

Chapter 2

The Discovery of GW Bodies

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Abstract Human autoantibodies were a key to the discovery of GW bodies and their integral protein, GW182. This publication marks the tenth anniversary of the discovery of GW182. As it turns out, the discovery of GW182 was quite timely because it coincided with the elucidation of the RNA interference (RNAi) pathway, which is now known to have a major role in post-transcriptional gene regulation. Following our publication of the essential features of GW182 in 2002, laboratories from around the world began investigations that led to the elucidation of the role of GW182 in RNAi and other pathways of mRNA processing and degradation. This chapter reviews the discovery of GW182 and the description of GWB and some of the observations that followed that still remain to be elucidated.

2.1 Introduction

Human autoantibodies have been key reagents in the identification and characterization of novel components and functions of cellular organelles and macromolecules. In turn, the elucidation of novel autoantibodies has led to new tools and diagnostic approaches in a variety of autoimmune conditions, providing the clinician with tools to make an earlier and more accurate diagnosis, predict prognosis and, in some cases, monitor disease activity (Table 2.1). For example, seminal

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Table 2.1 Some key proteins discovered and/or elucidated by use of human autoantibodies

Organelle	Target antigens	Molecular features/ functions	References
Spliceosome	snRNPs (U1-U6 RNP)	Splicing hnRNA	Lerner and Steitz (1979, 1981); Hardin et al. (1982)
Centromere/ kinetochore	CENP-A; -B, -C, -E, -F	Structure of metaphase chromosome, binding metaphase microtubules	Fritzler and Kinsella (1980); Moroi et al. (1980); Rattner et al. (1991, 1996); Earnshaw and Rothfield (1985); Earnshaw et al. (1987); Saitoh et al. (1992)
Small cytoplasmic RNP	SS-A/Ro60; SS-B/La; Ro52/ TRIM21	RNA quality control; RNA molecular chaperones; E3 ubiquitin ligase	Sim et al. (2009); Espinosa et al. (2006); Wada and Kamitani (2006)
Mitotic spindle apparatus	NuMA HsEg5	Movement of metaphase chromosomes	McCarty et al. (1981); Price et al. (1984); Whitehead et al. (1996)
Golgi complex	Golgins-95, -97, 160, 245 Macroglglin/ giantin	Post-translational processing and transport of newly synthesized proteins	Kooy et al. (1992); Seelig et al. (1994); Lindstedt and Hauri (1993); Fritzler et al. (1984, 1992, 1993, 1995); Griffith et al. (1997); Eystathiou et al. (1999)
Nuclear envelope	gp210, Tpr	Components of nuclear pore complex	Courvalin et al. (1990); Dagenais et al. (1988); Wesierska-Gadek et al. (1995); Enarson et al. (2004); Ou et al. (2004)
Nucleolus	Fibrillarin (U3-RNP) 7-2, 8-2 RNP NOR-90 (hUBF)	Processing ribosomal RNA	Busch et al. (1985); Reimer (1990); Reddy et al. (1983); Okano et al. (1992); Rodriguez-Sanchez et al. (1987)
Exosome	PM/ScI-75 PM/ScI-100	Degradation of selected mRNA	Brouwer et al. (2001, 2002)
Coiled bodies	p80 coilin	Multifunctional: RNA-related metabolic processes such as snRNPs biogenesis, maturation and recycling, histone mRNA processing and telomere maintenance	Andrade et al. (1991); Raska et al. (1990)
Ku	DNA-PK	Repair damaged DNA	Reeves and Stoecker (1989); Francoeur et al. (1986); Chan et al. (1989)

studies of Lerner and Steitz used human antibodies from a SLE patient to first identify key components of the spliceosome (U1-, U2-ribonucleoprotein (RNP)) (Lerner and Steitz 1979; Lerner et al. 1982). Antibodies from scleroderma patients were used to identify key components of the centromere/kinetochore (Fritzler and Kinsella 1980; Ren et al. 1998; Earnshaw et al. 1987; Fritzler et al. 2010) and nucleolus (Reimer et al. 1987). Human autoantibodies were also used to identify novel components of cytoplasmic targets in the mitotic spindle apparatus (McCarty et al. 1981; Price et al. 1984; Rattner et al. 1998), Golgi complex (Fritzler et al. 1984, 1993, 1995) and endosomes (Mu et al. 1995; Selak et al. 1999; Stinton et al. 2005).

The foundation for some of these discoveries were initially based on indirect immunofluorescence (IIF) analysis of tissue substrates but the use of tissue culture cells (i.e. HeLa, HEp-2) as superior diagnostic substrates became a turning point in the description and discovery of novel proteins (Nakamura et al. 1984; Fritzler 1986). Thereafter, there was a progression from IIF studies that suggested which cytoplasmic organelle was the target, to western immunoblot to determine some essential molecular features of the targets, to cloning that used immunoscreening of expression libraries, followed by sequencing and characterization of the DNA and the expressed protein. In more recent times, spectroscopic analysis and identification of immunoprecipitated proteins of interest in polyacrylamide gels derived from one or two dimensional electrophoresis as well as immunoscreening of protein microarrays have not only added important dimensions to understanding both the B cell repertoire in autoimmune diseases but also provided insight into the cell and molecular biology of target organelles and their cognate proteins, nucleic acids and lipids.

2.2 A Short History of the Discovery of GWB

The investigational approaches described above were keys to the discovery of GW182, its paralogs and ligands, all now known to be components of GW bodies (GWB). In the 1990s and early 2000s, our laboratories at the University of Calgary and The Scripps Research Institute in La Jolla, California turned their attention to human autoimmune sera that reacted with cytoplasmic components. Out of these investigations emerged the identification of unique targets in the mitotic spindle apparatus (Whitehead et al. 1997; Rattner et al. 1998), the Golgi complex (Fritzler et al. 1995, 2007) and endosomes (Selak et al. 1999; Stinton et al. 2005), which complemented much earlier studies of mitochondria (Fritzler and Manns 2002), cytoskeletal (Senécal et al. 1985), ribosome (Gordon et al. 1982; Meroni et al. 1984; Elkon et al. 1985), exosome/PM-Scl (Brouwer et al. 2002; Raijmakers et al. 2004; Mahler and Raijmakers 2007) and the neutrophil cytoplasmic (Bosch et al. 2006; Wiik 2009) autoantigens.

Since it was becoming apparent that antigens in virtually every cytoplasmic organelle were autoantibody targets, our attention turned to lysosomes because, for reasons that are still not clear, very little was known about autoantigen targets in this organelle (reviewed in (Stinton et al. 2004)). Thus, we were interested in sera that

produced staining patterns that suggested autoantigen targets in the lysosome. One such serum was from a 52-year-old female (pseudonym “Amy”) from Fort McMurray, Alberta who was referred to a neurologist at the University of Calgary Medical Centre because of progressive severe ataxia (loss of balance and fine coordinated movements of limbs) and was found to have a mixed motor and sensory neuropathy. The IIF pattern produced by her serum autoantibodies produced a cytoplasmic staining pattern (Fig. 2.1) and based on the size and distribution of these “dots”, we postulated that her autoantibodies targeted lysosomes. Shortly before Christmas 2000, a former graduate student who had identified the cytoplasmic linker protein, CLIP-170, as a target autoantigen (Griffith et al. 2002), returned to the University of Calgary lab looking for a short-term project and asked if he could attempt to identify the “Amy” target by immunoscreening a cDNA expression library. He set about the task working part time in evenings and weekends and shortly before Christmas, showed me the X-ray films of the first expression cloning experiments that revealed three positive signals that were thought to merit further analysis. Being quite excited about the possibility of discovering a unique lysosomal autoantigen target, the reactive phages were isolated when a strong signal at the very edge of the membrane was noticed (Fig. 2.2). In our hands, positive signals at the edge of the membrane “lifts” were fairly common in expression immunoscreening but, based on several years of experience, the rule of thumb was to “never pursue signals located at the edge of a filter because they inevitably turned out to be artefacts or false positive signals”. However, this particular signal was notable because its complementary signal on the duplicate X-ray film was particularly strong. So, four agar plugs (Fig. 2.2: identified as E.1, E.2, E.3 and E.4) were pulled from the cDNA expression plates and replated on smaller agar plates to make sure that the reactivity persisted (i.e. was not a false positive) and to eventually achieve 100% purity of the isolated phages. The first 3–4 h filter lift was performed and the next day overnight nitrocellulose membrane lift was processed using a conventional immunoblot protocol and the index serum “Amy” as the antibody source. As the first processed X-ray films were scanned the results were disappointing : E.1 negative; E.2 negative; E.3 negative (these three lifts were on one film because four filters did not fit into a single film). So, discouragement was palpable and it was thought that this particular adventure was certainly a dead end until the second X-ray film was processed and a high intensity and high density positive signals of the E.4 phage plaques were observed. Taking into consideration the lab rule about false positive clones at the edge of a plate, there was understandable scepticism that this represented a valid signal, so three E.4 plaques were isolated, replated and the series of filter lifts were repeated. To our amazement the signals of all three E.4 subclones remained highly positive. The date was December 24 and it seemed like an early Christmas gift: a cDNA clone that was anticipated to reveal a novel lysosomal autoantigen. The day after Boxing Day, the process of isolating pure phage, preparing the cDNA, expressing recombinant protein and testing it against the index serum “Amy” and other control sera began. By now, there was some time constraints because one of us (MJF) was scheduled to go on a sabbatical leave on January 5 at The Scripps Research Institute in La Jolla, California joining up with

Fig. 2.1 Anti-GWB antibodies were characterized as a cytoplasmic discrete speckled indirect immunofluorescence pattern (*red*) on HEp-2 as well as a variety of other tissue culture cells. Nuclei are counterstained with DAPI. Original magnification 400×

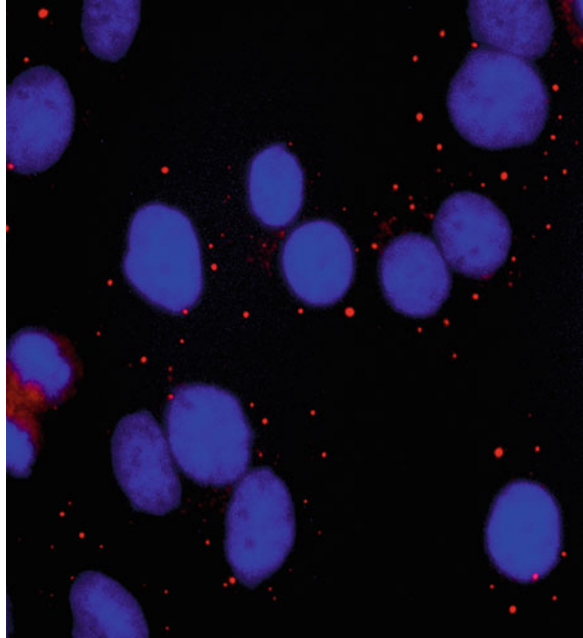
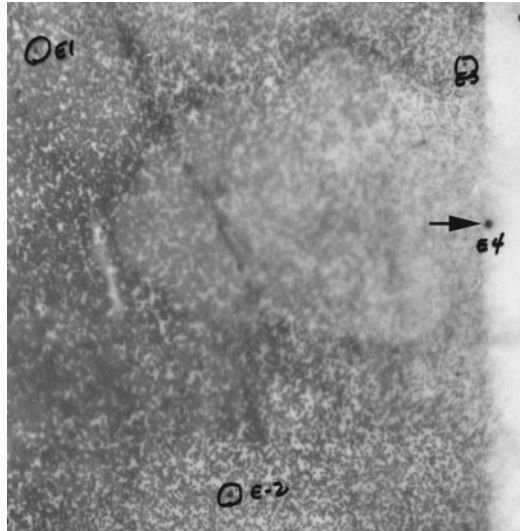


Fig. 2.2 Immunoscreening of a HeLa cDNA library (Stratagene, La Jolla, CA) with the index serum Amy identified four positive signals, originally identified as E1, E2, E3 and E4, on the X-ray film. On subsequent screening, only the clone represented as E4 (*black arrow*) retained reactivity and this was the source of the first cDNA sequence of GW182



my former mentor Dr. Eng Tan and working with a former University of Calgary graduate student, Dr. Ed Chan, the co-author of this chapter. As had been done with some earlier clones (Golgi, endosomes), the purified cDNA extracted from the E4 clone was sent to Dr. Ed Chan for sequencing. Several days later, Dr. Chan sent an email that had a guarded tone: there was confidence, based on a Kozak start sequence near the 5' terminus and a putative open reading frame and a 3' termination signal,

that the isolated cDNA was indeed coding a protein of interest. However, the deduced protein sequence indicated numerous GW (glycine-tryptophan) dipeptide repeats throughout much of the protein, a feature that was perplexing at the time. We wondered if this might be a sequencing error or an inherent cDNA artefact and decided to resequence that cDNA as well as other positive clones to determine if the same sequence was found.

By then, MJF was due to depart for the long drive from snow bound Calgary to sunny San Diego. About 5 days later, I had settled in the apartment in Solana Beach, California and was in Dr. Tan and Chan's laboratory ready to embark on a completely unrelated sabbatical project. However, at the first meeting Dr. Chan showed me the most recent sequence data that had validated the initial data and he thought we ought to give particular attention to this putative ~185 kDa target despite the fact there were no proteins with similar sequences in the GenBank and, in particular, no homologues with numerous GW dipeptide motifs (more on this later) as expressed in the cDNA of interest.

The next efforts concentrated on characterizing the reactivity of the Amy serum to determine the expression of GW182 in a variety of cells, tissues and organs, as well as candidate organelle targets by colocalization experiments using index antibodies that reacted with lysosomes, peroxisomes, endosomes and the Golgi complex (still believing this was a lysosomal target) only to find that the cytoplasmic structures reacting with the index serum did not co-localize with lysosomes or other known cytoplasmic organelles (Eystathiou et al. 2002). These puzzling observations prompted us to seek collaboration with electron microscopist Malcolm Wood in the Core Microscopy Facility at The Scripps Research Institute to see if we could validate these findings and learn more about the target organelle by immunoelectron microscopy. The technical expertise and experience of Malcolm Wood was pivotal to determining a key feature of the target since, much to our amazement, the gold labels were localized to 100–300 nm electron dense cytoplasmic structures that, unlike nearby mitochondria, lysosomes/multivesicular bodies or other cytoplasmic organelles, did not have a limiting bilayer membrane (Fig. 2.3). After some debate and review of the literature, we decided we would tentatively refer to these apparently unique cytoplasmic structures as GWB based on their apparent marker protein GW182. Shortly after we published our initial findings (Eystathiou et al. 2002), we became aware that distinct cytoplasmic foci similar to GWBs had been reported in 1997 by Bashkirov and colleagues who reported the cellular localization of mXRN1p in mouse E10 cells by IIF as distinct cytoplasmic domains (Bashkirov et al. 1997); these structures were later named P bodies, which are practically the same structures as they are labelled by both markers (Jakymiw et al. 2007).

At this point, we realized that the future of further studies relied heavily on a supply of "Amy" and/or other sera with identical reactivity. That led to an urgent email to Dr. Zochodne at the University of Calgary suggesting that when he saw the "Amy" patient at future visits to his outpatient clinic, it would be appreciated if he would obtain informed consent and additional sera to secure a source of human anti-GWB/GW182. Unfortunately, that never transpired because the patient "unexpectedly" passed away from heart failure. However, at about the same time, two other

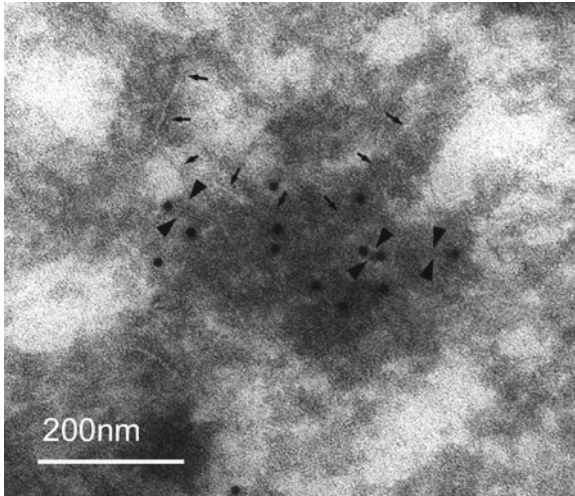


Fig. 2.3 Immunogold electron microscopy localization of GWBs in the cytoplasm of HeLa cells during interphase. Frozen sections of fixed and gelatin-embedded HeLa cells were incubated with the index human anti-GW182 serum diluted 1:400 and then post-immunolabelled with protein A-gold (10 nm). Representative gold-labelled cytoplasmic structures with diameters which vary from 100 to 300 nm. The gold labels are clustered on electron dense fibrils or strands, 8–10 nm in diameter (*arrowheads*). These fibrils appear to form the matrix that the gold decorates. Sometimes filaments are observed right through GWBs (*arrows*)

sera were referred to the Mitogen Advanced Diagnostics Laboratory for analysis. One we labelled IC-6 and it also had fairly typical GWB staining in combination with staining of the nuclear pore complex. Later studies would identify the nuclear pore complex targets as Tpr and gp210 (reviewed in (Ou et al. 2004; Enarson et al. 2004)). The second serum (18033) was referred by Dr. Zochodne's colleague, a neurologist in Barrie, Ontario from a female patient (identified as 18033) who was a clinical "carbon copy" of the index patient Amy and whose serum had high titer and virtual identical autoantibody reactivity. Subsequent to our first (Eystathiou et al. 2003b) and subsequent publication (Bhanji et al. 2007) of the clinical features of patients with GWB autoantibodies, we have identified over 300 sera with anti-GWB referred to us by colleagues in Japan (Dr. K. Miyachi), Brazil (Drs. L. Andrade and C. A. von Muhlen) and Australia (Dr. R. Wilson and W. Pollock), to name a few.

Shortly after the cDNA sequencing was virtually completed, Dr. Jack Keene from Duke University Medical Centre, gave an invited seminar at The Scripps Research Institute on his studies and mounting evidence for post-transcriptional gene regulation with a focus on key mRNA binding proteins such as HuR/Elav (Antic and Keene 1998; Keene 2001; Keene and Tenenbaum 2002); this at a time when evidence about the microRNA pathway was just starting to emerge. After his seminar, we patiently waited for him as he fielded questions and comments from the Scripps faculty and postdoctoral fellows. He kindly agreed to come to the lab so that

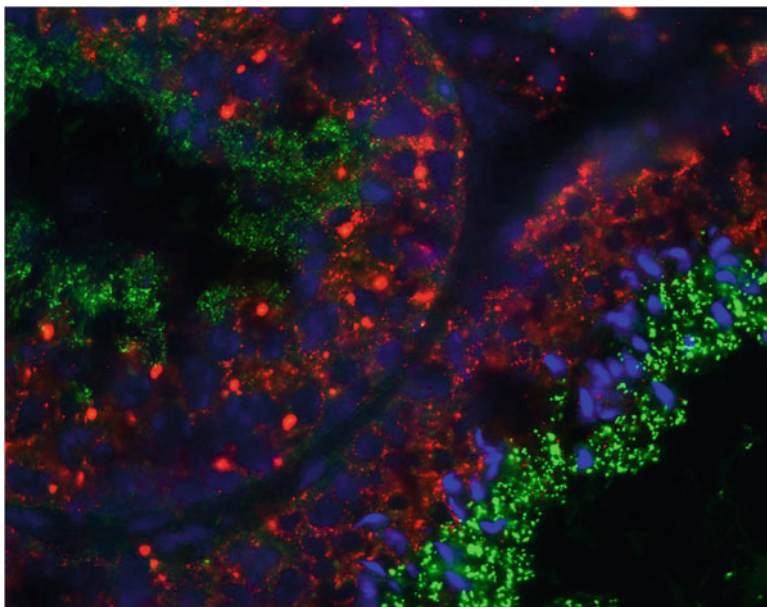
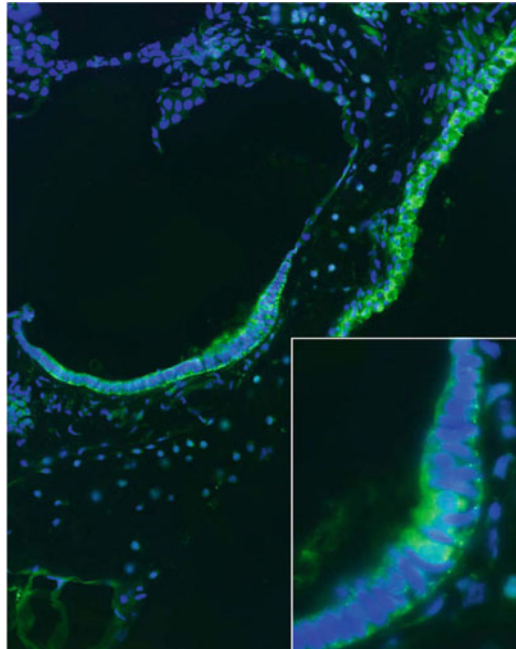


Fig. 2.4 Indirect immunofluorescence using the index anti-GWB serum Amy showed intense staining of mouse testis, particularly cells of the basal/germinative layers of the seminiferous tubules (*red*) compared to staining with a monoclonal antibody directed to golgin-97, a Golgi complex protein (*green*). Original magnification 400×

we could show him the sequence of an “interesting” protein and data on GWB; he took about 10 s to scan the sequence and noting the candidate RNA recognition motif (RRM) near the carboxy terminus (see (Li et al. 2008) and also Fig. 6.5 in Chap. 6) proclaimed “you likely have a mRNA binding protein”. This serendipitous meeting with Dr. Keene was the beginning of productive collaboration that included his postdoctoral fellow at the time, Dr. S. Tenenbaum, who had developed expertise in what became known as the RIP-Chip analysis of mRNAs bound to proteins of interest (Tenenbaum et al. 2000, 2002). This collaboration eventually led to determining the spectrum of mRNAs bound by GW182 in HeLa (Eystathiou et al. 2002) and breast cancer (Luft 2005) cell lines.

Still on sabbatical at Scripps and assisted by an MSc student, LeeAnne Luft, other studies focussed on determining the tissue distribution of GW182 and GWB in mouse tissues by indirect immunofluorescence. While GWB could be identified in virtually every tissue, by far the most remarkable was testis (Fig. 2.4) and certain regions of the brain. The latter observation would eventually spark the interest of Joanna Moser, a Ph.D. student who, based on the earlier studies of LeeAnne Luft in breast cancer (Luft 2005), initiated studies of GWB in astrocytes and astrocytoma cells (Moser et al. 2007; Moser and Fritzler 2010b). Based on observations that GWB were remarkably over-expressed in rapidly dividing cells such as HEP-2 (Eystathiou et al. 2002), HeLa (Yang et al. 2004) and breast cancer (Luft 2005), and

Fig. 2.5 Indirect immunofluorescence using the index anti-GWB serum Amy showed intense staining of the epithelium of *Xenopus* sp. embryos including the ocular cup (inset). Nuclei are stained blue with DAPI. Original magnification 200×

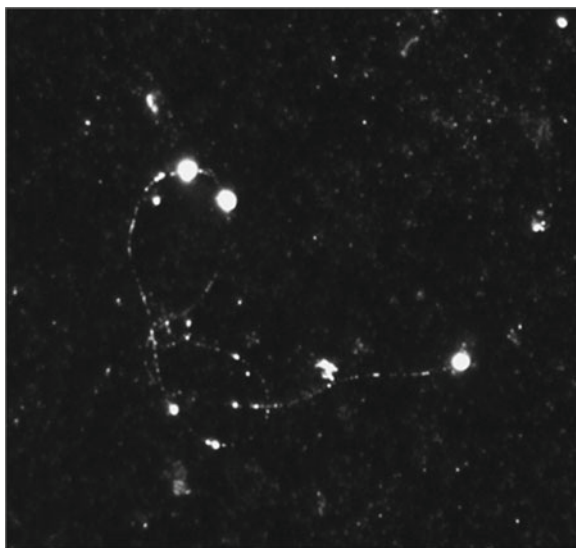


in collaboration with Dr. Leon Browder at the University of Calgary studies of *Xenopus* embryos were started, providing evidence that GWB were particularly highly expressed in the developing eye (Fig. 2.5).

After returning to Calgary from sabbatical leave and armed with a long list of potential projects, some of which would be taken up by Dr. Ed Chan at Scripps and then the University of Florida (Gainesville) and also a former laboratory assistant of Dr. Chan's, Theophany Eystathioy (aka Theo), who had several years earlier completed an MSc degree at the University of Calgary and in 2001 returned to complete a PhD in my laboratory. Keeping virtually weekly contact with Dr. Chan's laboratory, Theo initiated much of the early work that characterized GWB. A key observation at the time was that GW182 was a phosphoprotein that bound a unique subset of mRNAs (Eystathioy et al. 2002) and, in collaboration with Drs. B. Séraphin and N. Cougot (CNRS, Cedex, France) showed that GW182 co-localized with hDCp1 and hLSm4 (Eystathioy et al. 2003c). We also spent considerable effort attempting to purify GWB from cell lysates but the remarkable heterogeneity of size and their apparent tethering by a connecting filament (Fig. 2.6) made that approach less than ideal. In addition, the human index sera became helpful in studies by Dr. Nancy Kedersha who was interested in the association of GWB with stress granules (Kedersha et al. 2005) (see Chap. 12).

In appreciation that the human sera were not ideal reagents for the cell biology and biochemical studies, attempts were made to raise antibodies to GW182 in rabbits and murine monoclonal antibodies. While a number of rabbit sera were shown to have

Fig. 2.6 HeLa cells were gently lysed with detergent and then layered onto a discontinuous sucrose gradient and then centrifuged at $100,000 \times g$ for 2 h at 4 °C. Purified GWB, characterized as variable sized granules dispersed along a filament were stained with the 18033 human serum were found primarily at the 15% sucrose layer. Original magnification 600×



significant anti-GW182 activity, these sera were not particularly helpful for cell biology studies because they contained antibodies to other cellular components resulting in unsatisfactory IIF staining. At about the same time, with the assistance of the Southern Alberta Cancer Research Institute monoclonal laboratory, we successfully generated four monoclonal antibodies identified as 4B6, 2D6, 5C6 and 6D7 that were patented in 2008. Of these, 4B6 seemed to be the most useful because it was an IgG1 antibody and it replicated IIF staining that co-localized with the index human sera (Eystathioy et al. 2003a). Curiously, by IIF 5C6 showed that, in addition to cytoplasmic dot staining that co-localized with the index human sera, it also stained the nuclear envelope, a feature that was similar to another human serum, IC-6, a feature that became useful for a number of knock-down and cell biology experiments (Gavanescu et al. 2004; Pauley et al. 2006). These monoclonal antibodies quickly drew the interest of several companies such as Abcam and Santa Cruz Biologicals who eventually marketed them. However, it soon became clear that many investigators who purchased these antibodies had difficulties replicating their staining properties. In discussion with the companies and some of the researchers, it became clear that many investigators assumed they could dilute the monoclonal like monoclonals of different specificities. This notion despite clear statements in the legend to Fig. 2.1 of our related publication “All MABs were added undiluted, except for the 2D6 MAB, which was added at a 1/25 dilution” (Eystathioy et al. 2003a). This misunderstanding eventually led us to develop a protocol for the use of 4B6 that we freely distribute to all users of the antibody. Another problem referred to our laboratory was the difficulty detecting GW182 by western blotting and/or immunoprecipitation. It became apparent that the most common problem was failure to load sufficient protein on the gels to allow the detection of GW182 and to address this concern, we published a protocol for the detection of GW182 and related proteins in cell lysates (Moser et al. 2009) (Fig. 2.7).

Indirect Immunofluorescence (IIF)
Anti-GW182 mouse monoclonal antibody (4B6)

Overview:

Successful use of the monoclonal antibody 4B6 is dependent on three critical considerations:

- **Cells and tissues:** In our laboratory and in the literature a wide variety of cells have been probed successfully with the monoclonal antibody. These include HeLa, HEp-2, HepG2, MDCK, astrocytoma, breast cancer cell lines, pig skin cell lines and many others. Not widely tested on tissues but excellent staining obtained of testing on skin.
- **Fixation:** A number of fixatives are effective but for routine staining fixation in two changes of ice cold acetone:methanol (3:1, v/v) for 15 minutes works very well. In addition, fixation with buffered 3% Paraformaldehyde followed by permeabilization with 0.5% Triton X for 10min also works well. Other fixatives have been tried with varying success.
- **Incubation time of monoclonal antibody on cell substrates:** Incubation must be a minimum of 2 hours at room temperature for any effective staining. Incubation overnight at 4C taking care to avoid dehydration gives the best results.

Protocol:

1. Dilute stock monoclonal antibody 1/10 – 1/100 with phosphate buffered saline (pH 7.1 - 7.3). On most substrates, best results are obtained at lower antibody dilutions (i.e. 1/10). It is recommended that three or four dilutions starting at neat (undiluted) and proceeding to 1/100 be tested to determine optimal conditions for your cells, tissues and general protocols.
2. Add 30 µl of the diluted antibody to fixed cell monolayers such as HEp-2 cell substrates (ImmunoConcepts Inc., Sacramento, CA).
3. Incubate for a minimum of 2hr at room temperature. For optimal results incubate overnight at 4°C in a humid chamber (in sealed dish on top of moist paper towel).
4. Rinse away excess antibody solution with a gentle stream of 1xPBS (always direct the stream to the edge of the slide or coverslip or wells, never directly onto the cells) and then place the slide/cover slips in a glass jar (Coplin jar) containing 1xPBS and gently agitate on a shaker for 5 minutes; Repeat this wash step 3 times.
5. Taking care to ensure that the wells, slides or cover slips do not dry out, overlay the solution containing the secondary antibody (i.e. Alexa 488 conjugated anti-mouse IgG works well in our laboratory).
6. Incubate in a humid chamber in the dark (cover with foil or place in the cupboard) for 1 hr at room temperature.
7. Repeat step (4).
8. Overlay the moist cells with mounting medium (i.e. VectaShield containing DAPI: Vector Laboratories, Burlingame, CA) and then add a cover slip. Avoid bubbles under the cover slip.
9. If the intention is to keep the slides for a week or more, seal and adhere the cover slips to the slides with nail polish. After viewing with an appropriate UV microscope, slides are stored in a light tight holder at 4C.ensure the slides are stored in the fridge.

Fig. 2.7 Protocol for use of anti-GW182 monoclonal antibodies

As interest in GWB and GW182 paralogs (GW182, GW2 and GW3, named provisionally) increased, it became known that the original GW182 sequence that we submitted to GenBank based on our cDNA represented only the shorter of the two isoforms of GW182. In examining the genomic loci for GW182, a “TNRC6A” gene encoding a short transcript was identified ~50 kb upstream of GW182; this was reported in a study by investigators interested in identifying trinucleotide repeat containing gene in mammalian cells (Margolis et al. 1997). At the genetic level, the TNR region of the trinucleotide repeat containing the TNRC6A gene is encoded on exon 5 of chromosome 16p11.2 and is rich in CAG/CCA/G codons (reviewed in (Li et al. 2008)). We later showed that the TRNC6A gene can indeed be transcribed in the same transcript encoding the long isoform TNGW1 with an extra N-terminal QP-rich 253 amino acid sequence

upstream of the GW182 isoform (Li et al. 2008). In the meantime, GenBank had renamed the cognate genes as TNRC6A (GW182/TNGW1), TNRC6B (GW2) and TNRC6C (GW3) (see (Li et al. 2008) and also Fig. 6.5 in Chap. 6). It is noted that the TNRC6B and TNRC6C names can be distracting as they do not have a trinucleotide repeat domain as in TNRC6A; rather the most important similarity with these two proteins are their GW-rich domains and putative function in translational repression. Although the function of the TNR domain of the long form of GW182 proteins is not clearly understood, genetic aberrations of this type of TNR region have been associated with a number of neurological diseases including Huntington's disease, fragile X syndrome and spinocerebellar ataxia type 2 (SCA2) (Xuereb et al. 1996; Batra et al. 2010; Di Prospero and Fischbeck 2005). This is curious information in the context of the index patient Amy, patient 18033 and approximately one third of patients with anti-GWB who have a neurological disease (Eystathiou et al. 2003b; Bhanji et al. 2007). To that date, no studies were published determining if the TNR region of TNGW1 is the target of autoantibodies or if patients that produce anti-TNGW1 have a mutation of the TNR region, a feature that could conceivably render it immunogenic. To address this, we initiated a study of the TNR region, the key results of which are presented in Chap. 14.

During the course of our studies it became obvious that a number of cytoplasmic structures had one or more features of GWB (Moser and Fritzler 2010a). In addition, we have been intrigued that very few mammalian proteins contain repeat GW motifs, although a notable example is the GWG octapeptide repeat of the prion protein (Zahn 2003). This is also intriguing, since prion-related conditions in animals (i.e. "mad cow" disease, chronic wasting disease) and man (i.e. variant Creutzfeldt Jakob syndrome, Gerstmann-Straussler-Scheinker Disease) have some neurological features that were seen in some of the anti-GWB patients.

2.3 Summary and Conclusions

Human autoantibodies were a key to the discovery of GWB and their integral protein, GW182. This book marks the tenth anniversary of the discovery of GW182 and GWB. Serendipitously, the discovery of GW182 was quite timely because it coincided with the elucidation of the RNA interference (RNAi) pathway, which is now known to have a major role in post-transcriptional gene regulation. Following our publication of the essential features of GW182 in 2002, laboratories from around the world began investigations that led to the elucidation of the role of GW182 in RNAi and other pathways of mRNA processing and degradation. A review of the history of the discovery of GWB and GW182 paralogs and the subsequent plethora of research studies on these structures can be attributed to a clinician scientist, Dr. Doug Zochodne at the University of Calgary, who is very interested in patients with autoimmune neurological syndromes. This was followed by a good fortune, remarkable expertise and advice of collaborators in a number of centres, and the coincidental elucidation of the miRNA/RNAi pathways. The study of GWB has taken on broad implications in the post-translational control of gene expression leading to numerous avenues of fruitful investigation still to be explored.

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