

Extra View

GW Bodies, MicroRNAs and the Cell Cycle

Shangli Lian¹

Andrew Jakymiw¹

Theophany Eystathioy²

John C. Hamel¹

Marvin J. Fritzler²

Edward K.L. Chan¹

¹Departments of Oral Biology and Anatomy & Cell Biology; University of Florida; Gainesville, Florida USA

²Department of Biochemistry and Molecular Biology; University of Calgary; Calgary, Alberta Canada

*Correspondence to: Edward K. L. Chan; Department of Oral Biology; University of Florida; P.O. Box 100424; Gainesville, Florida 32610-0424 USA; Tel.: 352.392.6190; Fax: 352.392.4620; Email: echan@ufl.edu

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GW182, GW bodies, RNA interference, miRNA, cell cycle

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ABSTRACT

GW bodies (GWBs) are cytoplasmic foci initially identified through the use of an autoimmune serum targeting the marker protein, GW182. GWBs were first considered as both storage centers for a specific subset of mRNAs and degradation sites for mRNAs. Interestingly, they are known to vary in size and number throughout the cell cycle and are largest in size and most abundant in number during the late S and G₂ phases. Recent studies have linked RNA interference to GWBs, in that disruption or disassembly of GWBs was demonstrated to impair siRNA and miRNA silencing activity. As miRNAs are implicated in the regulation of cell cycle progression and cell proliferation, it is very likely that GWBs, the critical intracellular structures for miRNA function, may very well be also linked to this cellular process.

GW BODIES ARE SITES INVOLVED IN 5'-3' mRNA DEGRADATION

The GW182 protein was initially identified and cloned in 2002 by using autoimmune serum from a patient with motor and sensory neuropathy.¹ GW182 is a 182 kDa protein with a classical RNA recognition motif at its C-terminal domain and is characterized by unique glycine/tryptophan (GW) repeats in the N-terminal half of the protein.¹ GW182 was found to be associated with a specific subset of mRNAs and resided within unique cytoplasmic foci designated as GW bodies (GWBs), which were distinct from other known cytoplasmic organelles such as the Golgi complex, endosomes, lysosomes or peroxisomes.¹ It was speculated that GWBs were involved in the post-transcriptional regulation of gene expression by sequestering a subset of gene transcripts involved in cell growth and homeostasis.¹ In 2003, Sheth and Parker identified that multiple factors involved in 5'-3' mRNA degradation, including Dcp1/Dcp2 (mRNA decapping factors),² Xrn1 (5'-3' exonuclease),³ Ccr4 (deadenylase) and LSM proteins (stimulator of mRNA decapping),⁴ as well as mRNA degradation intermediates localized to discrete cytoplasmic foci in yeast designated as processing bodies (P-bodies). Accordingly, P-bodies were postulated as sites involved in the regulation of mRNA degradation.⁵ At about the same time, Eystathioy et al. demonstrated that Dcp1a and LSM4 colocalized with GW182 in GWBs in mammalian cells.⁶ As a result, GWBs were considered to be the mammalian analogue of P-bodies and were implicated in 5'-3' mRNA degradation.⁶

LINKS BETWEEN RNA INTERFERENCE AND GW BODIES

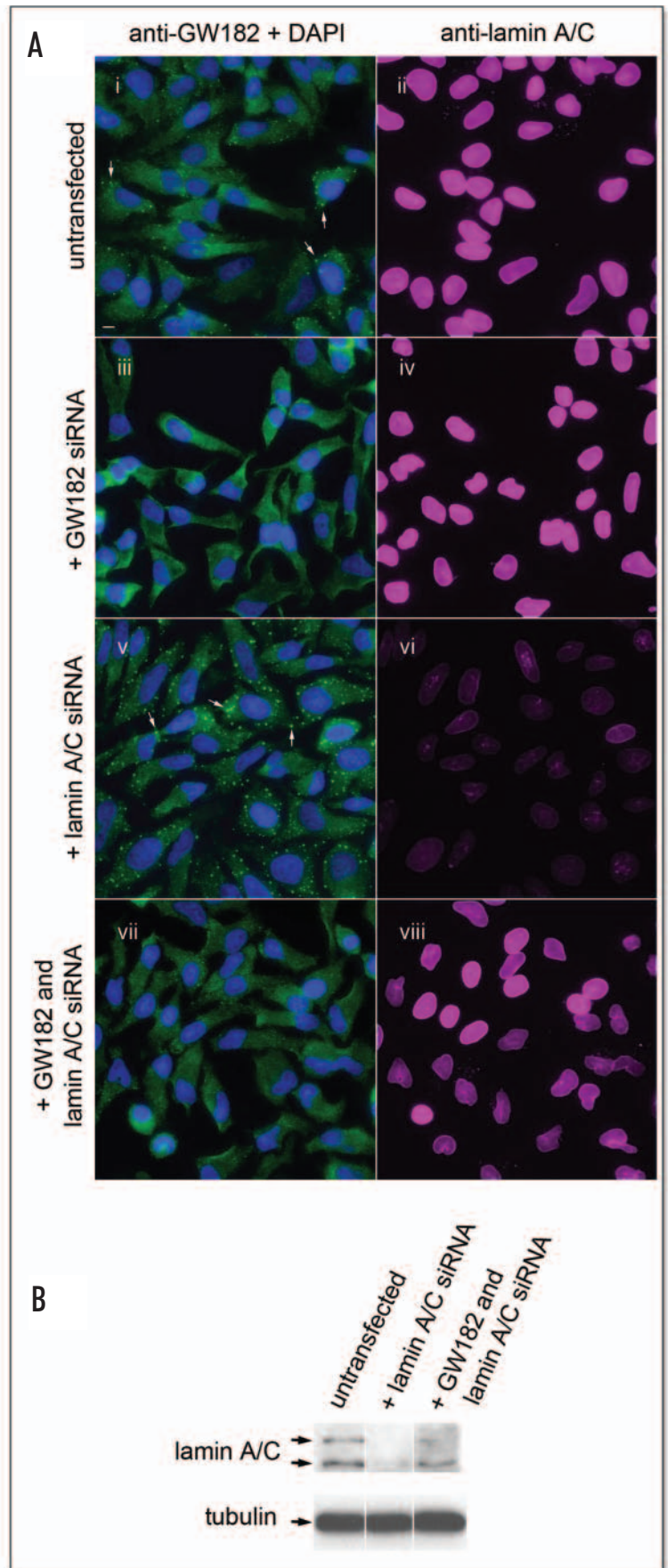
RNA interference (RNAi) was initially described in plants as a genetic control mechanism implicated in virus resistance,^{7,8} genome maintenance⁹ and developmental control.¹⁰ In 1998, it was further characterized in *C. elegans* by Fire et al. as a potent and sequence-specific mechanism that silenced endogenous genes.¹¹ Based on current studies, RNAi is considered to be an efficient mechanism for post-transcriptional, sequence-specific regulation of gene expression and is conserved from plants to animals. In this pathway, long double-stranded RNAs (dsRNAs) or pre-microRNAs (pre-miRNAs) are cleaved into small interference RNAs (siRNAs) or microRNAs (miRNAs), respectively by a RNase III enzyme known as Dicer. MiRNAs are endogenous noncoding small RNAs that are approximately 22 nucleotides in length and are known to negatively regulate the expression of protein-encoding genes. Subsequently, these single-stranded antisense siRNAs or miRNAs bind to Argonaute 2 (Ago2), a key enzyme in the RNA-induced silencing complex (RISC),¹² and this leads to either cleavage or translational repression of targeted mRNAs, and hence a loss of gene expression.¹³ As a potentially powerful tool for experimental gene

Figure 1. Cotransfection of GW182 and reporter lamin A/C siRNAs leads to the disassembly of GWBs and abrogation of RNA silencing activity. (A) HeLa cells were transfected with 100 nM siRNAs for GW182 (iii, iv), 100 nM siRNAs for lamin A/C (v, vi), or both (vii, viii) for three days, and were stained with both human anti-GW182 antibody (green) and mouse monoclonal antibody to lamin A/C (magenta). Nuclei were counterstained with DAPI (blue). Untransfected cells (i, ii). As expected, GW182 siRNA caused disassembly of GWBs (iii) without affecting the expression of lamin A/C (iv compared to ii) and lamin A/C siRNA efficiently silenced the expression of lamin A/C (vi compared to ii). Notably, cotransfection of siRNAs for GW182 and lamin A/C resulted in complete disassembly of GWBs (vii compared to i) and significant inhibition in knockdown of lamin A/C by lamin A/C siRNAs (viii compared to vi). Arrows, GWBs. Bar, 10 μ m. (B) Western blot analysis of lamin A/C expression demonstrating inhibition of siRNA silencing activity by cotransfection of siRNAs specific for GW182. Tubulin levels were monitored to confirm the equal loading of samples.

knockdown and clinical therapy, extensive research over the past few years has furthered our understanding of RNAi. However, many of these studies have only used biochemical techniques with little known about the cell biology of RNAi. Only recently has evidence been presented that links mRNA turnover and RNAi at the level of cell biology.

Studies from several groups have shown that Ago2 localizes to GWBs or mammalian P-bodies.¹⁴⁻¹⁷ Furthermore, miRNAs/siRNAs were demonstrated to be present in GWBs and miRNA-mediated mRNAs were found to localize to these foci in a miRNA-dependent manner.¹⁴⁻¹⁶ These studies suggested that there were at least two possibilities as to how GWBs were involved in RNAi function: (1) GWBs were primarily storage sites for Ago2, siRNAs/miRNAs, and storage/degradation sites for mRNAs targeted by miRNA/siRNA, but not involved in the actual RNA silencing function and (2) GWBs were directly involved in RNA silencing function mediated by siRNAs/miRNAs. Our data, together with two other independent studies, demonstrated that knockdown of GW182 and disruption of GWBs greatly impaired RNA silencing function mediated by siRNA or miRNA.^{14,18,19} This supported the hypothesis that GW182 and GWBs played a critical role in RNAi. Our data demonstrated that sequential transfection of the siRNA for GW182 two days prior to that of the reporter siRNA for lamin A/C resulted up to a 91% reduction of lamin A/C expression.¹⁴ In addition, we have also been able to demonstrate that cotransfection of both siRNA for GW182 and lamin A/C simultaneously could lead to the knockdown of GW182 and inhibition of lamin A/C silencing (Fig. 1). The knockdown of lamin A/C was monitored as a reporter for intracellular RNAi activity. As shown in (Fig. 1), the knockdown of lamin A/C was greatly inhibited by siRNA specific for lamin A/C in GW182- knockdown cells, where GWBs were disassembled, indicating that the RNA silencing function was disrupted without the presence of GW182 and GWBs. These results confirmed our initial findings¹⁴ and also demonstrated that the transfection method used was not a confounding factor in the experiment.

Two recently published papers also support the link between miRNA function and GWBs. In one report, AIN-1, a *C. elegans* protein with partial shared homology to GW182 was shown to interact with protein complexes containing an Ago protein, Dicer, and miRNAs.²⁰ Furthermore, AIN-1 targeted



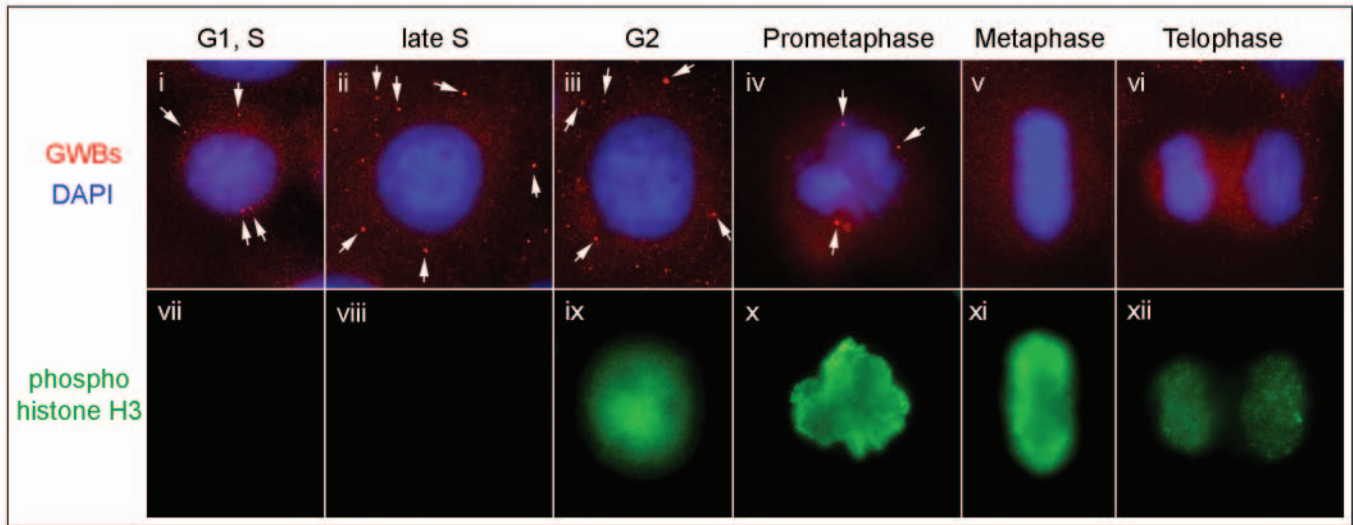


Figure 2. The number and size of GWBs are the largest in late S/G₂ phase of the cell cycle. HeLa cells were stained with mouse monoclonal anti-GW182 (red) to monitor the changes of expression of GWBs (i-vi). Phospho-histone H3 (green) served as a marker for G₂ phase (vii-xii). Nuclei were counterstained with DAPI (blue). GWBs are small in G₁ and early S phase (i), whereas they increase in size and number in late S and G₂ phases (ii and iii). A majority of GWBs disassemble prior to mitosis (iv), and appear to be absent through mitosis (v and vi), and reassemble after mitosis. Notably, there are several GWBs around condensed chromosomes in prometaphase. Arrows, GWBs.

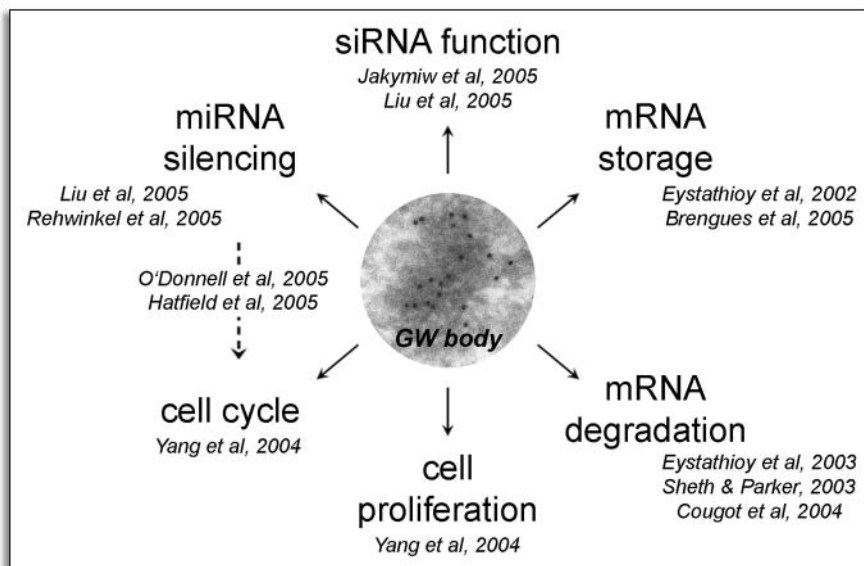


Figure 3. Multiple cellular pathways associated with GWBs.

the Ago protein to cytoplasmic P-bodies in *C. elegans*. In a second study,²¹ miR16 was demonstrated to be required for AU-rich element (ARE)-mediated mRNA degradation, a process that was dependent on the ARE binding protein tristetraprolin (TTP), a protein found to localize within GWBs.²² Interestingly, TTP did not directly bind to miR16, but instead interacted through association with Argonaute proteins to complex with miR16.²¹

MiRNAs in Cell Cycle Progression

Bioinformatic approaches have identified about 200–250 human miRNA genes, but more recent reports predict that there are up to 800 miRNA genes, which account for >3% of all human genes.²³ Current estimates suggest that about one-third of human mRNAs may be regulated by miRNAs.²⁴ The proposed functions of miRNAs

are diverse including the regulation of cellular differentiation, proliferation and apoptosis. Some of the evidence supporting miRNA function in cell cycle comes from studies that have shown that the expression of a cluster of six miRNAs can be activated by c-Myc, a transcription factor and a proto-oncogene that regulates cell proliferation, growth and apoptosis.^{25,26} Interestingly, expression of a transcription factor E2F1, a target of c-Myc that promotes cell cycle progression, was found to be negatively regulated by two miRNAs in this cluster, miR-17-5p and miR-20a.²⁵ The proposed model is that this cluster of miRNAs cooperates with c-Myc in regulating cell cycle progression. Further evidence in *Drosophila* shows that stem cell division is also regulated by the miRNA pathway based on the analysis of a germline stem cell mutant for *dicer-1*, the RNase III essential for miRNA maturation, revealing a marked reduction in the rate of stem cell division.²⁷ The *dicer-1* germline stem cell mutant was delayed in the G₁ to S transition. Interestingly, this specific transition is dependent on the cyclin-dependent kinase inhibitor Dacapo. These observations lead to the hypothesis that miRNAs were required for stem cells to bypass the normal G₁/S checkpoint by downregulating the expression of Dacapo.²⁷

CORRELATION OF GWB EXPRESSION AND miRNA ACTIVITY DURING THE CELL CYCLE?

When GWBs were first observed by indirect immunofluorescence in HEP-2 and HeLa cells, it was noted that there were larger and greater numbers of GWBs in some cells compared to others,²⁸ leading to our hypothesis that the number and size of GWBs was cell cycle dependent. To examine this hypothesis and further characterize this observation, the expression pattern of GWBs was analyzed in

cells that were synchronized in S phase by a double thymidine block.²⁸ The cells were costained with anti-PCNA to monitor the efficiency of the cell synchronization steps. It was demonstrated that GWBs were smaller in early stages, whereas larger and greater numbers were observed in the later stages of the cell cycle.²⁸ As shown in Figure 2, consistent with our published observations, the number and size of GWBs labeled by a monoclonal anti-GW182 antibody increased at the late S and G₂ phase. The majority of GWBs disassembled prior to mitosis and started to reassemble in early G₁. Notably, there were several large GWBs detected around the condensed chromosomes in prometaphase. Studies have reported that RNA-directed transcriptional silencing activities occur inside the nucleus²⁹⁻³¹ although Ago2 distributes exclusively in the cytoplasm.¹⁴ As the nuclear envelope disassembles in prometaphase, some GWBs may access the chromosomes and it is provocative to postulate that these GWBs may serve as sites for miRNA directed assembly of heterochromatin that were shown to be critical for proper chromosome segregation during cell division.

We have also previously analyzed the number and size of GWBs in proliferating cells.²⁸ One of the analyses was performed in mouse 3T3 cells synchronized by serum starvation, wherein very few GWBs were detected.²⁸ After the addition of serum, small GWBs started to appear after three to six hours and larger, brighter, and more numerous GWBs were detected at later time points. As an independent method to evaluate the number and size of GWBs in proliferating cells, Concanavalin A (ConA) was used to stimulate resting mouse splenocytes or isolated T cells. Increases in number and size of GWBs was consistently observed in splenocytes and isolated T cells after ConA stimulation, confirming the hypothesis that the number and size of GWBs was related to cell proliferation.²⁸ Based on the close association of GWBs with the miRNA pathway and the emerging links of miRNA biology to cell cycle and cell proliferation, we postulate that the number and size of GWBs may correlate with the activities of miRNA during the cell cycle. It is intriguing to speculate that GWBs may be involved in the regulation of the cell cycle and cell proliferation via miRNA-mediated mRNA degradation or translational inhibition of important factors involved in these cellular processes (e.g., regulation of cell cycle check point control factors).

In summary, GWBs appear to be involved in a number of cellular processes (Fig. 3). First, they may be potential storage sites for specific subsets of mRNAs.^{1,32} Second, GWBs appear to be sites involved in mRNA degradation.^{5,6,33} Third, recent reports demonstrate that GWBs are critical foci for siRNA and miRNA silencing functions.^{14,15,18,19} Lastly, the emerging evidence that GWBs and miRNAs are associated with the cell cycle and cell proliferation implicate GWBs as potentially important sites for miRNA-mediated regulation of these cellular mechanisms.^{25,27,28}

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