A HaloTag-based Gene Reporter System for Live-Cell Imaging and High-Throughput Screening

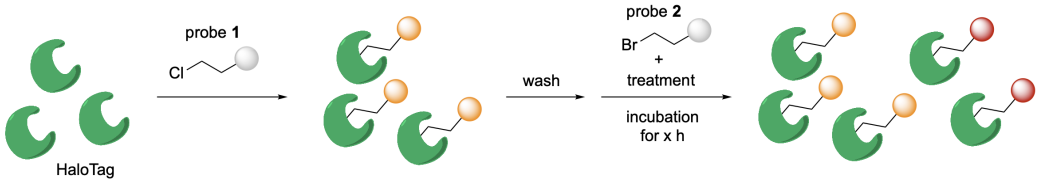
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Gene expression monitoring is a powerful tool to elucidate stress response mechanisms and dynamics. We developed a gene reporter system for BiP (binding immunoglobulin protein), a key mediator of the endoplasmic reticulum (ER) stress response.[1]

Upon accumulation of unfolded proteins in the ER, the unfolded protein response (UPR) is activated, which consists of three pathways that are initiated by IRE1, PERK, and ATF6.[2] The target BiP is a chaperone that assists protein folding. It is involved in the activation of the UPR as well as induced via the ATF6 pathway.[3]

Our system is based on the co-expression of the target BiP and the self-labeling HaloTag protein[4] in a stable cell line. The amount of HaloTag that is produced thus corresponds to the amount of BiP. By labeling HaloTag with two fluorogenic compounds with different spectral properties—a rhodamine and a silicon rhodamine—two gene expression levels can be time-stamped and visualized in a single pulse-chase experiment, providing additional information and increasing the robustness of the system. The method is suitable for live-cell fluorescence imaging as well as flow cytometry. Moreover, we are able to employ the system in high throughput screening experiments, searching for novel inducers of BiP expression.



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