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Spatial Control of Protein Synthesis

Previously used to provide temporal control over cellular processes, "photocaged" small molecules can impart spatial control to in vitro models. In this issue of *Chemistry & Biology*, the Dore and Schuman labs report a system to block protein synthesis in a spatially confined manner with a photo-caged form of anisomycin [1].

The actions of many proteins and biopolymers are often segregated into precise spatial and temporal patterns in tissues. However, in many cases, we lack the tools to study such exquisitely regulated events. New methods to spatially and temporally control biomolecular function, such as enzyme activation/inhibition, local control of gene transcription, and RNAi provide powerful tools for studying these important but more subtle aspects of coordinated cellular function. In this issue of *Chemistry & Biology*, the Dore and Shuman labs report a general method to spatially regulate protein translation with a photo-caged form of the ribosome inhibitor anisomycin.

Goard et al. selected the photo-cage 6-bromo-7-hydroxycoumarin-4-ylmethyl (Bhc) based on its photophysical properties compared to two other common caging groups. Bhc-anisomycin was synthesized and tested for its ability to inhibit protein synthesis in an in vitro translation system. The results presented demonstrate that Bhc has significant advantages over the more commonly used nitro benzyl or nitroveratryl (dimethoxynitrobenzyl) caging groups; its quantum efficiency is more than an order of magnitude greater. The release of micromolar levels of anisomycin was extremely rapid with inhibition observed after less than 2 s exposure to UV light.

With the goal of inhibiting ribosomes in a subset of cultured cells on a plate, GFP reporters were introduced into Chinese hamster ovary cells and into hippocampal neurons, and cellular fluorescence was monitored as the cells were pulse irradiated with UV via an optic fiber. The high quantum efficiency allowed photorelease of anisomycin in highly sensitive hippocampal neurons while avoiding the phototoxic effects of longer exposures. Cessation of protein synthesis was dependent on both the presence of the inhibitor and irradiation with UV light. Finally, Goard et al. established spatial specificity for ribosome inactivation by determining the range of the affected cells radiating out from the site of irradiation.

This study is not the first example of a photocaged enzyme inhibitor; inhibitors of nitric oxide synthase and carbonic anhydrase have previously been developed as a potential means to selectively deliver drug candidates to specific tissues [2–4]. However, this report is the first to actually demonstrate that caged inhibitors can be used to regulate cellular function in a spatially defined manner. Irradiation of cultured cells treated with caged anisomycin can be used to selectively block translation of reporter genes within a confined region of cells [1].

This is also the first report of a photocaged inhibitor of the ribosome that can be used to address a range of important biological questions involving the spatial and temporal expression of proteins. For example, localized inhibition of protein synthesis might be used to block synthesis of morphogens or other developmental cues in a subset of cells within a tissue. Thus far, caged anisomycin has been used to regulate protein synthesis in multicellular and single-cell systems but not at the subcellular level.

If the release of anisomycin can be controlled at higher resolutions, the ability to block protein synthesis within a cell might be used to assess the importance of subcellular regions of protein synthesis such as the local synthesis of protein within the dendrites of neurons [5]. Studies involving subcellular activation of inhibitors will likely require the use of two-photon uncaging techniques which can more precisely confine the region of activation [6]. It is important to note, however, that although anisomycin was caged with the Bhc group that was initially developed for its large two-photon cross-section [7], the reported studies of Bhc-anisomycin used more traditional one-photon deprotection methods.

Other methods used to downregulate protein activity involve the use of specific inhibitors of enzyme activity, antagonists of transcription factors [8], and photocaged oligonucleotides that control DNA transcription [9], RNA translation [10], or RNA interference [11]. Unlike these systems that are highly specific and need to be customized to target each protein of interest, the ability to regulate general protein synthesis can be used

to probe the functions of any locally expressed protein provided that it is turned over at an appreciable rate compared to the duration of the experiment. The ability to inhibit protein synthesis efficiently in a spatially discrete manner should open the way for a myriad of studies exploring the role of protein patterning in intra- and intercellular signaling.

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