Bioluminescence resonance energy transfer biology essay

Science, Biology

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\n[/toc]\n \nThe efficiency of this energy transfer is highly dependent upon the distance between donor and acceptor molecules, with the optimum distance for many standard interactions being between 10 and 50Å. If there are no interactions between the two proteins of interest, RLuc and GFP will be too far apart for significant energy transfer and hence the blue bioluminescent signal only will be detected and not the visible light emission of YFP or GFP. Such interactions are detected using an emission spectrum obtained from a Microplate Analyzer (a luminometer) for each of the donor and acceptor proteins at specific wavelengths. These include λ (wavelength) of 480nm for RLuc, λex/em (excitation/emission) at 514/530nm for YFP or GFP at λex/em of 400/510nm respectively. Fig 1. BRET can be detected using a luminometer (microplate or single-tube) or scanning spectrometer. The luminometer must have the capacity to sequentially or simultaneously detect filtered light within two distinct wavelength windows. http://cochin. inserm. fr/departments/emd/team-t.-issad-r.-jockers/techniques/bret-

bioluminescence-resonance-energy-transfer/images/BRET-figure. jpgFig 2. BRET1 notes the emission of blue light with the addition of coeletarazine when one protein is fused to the donor (Rluc). In BRET2 where YFP is replaced by GFP, the Rluc substrate is Deepblue C. The advantage of BRET2 is a superior separation of donor and acceptor peaks. In the presence of Deepblue C, Rluc emits light at 400nm, a wavelength that excites GFP, which, in turn, emits light at 510nm. The disadvantage of BRET2, compared to BRET1 is the 100-300 times lower intensity of emitted light. The advantage is that with a larger spectral resolution, BRET2 permits the selection of filters that absorb the entire bandwidth of the donor and acceptor emission peaks thereby increasing the robustness of the detection. BRET has successfully assisted in the study of signal transduction pathways, the dimerization of G-protein coupled receptors (GPCRs) and the detection of secondary messengers like cAMP. To achieve this, suitable cell lines are transfected into cDNA plasmids that are able to encode luciferase and recombinant fusion proteins. A fluorescent plate reader can be used to monitor the BRET signal that fluctuates according to agonist stimulation.

How is BRET used?

BRET's main function is to detect protein-protein interactions. The emission of light of a specific wavelength by the luminescent protein (GFP/YFP, etc) is detected and analysed, to show whether or not any interaction between the two proteins takes place. This data can also be used to obtain information about the type of association between the two proteins and their orientation at this time. This works because the position at which the donor and acceptor proteins are fused to the targets may affect whether the interaction

will be able to be visualized using BRET. Therefore conducting a series of experiments where bioluminescent/luminescent proteins are fused to either the target's N or C terminus will provide information about how the protein interaction actually occurs. More recently BRET has been able to be used to investigate protein-protein binding in deep tissue. This has required modifications to the type of fluorescent donor and the luminescent acceptor proteins used. This is necessary because red light being given out as fluorescence is better than green or yellow light, which are absorbed by blood cells before they can be detected. This type of experiment is extremely valuable as it is able to give data that is representative of the protein interactions taking place in real time in living tissue.

Hypotheses

BRET can be used to test:- That human β2-adrenergic receptors form homodimers when they are expressed in HEK-293 cells. (Angers, S et al.)- That the clock proteins KaiB encoded in the circadian clock genes KaiA and KaiB of cyanobacterium Synechococcus sp. strain PCC 7942 form dimers when they interact. (Xu, Y et al.)BRET cannot be used to test:- That- That a metal and a protein interact with high affinity.

Advantages

-The method of measurement done using BRET is non-invasive, therefore it can be used to determine if the interaction changes with time.-BRET is appropriate to assay the protein-protein interactions in different sub-cellular compartments or specific organelles of a native cell.-The dynamic process of protein-protein interactions in vivo, such as intracellular signalling, can be

monitored using BRET assays.-The BRET signal is a ratio-metric measurement. This type of detection eliminates data variability caused by fluctuations in light output due to variations in assay volume, cell types, the number of cells per well and/or signal decay across a plate.

Limitations

-Conformational states of the fusion proteins may set the dipoles into a geometry that is unfavorable for energy transfer and this shows that the efficiency of a BRET assay is dependent on the proper orientation of the donor and acceptor dipoles.-It is possible that some parts of the candidate molecules are interacting without allowing the luciferase tags to be close enough for energy transfer to occur.

BRET vs FRET

FRET (Fluorescence Resonance Energy Transfer) is a technique similar to BRET, but which doesn't incorporate a donor protein capable of bioluminescence as a result of breaking down its substrate. Instead the donor protein requires irradiation with an external light source, which will then cause the transfer of energy to the acceptor protein which will fluoresce with a light of a different wavelength that can be detected, as in BRET. In comparison to FRET, BRET does not require an external light source for the excitation of the donor. Therefore, problems such as auto-fluorescence, light scattering, photo bleaching, and photoisomerization of the donor half, or photodamage cells which are usually associated with FRET-based technologies can be prevented. In addition to this, the absence of contamination of the light output by the incident light results in a very low

background in BRET assays. This allows for the detection of smaller variations in the BRET signal, compared to FRET.