoting SG assembly.

**JMJD6 expression results in G3BP1 demethylation**

To test whether changes of ADMA signals on total proteins reflect levels of ADMA on G3BP1, we performed LC-MS analysis of changes in G3BP1 methylation after manipulating JMJD6 expression by plasmid overexpression or shRNA knockdown and stressing with oxidative reagents. Consistent with our previous work (25), we saw a decrease in methylation on Arg-435 (~30% and ~40%), Arg-447, and Arg-460 (~25% and 50%) in GFP- or shSc-expressing control cells during arsenite stress.
G3BP1 demethylation

stress (Fig. 5, a, and c, white bars). Both Arg-447 and Arg-460 consistently appear on the same tryptic peptide; however, analysis of fragment ion (MS-MS) spectra can effectively determine, which residue is mono- or dimethylated (Fig. 5, b and d). Expression of JMJD6 reduced mono- and dimethylation on Arg-435 (~50%), and monomethylation on Arg-477 (~50%) in arsenite-stressed cells (Fig. 5a, black bars). Demethylation of G3BP1 was reduced (~50% at Arg-435, ~20% at Arg-447), even during untreated conditions by expressing JMJD6 (Fig. 5a, light gray bars). Interestingly, G3BP1 containing dimethyl marks on both Arg-447 and Arg-460 were not demethylated under these conditions, suggesting a substrate preference for methyl arginines at Arg-435 and Arg-447, which are both ADMA modified (25). We assume that the reduction in G3BP1 methylation during arsenite is nearing the sensitivity of detection using LC-MS at these sites on G3BP1.

In contrast to JMJD6 expression, knockdown of JMJD6 resulted in more sustained monomethylation on Arg-447 in response to arsenite stress (Fig. 5c, compare white and black bars on the Arg-447-M, Arg-460-D peptide) and a slight shift on Arg-435. As we expected, in most cases JMJD6 silencing did not significantly change G3BP1 methylation status at the three methylation sites under unstressed conditions (Fig. 5c, light gray bars). Collectively, these data strongly suggest that expression of JMJD6 is linked to G3BP1 demethylation at residues Arg-435 and Arg-447.

**JMJD6 promotes SG formation and demethylation of G3BP1**

To confirm that demethylase activity of JMJD6 promotes SG assembly, we performed a JMJD6 rescue experiment in JMJD6 silenced cells to monitor ADMA, SDMA levels on G3BP1, and how SG assembly was affected under these conditions. shJMJD6 expression resulted in a 70% knockdown of JMJD6 compared with shSC control, and transgenes were expressed equivalently (Fig. 6a). We examined the expression levels of JMJD6 transgene, ADMA and, SDMA signals after 24 h of transient transfection of HeLa cells with silencing of endogenous JMJD6 (shJMJD6). Silencing of JMJD6 modestly increased ADMA levels in cells (1.3-fold), but cells with endogenous JMJD6 silenced and rescued with GFP-JMJD6 had almost the same levels of ADMA signals compared with GFP transfected control shRNA. However, cells expressing GFP-J6M failed to rescue the demethylation phenotype and contained 1.7-fold increased ADMA signal on G3PB1. There was an 80% reduction of ADMA levels on G3BP1 in shJMJD6 cells rescued with GFP-JMJD6M when compared with the GFP alone rescue in shJMJD6 cells, both of which displayed ~2-fold higher ADMA than the control (Fig. 6c, top panel). We also performed a reciprocal immunoprecipitation with G3BP1 and found similar results where silencing of JMJD6 increased ADMA signal on G3BP1. There was a 80% reduction of ADMA levels on G3BP1 in shJMJD6 cells rescued with JMJD6 compared with cells rescued with GFP, but not when GFP-JMJD6M was expressed (Fig. 6c, bottom panel).

Finally, we examined whether the changes in ADMA observed on G3BP1 resulting from manipulation of JMJD6 were sufficient to alter SG assembly. In agreement with previous results, expression of JMJD6 and JMJD6M did not induce SGs under unstressed condition (Fig. 6d, top panel). Knock-
down of JMJD6 impaired SG formation (~46%) during arsenite stress (Fig. 6, bottom panel, and e). Interestingly, there was a 1.6-fold increase in SG formation when GFP-JMJD6 was expressed in shJMJD6 cells (yellow arrows in Fig. 6, d, bottom panels, and e). However, GFP-JMJD6M expression did not rescue SG assembly in response to arsenite stress (Fig. 6, d, bottom panels, and e). Together, these results indicate that JMJD6 demethylates G3BP1 at Arg-435, Arg-447, and Arg-460 to nucleate SG assembly in response to arsenite stress.

Discussion

SG assembly is a dynamic process regulated by protein–protein and protein–RNA interactions that may involve LLPSs (23, 31, 53–57). SG–nucleating proteins are thought to play critical roles in SG condensation by recruiting other SG constituents, thus acting as platforms for SG assembly (31). SG–nucleating proteins are enriched in IDRs that might facilitate the dynamic interactions between other SG constituents and the cellular milieu and are decorated by PTMs that potentially regulate SG assembly (3, 32–34, 36, 37). Few studies describe how PTMs serve as molecular switches to govern SG–nucleating protein activity in SG assembly (3, 25, 35). In our previous study, we reported that arginine demethylation of G3BP1 promotes SG assembly. Here, we extend our work to identify a candidate G3BP1 demethylase that promotes SG formation. Our data strongly suggests that asymmetric dimethyl argine modification on G3BP1 is reversible, similar to DNA methylation. Given the location of the methylation sites on G3BP1 within an IDR, this work emphasizes the importance of IDRs in SG proteins. Dephosphorylation of Ser-149 is another example of a PTM within an IDR that promotes SG assembly. We propose that phosphate- and methyl-groups are removed during stress to expose IDR, perhaps promoting interaction with other SGs proteins or 40s ribosomes (26). This raises the question of possible synergy between demethylation and dephosphorylation of G3BP1 to promote SG-related functions.

The RGG domain in the C terminus of G3PB1 has critical functions in SG assembly and interacts with 40S ribosomal subunits during translation inhibition (26). The N-terminal NTF2 domain of G3BP1 interacts with other SG components, such as
Caprin1, and brings together RGG-bound 40S subunits (26). Therefore, SG assembly requires both the NTF2 and RGG domains of G3BP1. Post-translational modification of G3BP1 could alter SG condensation by changing the affinity of G3BP1 for other SG constituents through the N terminus or the 40S ribosome through the C-terminal RGG domain.

Here, we show that the enzymatic activity of JMJD6 is also positively correlated with SG assembly. Together, these results suggest that JMJD6 enhances SG condensation by demethylating the RGG domain and possibly promoting interaction with the 40S subunit. Deletion of the RGG domain from G3BP1 abolishes association of JMJD6 in G3BP1 complexes (data not shown).

Previous work has suggested a two-staged assembly of SGs, nucleated in the first stage by G3BP1-formation of many small granules that then merge into larger granules in the second stage (24, 58). Formation of small SGs seems to be independent of translational repression in other studies but disrupts SG-PB interactions (15, 59). We noticed the size of SGs is smaller when JMJD6 is depleted in arsenite-stressed cells, despite the increased number per cell (Figs. 3b, 4b, and 5d). SGs in PRMT1 and PRMT5 knock-out cells also showed a small SG phenotype,
and those small SGs still correlated with translational repression in response to arsenite stress (25). These data indicate that the link between SGs and the translational apparatus is more complicated than previously thought and is probably a context-dependent relationship.

JMJD6 has been reported to be involved in an array of biological processes, but to our knowledge nobody has shown a link to SG biology. Our data support a role for JMJD6 in SGs because we show cytoplasmic JMJD6 concentrates in SGs and is involved in SG assembly by demethylating G3BP1 at Arg-435, Arg-447, and Arg-460 during arsenite stress. FMDV causes SG formation early in infection but then eliminates SGs for viral replication (60). Intriguingly, Lawrence et al. (44) has observed JMJD6 foci in the cytoplasm during FMDV and BEV-1 infection early in infection, which could be SGs as many enteroviruses initially promote SG formation (61). This idea is supported by our findings that JMJD6 is recruited to SGs during stress. JMJD6 has also been shown to interact with RNA in vitro (62), suggesting that JMJD6 interacts with other RNA-binding proteins or is an RNA-binding protein itself. Indeed, mass spectrometry data from Poulard et al. (43) reveals that JMJD6 interacts with SG components Tia1, PABP1, PABP4, and DDX3. These proteins or G3BP1 itself might be involved in recruiting JMJD6 to SGs. Collectively, these data strongly implicate JMJD6 in SG biology. HSP70 is a substrate of JMJD6, suggesting that JMJD6 may have other roles in the stress response (45). JMJD6 is also involved in regulation of a multitude of biological processes, including embryonic development (63–65), cell proliferation (66), cell cycle (50), cell mobility (67), adipocyte differentiation (68), and the emerging roles in human cancers (50, 66), cell cycle (50), cell mobility (67), adipocyte differentiation (68), and the emerging roles in human cancers (50, 66, 67, 69, 70). Despite this, however, further work is required to characterize the function of JMJD6 in the stress response.

Our results demonstrate that the enzymatic activity of JMJD6 promotes G3BP1 demethylation. JMJD6 expression only alters ADMA modified proteins, but not SDMA. Similar results have been shown by other groups on different proteins such as ERα, RNA helicase A, HSP70, and TRAF6 (43–46). Interestingly, these proteins are all substrates of PRMT1, which is the major type I PRMT that generates ADMA. G3BP1 is also a substrate of PRMT1 (25), supporting the notion that JMJD6 is an important regulator of a stress-dependent G3BP1 methylation cycle. The literature has debated whether JMJD6 is a true arginine demethylase or a hydroxylase because it has a JmJC domain similar to lysine hydroxylases and lacks a consensus arginine demethylase domain. However, evidence from some studies also point out that JMJD6 requires other proteins as cofactors (42, 71). This is also reported for PRMT5 and for the lysine demethylase LSD1, which require MEP50 and Co-REST to become fully active (72, 73). Additionally, two methylation readers TDRD3 (74) and Tudor-SN (75) are components of SGs and could direct JMJD6 to methylated residues in SGs. Although we detected demethylation of G3BP1 during JMJD6 overexpression, our data do not distinguish between the effects of JMJD6 itself or as a complex requiring the use of cofactors.

In conclusion, our findings link the arginine demethylase JMJD6 with SG assembly by modulating the SG-nucleating protein G3BP1. Gain- and loss-of-function assays, MS analysis and data from other groups strongly suggest that JMJD6 mediates demethylation of G3BP1 and promotes SG formation. However, arginine demethylation is not sufficient to induce SGs because we did not observe any SG formation in untreated GFP-JMJD6 overexpression cells. Similarly, dephosphorylation of G3BP1 under unstressed conditions cannot drive SG assembly. These data suggest that multiple PTMs on G3BP1 are required to effectively drive SG formation.

Experimental procedures

**Cell culture, transfections, and JMJD family inhibitor**

The cells were cultured under standard conditions of 10% FBS in DMEM. All expression constructs were transfectioned into cells by Lipofectamine 3000 (Thermo Fisher Scientific) in accordance with the manufacturer’s instructions. The JMJD inhibitor NOG was purchased from SIGMA (o-9390). The cells were pretreated with 5 mm NOG for 24 h before use in experiments.

**RNA interference with shJMJD6**

HeLa, U2OS, or G3BP1-KO U2OS cells were plated and infected with lentiviruses expressing shJMJD6 (TRCN0000303286, SIGMA) or shScramble in the presence of 8 g/ml protamine sulfate for 24 h followed by puromycin (2 g/ml; 48 h) selection. RT-PCR and/or Western blotting were performed to validate the knockdown efficiency.

**Plasmid constructs**

JMJD6 cDNA was amplified by PCR from HEK293 cell cDNA and then subcloned into pAC-GFP plasmid. JMJD6 catalytic domain mutant was generated by site-directed mutagenesis at H187A and D189A (forward primer, CTCGGGAACCTGGGA- TTGCGATGCACCTCTGGGAAACGATG; reverse primer, CAGGCACTGGTTCCCAGAGGTGCGATCGCAATCCCA- GTTCCGGAGCGTG). Site-directed mutagenesis was performed with Herculase II fusion DNA polymerase (Agilent Technologies) as described by the manufacturer.

**Mass spectrometry analysis**

G3BP1 was immunoprecipitated from untreated and arsenite-treated cells that were overexpressing or silencing JMJD6, subjected to SDS-PAGE, and excised for trypsin digestion. The resulting peptide fragments were analyzed by LC-MS using an LTQ ion-trap mass spectrometer (Orbitrap Elite™; Thermo Fisher Scientific) equipped with a nano LC electrospray ionization source. Obtained MS/MS spectra were searched in Proteome Discoverer 1.4 interface (Thermo Fisher Scientific) with Mascot algorithm (Mascot 2.4; Matrix Science) to determine arginine methylation of G3BP1 peptides. Approximately 85% of total G3BP1 peptide coverage was achieved in each independent experiment.

**Immunofluorescence assay**

Microscopy was performed essentially as described previously (25). Primary antibodies were incubated overnight at 4 °C. The primary antibodies used were anti-JMJD6 (Cell Signaling, catalog no. 2449), anti-G3BP1 (28) (Abcam, catalog no. ab56574), anti-Tia1 (Santa Cruz, catalog no. SC11386), anti-HuR (Santa Cruz, catalog no. SC-5261), anti-eIF3b (Santa Cruz,
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