EXPERT REVIEWS

GW bodies: from RNA biology to clinical implications in autoimmunity

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Evaluation of: Lian S, Fritzler M, Katz J *et al*. Small interfering RNA-mediated silencing induces target-dependent assembly of GW/P bodies. *Mol. Biol. Cell* 18, 3375–3387 (2007).

GW bodies (GWBs) are also known as mammalian processing bodies and are involved in 5′–3′ mRNA degradation. Conversely, siRNA is a powerful tool for silencing genes. Recently, components of RNAi have been associated with GWBs, but as more components of this complex pathway become known, such relationships remain to be clarified. This paper evaluates the induction of GWBs by siRNA transfection. The main results of these studies indicate that siRNA increased the GWBs, such an increase is also dependent on the endogenous expression of the target mRNA; siRNA increases require GW182 or Ago-2 proteins, but not rck/p54 or LSm1. Results of the present studies propose a regulatory function of RNAi in GWB assembly; therefore, cell biology implications of GWBs may open a new area in pathogenic mechanisms of autoimmunity.

KEYWORDS: GW 182 • GW bodies • neuropathy • siRNA

The GW bodies (GWBs) are the equivalent to mammalian processing (P) bodies, located in cytoplasmic foci that have multiple decay factors involved in the 5′–3′ mRNA degradation pathway. Their name is derived from the GW182 marker protein, which contains repeats of glycine (G) and tryptophan (W) and a RNA-binding domain at the carboxyl end. Decay and processing factors localized to GWBs include the deadenylase Ccr4, the decapping complex Dcp1a/1b/Dcp2, the LSm1–7 complex, Ge-1/Hedls, rck/p54, RAP55 and exonuclease Xrn1. GWBs colocalize and interact with the stress granules (SGs), which process aggregates of delayed translational complexes that accumulate during cell stress. Therefore, SGs share some components with GWBs. Interestingly, it was found that GWBs are associated with components of RNAi, which is a post-transcriptional gene-silencing mechanism that uses specific dsRNA molecules to silence genes in a sequence-specific manner. These seminal observations gave rise to the observations of intracellular RNAi processing and that the mRNA degradation mediated by RNAi requires the GWB components. The fate of GWBs is dynamic, and the size and number of GWBs depends on the cell cycle phase, nutrient conditions and other variables, for instance small GWBs, are observed in the early S phase but are larger in late S and G2 phases, GWBs are disassembled before mitosis and reassembled in early G1. In addition, the size and number of GWBs are affected by the amount of mRNA and its blocking-decay intermediates, by deadenylation, decapping, and 5′–3′ mRNA degradation or translation. Finally, an important observation of these authors is the demonstration that if RNAi is blocked, the GWBs are disassembled, whereas the introduction of siRNA into the cells led to GWB reassembly.

Methods & results

The aim of the study was to investigate the relationship between RNAi activity and the formation of GWBs. The following antibodies were used in the study: anti-GW182; anti-Ago2; anti-Dcp1a; anti-LSm4; anti-lamin A/C 636; anti-TIAR; anti-tubulin, anti-green fluorescent protein (GFP); and anti-rck/p54 and anti-LSm1. siRNA duplexes designed for hAgo2, hrck/p54, luciferase GL2-duplex EGFP and human lamin A/C as control were also used.

Construction of inducible GFP3 fibroblast (TRE-GFP3T3) cells was required. pCAG20–1 and pUHD10–3Puro constructs were transfected

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into the 3T3 fibroblast cell line to express tTA under selection with puromycin. The open reading frame of enhanced GFP (EGFP) was amplified by PCR with specific primers and the PCR fragment was ligated into the MluI–NotI restriction site of the pTRE2hyg expression vector. HeLa, HSG, NIH3T3 and GFP3T3 cells were cultured in Dulbecco's modified Eagle's medium, transiently transfected with siRNA and, in some cases, were cotransfected with two different siRNAs (24 h of difference between transfections). The transfected cells were analyzed by indirect immunofluorescence (IIF) and western blot. Statistical analysis was performed with the PRISM 4.0 program.

The main results of the present investigation were:

- The size and number of GWBs increased in siRNA-transfected cells. The induction of RNA silencing after transfection of siRNAs directed to endogenous targets indicated that the increase in GWBs is target dependent;
- The increase of GWBs induced by siRNA started the first day of transfection and was maintained over the following 4 days, peaking on day 3;
- The presence of GW182 and Ago2 is required to induce siRNA activity and increase GWB; furthermore, Ago2 expression correlates with the siRNA activity. Additionally, a decrease of GW182 inhibited GWB assembly;
- The decrease of LSm1 or rck/p54 did not inhibit the assembly of GWBs induced by siRNA.

Discussion & significance

The GWB regulation under conditions of induced siRNA is highly reproducible and GWBs may be considered a marker of siRNA activity, since GWB formation implicates mRNA silencing. The number of GWBs is dependent on mRNA fragments. The pre-existing GWBs are the targets of siRNA, and these structures can be observed under light microscopy. Finally, rck/p54 contributes to the assembly of recently formed GWBs [1].

Conclusion & expert commentary

GW bodies are cellular structures located in the cytoplasm that play a key role as gene regulators. This control mechanism switches off a number of genes and may modulate cell reproduction and development. The GWBs are conserved structures in mammalian cells (Dcp-containing bodies or P mammalian bodies), in yeast there are P bodies; however, there is no clear evidence that all yeast have GWBs or equivalent GW182. In human cells, gene regulation via RNAi is important in the pathophysiology of cancer and autoimmunity.

RNA-silencing pathway

In the last decade, RNAi has become known as a natural mechanism for silencing of gene expression. This primordial cellular response can inhibit the function of any chosen target gene, and such mechanisms include the genes involved in cancer, AIDS, hepatitis and other diseases. The knowledge in this area has increased quickly, and is actually a useful tool in the functional characterization of different genes and has rapidly evolved to the development of technologies that are currently applied as therapeutics [2].

Foundations of mechanisms involved in gene silencing arose in the early 1980s when the group of Jorgensen attempted the genetic modification of plants to get colorful petunias. Nevertheless, it was not until 1990 when two groups of investigators led by Van der Krol and Napoli [3,4], manipulated the violet tone of petunias by increasing the expression level of the chalcone synthase gene, which is involved in violet pigmentation. These experiments unpredictably resulted in white flowers; furthermore, the introduction of extra copies of the gene caused a decrease rather than the expected increase in the pigment. Later, similar observations were obtained in *Neurospora crassa* [5] during attempts to introduce extra copies of the genes involved in carotene production. Interestingly, the results of these experiments confirmed the seminal observations in petunias and, therefore, the transgenic fungus lost their orange color while acquiring a whitish phenotype. Another group of investigators from Cornell University (NY, USA), who inhibited the expression of genes involved in development by introducing antisense sequences in *Caenorhabditis elegans*, also found that gene suppression was more efficient if sense and antisense sequences were introduced simultaneously [6].

In 1998, Fire and Mello demonstrated that gene-specific post-transcriptional silencing (PTGS) was archived when a dsRNA was processed into the cell, and was converted into an siRNA, which in turn bound its single stranded complementary sequence [7]. In doing so, siRNA is capable of directing the degradation of a target mRNA. It is believed that RNAi constitutes a mechanism developed by cells to eliminate undesirable genes present in high copy numbers, such as viral genes, transposable elements, or experimentally transfected genes. Gene silencing may take place during transcription; gene suppression is chiefly archived post-transcriptionally.

Mechanism of siRNA

RNAi is initiated with the production of dsRNA that is complimentary to a specific mRNA. The introduction of a dsRNA triggers a cascade of events followed by the recognition and processing to produce small RNA fragments of 21–25 nucleotides called siRNAs. After processing, these siRNAs recognize the cognate mRNA target by base pair-dependent binding, and then a selective degradation of mRNA can occur. Events leading to this follows the cleavage of the dsRNA into 21–25 nucleotide double-stranded fragments by an ATP-dependent, conserved nuclease known as Dicer, which is a member of the RNase III family of double strand-specific ribonucleases [8]. The siRNA induce the formation of a multiprotein complex designated as RNA-induced silencing complex (RISC), which is involved in the recognition and silencing and, in some cases,

degradation of the mRNA target. The RISC components include different members of the Argonaut (Ago) family of proteins eIF2C1 and eIF2C2, as well as Ge-1/Hedls and other enzymes implicated in mRNA degradation and silencing. The RISC also contains a helicase activity responsible for unwinding both strands of RNA. The RISC then chooses a single antisense strand; a homolog protein named Dicer 2 with nuclease activity cleaves the strand of mRNA, which is complementary to the siRNA sequence (Figure 1) [9].

There are other related mechanisms to suppress gene expression; among them are the noncoding RNAs, including tRNAs, siRNAs and rRNA, and the micro-RNA (miRNA), which are constituted by small fragments of approximately 22 nucleotides. Some miRNA are conserved from *C. elegans* through to humans and are able to block the translation of specific mRNAs. In contrast to siRNA, the mRNA targeted by the miRNA is not necessarily destroyed during this process. Additionally, the nonsense-mediated mRNA decay, is a process that probably participates in the quality-control mechanism to remove nonsense transcripts, such as mRNA with premature termination codons. PTGS and RNAi constitute the same phenomenon, and are highly conserved along the phylogenic scale from yeast to mammals, including humans.

Micro-RNAs

miRNAs are small noncoding RNAs of 21–23 nucleotides that target specific mRNAs to prevent their translation via the RNAi pathway. The genes encoding for miRNAs are transcribed by RNA polymerase II. In animals, most miRNAs regulate gene expression utilizing mechanisms of RNAi; however, this mechanism is accomplished in the absence of siRNA-directed mRNA cleavage. As previously mentioned, Ago proteins are a crucial part of the machinery required for miRNAs function and their target mRNAs are localized in the cytoplasm as scattered foci known as P bodies or GW bodies. In this cellular compartment, the Ago proteins physically interact with GW182, and it has been demonstrated that GW182 is critical for GWB formation. In addition, silencing of GW182 dissociated the resident P-/GW-body proteins and impaired the silencing of miRNA reporters [10,11]. The multicomponent clustering of Ago2 and GW182 is important for the interaction as was demonstrated by investigators from the University of Florida (FL, USA) and Calgary (AB, Canada), who showed that Ago2 and transfected siRNAs were present within the same cytoplasmic bodies where this interaction appeared to take place. Further, disruption of GWB interfered with the silencing ability of a given siRNA, an observation that supported the notion that GW182 and/or the microenvironment of the cytoplasmic GWBs contribute to the RNA-induced silencing complex and to RNA silencing via miRNA. Subsequent studies by the same group substantiated this notion in that the miRNA localization to GWBs was important for the generation of these structures as well as for silencing the target gene [11].

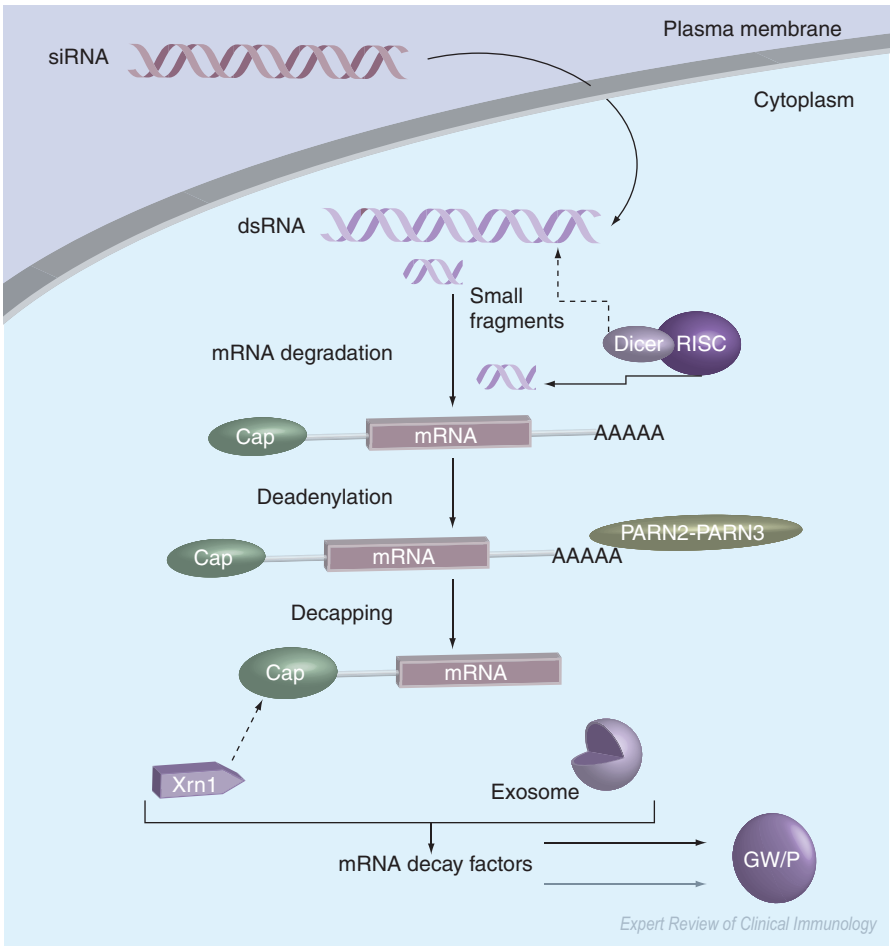

Figure 1. mRNA degradation by siRNA and formation of GW/P.
PARN: Deadenylation initiator; RISC: RNA-induced silencing complex.

GWB as targets in autoimmune diseases

The GW182 protein was discovered using an autoimmune serum from a patient with motor and sensory neuropathy to screen an expression cDNA library. The identified gene possessed an open reading frame with numerous GW repeats and a single RNA recognition motif. Both the patient's serum and a rabbit serum raised against the recombinant

GW protein stained discrete cytoplasmic speckles named GWBs, reactivity that did not overlap with the Golgi complex, endosomes, lysosomes or peroxisomes.

Five-year view

The contribution of this group of investigators from Calgary and Florida in the year of 2002 constitutes a tremendous advance in biology, all of which started with the simple process of screening an expression cDNA library with human autoantibodies [12]; this initial observation was followed by a large and rapidly growing body of information and all of which has resulted in a better understanding of the biology of post-translational control of gene expression via mRNA degradation and silencing. Studies by this group into the clinical importance of anti-GW182 autoantibodies indicate that approximately a third of patients possessing this autoantibody have a motor and sensory neuropathy. In another investigation of a cohort of autoimmune patients, they found a link between anti-GW182 antibodies and Sjögren's syndrome also accompanied by mixed motor/sensory neuropathy, systemic lupus erythematosus and other conditions. It is remarkable that this autoantibody distinguishes a subset of autoimmune conditions with neurological pathology [13]. The pathogenic role of anti-GW182 autoantibodies in autoimmune disease or with neurological sequelae is still unknown. A study of cells from neurological tumors may provide a clue because GWBs are present in astrocytes and astrocytoma cells within cell bodies and cytoplasmic projections [14]. Furthermore, the astrocytoma GWBs exhibit a complex heterogeneity with combinations of LSm4 and XRN1, as well as Ago2 and Dicer, key proteins involved in mRNA degradation and RNAi, respectively. Also the GWBs present in astrocytes contained the mRNA transport and stabilization proteins SYNCRIP, hnRNPA1 and FMRP. In addition, antiGW182 autoantibodies are also found in a particular subset of patients with primary biliary cirrhosis. Taken together, these observations suggest the possible origin of anti-GWB production are siRNA/miRNA complexes that may be presented to the immune system during dysregulated apoptosis and the inefficient clearance of apoptotic bodies described in lupus erythematosus and allied conditions. In previous studies, apoptotic bodies have been shown to contain other target autoantigens, such as small cytoplasmic ribonucleoproteins, RNA-processing components and, perhaps, even GWB elements. These authors have also demonstrated that other components of the RNAi pathway that are targets of the human autoimmune response include Ago2, Ge-1/Hedls (58%), GW182 (40%) and Ago2 (16%) [15,16]. GWB autoantibodies targeted epitopes of the N-terminus of Ago2 and the nuclear localization signal-containing region of Ge-1/Hedls [16].

Finally, in spite of the aforementioned advances, the significance of autoimmunity to GWBs and RNAi are still under investigation. The relationship between inflammation, innate immunity and miRNA expression is just beginning to be explored. Interesting results appeared recently indicating that murine macrophages exposed to polyriboinosinic:polyribocytidylic acid or the cytokine IFN-β upregulate a miRNA (miR-155) and such an effect was induced by several Toll-like receptor ligands. In addition, upregulation by interferons was shown to involve TNF-α autocrine signaling, the inhibition of the kinase c-Jun N-terminal kinase (JNK) blocked induction of miR-155, suggesting that miR-155-inducing signals use the JNK pathway. These findings suggest that miR-155 is a common target of different inflammatory mediators [17], and this result may constitute a possible link between RNA silencing and the inflammatory response of autoimmune diseases.

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Key issues

- GW bodies (GWBs) are involved in 5′–3′ mRNA degradation.
- Components of RNAi are associated with GWBs.
- GW182 is critical for GWB formation.
- Disruption of GWBs interfered with the silencing ability of a given siRNA.
- RNAi possesses a regulatory function in GWB assembly.
- The clinical importance of anti-GW182 autoantibodies indicate that a third of patients possessing this autoantibody have a motor and sensory neuropathy.
- Cell biology implications of GWBs may open a new area in pathogenic mechanisms of autoimmunity.

References

1 Lian S, Fritzler MJ, Katz J *et al.* Small interfering RNA-mediated silencing induces target-dependent assembly of GW/P bodies. *Mol. Biol. Cell.* 18(9), 3375–3387 (2007).

2 Downward J. RNA interference. *BMJ* 328(7450), 1245–1248 (2004).

3 van der Krol AR, Mur LA, de Lange P, Mol JN, Stuitje AR. Inhibition of flower pigmentation by antisense CHS genes: promoter and minimal sequence requirements for the antisense effect. *Plant Mol. Biol.* 114(4), 457–466 (1990).

4 Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* 2(4), 279–289 (1990).

5 Romano N, Macino G. Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. *Mol. Microbiol.* 6(22), 3343–3353 (1992).

6 Guo S, Kemphues KJ, Molecular genetics of asymmetric cleavage in the early *Caenorhabditis elegans* embryo. *Curr. Opin. Genet. Dev.* 6(4), 408–415 (1996).

7 Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391(6669), 806–811(1998).

8 Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature* 431(7006), 343–349 (2004).

9 Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes Dev.* 20(5), 515–524 (2006).

10 Liu J, Rivas FV, Wohlschlegel J, Yates JR 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. *Nat. Cell Biol.* 7(12), 1261–1266 (2005).

11 Jakymiw A, Pauley KM, Li S *et al.* The role of GW/P-bodies in RNA processing and silencing. *J. Cell. Sci.* 120(Pt 8), 1317–1323 (2007).

12 Eystathioy T, Chan EK, Tenenbaum SA, Keene JD, Griffith K, Fritzler MJ. A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. *Mol. Biol. Cell.* 13(4), 1338–1351 (2002).

13 Eystathioy T, Chan EK, Takeuchi K *et al.* Clinical and serological associations of autoantibodies to GW bodies and a novel cytoplasmic autoantigen GW182. *J. Mol. Med.* 81(12), 811–818 (2003).

14 Moser JJ, Eystathioy T, Chan EK, Fritzler MJ. Markers of mRNA stabilization and degradation, and RNAi within astrocytoma GW bodies. *J. Neurosci. Res.* 85, 3619–3631 (2007).

15 Jakymiw A, Ikeda K, Fritzler MJ, Reeves WH, Satoh M, Chan EK. Autoimmune targeting of key components of RNA interference. *Arthritis Res. Ther.* 8(4), R87 (2006).

16 Bhanji RA, Eystathioy T, Chan EK, Bloch DB, Fritzler MJ. Clinical and serological features of patients with autoantibodies to GW/P bodies. *Clin. Immunol.* 125, 247–256 (2007).

17 O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc. Natl Acad. Sci. USA* 104(5), 1604–1609 (2007).

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