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G-protein-coupled receptors participate in cytokinesis

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Abstract

Cytokinesis, the last step during cell division, is a highly coordinated process that involves the relay of signals from both the outside and inside of the cell. We have a basic understanding of how cells regulate internal events, but how cells respond to extracellular cues is less explored. In a systematic RNAi screen of G-protein-coupled receptors (GPCRs) and their effectors, we found that some GPCRs are involved in cytokinesis. RNAi knockdown of these GPCRs caused increased binucleated cell formation, and live cell imaging showed that most formed midbodies but failed at the abscission stage. OR2A4 localized to cytokinetic structures in cells and its knockdown caused cytokinesis failure at an earlier stage, likely due to effects on the actin cytoskeleton. Identifying the downstream components that transmit GPCR signals during cytokinesis will be the next step and we show that GIPC1, an adaptor protein for GPCRs, may play a part. RNAi knockdown of GIPC1 significantly increased binucleated cell formation. Understanding the molecular details of GPCRs and their interaction proteins in cytokinesis regulation will give us important clues about GPCRs signaling as well as how cells communicate with their environment during division.

Introduction

Cytokinesis is the last step of cell division, in which cells physically separate their content into two daughter cells. It involves many cellular structures and compartments, including microtubules and its associated proteins, a contractile ring that is composed of actin, myosin II and many other proteins, intracellular vesicles as well as cell membrane [Atilla-Gokcumen et al. 2011; Atilla-Gokcumen et al. 2010; Eggert et al. 2006; Normand and King 2010; Rappaport 1986; Rappaport 1996]. Successful cytokinesis requires temporal and spatial control of multiple cellular events. The cell needs to accurately coordinate these different components to ensure the proper positioning of the contractile ring, ingression of the furrow, equal partitioning of cellular contents, and the membrane sealing between two daughter cells. For most cell types, cytokinesis is a symmetric process in which genetic materials and cellular contents are divided evenly. In some specialized cells, for example

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stem cells, cytokinesis needs to be asymmetric so that daughter cells can be different sizes and adopt different fates, which is important for organism development and tissue homeostasis in multicellular organisms [Oliferenko et al. 2009]. For single cell organisms, one obvious example is budding yeast, which uses asymmetric cytokinesis to sequester damaged proteins in aging mother cells [Aguilaniu et al. 2003].

In multicellular organisms, cells mostly undergo cytokinesis in a three dimensional tissue environment. Although cytokinesis has been studied for decades, not much is known about how signals from outside of the cell communicate with intracellular events. The contractile ring lies right beneath the plasma membrane, a key module in cleavage furrow positioning and ingression. While it is known that extracellular matrix proteins are required for cytokinesis [Xu and Vogel 2011], how or if cells respond to extracellular signals is not known. How or if the cell membrane passes along signals from the outside to the inside of the cell is also unclear. One class of obvious candidates for such signal transduction events are the G-protein-coupled receptors (GPCRs), the most abundant integral membrane protein superfamily in mammalian cells. We show that several GPCRs appear to play a role during cytokinesis, suggesting that external cues do play a role in this important process.

GPCRs are also known as 7-transmembrane receptors because they share similar cross membrane structures. It is estimated that the human genome has around 1000 GPCRs and they are one of the most studied target families in the pharmaceutical industry [Filmore 2004; Gilchrist 2010; Overington et al. 2006]. GPCRs can be found in eukaryotes from amoeba and fungi to plants, invertebrates and vertebrates. Upon ligand binding at the cell surface, GPCRs undergo conformational changes and send signals across the cell membrane by interacting with heterotrimeric G proteins. Different subclasses of Gα proteins, such as Gαs, Gαi, Gαq and Gα12, signal through distinct pathways [Neubig and Siderovski 2002]. G proteins transmit signaling cascades in cells through a large number of effectors, including adenylyl cyclases, ion channels, calcium, protein kinase C (PKC) and Rho GTPases.

In addition to the traditional G-protein dependent second messenger signaling cascades triggered by GPCR activation, GPCRs can also stimulate G-protein independent signaling events such as arrestin recruitment [Defea 2008] and activate a broad set of intracellular signaling molecules, such as JNK, Akt, PI3 kinase and RhoA [DeWire et al. 2007]. Upon ligand binding, GPCR kinases (GRKs) phosphorylate GPCRs and recruit β-arrestins, which results in termination or attenuation of signaling by blocking G-proteins from further interaction with the receptors [Hupfeld and Olefsky 2007]. Thus, the β-arrestins are central players for desensitization, sequestration and intracellular trafficking of GPCRs, which prevents cells from undergoing excessive receptor stimulation. Recent findings also show that β-arrestins have additional functions, such as to interacting with and controlling cytoskeletal actin and the F-actin severing protein cofilin [Min and Defea 2011; Pontrello et al. 2011], which may mediate some GPCRs' function in actin regulation.

GPCRs are the major way for cells and organisms to sense the environment and their ligands include neurotransmitters, nonsteroid hormones, biogenic amines, odorants and light. Some of the best characterized examples of GPCR-mediated signaling cascades are in neuronal

function (e.g. dopamine, serotonin and adrenergic receptors) and in sensory responses, for example, to taste, smell and light. GPCRs are usually categorized by the ligands they bind to or by their functions, but both ligands and functions remain unknown for many receptors. Many of these GPCRs of unknown function are named by the tissues in which they were originally identified, by analogy to known GPCRs, or because they are genetically linked to loci that respond to and influence a certain type of perception. For example, olfactory receptors' classical function appears to be interaction with odorant molecules and initiation of neuronal responses to smells. Taste receptors respond to different tastes and were originally found in taste receptor cells of the tongue and palate epithelia. We conducted a screen to investigate if GPCRs play a role in cytokinesis and report that knockdown of several GPCRs that were traditionally thought to be involved in sensory signal transduction also cause cell division defects, suggesting that some of these GPCRs may play broader roles.

Results and discussion

To systematically investigate whether GPCRs play a role in cytokinesis, we screened a GPCR siRNA library provided by Dharmacon for the ICCB-Longwood screening center at Harvard Medical School (Figure 1A). This library includes siRNA SMARTpools (a mixture of four individual siRNAs) for 516 GPCRs and related proteins (423 GPCRs, 19 G proteins, 42 GPCR regulators and effectors, 11 other receptors and 21 ligands). We used an increased number of bi- and multinucleated cells as a readout for cytokinesis failure [Castoreno et al. 2010; Eggert et al. 2004]. After siRNA transfection, HeLa cells were incubated for 3 days before fixation. Whole cells were then visualized with amine reactive tetramethyl rhodamine-*N*-hydroxysuccinimide (TAMRA-NHS) ester and DAPI was used to visualize DNA. We then imaged the screening plates by automated high content confocal fluorescence microscopy, and identified wells with elevated levels of binucleated cells by visual inspection. To control for potential off-target effects due to unintended knockdown of mRNAs corresponding to other genes, we confirmed positive hits from the screen by testing the individual siRNAs from the SMARTpools in subsequent cherry picks. We only followed up potential hits where several individual siRNAs targeting different regions on the target gene resulted in a failed cytokinesis phenotype.

After examining all 516 gene knockdown wells in duplicate experiments, we found that RNAi knockdown of 27 genes resulted in an increased number of bi- and multinucleated cells. These include both GPCRs and interacting proteins, and gene symbols, number of confirmed individual siRNAs and the siRNA sequences used for follow-up are listed in Table S1. For follow-up experiments, we focused mostly on GPCRs rather than effector proteins and selected those genes that resulted in the highest percentage of bi- and multinucleated cells per well. Using these criteria, we selected 13 genes (12 GPCRs and a GPCR interacting protein GIPC, see below), where at least 2 out of 4 confirmed individual siRNAs showed obviously increased bi- and multinucleated cells. Quantification revealed that knockdown of these GPCRs increased bi- and multinucleated cells by 4–10 fold relative to the control (Figure 2B). We subsequently confirmed that all GPCRs are expressed in HeLa cells and used live imaging to evaluate their effects on cytokinesis.

The 12 GPCRs we selected for follow-up range from taste receptors, opsins, olfactory receptors, to dopamine receptors. They include: BLR1/CXCR5 (Burkitt lymphoma receptor 1/C-X-C chemokine receptor type 5), DRD2 (dopamine receptor D_2), DRD3 (dopamine receptor D_3), MRGPRX2 (MAS-related GPR, member X2), OPN1MW (opsin 1, medium-wave-sensitive), OPN1LW (opsin 1, long-wave-sensitive), OR1A2 (olfactory receptor, family 1, subfamily A, member 2), OR2A4 (olfactory receptor, family 2, subfamily A, member 4), RXFP3 (Relaxin/insulin-like family peptide receptor 3), SSTR5 (somatostatin receptor 5), TAS1R2 (taste receptor, type 1, member 2) and TAS2R13 (taste receptor, type 2, member 13) (Figure 2A; Table S1). Other than our recent report showing that DRD3 is involved in endocytic sorting and cytokinesis [Zhang et al. 2012], to our knowledge, none of these receptors have previously been connected to cytokinesis, although the Mitocheck project reported a lethal phenotype for MRGPRX2 knockdown (www.mitocheck.org) [Neumann et al. 2010]. Surprisingly, many of these GPCRs belong to subfamilies that function in sensation. Some receptors have well established and characterized roles in transmitting sensory signals, for example, taste receptor TAS1R2 is highly expressed in specialized cells on the tongue and is responsible for sensing sweet tastes [Hoon et al. 1999; Matsunami et al. 2000; Zhao et al. 2003]. The Opsins OPN1LW and OPN1MW are important for color vision and are highly expressed in optical cones and mutations in these receptors are associated with eye diseases [Gardner et al. 2012]. Interestingly, the third Opsin, OPN1SW, responsible for processing short-wave light, did not score in our screen. Our data suggest that these GPCRs have additional functions independent of their sensory roles. Other GPCRs on our list, for example, the putative odorant receptor OR2A4, are uncharacterized and appear to have been named mainly based on similarity. It is very possible that they have unknown cellular functions other than their predicted role in sensation.

Most of what is known in the literature about the GPCRs we identified here is focused on their roles in specialized tissues such as sensory organs or the brain. However, we discovered that they are involved in cytokinesis in HeLa cells, a human cervical cancer cell line. To test the generality of our results, we investigated if knocking down these 12 GPCRs in other cancer cell lines also caused cytokinesis failure (Supplementary Table 3). As expected, knowing that different tissues express different GPCRs, we found that there was partial overlap between cell lines. Knockdown of 8/12 GPCRs in a colon cancer cell line (HCT116) and 5/12 in a prostate cancer cell line (DU145) resulted in cytokinesis failure, while knockdown 3/12 GPCRs caused cytokinesis failure in all three cell lines. The plasticity of GPCR expression and function amongst different cell lines might be an interesting consideration in the development of specific cancer therapeutics.

We next tested the GPCRs' expression in HeLa cells. Since it can be difficult to obtain sensitive ligands and/or specific antibodies to determine protein levels, we used Real-Time quantitative PCR (RT-qPCR, or qPCR) to examine the expression as well as the knockdown efficiency of the GPCR hits from the screen (Figure S1A). Primer/probe sets were validated as described in the Materials and Methods and their sequences are listed in Table S2. qPCR confirmed expression and knockdown of all 12 GPCRs in HeLa cells. To rule out the possibility that the effects we observed were due to a general perturbation of GPCR

signaling, we also used qPCR to confirm the expression in HeLa and knockdowns of several GPCRs that did not cause cytokinesis failure after RNAi (Figure S1B).

As expected for proteins of low abundance, the GPCRs' threshold cycle (Ct) values, which are the numbers of PCR cycles required for the sample fluorescence to reach the threshold level in qPCR, were relatively high (~ 25 to 37), but consistent with ~ 40 cycles of qPCR usually recommended for GPCRs [Brattelid and Levy 2011; Regard et al. 2008]. This indicates that their expression levels are hundreds to thousands fold lower than abundant proteins such as tubulin (Ct value ~18). The different Ct value among these GPCRs also indicates their differential expression. For example, the olfactory receptor OR2A4 (Ct value ~26) seems to express at higher level than dopamine receptor DRD2 (Ct value ~37). We also confirmed knockdown efficiency of the different GPCRs by qPCR. All of the GPCRs showed knockdown, but were only partially depleted. This could be due to high protein stability, and was amplified by using an iterative method such as qPCR to measure changes in a population of mRNA corresponding to proteins that are not highly abundant in control cells. For example, OR2A4, for which we could obtain a functional antibody, appears to be quite efficiently knocked down at the protein level (Figure 4A).

While the formation of binucleated cells is a general readout for cytokinesis failure, live imaging is needed to determine which step of cytokinesis is affected, for example, cleavage furrow positioning, furrow ingression, or abscission. We analyzed cell division in control or the 12 GPCR knockdown cells by live imaging for 48 hours, which allowed each cell to complete at least one full cell cycle. We found that knockdown of the GPCRs (except for OR2A4, see below) caused cytokinesis failure at a very late stage (Figure 3 and Supplementary Movies 1–4). Control cells usually divide quickly after furrow ingression and midbody formation (Figure 3 and Supplementary Movie 1). GPCR RNAi knockdown cells (expect for OR2A4) were able to go through normal mitosis, furrow ingression and midbody formation until the final cutting step. The two daughter cells then remained connected by the midbody bridge for extended periods of time, ranging from 1–20 hours, before their cleavage furrows regressed to form binucleated cells (Figure 3, Supplementary Movies 2 and 4). The times cells spent at the abscission stage before binucleated cell formation were not correlated with the GPCR that was knocked down and are likely due to cell by cell variations in RNAi efficiency and the stage of the cell cycle when knockdown was first achieved. To better understand how these GPCRs might participate in cytokinesis, we used immunofluorescence to assess the localization of several key players of cytokinesis in control or GPCR RNAi-treated cells. Except for OR2A4 (see below), the actin cytoskeleton was not affected. Microtubule structures, including the mitotic spindle and the midzone, were also not affected, and neither were microtubule associated proteins with functions in cytokinesis, such as the mitotic kinesin CENPE (centromere-protein E) and MKLP1 (mitotic kinesin-like protein 1) [Liu et al. 2006; Raich et al. 1998; Zhu et al. 2005]. Ray Rappaport showed that microtubule structures are essential during early cytokinesis [Rappaport 1996], but they are less important during the final steps of cytokinesis, so a lack of perturbation is consistent with our observation that most GPCR RNAi cells fail cytokinesis at the abscission stage. GPCRs are membrane proteins and enter cells through the endocytic pathway. Endocytic vesicles and proteins are involved in the abscission process [Carlton and Martin-Serrano 2007; Horgan and McCaffrey; Lee et al. 2008; Morita

et al. 2007; Strickland and Burgess 2004]. It is possible that the GPCRs we identified here are transported to the midbody via vesicle trafficking, where they may play a role in regulating the final steps of cytokinesis.

Knockdown of OR2A4 caused a minority of cells (~20–30%) to fail to form midbody bridges, but the majority of remaining cells failed during early cytokinesis (Figure 3 and Supplementary Movie 3). OR2A4 has been predicted to belong to the olfactory receptor family, but it is completely uncharacterized (0 PubMed entries in July 2012). Western blot and immunofluorescence with an OR2A4 antibody confirmed the expression and knockdown of OR2A4 (Figure 4A–B). Cell cycle analysis of OR2A4 RNAi cells using flow cytometry shows an increase in G2/M (4n) cells as well as a peak of polyploid/multinucleated cells (8n or more), an indication of cytokinesis failure (Figure S2). The increased G2/M peak in flow cytometry is likely due to an increase in binucleated cells, which have the same cellular DNA content as and are therefore indistinguishable from G2 phase or normal mitotic cells (Figure S2). Immunofluorescence of OR2A4 shows spindle pole, midzone and midbody ring localizations (Figure 4B), further supporting a role for this GPCR in cytokinesis. While it may seem counterintuitive that an integral membrane protein would localize to cytokinetic structures, this is not uncommon because membrane trafficking plays a key role during cell division [Montagnac et al. 2008]. We investigated if OR2A4 associated with specific vesicle types and found that it co-localizes at the spindle poles and the midbody with the vesicle-associated membrane protein 3 (VAMP3) and transferrin receptor (TfR), markers of recycling endosomes and endosome-derived vesicles (Figure S3), suggesting that these vesicles may be involved in signal transduction in these compartments.

We examined F-actin by phalloidin staining and found that the actin cytoskeleton is perturbed in OR2A4 knockdown cells. Compared to control cells and other GPCR knockdown cells at the same stage, these cells have reduced polar blebs, which are dynamic actin-rich cell protrusions on membranes, in early cytokinesis (Figure 4C–D). While actin-enriched membrane blebs were traditionally considered as a characteristic of apoptosis, they have been more recently observed under other circumstances, for example during cell division and migration [Charras and Paluch 2008]. Blebs are produced by actomyosin contractions at the cell membrane cortex and are thought to be involved in a spindle-independent mechanism that cells use to regulate cleavage furrow positioning. Disturbing the actin polymer by actin stabilizing reagents prevents cells from forming polar blebs during early cytokinesis [Sedzinski et al. 2011]. Increased brightness of F-actin staining and the appearance of more highly bundled actin filaments in OR2A4 knockdown interphase cells indicate that actin polymers may have been stabilized (Figure 4D, E), while actin protein expression was not affected (Figure 4A). As other GPCRs have been shown to play a role in actin organization [Cotton and Claing 2009; Davies et al. 2006], we propose that OR2A4 is involved in cytokinesis by exerting a regulatory role on the actin cytoskeleton. Given that OR2A4 is a GPCR, it is likely to be upstream in any signaling cascades it might control. Therefore, actin regulation is probably just one of the pathways that OR2A4 affects during cytokinesis. Based on its localization at the spindle pole, midzone and midbody, it is likely that it participates in additional pathways during in cytokinesis.

We show here that some GPCRs play a role in cytokinesis, but how they send signals into cells is an open question. It is highly likely that there are other proteins, or indeed entire signaling cascades, that regulate crosstalk between these GPCRs and their final effectors. We showed recently that small molecule agonists/antagonists of dopamine receptor D_3 do not induce cytokinesis failure while RNAi depletion of this GPCR does, suggesting that some of the signaling may be non-traditional [Zhang et al. 2012]. Some GPCR effector proteins were included in our RNAi library, including one strong hit named GIPC1 (GIPC PDZ domain containing family, member 1). GIPC1, also called synectin, is an adaptor and scaffolding protein that interacts with many GPCRs [Hu et al. 2003; Jeanneteau et al. 2004b; Katoh 2002]. Western blot and qPCR show that it is expressed in HeLa cells and that knockdown is efficient (Figure 5A–B). The low Ct value of GIPC (Ct value ~21) in our qPCR analysis indicates that it expresses at higher levels than the tested GPCRs. GIPC1 knockdown significantly increases bi- and multinucleated cell formation from 4% to 64% (Figure 5D). GIPC1 knockdown also caused penetrant cytokinesis failure in the other cell lines we tested (colon and prostate cancer, Fig 5D). Live cell imaging in HeLa shows that GIPC RNAi caused late cytokinesis failure, as did all of the GPCRs we identified except for OR2A4. GIPC may act as a mediator between GPCRs and their downstream players in cytokinesis regulation. This is consistent with the observation that GIPC interacts with DRD2 and DRD3, but not DRD4 [Jeanneteau et al. 2004a]. We showed that knockdown of DRD2 and DRD3, but not DRD4, results in cytokinesis defects. GIPC not only interacts with different GPCRs, but also with numerous other proteins, such as MyoGEF [Wu et al. 2010], suggesting that it may serves as a bridge between GPCRs and their effectors during cytokinesis.

This study opens a new door for cytokinesis regulation. Not only do we add to the list of proteins that play a role during cytokinesis, but we also show that GPCR signaling is involved, suggesting a role for communication between the exterior and interior of the cells. Examining GPCRs and their interaction with extracellular stimuli and intracellular proteins will help us understand how cells divide in a three dimensional context, where external cues are likely to be important. Our results raise many questions about how GPCRs might participate in cytokinesis that will be important to address in the future. For example, what are the downstream components that mediate the cytokinesis effects of these GPCRs? Do they involve different G proteins, β-arrestin or Rho family GTPases? Giα proteins localize to centrosomes, the spindle midzone and midbodies and are involved in cell division [Cho and Kehrl 2007]. A role for some heterotrimeric G proteins in cell division has also been proposed in some model organisms [Bringmann 2008; Schaefer et al. 2001]. It is possible that the GPCRs we found exert their effects through one of these G proteins. Do GPCRs sit on the membrane and send signals into the cells to regulate cytokinesis or do they need to be endocytosed inside the cells to participate? GPCRs are internalized through endosomes, which can localize to the mitotic apparatus including spindle poles and midbodies [Emery et al. 2005; Fielding et al. 2005; Montagnac et al. 2008; Morita et al. 2010; Neto et al. 2011]. OR2A4 localizes to spindle poles and midbodies, indicating that it may function at these locations, instead of at the distant cytoplasmic membrane.

Although it is estimated that up to 40% of the drugs used in the clinic act via GPCRs [Filmore 2004] many of the older drugs were not originally designed as GPCR ligands, but

were developed based on functional activity observed. GPCR signaling is far more diverse than originally thought. One GPCR can couple to multiple G-proteins, as well as signal through other adaptor proteins independently of G-protein coupling [Defea 2008; Marinissen and Gutkind 2001; Seasholtz et al. 1999]. A better understanding of the physiological roles of GPCRs will be essential in the design of next generation therapeutics, not only in aiming to selectively modulate GPCRs for drug development, but also in understanding the consequences of GPCR modulation on different biological systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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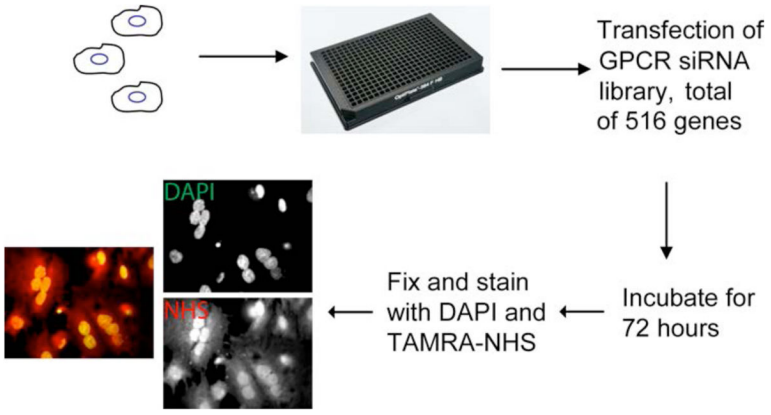

Figure 1. Systematic screen for cytokinesis inhibition with siRNAs targeting GPCRs and effector proteins

Schematic flow chart illustrates our systematic GPCR RNAi screen. HeLa cells were plated in 384 well plates before they were transfected with GPCR siRNAs at ICCB-Longwood. Cells were incubated for 72 hours before fixing and staining. Images were acquired using automated microscopy and wells containing binucleated cells were identified using visual inspection.

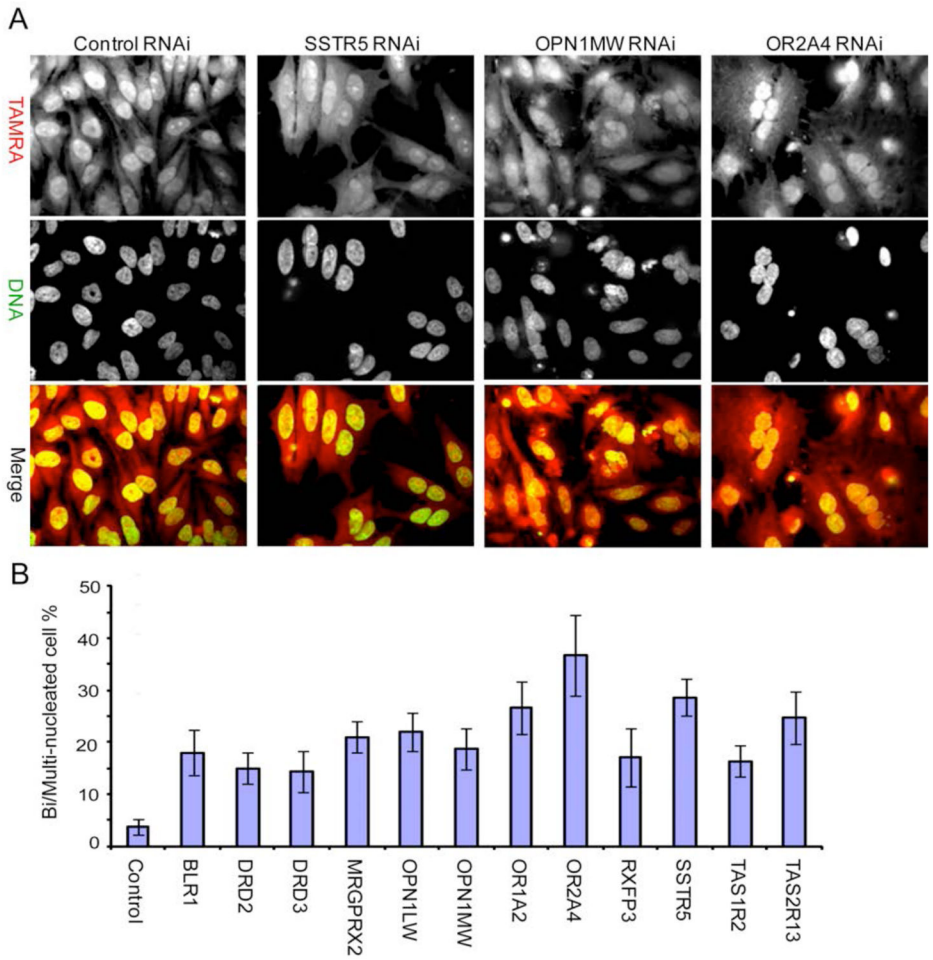

Figure 2. Knockdown of several GPCRs causes binucleated and multinucleated cell formation

(A) Control or GPCR RNAi cells were stained with TAMRA-NHS (shown in red) to visualize whole HeLa cells, and DAPI (shown in green) for DNA. Images were taken using an OPERA high content confocal microscope. Representative images for RNAi of SSTR5, OPN1MW and OR2A4 are shown.

(B) Quantification of binucleated and multinucleated cells in RNAi knockdown cells of 12 selected GPCRs. Quantification was done by counting 100 HeLa cells each in two duplicates from two independent experiments. Error bars show standard deviations.

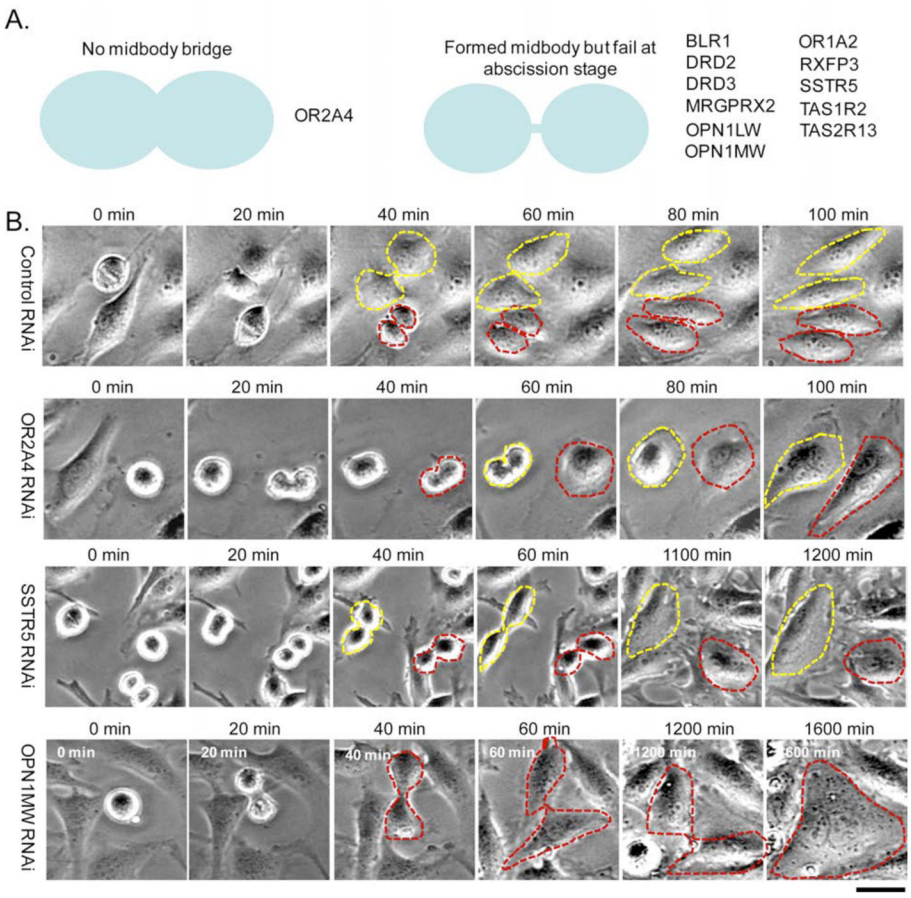

Figure 3. GPCR knockdowns cause cytokinesis failure at early (OR2A4) and late stages (other GPCRs)

(A) Schematic illustration of cytokinesis failure after RNAi depletion of the 12 hit GPCRs. 11/12 GPCR knockdowns show cytokinesis failure at the abscission stage, where midbody bridges were formed between two daughter cells before they bounced back and became binucleated. For OR2A4 knockdowns cells, a majority of cells fail cytokinesis at an earlier stage, where cleavage furrows formed but did not progress to form midbodies.

(B) Images from live imaging of OR2A4, SSTR5, OPN1MW RNAi. Dotted lines outline cell boundaries, two different colors are used (yellow and red) to illustrate individual cells within the same field. Live imaging was done every 20 minutes for 48 hours, starting 24 hours after time of transfection. The complete movies are available in the Supplementary Materials. Scale bar: 10 μm.

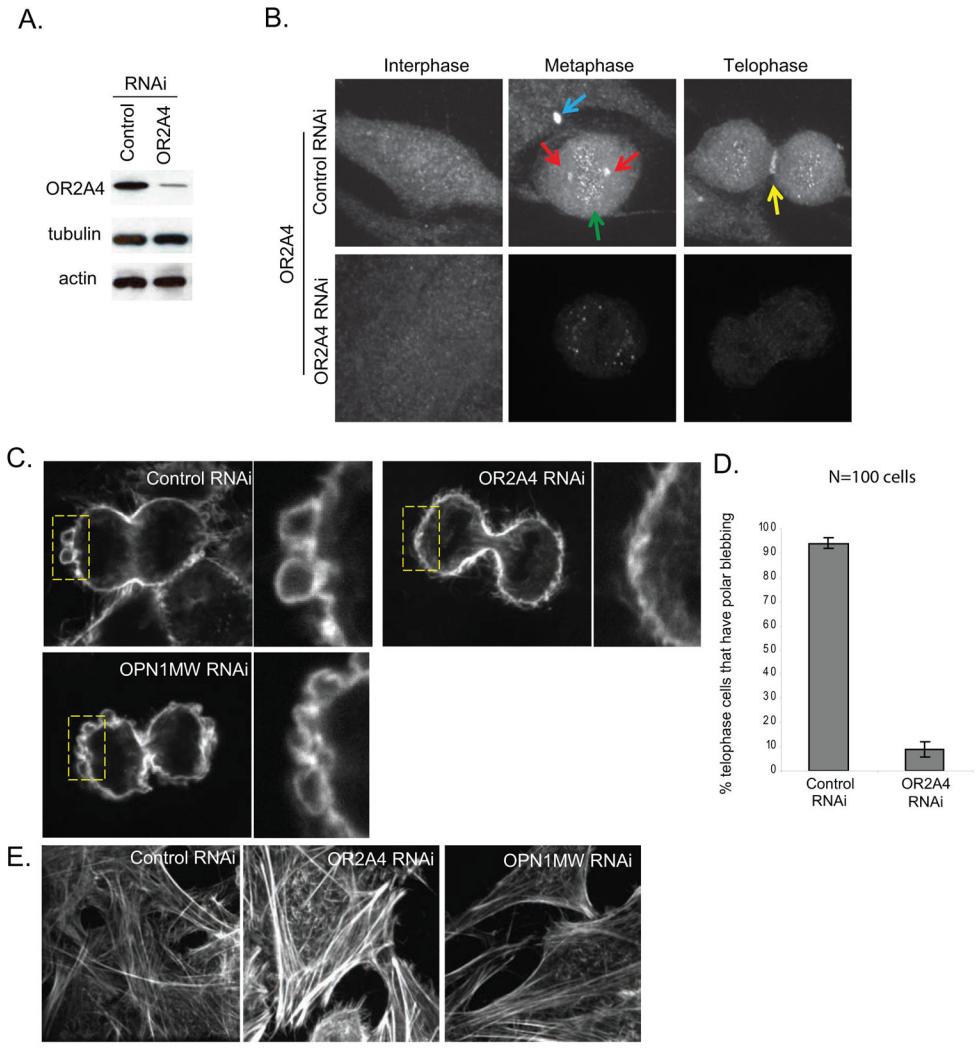

Figure 4. OR2A4 localizes to cytokinetic structures and affects the actin cytoskeleton

(A) Western blots with OR2A4 antibody show the knockdown of OR2A4. Tubulin and Actin were used as loading controls.

(B) OR2A4 localizes to the spindle poles (red arrows) and chromosomes (green arrow) during mitosis and to the cleavage furrow (yellow arrow) and midbody ring/Flemming body (blue arrow in cell adjacent to metaphase cell) in cytokinesis. OR2A4 immunofluorescence in control and OR2A4 knockdown HeLa at different cell cycle stages are shown.

(C) OR2A4 RNAi inhibits polar blebbing in cytokinesis cells. TRITC-phalloidin staining to visualize F-actin in representative control, OR2A4 or OPN1MW RNAi telophase/early cytokinesis cells is shown. Inserts magnify marked regions.

(D) Quantification of percentage of cells in cytokinesis with polar blebbing in control or OR2A4 RNAi cells. 50 cells in each condition were counted in two independent experiments.

(E) OR2A4 RNAi affects actin structures in interphase cells. TRITC-phalloidin staining to visualize actin in control, OR2A4 or OPN1MW RNAi interphase cells is shown,

representative images were taken at the same exposure times. Note the increase in the brightness of actin structures in OR2A4 RNAi cells.

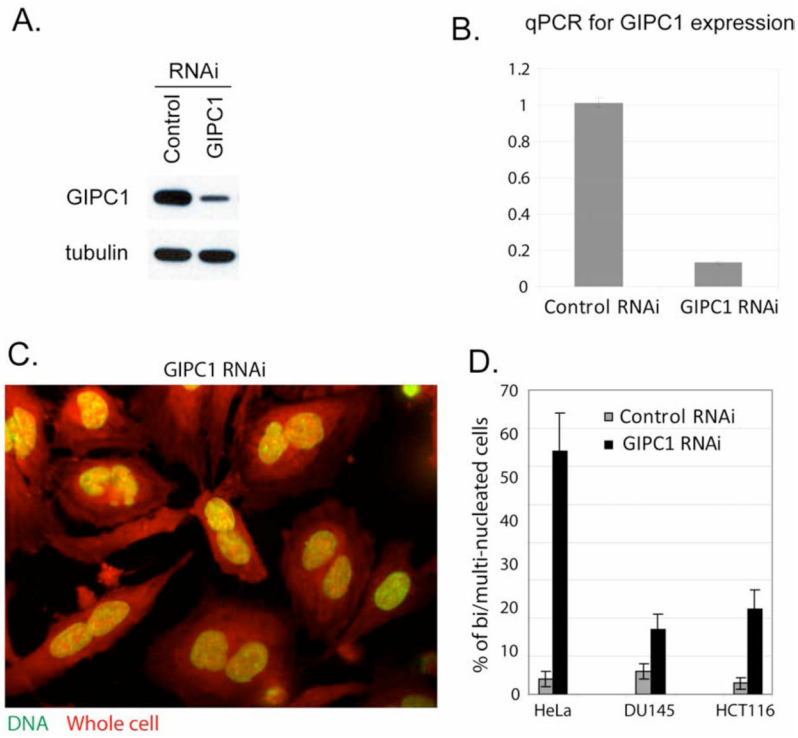

Figure 5. GIPC1 RNAi knockdown causes cytokinesis inhibition

(A) Western blots with GIPC1 antibody show knockdown efficiency of GIPC1 RNAi. Tubulin was used as loading control.

(B) Real-Time quantitative PCR shows knockdown efficiency of GIPC1 RNAi. 28S RNA was used as internal control.

(C) GIPC1 RNAi induces binucleated cell formation. HeLa cells were stained with TAMRA-NHS (shown in red) to visualize whole cells, and DAPI (shown in green) for DNA. Images from the screen were taken on an OPERA high content confocal microscope. Representative images for RNAi of GIPC1 are shown.

(A–C) are in HeLa cells.

(D) Quantification of binucleated and multinucleated cells in control *vs*. GIPC1 RNAi - treated HeLa, DU145 and HCT116 cells. Quantification was done by counting 100 cells each from two duplicates from two independent experiments. Error bars show standard deviations.