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REVIEW

The Wnt/Frizzled GPCR Signaling Pathway

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Abstract—G protein-coupled receptors (GPCRs) represent the biggest transmembrane receptor family. The Frizzled group of GPCRs is evolutionarily conserved and serves to transduce signals from the Wnt-type lipoglycoprotein growth factors. The Wnt/Frizzled signaling cascades are repeatedly used during animal development and are mostly silent in the adult. Improper activation of these cascades, e.g. through somatic mutation, underlies cancer development in various tissues. Our research over the past years has identified the trimeric G proteins as crucial transducers of the Wnt/Frizzled cascades in insect and mammalian cells. The current mini-review summarizes our findings on the role of G proteins in Wnt/Frizzled signaling, as well as on identification of other signaling intermediates in this physiologically and pathologically important type of intracellular signal transduction.

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INTRODUCTION INTO GPCR SIGNALING

G protein-coupled receptors (GPCRs) represent the biggest receptor family in the animal kingdom [1]. More than one thousand GPCR genes are encoded by the human genome, and more than half of all marketed drugs target GPCRs and their signaling pathways [2]. The evolutionary success of GPCRs has allowed diversification of their sequences, building structures competent to recognize and transduce across the cell membrane a wide variety of signals, from quanta of light, ions, small organic molecules, to large macromolecules [3]. Yet the principal organization of GPCRs is stable: they have the extracellular N- and the intracellular C-terminus, and seven transmembrane helixes connected by three extracellular and three intracellular loops [1, 3].

Ligand binding is mediated by receptor regions distributed throughout the extracellular and transmembrane domains and causes conformational changes in the receptor, most noticeable of which is the outward movement of transmembrane helix VI [4, 5]. The consequence of these changes is the release of intracellular protein recognition sites, hidden in the inactive receptor state and for different GPCRs located on different intracellular parts of the receptor [5, 6]. Interaction with and resultant activation of the intracellular effector molecules is the first step in the series of signal transduction events culminating at various GPCR-induced cellular responses, e.g. transcription regulation, cell shape change or motility, proliferation, or death.

The main intracellular GPCR effector molecules are the heterotrimeric G proteins, which provide the name for this receptor group. A heterotrimeric G protein consists of three subunits, α, β, and γ, of which the α-subunit is responsible for binding to guanine nucleotides. Four main subgroups of Gα-subunits can be identified: Gαs, Gαq, Gαi/o, and Gα12/13 [7].

In the GDP-bound state, the G protein can exist as the heterotrimer and is competent to interact with the cognate GPCR. The activated receptor acts as a GEF (guanine nucleotide exchange factor), catalyzing the exchange of GDP for GTP on the Gα. In most cases, this event is followed by dissociation of the G protein trimer into Gα-GTP and the βγ-heterodimer; both also typically lose their contact with the GPCR. The two active halves of the G protein bind and activate various downstream transducer proteins [8]. With time, GTP on Gα is hydrolyzed back to GDP; this reaction is strongly stimulated by the group of GAP (GTPase activating proteins) regulators, most of which belong to the RGS (regulator of G-protein signaling) family of proteins [9]. In the conventional view, GTP hydrolysis is immediately followed by the re-association of the inactive Gαβγ trimer, which is ready for the new cycle of activation by the GPCR.

KINETIC DIVERSITY IN GPCR SIGNALING

Thousands of GPCRs are encoded by animal genomes, yet the number of G-protein subunits is much more limited. For example, six Gα genes are present in *Drosophila melanogaster* and 16 in human [10]. As the βγ-identity of the trimeric G protein complex is at large irrelevant for the GPCR–G protein coupling [11], this mere comparison of numbers poses the issue of how signal specificity is achieved in the GPCR signaling. This issue is further aggravated by the GPCR promiscuity, when any given receptor can efficiently signal through multiple Gα-proteins [12]. So, how does the cell know which GPCR has just been activated and which response program to choose, if, simply stated, numerous GPCRs expressed on its surface all signal through the same G proteins?

Our analysis has provided a possible answer to this question. We performed mathematical modeling of the early events in GPCR signal transduction, relying on previous rigorous experimental measurements of GPCR, G protein, and RGS intracellular concentrations, as well as of their kinetic interaction constants [13]. We came across a number of unexpected predictions concerning the kinetics of GPCR signaling. First of all, we found that the conventional view of the G protein activation, sketched above, is just one of the several predicted signaling modes. Indeed, high concentrations of free GTP-loaded Gα and Gβγ are predicted to result from GPCR activation in one subset of experimentally measured concentrations and constants. However, in another subset, the predicted mode of system response is the production of high concentrations of GDP-loaded but monomeric Gα (plus free Gβγ), while concentration of Gα-GTP is low in this regime. Interestingly, this response mode is predicted to occur in case of Gαo-mediated signaling [13], which has multiple implications for the rest of this mini-review. One of such implications is the prediction that Gαo-GDP should be at least as effective in its interactions with the effector molecules as Gαo-GTP. This prediction is confirmed by the subsequent experimental analysis (see below).

Detailed modeling also showed the possibility of oscillations in the GPCR–G protein–RGS signaling system [13]. The frequency and the amplitude of these oscillations depend on the exact parameters of the participating components, most importantly – of the GPCR involved. Thus, we predicted that the signal specificity in GPCR signaling is mediated not by the exact identity of the G protein transducer, but by the kinetic "signatures" of the signaling elicited by the given GPCR. These kinetic "signatures" allow the cell to "know" which signal it has received and how to properly respond to this signal.

The simple forms of kinetic diversity of GPCR signaling predicted by our analysis (the existence of multiple response modes, as well as existence of multiple steady-states with different flux rates) are the result of application of basic Michaelis–Menten-like enzyme–substrate analysis. In contrast, oscillations are predicted to occur upon inclusion of the positive and the negative feedback loops in our analysis [13], or of the negative feedback with time-delay (not shown). Such regulations are well known to exist in the GPCR signaling [14]. However, the direct demonstration of oscillations in the component concentrations of the GPCR signaling is still missing. One of our goals is to test experimentally whether such oscillations indeed exist. To this end, we had to develop an efficient read-out system using a selected GPCR. Many of our experiments in this direction utilize the somewhat atypical GPCR subfamily—the Frizzled family of receptor proteins.

THE Wnt/FRIZZLED SIGNALING PATHWAY

Frizzled (Fz) proteins serve as receptors to the Wnt family of secreted lipoglycoproteins [15]. The human genome contains 19 genes for the Wnt ligands and 10 genes for Fz receptors. The Wnt/Fz signaling cascades are well conserved in animal evolution, with the basic design principles of this pathway present already in sponges [16]. In the absence of pathway activation, the cytoplasmic protein Axin organizes the so-called destruction complex of proteins which additionally includes the adenomatosis polyposis coli protein (APC), glycogen synthase 3 kinase (GSK3), and casein kinase [15]. The function of this destruction complex is to bind and phosphorylate cytoplasmic β-catenin, which is followed by ubiquitination and proteasomal degradation of the latter. Binding of the Wnt ligand to Fz and the single-pass transmembrane coreceptor LRP5/6 leads to reorganization of the Axin-based destruction complex. An important role in this process is played by the cytoplasmic protein Dishevelled [17]. As a result of reorganization, the complex can no longer bind and phosphorylate β-catenin. Thus, cytoplasmic β-catenin levels rise, and it can diffuse into the nucleus and bind a number of cofactors to trigger transcription of a set of Wnt target genes [18]. Among these genes are cell cycle regulators [19, 20]; in general, the Wnt signaling is responsible for the transcriptional activation of cell proliferation.

Such cellular effect of the Wnt/Fz signaling on cell proliferation underlies the main function of the pathway: it is repeatedly used in organism development, being responsive for multiple developmental programs from early embryonic patterning to later organogenesis processes [21]. In the adult, the Wnt/Fz signaling is mostly silent. As may be predicted, improper activation of the pathway, e.g. through somatic mutations in the pathway components, leads to uncontrollable growth and ultimately cancer [22]. Many tissues are susceptible to car-

cinogenesis due to activation of the Wnt signaling; among the most medically relevant cases are colon cancer and breast cancer: ca. 90 and 50%, respectively, of all individual cases of these cancers are associated with overactivation of the Wnt pathway [22, 23].

During development, the physiological function of Wnt ligands is to serve as morphogens [24, 25]. Morphogens are secreted molecules whose production occurs in a spatially restrictive manner. Upon diffusion through the developing tissue, morphogens create a gradient of concentration. Morphogen-receiving cells then "read" the morphogen concentration and respond differently depending on the concentration received. Thus, Wnt secretion and diffusion must be a very precise and controllable process. A very sophisticated route of Wnt secretion has been developed, where multiple posttranslational modifications are sequentially added on the morphogen. These modifications allow creation of a number of quality control steps during Wnt secretion [26, 27]. They are also required for the biological ligand activity of Wnts.

REGGIE/FLOTILLIN IN LONG-RANGE Wnt DIFFUSION

The posttranslational modifications help build the controllable diffusion of Wnts through the receiving tissue and establish the precise morphogen concentration gradient. Two to three lipid moieties added on Wnt molecules drastically change Wnt diffusion properties, making it highly hydrophobic and "sticky" for the cell membrane and extracellular matrix components [28, 29]. This feature ensures creation of very high Wnt concentrations close to the source of production, required for expression of specific "high-threshold" target genes by the tissue located close to the Wnt production zone. However, Wnt is known to diffuse over large distances *in vivo*, allowing patterning of tissues on long scales, as happens e.g. during limb development [30]. How does this hydrophobic sticky Wnt penetrate far through the receiving tissue? The answer came upon analysis of the form(s) in which Wnt is secreted by the producing cells. It was discovered that in addition to the monomeric (and poorly diffusive) form, Wnt molecules can be packaged into lipoprotein particles, where the hydrophobic parts of the morphogen are hidden inside the particle [31]. Such lipoprotein particles charged with Wnt can diffuse over long distances and are responsible for target gene induction in cells located far from the region of Wnt production.

The mechanism(s) mediating Wnt loading on lipoprotein particles are poorly described. Our work has provided compelling evidence that the lipid raft-organizing protein reggie/flotillin plays an important role in this process within the Wnt-producing cells [32]. Reggie is the first protein described to function *in cis* to specifically package Wnt for the long-range diffusion. Loss of reggie reduces Wnt diffusion range, while reggie overexpression expands Wnt diffusion. Such changes differently affect the expression of the high-threshold versus the low-threshold Wnt target genes throughout the receiving tissue. Modeling predicts that the overall diffusion capacity of Wnt upon loss of reggie is decreased by an order of magnitude [32]. These results are explained by the idea that two secretion routes exist within the Wnt-producing cell. The first directs secretion of Wnt monomers, while the second ensures Wnt packaging into highly diffusive particles. Reggie likely acts as a pointsman on the branch point of Wnt secretion, determining the selection of the secretion route of the morphogen.

FRIZZLED PROTEINS SIGNAL THROUGH THE TRIMERIC Go-PROTEIN

Regardless of the molecular form by which Wnt is delivered, essentially the same signaling cascade is initiated in the receiving cells. As mentioned above, this cascade starts with the atypical GPCR Fz. Although Fzs have the normal GPCR topology, their sequences bear almost no similarity to the largest rhodopsin-like subgroup of the GPCR family. The GPCR nature of Fz proteins has been long debated, not the least due to the absence of experimental evidence from model organisms of the involvement of trimeric G proteins in Fz signaling. We have closed this gap by unequivocally demonstrating that in *Drosophila*, Fz pathways are mediated by the trimeric Go protein, which acts as an immediate transducer of these receptors [33, 34]. Analysis of Gαo mutant alleles has revealed that the G protein-defective tissue failed to normally transduce the Wnt/Fz signals. $G\alpha o^{-/-}$ cells were unable to properly activate expression of the Wnt target genes, which resulted in morphological abnormalities in the adult mutant structures [33, 35]. Similar defects could be induced by expression of pertussis toxin (Ptx), whose enzymatic activity results in ADP-ribosylation of *Drosophila* Gαo, leading to its uncoupling from cognate GPCRs [33, 36, 37]. Subsequent experiments also revealed the identity of the Gβγ subunits required for the Wnt/Fz/Go signaling [35, 38].

In vitro PLATFORM TO MONITOR Wnt/FRIZZLED ACTIVATION

We also wished to prove biochemically the GPCR nature of Fz proteins. A receptor can be considered a GPCR, if it can physically bind a trimeric G protein and – upon ligand binding – activate the guanine nucleotide exchange on its Gα-subunit. We performed a series of biochemical experiments, demonstrating that Fz proteins indeed bind the trimeric Go protein and activate it in the

presence of Wnt ligands [39]. While multiple Wnt ligands and 10 Fz proteins are encoded by the human genome, very limited information is available as to which Wnts can bind and activate which Fz receptors. Our experiments lay the ground for the systematic analysis of this kind. Out of multiple Wnts and Fzs analyzed, we establish the following highly efficient human Wnt–Fz pairs: Wnt3a–Fz1, Wnt5a–Fz7, and Wnt7–Fz6 [39].

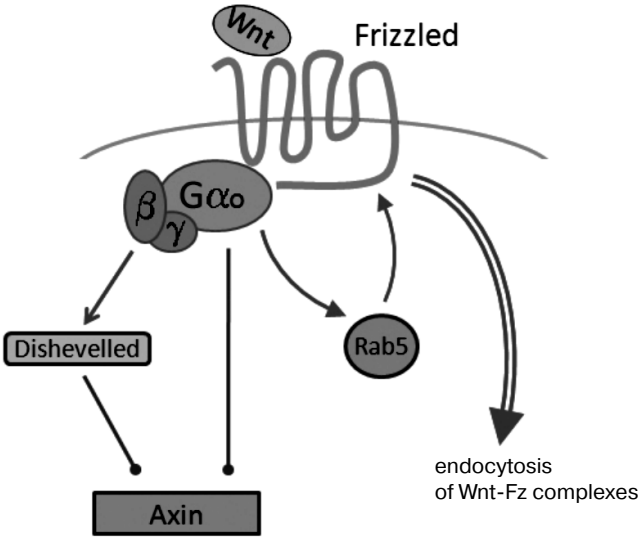
In the course of our experiments, we established a novel assay to monitor G protein activation. Traditionally, the radioactive nonhydrolyzable GTP analog [^{35}S]GTPγS has been used for this purpose [40, 41]. However, the usage of [^{35}S]GTPγS has clear limitations as this assay is very low throughput and involves handling of radioactive compounds and waste. As the alternative to the traditional assay, we expanded the usage of europium-labeled GTP analog (Eu-GTP), originally developed by Perkin Elmer [42], to any type of assay where radioactive analogs were previously utilized [43]. This new format of monitoring G protein activation is ideal for high-throughput analysis. Thus, our experiments establish an innovative *in vitro* platform suitable for the screening of chemical libraries for GPCR modulators. We currently apply this platform to identify small-molecule agonists and antagonists of the Wnt/Fz pathway. Given the high medical importance of this pathway, the identified antagonists could become leads to novel anticancer therapies [44]. On the other hand, artificial agonists of the Wnt/Fz pathway could become highly useful for the proliferation of various stem cells in culture [45] or as potential regeneration activators [46].

AXIN IS A TARGET OF Gαo SIGNALING

We next questioned, what could be the downstream effectors of Gαo in Fz signaling? One of the possible targets was Axin, the key negative player in cytoplasmic Wnt signaling [47]. Axin was a likely candidate due to the presence of the RGS domain in its sequence, known in other proteins to bind Gα-subunits and catalyze GTP hydrolysis on them [9]. However, previous studies showed the absence of key residues responsible for this GAP activity in the RGS domain of Axin [48].

To investigate whether Axin could be the target of Gαo, we first performed biochemical investigation with purified proteins. We found that the RGS of Axin could efficiently interact with Gαo. However, as predicted, Axin failed to speed up GTP hydrolysis by this G protein. We next moved to cells and found that Gαo, especially in its GTP-bound state, could efficiently relocalize Axin from cytoplasm to plasma membrane; such relocalization has been observed before in cells stimulated by Wnt ligands and is considered to be the first step towards reorganization of the Axin-based β-catenin destruction complex [49, 50]. Finally, we performed experiments on developing wing tissue of *Drosophila* and found that Gαo, in its activated state, can suppress negative activity of Axin towards the Wnt/Fz signaling. Crucially, the RGS domain of Axin was absolutely required for this negative interaction between Axin and Gαo, replicating our biochemical observations. Thus, we concluded that Axin is one of the targets of the G protein-mediated signaling (see figure).

Interestingly, we found that the other half of the initially trimeric Go complex, the Gβγ heterodimer, also played an active role in Wnt signal propagation. Gβγ is localized to the plasma membrane through its lipid modifications, and upon liberation from the trimeric complex it serves to attract to the plasma membrane another cytoplasmic component of Wnt signaling, Dishevelled [38]. We predict that such relocalization ensures activation of Dishevelled, which is then expected to result again in inhibition of Axin, which Dishevelled binds through the heterophilic interaction of the DIX domains present in both proteins [51, 52]. Thus, the trimeric Go protein, after dissociation by the GPCR activity of Fz receptors, produces two moieties both acting to suppress Axin: Gαo producing the direct, and Gβγ producing the indirect (Dishevelled-mediated) impact on Axin [38]. Such double action possesses a certain elegancy and probably has been developed to guarantee Axin inhibition for the efficient Wnt/Fz signal propagation (see figure).



A general scheme of the early signal transduction events in Wnt/Frizzled signaling. Upon binding of the Wnt ligand to Frizzled, the trimeric Go complex is activated. The two components of the complex play an active role in signal transduction. Gαo can directly act on Axin, and Gβγ activates Dishevelled. Both Gαo and Dishevelled then converge on Axin to inhibit this negative regulator of Wnt signaling. Additionally, Gαo recruits Rab5 to promote endocytosis of the Wnt–Frizzled complexes, which serves as amplification of the initial input from the ligand–receptor interaction. See text for more detail

Rab5 IS ACTIVATED BY Gαo TO AMPLIFY THE Wnt/FRIZZLED SIGNAL TRANSDUCTION

Another – less expected – target of Gαo in Fz and probably other GPCR signaling is Rab5. Rab5 is a small (monomeric) GTPase, famous for its involvement in the control of early endocytic events: clathrin-coated vesicle formation, fusion of the endocytic vesicles and early endosomes, as well as homotypic fusion between early endosomes [53]. We originally found a robust genetic interaction between Rab5 and Gαo [54], which prompted us to test whether the two proteins could physically bind each other. To this end, we prepared Gαo and Rab5 as recombinant proteins and found a robust binding of Gαo to Rab5; Gαo was also found to bind the fast-recycling Rab4 but not the slow-recycling Rab11 [54]. Curiously, Rab5-GDP was a preferential binding partner of Gαo. This might have suggested that Gαo acted as an activator of Rab5, and indeed in cellular assays we found that Gαo-GTP was able to activate Rab5 and induce massive endosome fusion. Fz receptors had a similar effect on Rab5, which was Gαo-dependent. What could be the mechanism of Gαo-mediated activation of Rab5? We excluded direct activation, as Gαo failed to activate purified Rab5 *in vitro*. Instead, we showed that *in vivo* activated Gαo was able to recruit Rab5 from the cytoplasm to plasma membrane, where endogenous Rab5 activators are located [54]. Thus, we built the model where Fz activation of Gαo resulted in local recruitment and activation of Rab5, which in turn promoted Wnt/Fz internalization (see figure). Such internalization of the ligand/receptor complexes was found to serve to markedly strengthen the signal propagation [54-56]. Thus, the Gαo–Rab5 link is a novel mechanism ensuring amplification of the signal initially provided by Wnt/Fz binding.

BROAD SCREENING FOR Gαo TARGET PROTEINS

We next decided to perform a broad whole-genome/whole-proteome identification of Gαo target proteins. To achieve this goal, we designed and accomplished in parallel three screening projects. The first was genetic: Overexpression of Gαo in the eyes of *Drosophila* leads to a clear rough-eye phenotype due to developmental aberrations. We then used this phenotype to look for mutations, which would suppress or enhance its strength. Such mutations represented genes whose products play a function in the Gαo-dependent pathways. The second screen was biochemical/proteomic. We immobilized different forms of Gαo on a matrix, followed by incubations with *Drosophila* protein extracts. Proteins specifically retained by Gαo were then identified by peptide mass-fingerprinting. The third approach to identify Gαo-interacting proteins was the yeast two-hybrid screen. The completion of these three whole-genome/proteome screening strategies has provided us with an unprecedented thoroughness of identification of the binding/signaling partners of a trimeric G protein. We currently apply an intense bioinformatics analysis of this Gαo-interaction net in order to identify functional modules in Gαo-mediated cellular responses. Partial results of these screens have been published [37, 57]. Apart from the interesting Gαo target proteins identified, these screens reveal one interesting feature of Gαo signaling, namely the great degree of overlap of the target proteins interacting with the GDP-bound (and traditionally considered inactive) form of Gαo and its GTP-bound (activated) form [37, 57]. In other words, many Gαo targets do not discriminate between Gαo-GDP and Gαo-GTP. This feature is unexpected within the dogmatic view of trimeric G protein signaling. However, as described above, mathematical modeling predicts that one of the modes of cellular G protein activation produces high concentrations of Gβγ and free Gα-GDP (with low concentrations of Gα-GTP) [13]. Importantly, this mode of activation was predicted to be selected by Go due to specific kinetic properties of this G protein [13]. It is thus not surprising that Gαo, which inside the cell preferentially resides in the monomeric GDP-bound state upon GPCR activation, efficiently binds many effector proteins in this GDP state. Thus, our modeling predictions [13] are neatly confirmed by experimental data ([37, 57] and unpublished data).

GoLoco-PROTEIN Pins IS A TARGET OF Gαo DURING ASYMMETRIC CELL DIVISIONS

One of the effector proteins of Gαo, identified in our screens, is Pins (also known as Rapsynoid). Pins and its homologs from nematodes to mammals are well-established regulators of asymmetric cell divisions [58]. Through multiple GoLoco domains located in the C-terminal half of the protein, Pins binds Gα-subunits of trimeric G proteins [59], and through the N-terminal tetratricopeptide repeats—the microtubule anchoring protein NuMA (Mud in flies, LIN-5 in nematodes) [60, 61]. Previously, the GoLoco-mediated interaction of Gαi to Pins had been reported [62], while we revealed the interaction of Gαo and Pins in the asymmetric divisions of the sensory organ precursor lineage of the *Drosophila* peripheral nervous system [35]. However, the crucial feature of the GoLoco–Gα interactions has been known as the exclusive binding of GoLoco to the GDP-loaded form of Gα-subunits, while GTP-bound forms of G proteins failed to bind GoLoco-containing proteins [59]. This and other observations have led to the conventional view that GoLoco proteins serve not as targets of Gα signaling, but instead as modulators of this signaling [59, 63]. This view became challenged by our identification – in the yeast two-hybrid screen – of Pins as the binding

partner of the GTPase-deficient (and thus constitutively GTP-bound) mutant form of Gαo [37]. Yeast two-hybrid and subsequent biochemical data have pinpointed this unusual interaction to the GoLoco1 domain of Pins. In contrast, GoLoco3 domain showed the conventional exclusive interaction with Gαo-GDP. Analysis of the GoLoco1 sequence revealed an additional positively charged amino acid (lysine 15) in the center of GoLoco1 sequence. Mutation of lysine 15 for the amino acid, located in the same position in GoLoco3, abrogated binding to Gαo-GTP, leaving binding to Gαo-GDP unaffected [37]. We performed numerous additional biochemical and genetic experiments, which established Pins as a target of Fz-Gαo signaling in the sensory organ precursor cells. As Pins and its homologs are expressed in other tissues, most notably the brain [64, 65], where Gαo represents the major G protein in insects and mammals [66, 67], our data suggest that Pins is a general target of GPCR signaling mediated by the trimeric Go protein [37].

This completes the overview of the research in the field of GPCR signaling in general and Wnt/Fz signaling in particular, with the special focus on the contribution of investigations of my laboratory. More research projects are on the way in my laboratory, and multiple new exciting projects are being born along the progression of our research. These ongoing investigations aim at characterizing newly identified Gαo targets, as well as the whole Gαo-mediated GPCR signaling network at the systems level. I wish to round up this mini-review by expression of the idea that GPCR signaling, despite its vast biological and medical importance and many years of investigation in numerous labs, is still waiting for the understanding of its fundamental organizing principles. My research aims at approaching the perception of these principles, which – I strongly believe – can only be achieved through the intense combination of experimentation and mathematical modeling.

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REFERENCES

1. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) *Nat. Rev. Mol. Cell Biol.*, **3**, 639-650.
2. Nambi, P., and Aiyar, N. (2003) *Assay Drug Dev. Technol.*, **1**, 305-310.
3. Fredriksson, R., and Schioth, H. B. (2005) *Mol. Pharmacol.*, **67**, 1414-1425.
4. Scheerer, P., Park, J. H., Hildebrand, P. W., Kim, Y. J., Krauss, N., Choe, H. W., Hofmann, K. P., and Ernst, O. P. (2008) *Nature*, **455**, 497-502.
5. Oldham, W. M., and Hamm, H. E. (2008) *Nat. Rev. Mol. Cell Biol.*, **9**, 60-71.
6. Oldham, W. M., and Hamm, H. E. (2007) *Adv. Protein Chem.*, **74**, 67-93.
7. Milligan, G., and Kostenis, E. (2006) *Br. J. Pharmacol.*, **147**, Suppl. 1, S46-55.
8. Gilman, A. G. (1987) *Annu. Rev. Biochem.*, **56**, 615-649.
9. Ross, E. M., and Wilkie, T. M. (2000) *Annu. Rev. Biochem.*, **69**, 795-827.
10. Malbon, C. C. (2005) *Nat. Rev. Mol. Cell Biol.*, **6**, 689-701.
11. Clapham, D. E., and Neer, E. J. (1997) *Annu. Rev. Pharmacol. Toxicol.*, **37**, 167-203.
12. Hermans, E. (2003) *Pharmacol. Ther.*, **99**, 25-44.
13. Katanaev, V. L., and Chornomorets, M. (2007) *Biochem. J.*, **401**, 485-495.
14. Ferguson, S. S. (2001) *Pharmacol. Rev.*, **53**, 1-24.
15. MacDonald, B. T., Tamai, K., and He, X. (2009) *Dev. Cell*, **17**, 9-26.
16. Petersen, C. P., and Reddien, P. W. (2009) *Cell*, **139**, 1056-1068.
17. Gao, C., and Chen, Y. G. (2010) *Cell Signal.*, **22**, 717-727.
18. Vlad, A., Rohrs, S., Klein-Hitpass, L., and Muller, O. (2008) *Cell Signal.*, **20**, 795-802.
19. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) *Science*, **281**, 1509-1512.
20. Tetsu, O., and McCormick, F. (1999) *Nature*, **398**, 422-426.
21. Logan, C. Y., and Nusse, R. (2004) *Annu. Rev. Cell Dev. Biol.*, **20**, 781-810.
22. Giles, R. H., van Es, J. H., and Clevers, H. (2003) *Biochim. Biophys. Acta*, **1653**, 1-24.
23. Brennan, K. R., and Brown, A. M. (2004) *J. Mammary Gland Biol. Neoplasia*, **9**, 119-131.
24. Tabata, T., and Takei, Y. (2004) *Development*, **131**, 703-712.
25. Lander, A. D. (2007) *Cell*, **128**, 245-256.
26. Bartscherer, K., and Boutros, M. (2008) *EMBO Rep.*, **9**, 977-982.
27. Mikels, A. J., and Nusse, R. (2006) *Oncogene*, **25**, 7461-7468.
28. Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R., 3rd, and Nusse, R. (2003) *Nature*, **423**, 448-452.
29. Papkoff, J., and Schryver, B. (1990) *Mol. Cell Biol.*, **10**, 2723-2730.
30. Geetha-Loganathan, P., Nimmagadda, S., and Scaal, M. (2008) *Organogenesis*, **4**, 109-115.
31. Panakova, D., Sprong, H., Marois, E., Thiele, C., and Eaton, S. (2005) *Nature*, **435**, 58-65.
32. Katanaev, V. L., Solis, G. P., Hausmann, G., Buestorf, S., Katanayeva, N., Schrock, Y., Stuermer, C. A., and Basler, K. (2008) *Embo J.*, **27**, 509-521.
33. Katanaev, V. L., Ponzielli, R., Semeriva, M., and Tomlinson, A. (2005) *Cell*, **120**, 111-122.
34. Egger-Adam, D., and Katanaev, V. L. (2008) *Front. Biosci.*, **13**, 4740-4755.
35. Katanaev, V. L., and Tomlinson, A. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 6524-6529.
36. Katanaev, V. L., and Tomlinson, A. (2006) *Cell Cycle*, **5**, 2464-2472.
37. Kopein, D., and Katanaev, V. L. (2009) *Mol. Biol. Cell*, **20**, 3865-3877.

38. Egger-Adam, D., and Katanaev, V. L. (2010) *Dev. Dyn.*, **239**, 168-183.
39. Katanaev, V. L., and Buestorf, S. (2009) Available from *Nature Precedings* (http://hdl.handle.net/10101/npre.2009.2765.1).
40. Carty, D. J., and Iyengar, R. (1994) *Meth. Enzymol.*, **237**, 38-44.
41. Weiland, T., and Jakobs, K. H. (1994) *Meth. Enzymol.*, **237**, 3-13.
42. Frang, H., Mukkala, V. M., Syysto, R., Ollikka, P., Hurskainen, P., Scheinin, M., and Hemmila, I. (2003) *Assay Drug Dev. Technol.*, **1**, 275-280.
43. Koval, A., Kopein, D., Purvanov, V., and Katanaev, V. L. (2010) *Anal. Biochem.*, **397**, 202-207.
44. Barker, N., and Clevers, H. (2006) *Nat. Rev. Drug Discov.*, **5**, 997-1014.
45. Nusse, R., Fuerer, C., Ching, W., Harnish, K., Logan, C., Zeng, A., ten Berge, D., and Kalani, Y. (2008) *Cold Spring Harb. Symp. Quant. Biol.*, **73**, 59-66.
46. Zhao, J., Kim, K. A., and Abo, A. (2009) *Trends Biotechnol.*, **27**, 131-136.
47. Luo, W., and Lin, S. C. (2004) *Neurosignals*, **13**, 99-113.
48. Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vasicek, T. J., Perry, W. L., 3rd, Lee, J. J., Tilghman, S. M., Gumbiner, B. M., and Costantini, F. (1997) *Cell*, **90**, 181-192.
49. Cliffe, A., Hamada, F., and Bienz, M. (2003) *Curr. Biol.*, **13**, 960-966.
50. Schwarz-Romond, T., Metcalfe, C., and Bienz, M. (2007) *J. Cell Sci.*, **120**, 2402-2412.
51. Kishida, S., Yamamoto, H., Hino, S., Ikeda, S., Kishida, M., and Kikuchi, A. (1999) *Mol. Cell Biol.*, **19**, 4414-4422.
52. Fagotto, F., Jho, E., Zeng, L., Kurth, T., Joos, T., Kaufmann, C., and Costantini, F. (1999) *J. Cell Biol.*, **145**, 741-756.
53. Zerial, M., and McBride, H. (2001) *Nat. Rev. Mol. Cell Biol.*, **2**, 107-117.
54. Purvanov, V., Koval, A., and Katanaev, V. L. (2010) *Sci. Signal.*, **3**, ra65.
55. Blitzer, J. T., and Nusse, R. (2006) *BMC Cell Biol.*, **7**, 28.
56. Seto, E. S., and Bellen, H. J. (2006) *J. Cell Biol.*, **173**, 95-106.
57. Katanayeva, N., Kopein, D., Portmann, R., Hess, D., and Katanaev, V. L. (2010) *PLoS One*, **5**, e12331.
58. Gonczy, P. (2008) *Nat. Rev. Mol. Cell Biol.*, **9**, 355-366.
59. Willard, F. S., Kimple, R. J., and Siderovski, D. P. (2004) *Annu. Rev. Biochem.*, **73**, 925-951.
60. Du, Q., and Macara, I. G. (2004) *Cell*, **119**, 503-516.
61. Siller, K. H., and Doe, C. Q. (2009) *Nat. Cell Biol.*, **11**, 365-374.
62. Schaefer, M., Petronczki, M., Dorner, D., Forte, M., and Knoblich, J. A. (2001) *Cell*, **107**, 183-194.
63. Hampoelz, B., and Knoblich, J. A. (2004) *Cell*, **119**, 453-456.
64. Blumer, J. B., Chandler, L. J., and Lanier, S. M. (2002) *J. Biol. Chem.*, **277**, 15897-15903.
65. Caussinus, E., and Gonzalez, C. (2005) *Nat. Genet.*, **37**, 1125-1129.
66. Sternweis, P. C., and Robishaw, J. D. (1984) *J. Biol. Chem.*, **259**, 13806-13813.
67. Wolfgang, W. J., Quan, F., Goldsmith, P., Unson, C., Spiegel, A., and Forte, M. (1990) *J. Neurosci.*, **10**, 1014-10s24.