Clontech

Rapid, on-demand protein stabilization and destabilization using the ProteoTuner™ systems

Clontech's new ProteoTuner™ system allows one to regulate the quantity of a protein of interest present in the cell, quickly and on demand. It uses a ligand-dependent destabilization domain that is fused to a protein of interest. Addition of a membrane-permeant small molecule, Shield1, reversibly stabilizes the fusion protein in a rapid, predictable and dose-dependent manner. The system's rapid kinetics and direct control enable characterization and functional studies of a specific protein in live cells.

Analyzing protein function is a key focus in discovery-based cell biology research. Clontech's revolutionary new ProteoTuner system allows direct investigation of the function of a specific protein of interest by rapidly changing the abundance of the protein itself. This technology has already been successfully used in a variety of organisms and cell types[1–4].

Rapid and precise mechanism of control

The ProteoTuner system consists of two components: a vector encoding the destabilization domain (DD) upstream of a multiple cloning site, and the DD's stabilizing ligand, Shield1. The DD is a 12-kDa (107-amino-acid) tag based on a mutated FKBP protein. When a protein of interest is expressed as a fusion with the DD tag, it is destabilized and rapidly degraded in the cell by proteasomes. However, when the DD's small (750 Da), membrane-permeant ligand (Shield1) is added to the culture medium, it reversibly binds to the DD tag. This protects the DD-tagged protein from degradation and leads to its rapid accumulation in the cell (**Fig. 1**; ref. 4). ProteoTuner systems are available in plasmid, retroviral and lentiviral delivery formats.

The ProteoTuner system controls the amount of the protein of interest by acting directly on the post-translated protein—unlike other systems that act at either the transcriptional (inducible gene expression systems) or translational level (RNA interference). The rate of protein accumulation is proportional to the amount of Shield1 added to the culture medium. For example, when we treated cells expressing DD–DsRed-Express with different concentrations of Shield1, we observed dose-dependent accumulation of the DD–DsRed-Express present in the cells, as assayed by fluorescence microscopy (**Fig. 2**) and confirmed by western blot (data not shown; *Clontechniques* XXIII(2), 1–2; 2008). Thus, it is possible to precisely 'tune' the amount of stabilized, DD-tagged protein present in the cell by varying the amount of Shield1 added to the culture medium, within the range of ~50–1,000 nM.

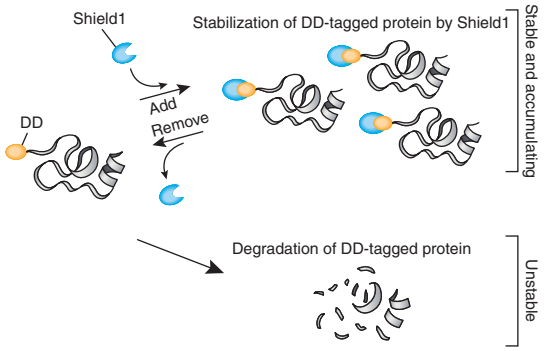

Figure 1 | Ligand-dependent, targeted and reversible protein stabilization. The default pathway for the ProteoTuner system is degradation of the DD-tagged protein (bottom), unless Shield1 is present to stabilize it.

Rapid kinetics of ProteoTuner control

ProteoTuner System's fast kinetics make it possible to cycle the amount of the DD-tagged fusion protein rapidly and sequentially by adding Shield1 to, or removing it from, the culture medium. When Shield1 is added to the medium, the fusion protein can accumulate to detectable levels within 15–20 minutes after addition of Shield1 (ref. 4). Conversely, upon Shield1 removal, the half-time for the protein's degradation can be as short as 30 minutes[4].

Protein function analysis with the ProteoTuner system

ProteoTuner technology has already been used for several types of protein function analyses, including observing cellular phenotype changes in response to the expression of specific proteins[4]. It has also been used to develop controllable dominant-negative phenotypes[1] and to study a protein's role against a knockout background, by maintaining

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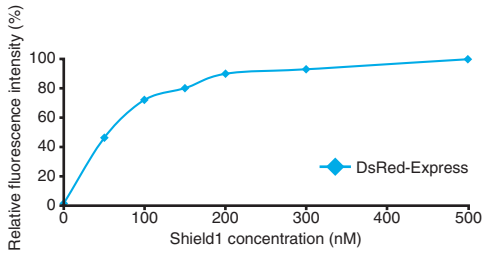

Figure 2 | Levels of DD-tagged proteins are directly related to the concentration of Shield1. Cells were infected with pRetro-X PTuner DsRed-Express and treated with the indicated concentrations of Shield1. The amount of DsRed-Express stabilized by different concentrations of Shield1 was measured 18 hours later by flow cytometry.

Plasmodium falciparum parasites without Shield1 and 'rescuing' protein function by adding Shield1 (ref. 2).

Monitoring rapid events with the ProteoTuner system

The fast kinetics of the ProteoTuner system make it possible to study rapid and essential cellular processes by quickly changing the stability of a specific protein involved in these processes. For example, actin polymerization and depolymerization is a quick, dynamic process, and studying its timing has been laborious and difficult. Existing visualization methods are limited to capturing a single snapshot of the state of the actin filament network. Until now, the only technique used to monitor the dynamics of actin rearrangement was microinjection of *in vitro*–labeled actin[5]. Unfortunately, microinjection is very labor-intensive, can only be performed on a limited number of cells and requires specific instrumentation and skills.

Recently, we developed a simpler method to visualize actin behavior, using the ProteoTuner systems. We infected HeLa cells with two constructs: one encoding mCherry-labeled (Clontech) human α-actin, which constitutively labels all actin filaments in the infected cells, and the other encoding human α-actin fused to AcGFP1 (Clontech) and the DD. This DD-AcGPF1-actin fusion was only stable in the presence of Shield1 (**Fig. 3**).

As predicted, in the absence of Shield1, we observed cytoskeletal labeling (from mCherry-actin), but DD-AcGFP1-actin was degraded too rapidly to integrate into the dynamically changing actin filament network (**Fig. 3b–d**). Then we added Shield1 to the culture to stabilize DD-AcGFP1-actin, and within 1 hour after adding Shield1, DD-AcGFP1-actin was stabilized and integrated into the same filaments that incorporated mCherry-actin (**Fig. 3e–g**). In fact, we detected Shield1-stabilized DD-AcGFP1-actin by fluorescence microscopy as early as 15 minutes after adding Shield1 to the cells (data not shown). We also obtained similar data using human neural progenitor cells. Our results are in agreement with a previous report that the actin filament network rearranges completely in 1 hour in PtK2 epithelial cells[5], but we used the extremely simple ProteoTuner-based method, rather than the extremely laborious microinjection method. We also observed that the DD did not interfere with the proper incorporation of DD-tagged actin monomers into actin filaments (**Fig. 3e,f**). Furthermore, we observed no toxicity in the presence of Shield1.

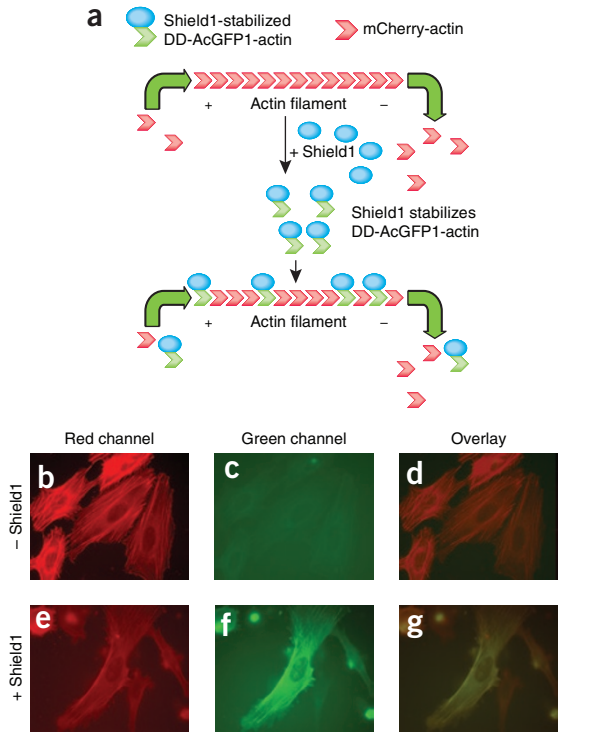

Figure 3 | Controlling actin stability with the ProteoTuner system. (**a**) Self-assembly of actin filaments occurs at the plus end of an existing actin filament as monomeric actin is incorporated. Conversely, disassembly occurs at the minus end where actin monomers depolymerize from the filament, causing a continuous rearrangement of the actin filament network. In the absence of Shield1, only mCherry-actin is present to be incorporated into newly forming actin filaments. When Shield1 is added, DD-AcGFP1-actin is stabilized and can be incorporated into newly formed actin filaments together with mCherry-actin. In the absence of Shield1, no DD-AcGFP1-actin is observed despite the presence of a normal, mCherry-labeled actin filament network. (**b–g**) Fluorescence micrographs 1 hour after addition of Shield1 (**e–g**) or without Shield1 (**b–d**). Cells were fixed using 4% paraformaldehyde and imaged with a ×40 objective.

Conclusion

The ProteoTuner system makes it possible to change the amount of a protein of interest rapidly, so it is possible to observe even the quickest cellular processes, such as cytoskeletal rearrangement in cells, by simply expressing the protein of interest as a DD-tagged fusion and controlling its stability by adding or removing Shield1.

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