Signal transduction exam 2018

This figure is from Labasque et al., 2008 (GPCR week).

A) Describe the experiment shown here and what was learned as a result.

B) Design a negative control for this experiment and describe why it is a good control.

C) Design an experiment to confirm this result in living cells. Please include controls in your experimental design.

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A) The experiment was performed on human embryonic kidney (HEK 293) cell line. The extracts of solubilized proteins were taken under consideration for this experiment. These were then transfected with different vectors: CTL (empty vectors), co-transfected with GFP fused Calmodulin(GFP-CaM) and serotonin receptor either wild type 5-HT2C or 5-HT2CR376/377A which were treated with a vehicle or 5-HT for five minutes, they analyzed the binding reaction by Western Blot and Immunoprecipitation (IP) techniques. Immunoprecipitation was done of these soluble proteins with polyclonal antibody: anti-GFP.

Furthermore, these immunoprecipitated proteins were blotted using a monoclonal anti-GFP antibody along with anti-5-HT2C receptor antibody. As a result of this experiment, it was found that the absence of agonist, 5-HT2C receptor did not co-immunoprecipitate with calmodulin in solubilized proteins that were co-transfected with Myc-5-HT2C receptor and GFP-CaM. However, serotonin receptor co-immunoprecipitated with GFP-CaM after treatment with 5-HT for 5 minutes.

But the exposure with 5-HT2CR376/377A did not co-immunoprecipitate GFP-CaM with serotonin receptor. It proves that 5-HT2C receptors are linked with CaM depending on the presence of agonist. The experiment performed is to check the agonist dependent precipitation of GFP-CaM and serotonin receptors in the presence of Myc-5HT2C 5-HT exposure. So, the negative control to be designed here for this experiment can be simply of serotonin receptor and CaM.

This would result in no immunoprecipitation and prove as a good negative control. On analyzing it through western blotting it could be proved that no immunoprecipitation had occurred in this control. To confirm the results in living cells the same experiment could be used to check the that whether the immunoprecipitation is agonist dependent or not. Take solubilized proteins from living cells and co-transfect them with GFP-CaM and 5-HT2C receptor followed by exposure to 5-HT, then analyze immunoprecipitation via western blotting.

The negative control for this experiment will include only CaM and serotonin receptor, and further analyzing it with western blotting, the results of blotting will show no precipitation in negative. 2. These figures are from Fig. 6A, C of Fan et al. (src kinase folder). (A) Explain the experiments shown here.

What was being tested? What conclusions can be drawn? How can it be improved? (B) Design another experiment to test the same concept/hypothesis? Include your controls. 4839335107886500A) In figure A: Human embryonic kidney cells were transfected with constructs for carrying

out this experiment. Immuno-precipitation was carried out of tagged PAG with an antibody against regulator MYC, its interaction is studied here with SRC and BRK followed by immunoblotting analyzation.

The result of this experiment was delaying in electrophoretic mobility of tagged PAG protein, when it was co-expressed with SRC. This delay is considered due to the hyper-phosphorylation. However, on co-expression of PAG and BRK this band shift was less evident. In figure C: Co-transfection of HEK 293 cells with MYC-PAG and SRC followed by treatment in the absence or presence of SRC kinase inhibitor SU6656 (5 M) for at least 1 hour.

PAG was immunoprecipitated by antibody against MYC, and the binding of CSK was compared by immunoblotting. On the other hand, the disturbance of link b/w CSK and PAG was observed on weakening SRC activity by a small molecular inhibitor SU6656, this experiment concluded the importance of SRC kinase activity for functioning of CSK.

Also, this study shows that distinct mechanisms have evolved to regulate the activities of two structurally similar and functionally related kinases, BRK and SRC, understanding of the signaling function of protein phosphatases, including definition of their substrate specificities, will allow us to exploit a greater spectrum of the changes in signaling in disease and to generate new and more effective strategies for therapeutic intervention in major human diseasesB) To test the same hypothesis with controls, take breast cancer frozen samples of different patients, divide the sample in two groups one will be the test group other the control group. The control group will be given doses of anti-tumor medication (like tamoxifen for breast cancer).

RNA will be extracted from both groups using Trizol and will be followed by purification assay. The breast cancer cell lines will be transfected with empty vectors or pcDNA3-MKP3-V5. Furthermore, the transfectants will be placed in media like minimum essential media MEM with phenol red, followed by SDS PAGE electrophoresis, and transfer to nitrocellulose membrane. After transferring they will be incubated with primary antibody for an hour or more then with the secondary antibody to observe the chemiluminescence with a reagent.

The cells will then be lysed in a buffer, phosphatase reaction carried out will be observed by assays and transfected cells will be compared with control ones to whom tamoxifen was given. 36734750003. This is figure 4b from Gresset et al (phospholipases folder). (A) What is the hypothesis being tested in this experiment? (B) Describe how the experiment was done, including results. (C) Predict what might happen to PLC? activity if Tyrosine783 was 'permanently' phosphorylated and explain why.

The hypothesis being tested here is the enhancement in the lipase activity of phospholipaseC-? 1 via phosphorylation of one or both specified tyrosine residues (Y775 and Y783) in the downstream process of signal transduction. To perform the experiment, equal concentrations of purified phospholipase-C-? 1 were set on incubation with the active kinase domain of the Fibroblast growth factor receptor FGFR2 and ATP in bovine serum albumin containing buffer, the samples of this reactions were tested for two activities: 1) for lipase activity in the phospholipid vehicles indicated in the figure on left Y axis.

Secondly the phosphate incorporation in phospholipase-C-? 1 was studied, illustrated at right Y axis of the figure. 4 moles of phosphate were added to each test samples, the Wildtype inactive PLC? 1 was taken as a normal control with its basal phospholipase activity, active PLC? 1 with wildtype FGFR2K served as a positive control with phosphate and PLC? 1 knockout mutant served as negative control to quantify the phospholipase activity among the mutants.

This was performed to check the phosphorylation of tyrosine and auto inhibition of PLC-? isozymes, 775/783 of PLC-? 1 were substitutes at the place of phenylalanine, they could be used individually or together, but in the experiment tyr783 is used individually. Phospholipase activity of resulting mutant after purification was quantified with active domain of FGFR2K (helps in phosphorylation and activation of phospholipase). Certain known moles of phosphates were added into purified PLC-? 1 in wild type under above mentioned conditions and observed that phospholipase activity was enhanced 10 times.

The mutation of tyr783 completely nullified the kinase stimulated acceleration of phospholipase activity along with reduction in FGFR2K-promoted phosphorylation of PLC-? 1. This experiment proves that, phosphorylation of Tyr783 is vital for relief of auto-inhibition. Studies reveal that Tyr-783 was essential for auto inhibition. As discussed above, permanent phosphorylation of tyr-783 will completely nullify the kinase stimulated and FGFR2K stimulated phosphorylation of PLC-? 1.

Lipase activity of PLC-? 1 will be enhanced across its limits and over-expression of PLC-? 1 can induce malignant transformation. The results could be leading to production of carcinoma cells. It has been found in various studies that activity of PLC-? 1 is more in cancerous cells as compared to normal cells. So, permanent phosphorylation tyr783 could be a way leading to malignant cancers. 3416300-254000004. This figure is from Tsui et al. 2015. (lipid raft folder). (A)

Explain the relationship between GFR and Ret51 and what they are testing in this paper (the overall idea). (B) Explain the experiment shown in this figure and what was learned. (C) What is a negative control that could improve the conclusions from this experiment? GFR and Ret51 both are receptors, GDNF is found to promote PNS development and kidney morphogenesis through the receptor complex consisting of GDNFfamilyreceptor 1 (GFR1) and the other receptor tyrosine kinase (Ret).

Ret signal transduction is increased by translocation of GFR. GFR-mediated Ret activation is essential too for the kidney morphogenesis and for various other functions of abdominal precursors that form abdominal nervous system. Also, GFR has many lipid rafts because its GPI anchorage, but Ret is expelled from lipid rafts. In this paper, the gene replacement for GFR in mice results GDNF resulting in Ret activation but prevented its translocation into lipid rafts.

These mice showed renal agenesis, and other disorders including loss of the enteric nervous system, and defects in motor neuron axon path similar to GFR mice that was knocked out, all this provided evidence along with lipid

rafts GFR is also needed for neurotrophic factor signaling. Primary considerate neurons secluded from Gfr1and Gfr1TM/TM mice were maintained in vitro for some days. Then they treated the neurons with GDNF or medium for exact time of 15 minutes.

The Detergent-resistant membranes quarantined from the neurons were examined by immunoblotting for Ret51. The comparative purity of detergent resistant and detergent soluble fractions was confirmed by using immunoblotting for caveolin and transferrin receptor, respectively B, the experiments shown in A, were computed and graphed. Otherwise, Substantial decline in the amount of Ret51 was recorded statistically that translocated into lipid rafts while GDNF stimulation in Gfr1TM/TM neurons compared with Gfr1 neurons. Similar Results were obtained after performing the experiment four times

. Moreover, Lipid raft translocation experiments were performed to prove the concept that GDNF/GFR1/Ret complex does not translocate into lipid rafts in Gfr1TM/TM mice. Primary sympathetic neurons from Gfr1/ and Gfr1TM/TM mice were used to extract detergent-resistant membranes. Upon stimulation of Gfr1/ neurons with GDNF, Ret translocated quickly into lipid rafts.

This was a contrast to Gfr1TM/TM neurons that an evident reduced movement of Ret into the detergent-resistant was recorded because of GDNF exposure. A small portion of Ret that did translocate into lipid rafts while stimulation may be owing to Ret kinase-dependent translocation of Ret into rafts that occurs with slower movements. There was a significant, 75% reduction in the kinetics of the Ret receptor complex into lipid rafts during

GDNF exposure in Gfr1TM/TM neurons according to computation made by these experiments.

The negative control design here for confirming the results that Ret doesn't translocate into lipid rafts during GFL activation in Gfr1TM/TM neurons, the primary sympathetic neurons isolated from Gfr1/and Gfr1TM/TM mice will be grown in the same way as test ones, with the only difference that there will be no treatment with GDNF or medium for 15 minutes, and the impact of this will confirm the result to much greater extent upon immunoblotting.