CHAPTER 5

IDENTIFICATION AND CHARACTERISATION OF IGF2R AS AN ENDOSOMAL RECEPTOR FOR GABBR2

5.1 Introduction

This chapter describes the application of the genome-scale screening approach to identify the interaction between a mannose-6 phosphate receptor (IGF2R) on the surface of HEK-293-E cells and the recombinant protein corresponding to the ectodomain of the B2 subunit of the gamma-aminobutyric acid (GABA) type B receptor (GABBR2 or $GABA_{B2}$). The interaction was subsequently validated using biochemical approaches.

5.1.1 Introduction to GABA-B receptors

Communication between neurons occurs via the release of small chemical molecules called neurotransmitters into the junction between two neuronal cells (called synapses). Neurotransmitters are of two types (i) excitatory, which increase the excitability of neurons, and (ii) inhibitory, which reduce the excitability of neurons. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the mammalian central nervous system (CNS) [275]. $GABA_B$ receptors are widely expressed metabotropic transmembrane receptors for GABA that mediate slow inhibitory neurotransmission in the CNS to control the excitability of neurons [276]. The $GABA_B$ (or GABAB) receptor is composed of two subunits, $GABA_{B1}$ and $GABA_{B2}$, both of which are members of the GPCR family and contain the characteristic GPCR seven transmembrane (7-TM) regions. Both subunits also contain an extracellular domain called the 'venus fly trap domain' (VFTD), which acts as the binding site for GABA. Functional $GABA_B$ receptors are generated through the combination of either of the isoforms of the $GABA_{B1}$ subunit ($GABA_{B1a}$ or $GABA_{B1b}$) together with the $GABA_{B2}$ [277].

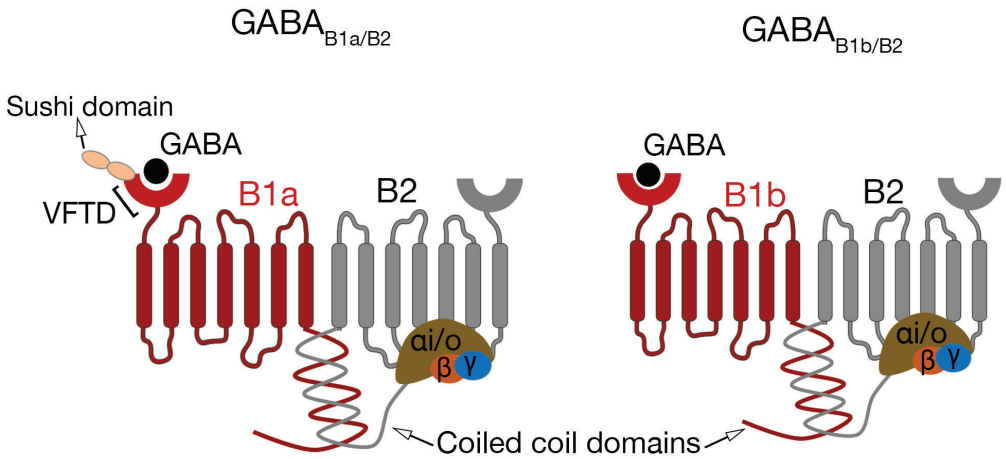

Fig. 5.1 Schematics of GABA$_B$ receptors. Formation of functional GABA$_B$ receptors requires dimerisation of the B1 and B2 subunits [278]. There are two isomers of B1 subunits that differ by the presence of two sushi domains on the B1a but not on the B1b subunit. GABA$_{B1a/B2}$ are preferentially found presynaptically whereas GABA$_{B1b/B2}$ are mainly localised postsynaptically [279]. The subunits interact via the coiled coil domains present on the C-terminus of the proteins. This interaction has been shown to be required for the transport of the GABBR1 subunit to the plasma membrane. The GABBR1 subunit contains an ER retention signal which is masked when it interacts with the GABBR2 subunit, thereby allowing it to reach the cell surface. The binding of GABA is medaited by the GABBR1 subunits whereas the GABBR2 subunit couples with the subset of heterotrimeric G-proteins (pertussis toxin sensitive Gi/o family) to regulate voltage-gated Ca(2+) (Ca(V)) channels, G-protein activated inwardly rectifying K(+) (GIRK) channels, and adenylyl cyclase activity [280].

Internalisation of GABA$_B$ receptors

The balanced expression of excitatory and inhibitory receptors in neurons is crucial for normal brain function. Disruption to such balances has been been implicated in a wide range of neurological disorders including anxiety, depression, epilepsy and neuropathic pain [281]. Internalisation of GPCRs receptors has been most comprehensively studied in the context of β-adrenergic receptors, the expression of which on the surface of cells is tightly controlled by agonist desensitization. In this model, upon a prolonged stimulation of GPCRs by an agonist, the agonist-bound GPCRs are phosphorylated by G protein-coupled receptor kinases (GRKs), which leads to decreased effector coupling and recruitment of arrestins and clathrins, in turn leading to the endocytosis of receptors followed by recycling or degradation [282, 283]. However, unlike the other GPCRs, GABAB are not GRK substrates [284, 285] and thus the mechanisms by which the plasma membrane expression of GABA$_B$ receptors are regulated have been suggested to follow other mechanism of internalisa-

tion rather than the classical pattern of agonist-induced desensitisation and internalisation[1]. While initial studies suggested that $GABA_B$ receptors are stably expressed on the surface of cells with very low rate of constitutive internalisation [285, 290, 284], it is now generally accepted that $GABA_B$ receptors are highly mobile and can undergo constitutive internalisation in both heterologous expression systems (including HEK293 cells) and cultured neurons even in the absense of an agonist [289, 291, 292, 288]. The internalisation of the receptor heterodimer has been reported to be carried out via clathrin- and dynamin-dependent pathways. Internalised receptors are either targeted to endosomes [289, 288] or degraded in the lysosomes [289]. Receptors destined for endosomes have been reported to be recycled back to the surface of the cells.

Several mechanisms mediated by post-translational modifications of heterodimer subunits have been proposed for the regulation of the expression of GABAB receptors on the surface of cells. It has been suggested that sustained activation of the N-methyl-D-aspartate (NMDA) receptor by glutamate causes the activation of the AMP-dependent protein kinase (AMPK) and the protein phosphatase 2A (PP2A). This in turn leads to the phosphorylation of serine 783 (S783) residue on the C-terminus of GABBR2 subunit, which causes the change in the fate of internalised GABAB receptors—rather than being recycled back to the surface of the cells, the receptors are instead directed towards lysosomal degradation [293]. Another post-translational mechanism that is known to contribute to receptor internalisation and regulation is ubiquitination. The role of ubiquitination in GPCR internalisation is well-studied in case of β-adrenergic receptors in which there exists an agonist-dependent ubiquitination of both β-arrestin and the receptor. It has been shown that ubiquitination on β-arrestin and on β-adrenergic receptors serve distinct signals. While the ubiquitination of β-arrestin is required for receptor internalisation, that of the receptor is required for receptor degradation [294, 295, 296, 297]. Although it is known that GABAB receptors do not engage β-arrestins, Lysine-63-linked ubiquitination of the GABBR1 subunit and its interaction with USP14 (ubiquitin-specific protease 14) has recently been reported to play a role in lysosomal targeting of GABAB receptors [298, 299]. While these studies provide evidence for ubiquitination mediated regulation of an amount of receptors present

[1]Studies regarding the effect of an agonist are conflicting as there are some studies that have shown agonist-induced internalisation [286, 287] and others that show no influence of an agonist on internalisation [288, 289].

on the plasma membrane, the mechanism by which GABAB receptors are constitutively internalised using clathrin-dependent endocytosis pathway still remains unknwown. The GABBR2 subunit of the GABAB receptors has also been suggested to play a regulatory role for mediating cell surface receptor stability of GABAB receptors, but the precise mechanism for this is also not known [292].

5.1.2 Introduction to IGF2R

IGF2R (also known as cation-independent mannose-6-phosphate receptor (CI-MPR) or CD222) is a multifunctional membrane-bound glycoprotein. It is mainly localised in the trans-Golgi network (TGN) and endosomes (90-95%) with 5–10% of the receptor expressed on the plasma membrane [300]. IGF2R, together with CD-MPR (cation-dependent mannose-6-phosphate receptor), makes up the class of 'P-type' lectins that primarily function in sorting mannose-6-phosphate- (M6P-) bearing glycoproteins from TGN to endosomes and lysosomes (figure 5.2A, the schematic representation of IGF2R is shown in figure 5.2B) [301].

Non-lysosomal targets of IGF2R

Apart from the transport of enzymes destined for lysosomes, IGF2R also functions in regulating other cellular functions by interacting with multiple non-lysosomal proteins. One of the first identified non-lysosomal protein bound by IGF2R was the insulin growth factor II (IGF-II, hence the name IGF2R). IGF2R has been suggested to be a 'sink' for excess IGF-II in the extracellular fluid [301]. The interaction with IGF2R leads to internalisation and subsequent degradation of IGF-II in the lysosomes. IGF-II does not contain M6P modifications and binds to a site different to the M6P binding sites of IGF2R. As IGF-II is important for cell growth, survival, and migration, maintenance of correct levels of IGF-II in the extracellular space has been shown to be crucial for normal growth and development of cells [302]. In addition to IGF-II, IGF2R interacts with multiple non-lysosomal ligands in both a M6P-dependent (such as Transforming growth factor-β precursor (TGFβ1), Leukemia inhibitory factor (LIF), Proliferin, Granzyme B, CD26, Herpes simplex virus glycoprotein D (HSV-glyD)) and -independent (such as Retinoic acid (RA), uPAR: urokinase-type (plasminogen activator) receptor, Plasminogen) manner. A summary of the cellular context in which the some of the interactions

with IGF2R occur, as well as the consequences of the interactions, is provided in table 5.1 (reviewed in [300]).

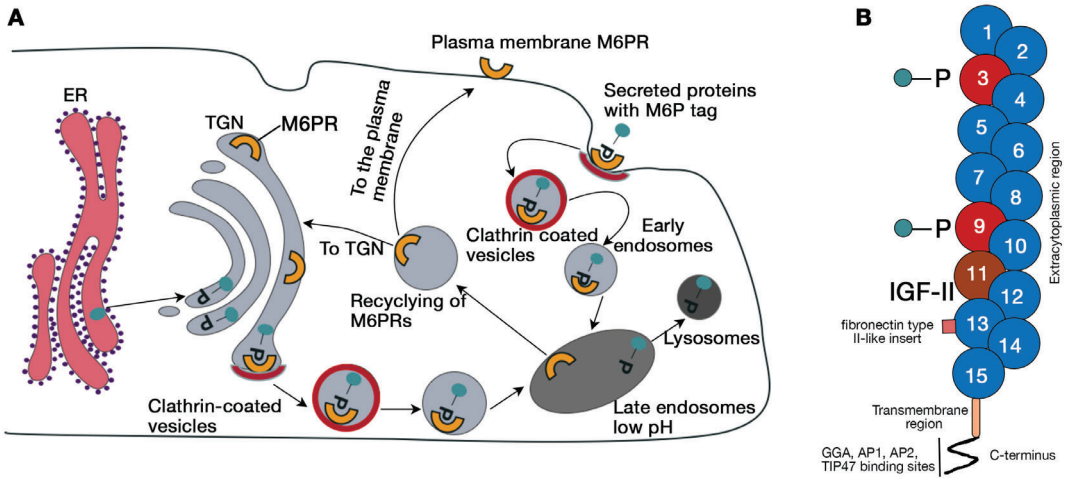

Fig. 5.2 Schematics of mannose-6-phosphate cellular transport pathway and structure of IGF2R. A. Newly synthesised enzymes such as soluble acid hydrolases that are destined for the acidic compartments in cells are post-translationally modified to contain M6P residues on their N-linked oligosaccharides in the golgi network (represented with -P). These residues are recognised by cellular M6P receptors (CI-MPR and CD-(cation-dependent)-MPR), which causes the receptors and their ligands to cluster into clathrin-coated transport vesicles at the trans-Golgi-network (TGN). Clathrin coated vesicles bud-out of the TGN and travel to an acidic late endosomal compartment in which the low pH causes dissociation of the receptor–ligand complex. The unbound M6PRs are then trafficked back to the TGN or trafficked to the plasma membrane. In some cases, lysosomal enzymes that carry the sorting tag escape the transport to the endosomes and are instead secreted. M6PRs (mainly IGF2R) present on the surface of cells are required for the 'secretion-recapture' pathway where such escapees are captured and are brought back into the cell via the clathrin dependent endocytosis pathway. These proteins are also eventually transported to the late endosomes (schematics is based on [303]). **B.** IGF2R is a 300 kDa glycoprotein that contains 15 repetitive extracytoplasmic domains each with 147 amino acids that share $\sim$14 % - 38 % sequence similarities. Monomeric receptors are found on the membrane surfaces; however, weak dimeric complexes formed upon ligand binding have also been reported. IGF-II binds to a site (domain 11) separate from the M6P binding sites (domain 3 and 9) of IGF2R. The c-terminus of CI-MPR contains important amino acid sorting signals (for example, GGA, Golgi-localized, gamma-ear-containing, ADP-ribosylation factor-binding protein TIP47, tail-interacting protein; AP1 and AP2, clathrin adaptor proteins) [303].

Table 5.1 Non-lysosomal interaction partners of IGF2R

Ligand	Context of interaction	Ref
	M6P bearing ligands	
TGF-β	The interaction leads to plasmin-mediated proteolytic activation of the precursor to generate active growth factors at the cell surface	[304]
LIF	Endocytosis and degradation in lysosomes	
Proliferin	Interaction induces endothelial cell migration and angiogenesis	[305, 306]
CD26	Internalisation of CD26 and role in T cell activation	[307]
Granzyme B	Internalisation and induction of apoptosis	[308]
HSV-glyD	Facilitation of viral entry into cells and transmission between cells	[309]
	Non-M6P bearing ligands	
IGF-II	Internalisation and degradation in lysosomes	[310, 311, 312]
RA	Growth inhibition and/or induction of apoptosis	[313]
uPAR	Binds to IGF2R to convert plasminogen into plasmin	[314, 315]
Plasminogen	Activated by uPAR to form plasmin and consequently activate the IGF2R bound TGF-β	[315]

5.1.3 Scope of this chapter

In this chapter I will describe the interaction that I identified between IGF2R and GABBR2 with the genome-scale screening approach developed in chapter 4 using an avid GABBR2 (ectodomain of GABBR2 conjugated to streptavidin-PE) as a screening probe. The interaction was subsequently validated using targeted gene knockout and biochemical approaches.

5.2 Results

5.2.1 IGF2R is required for the binding of GABBR2 to HEK-293-E cells

One of the proteins from the screening pipeline that was identified as binding HEK-293-E cells in a *SLC35B2* independent manner was the ectodomain of gamma-aminobutyric acid (GABA) type B receptor subunit 2 (GABBR2) (figure 5.3A). To identify the cellular components contributing to this interaction, a genome-scale screening approach was carried out and mutant cells that had lost the ability to bind the GABBR2 ectodomain were sorted (figure 5.3B). Enrichment analysis revealed a clear enrichment of gRNAs targeting *IGF2R* together with genes involved in endosomal function and trafficking in the sorted population (figure 5.3C). IGF2R is a known cargo receptor which transports mannose-6-phosphate (M6P)-modified proteins between the TGN, endosomes,

pre-lysosomal compartments, and the plasma membrane; it might therefore provide a mechanism for the known internalisation and lysosomal degradation of GABAB receptors through interactions with the GABBR2 subunit.

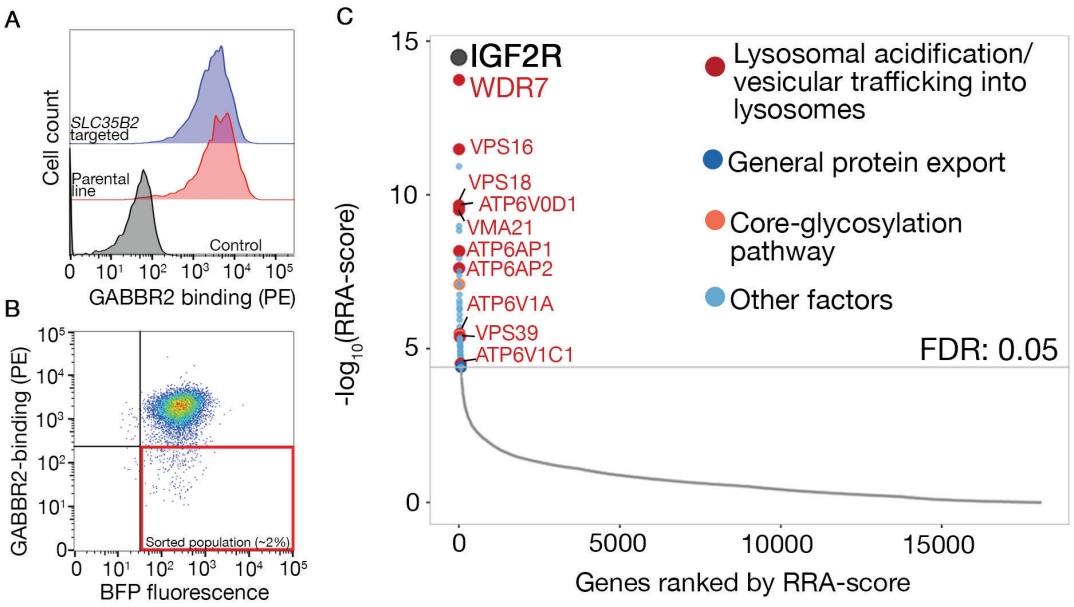

Fig. 5.3 A genome-scale screen using the ectodomain of GABBR2 identifies IGF2R and genes involved in endosomal function and trafficking. A. Monomeric biotinylated GABBR2 ectodomain conjugated to streptavidin-PE binds to HEK-293-E cells in a *SLC35B2*-independent manner. **B.** Cells lacking the surface staining from GABBR2 ecotodomain but expressing BFP fluorescence (from lentiviral transduction) were collected. Approximately 800,000 cells were collected during this sort. **C.** RRA-score rank-ordered genes identified from gRNA enrichment analysis from sorted mutant cells that had lost GABBR2 binding activity. Enriched genes encoded the IGF2R receptor and proteins involved in endo/lysosomal biology.

To investigate this further, I first validated the screen results using individual gRNAs targeting *IGF2R*, which resulted in the loss of binding of the GABBR2 ectodomain (figure 5.4A). IGF2R expression on the surface of cells is known to be dependent on lysosomal acidification, as cells treated with compounds that increase lysosomal pH cause IGF2R to accumulate in endosomes, with a consequent loss from the cell surface. This provides an explanation for why genes known to be required for endosomal function (such as the components or associated factors of the vacoular-type ATPases that pump protons into the acidic compartments of the cells: *ATP6VOD1, ATP6AP1, ATP6AP2, ATP6V1A1, ATP6V1C1, VMA21* [316], and genes encoding for endosomal trafficking proteins: *VPS16, VPS18 and VPS39* [317]) were also enriched [318]. One of the genes (*WDR7*) identified in this screen encodes for a poorly-

characterised protein implicated to be involved in lysosomal acidification [319]. Consistent with the suggested role, *WDR7* targeted HEK-293-E cells also demonstrated a decrease in the surface level expression of IGF2R (figure 5.4B) and a corresponding partial loss in GABBR2 ectodomain binding (figure 5.4A) .

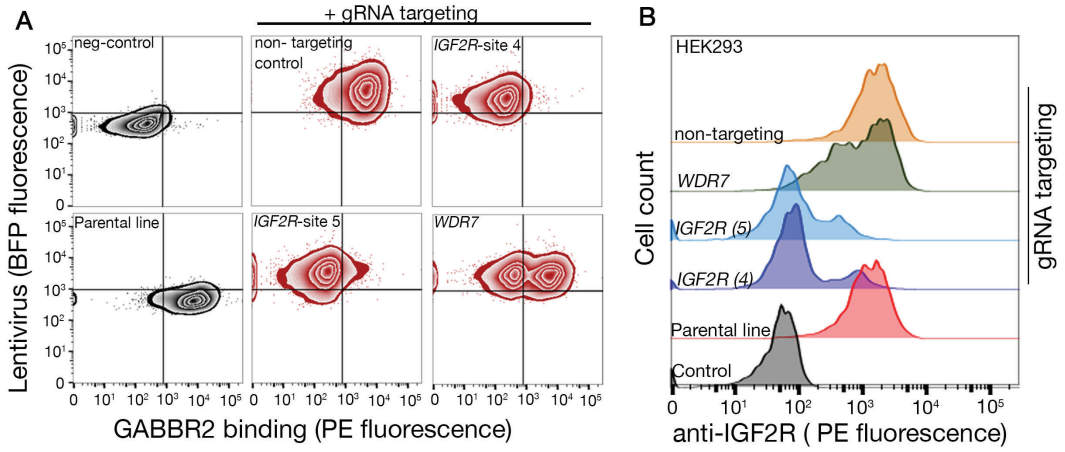

Fig. 5.4 Targeting *IGF2R* and *WDR7* on HEK-293-E cells leads to the loss of binding of GABBR2 ectodomain. A. Binding of GABBR2 was quantified on HEK-293-E cells transduced with two gRNAs targeting different exons of *IGF2R* and one gRNA targeting *WDR7*. A near complete loss of binding was observed on *IGF2R*-targeted cells and a partial loss on *WDR7*-targeted cells; targeted cells were maintained as polyclonal lines. **B.** Cells transduced with lentiviruses encoding individual gRNAs targeting *IGF2R* and *WDR7* show reductions in cell surface IGF2R levels. Cells were transduced with lentiviruses encoding the indicated gRNAs and stained 12 days later with a mouse anti-human IGF2R mAb. Cells stained with secondary antibody alone were the negative control and WT indicates untransduced cells as a positive control. In both cases, a representative of three technical replicates is shown.

5.2.2 Plasma membrane expression of IGF2R is required for the binding of GABBR2 ectodomain

I next set out to explore whether IGF2R was involved directly in the binding of the GABBR2 ectodomain, or whether, it was playing an indirect protein trafficking function in the cell, thereby assisting some other protein to the surface of the cells to mediate the binding. To test whether the ectodomain of IGF2R itself was required for the interaction with GABBR2, a IGF2R expression plasmid was constructed in which the cytoplasmic region of the protein, which contains the important amino acid sorting signals, was replaced with an eGFP reporter protein and then displayed at the surface of human cells by transiently transfecting the NCI-SNU-1 cell line. NCI-SNU-1 cells were chosen as they

exhibited very low levels of plasma membrane IGF2R compared to the HEK-293-E cells and did not bind the GABBR2 ectodomain (figure 5.5A). The NCI-SNU-1 cells transfected with the IGF2R-eGFP fusion protein but not the control-eGFP fusion protein showed surface expression of IGF2R as indicated by the presence of double-positive (GFP^+/PE^+) population when tested with an anti-IGF2R mAb (figure 5.5B, left panel). Correspondingly, the binding of the GABBR2 ectodomain could be conferred to the NCI-SNU-1 cells that expressed the IGF2R-eGFP fusion protein but not the control-eGFP fusion protein on the surface of cells (figure 5.5B). These data suggest that GABBR2 binding to cells was mediated by the ectodomain of IGF2R on the cell surface.

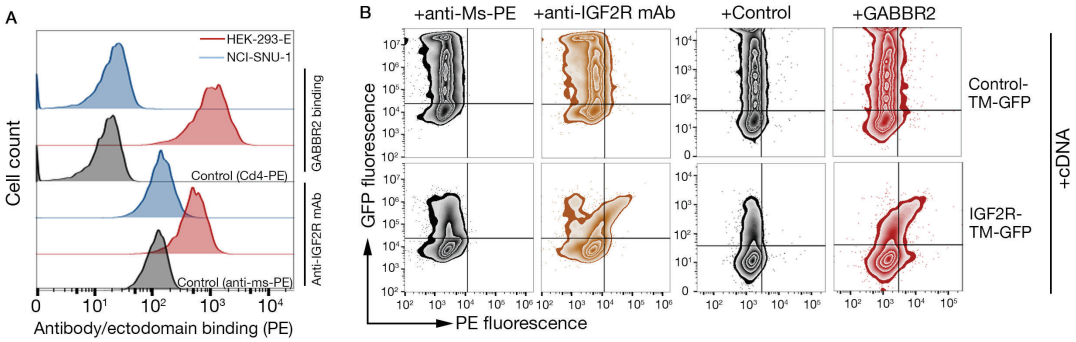

Fig. 5.5 Binding of GABBR2 can be conferred to NCI-SNU-1 cell line that do not display IGF2R on their surface. A. NCI-SNU-1 cell line was tested for IGF2R expression and GABBR2 ectodomain binding using an anti-IGF2R antibody and the avid GABBR2 probe respectively; neither the expression of IGF2R nor the binding to the ectodomain was observed. HEK-293-E cells were used as a positive control. **B.** Gain of IGF2R-eGFP expression (left panel) and GABBR2 binding (right panel) in cells transfected with a cDNA encoding IGF2R ectodomain. NCI-SNU-1 cells were transfected with either a cDNA construct encoding the entire ectodomain of IGF2R fused to a transmembrane (TM) region and an intracellular eGFP or a control TM-GFP tagged receptor and tested for their ability to bind a fluorescently labelled GABBR2 binding probe; only the IGF2R-GFP positive cells and not the GFP negative or control-TM-GFP bound GABBR2. In the case of anti-IGF2R antibody staining, a small fraction of cells that were GFP positive were PE-negative, suggesting that not all expressed IGF2R was displayed at the surface of the cells. In all cases, data is representative of three technical replicates.

5.2.3 IGF2R ectodomain and GABBR2 ectodomain directly interact

To further demonstrate that IGF2R and GABBR2 directly interact, I next expressed the entire ectodomain of IGF2R as a soluble beta-lactamase-tagged 'prey' and tested whether it could be captured specifically by a biotinylated GABBR2 ectodomain 'bait' in a plate-based ELISA-style assay (schematic

depicted in figure 5.6A). In the assay, biotinylated bait proteins are first immobilised on a streptavidin coated mictotitre plate and β-lactamase tagged prey proteins are added to the wells. Following wash steps, if an interaction with the bait has occurred, the prey proteins will be captured in the corresponding wells and this can be detected by addition of a β-lactamase substrate 'nitrocefin' the hydrolysis products of which absorb at 485 nm. Using this assay, I observed a specific capture of the IGF2R ectodomain by the GABBR2 ectodomain (figure 5.6B), demonstrating that the interaction between IGF2R and GABBR2 is direct.

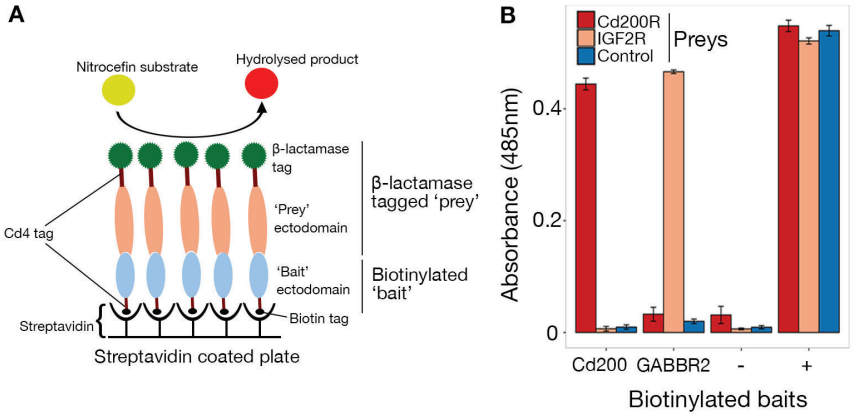

Fig. 5.6 Interaction between IGF2R and GABBR2 is direct. A. Schematic of plate-based ELISA-style assay to test direct binding between ectodomains. The biotinylated ectodomains are captured on streptavidin-coated plates and tested for direct binding using a beta-lactamase-tagged 'prey' ectodomain. **B.** In the plate based assay, the GABBR2 ectodomain was used as a bait and IGF2R ectodomain was used as a prey. Positive control was the Cd200-Cd200R interaction; control 'prey' is an unrelated ectodomain; positive (+) represents total capture of all preys with an anti-prey antibody and negative (-) represents a tag only bait control. Data points are mean $\pm$ sem, n=3.

5.2.4 GABBR2 interacts with IGF2R in a M6P-dependent manner

Given the known function of IGF2R to interact with multiple proteins in a M6P-dependent manner, I next investigated whether the interaction that was identified here also depended on the presence of M6P residues on GABBR2. In a plate-based assay, the interaction between the ectodomains of GABBR2 and IGF2R could be prevented completely by the preincubation of IGF2R with soluble M6P but not with a related monosaccharide, mannose (figure 5.7A). This suggested that the GABBR2 interaction with IGF2R was mediated by the M6P binding domains of IGF2R. Furthermore, GABBR2 binding to IGF2R was also dependent on the N-linked glycans as the interaction was abolished by treating the GABBR2 ectodomain with PNGaseF (an enzyme that

specifically removes N-glycans in a protein) (figure 5.7B). Together these data demonstrate that the interaction between IGF2R and GABBR2 is dependent on the M6P-modified N-linked glycans of GABBR2.

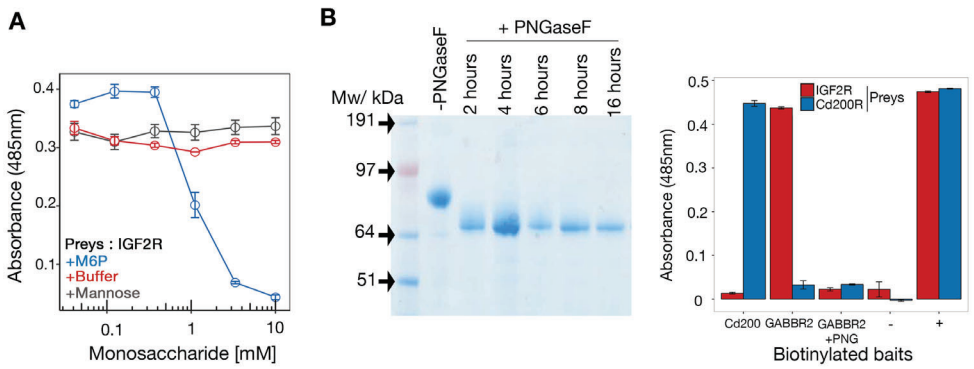

Fig. 5.7 Interaction between IGF2R and GABBR2 is dependent on the M6P-modified N-linked glycans of GABBR2. A. The interaction between IGF2R and GABBR2 can be completely inhibited by soluble M6P. The binding dependency on M6P was established by tested by adding serial dilutions of mannose, M6P, or buffer alone. **B.** Treating GABBR2 with PNGaseF ablates the interaction with IGF2R. Left panel: The purified ectodomain of GABBR2-Cd4-bio was treated with PNGaseF for the indicated times at 37 °C before aliquots were taken, resolved by SDS-PAGE under reducing conditions and stained with Coomassie Blue. No further reductions in molecular mass due to PNGaseF treatment occurred after eight hours incubation, suggesting that the vast majority of glycans had been removed from the protein. Right panel: The indicated biotinylated bait proteins were immobilised on a streptavidin-coated microtitre plate and probed for interactions with the beta-lactamase-tagged prey proteins. Prey binding was quantified by measuring the absorbance of the hydrolysis products of nitrocefin — a β-lactamase substrate — at 485 nm. The known interaction between Cd200-Cd200R was used as the positive control in the assay. Negative control (-) was prey capture by a biotinylated Cd4 tag only control and positive control (+) was total capture of all preys with an anti-prey antibody. Bars represent means $\pm$ sem, n=3. In **A** and **B**, data is representative of three technical replicates.

These data provide evidence that IGF2R directly interacts with the ectodomain of the GABBR2 receptor subunit via the M6P residues present on GABBR2; they may therefore provide mechanistic insights for the known constitutive internalisation of GABAB receptors.

5.3 Discussion

In this chapter, I have demonstrated the utility of the genome-wide screening approach developed in Chapter 4 by identifying IGF2R as a binding partner for GABBR2 receptor. GABAB receptors are expressed abundantly in almost all types of neurons and glia throughout the central nervous system and mediate slow-acting control of neuron excitability by inhibiting neurotransmitter release. This expression pattern overlaps with that of IGF2R, which is also widely distributed throughout the nervous system with particular enrichment in cortical areas, hippocampus and cerebellum [320]. The regulation of the surface level of GABAB receptors by endocytosis is an important mechanism to attenuate signal strength and can be modelled in HEK293 cells, where GABAB receptors have been shown to rapidly and constitutively internalise by the clathrin-dependent pathway to endosomes [289, 292]. The finding that IGF2R can interact directly with the GABBR2 subunit of the GABAB receptor complex provides a mechanism for the internalisation because IGF2R is itself constitutively endocytosed and trafficked to the endosomal compartments through clathrin-mediated uptake via 'YSKV' motifs in its cytoplasmic region [321]. This is also consistent with the regulatory role for the GABBR2 subunit in the uptake of the GABAB receptor complex, and the fact that antibodies directed against the extracellular region, but not receptor agonists, can inhibit GABAB receptor endocytosis [289]. A suggested physiological role of this interaction is depicted in figure 5.8.

A similar role for IGF2R in interacting with M6P-bearing ligands to down-regulate receptors from the cell surface has been shown for CD26 in activated T-cells [307]. CD26 expressed on the surface of activated T cells (activated with phytohemagglutinin), but not expressed on resting T cells was found to be mannose-6-phosphorylated. One of the key questions, which has not been addressed in this work, is the possible physiological mechanism by which GABBR2 would be mannose-6-phosphorylated. To address this, it will be important to first establish whether GABBR2 is constitutively mannose-6-phosphorylated or whether it is a regulated process. One way of addressing this experimentally would be to use the ectodomain of IGF2R to 'pull-down' endogenous GABBR2 from mouse (or rat) brain lysates. If GABBR2 exists in a constitutively mannose-6-phosphorlated state, it should, in principle, be possible to detect the interaction of IGF2R with endogenous GABBR2 using such an approach.

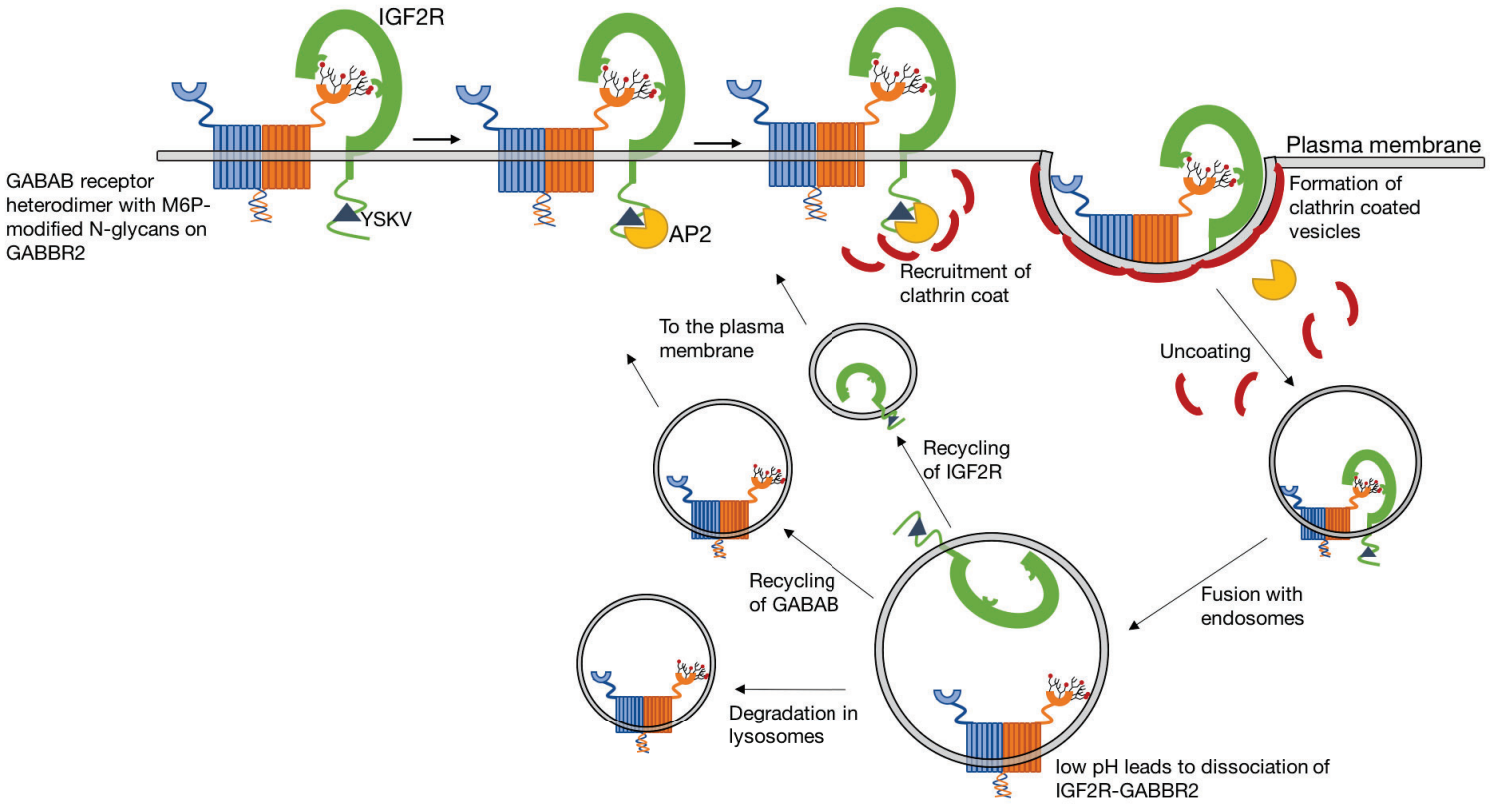

Fig. 5.8 Proposed mechanism of IGF2R mediated GABAB receptor internalisation. In this model, cell surface IGF2R associates with M6P-modified N-glycans of GABBR2 subunit on the plasma membrane. The 'YKSV' motif on IGF2R then recruits adaptor protein complex 2 (AP2 complex), which is a plasma membrane-localised clathrin adaptor composed of α, β2, μ2, and σ2 adaptin subunits [321]. This is consistent with the reported co-localisation of GABAB receptors with AP2 subunits in HEK293 cells [289, 322]. This is followed by clustering of IGF2R and its cargo (GABAB in this case) into clathrin-coated vesicles (CCVs). Following internalisation and uncoating of the clathrin coat proteins, the vesicles are fused with endosomes. The low pH of endosomes induces dissociation of cargo from IGF2R, thereby releasing GABAB receptors into endosomal lumen. IGF2R is recycled back to TGN or to the cell surface, whereas, GABAB receptors are either recycled back to the cell surface membrane or trafficked into lysosomes for degradation. This model is consistent with the observations in HEK293 cells where constitutively internalised GABAB receptors have been shown to be targeted to endosomes from where they are either recycled back to the cell surface or degraded in the lysosomes [289].

The genome-scale approach here has once again demonstrated how this method can be utilised to identify not only the receptor that directly interacts with the recombinant protein probe but also provide valuable information regarding the receptor biology. In the example here, I was able to identify cellular components involved into intracellular vesicle acidification and transport into endo/lysosomal compartments consistent with the known biology of the IGF2R receptor. In addition, I was also able to identify *WDR7* as an important factor mediating the surface expression of IGF2R. Recently, it has been shown that cells with RNAi mediated knock-down of WDR7 are unable to re-acidify intracellular vesicles during recovery from compounds that reversibly block acidification (bafilomycin A1), suggesting a role for regulation of vesicuar acidification for WDR7 [319]. The results here are consistent with this observation and further reveal the role of WDR7 in surface expression of mannose-6-phosphate receptors.