Dualsystems Biotech

Finding new protein interactions using the DUALhunter system

The identification of protein interactions has been greatly simplified by the invention of yeast-based screening assays such as the yeast two-hybrid system. However, yeast two-hybrid assays cannot be used to analyze several important protein classes, such as integral membrane proteins or transcription factors. Here we demonstrate the ability of the DUALhunter system to detect interactions between transcriptionally active proteins and to identify protein interactions by library screening.

Traditionally, biochemical methods such as coimmunoprecipitation or affinity purification have been used to characterize protein complexes, but the invention of yeast-based screening assays such as the yeast two-hybrid system[1] has resulted in a dramatic increase in the number of protein interactions described in the literature because these assays enable quick identification of new protein interactions by virtue of cDNA library screening. In these assays, a protein of interest is assayed against a complex mixture of full-length proteins, protein domains and/or protein fragments expressed from a cDNA library, and the protein's interacting partners are isolated. No prior knowledge of the interaction partner is needed, making these systems extremely powerful tools for protein-interaction discovery.

However, the yeast two-hybrid system is limited to studying proteins that can translocate to the nucleus and do not display any autonomous capacity for activating transcription[2]. Thus, this system cannot be used to study several important protein classes, such as integral membrane proteins, transcription factors or strongly acidic proteins. To circumvent these limitations we developed a flexible screening system, called the DUALhunter system, that would allow the use of potentially any cytoplasmic protein as a bait for screening.

Principle of the DUALhunter system

The DUALhunter system is based on the well known split-ubiquitin complementation assay[3–5]. To screen a protein of interest for new interactors, the protein of interest (the bait) is fused at its N terminus to the small membrane protein Ost4p (which anchors the bait at the membrane) and at its C terminus to a reporter module encompassing the C-terminal half of ubiquitin (Cub) followed by a transcription factor (LexA-VP16; **Fig. 1a**). Potential interactors (preys) are expressed from a cDNA library as fusions to a modified N-terminal half of ubiquitin (NubG; **Fig. 1b**).

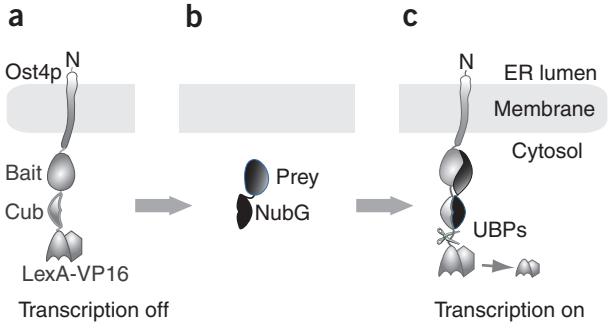

Figure 1 | Principle of the DUALhunter system. (**a**) A protein of interest (bait) is inserted between the membrane protein Ost4p, which anchors the protein in the endoplasmic reticulum (ER) membrane, and the C-terminal half of ubiquitin (Cub) followed by the artificial transcription factor LexA-VP16. (**b**) The prey is fused to the mutated N-terminal half of ubiquitin (NubG). (**c**) If bait and prey interact, Cub and NubG complement to form split ubiquitin, which attracts cleavage by ubiquitin-specific proteases (UBPs). As a result, the transcription factor is released and translocates to the nucleus, where it can activate transcription of endogenous reporter genes.

Interaction between the bait and a prey results in formation of split ubiquitin from Cub and NubG. Ubiquitin-specific proteases present in the yeast cell recognize the split ubiquitin and release the attached transcription factor by cleavage of the polypeptide chain after Cub. The transcription factor then translocates to the yeast nucleus and activates a set of cognate reporter genes, thereby converting the protein interaction into a transcriptional readout, which is easily measured—for example, as growth on a selective minimal medium (**Fig. 1c**).

The DUALhunter kit supplies bait and prey vectors, appropriate controls and the reporter strain NMY51 for screening a protein of interest against a NubG cDNA library to identify novel interactors.

Pairwise interaction assays between known proteins

We chose Uri1p (ref. 6), a protein with proposed roles in protein translation and folding, as a model protein because our previous

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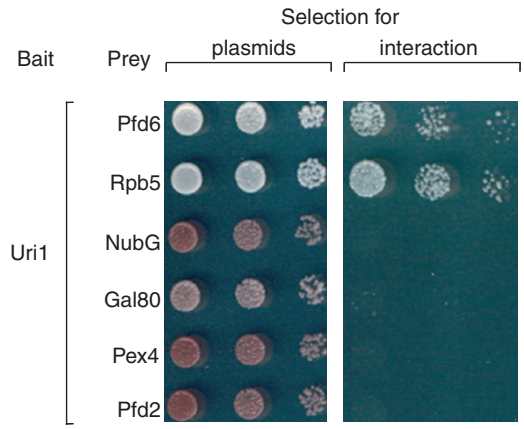

Figure 2 | Pairwise interactions in the DUALhunter system. Yeast coexpressing the indicated baits and preys was plated either on medium selecting for the presence of both bait and prey (left) or on medium selecting for a protein interaction (right). NubG, empty vector expressing only NubG. Gal80p, Pex4p and Pfd2p are noncognate proteins used as negative controls.

Table 1 | Uri1p interactors identified by DUALhunter screening

Protein class	Identity	Function	Confirmed
Translation	TEF1/2	Translation elongation factor 1α	Yes
	TEF4	Translation elongation factor 1γ	Yes
	TIF51A	Translation initiation factor 5A	No
	TIF11	Translation initiation factor 1A	ND
	RPS4A	Ribosomal protein of the 40S subunit	ND
Protein folding	SSB1	Hsp70 family	Yes
	SIS1	Hsp40 family, dnaJ homolog	Yes
	CPR1	Cyclophilin, prolyl-isomerase	No
	FPR1	Rapamycin binding, prolyl-isomerase	Yes
	EGD2	NAC subunit	ND
Other	3-PGK	3-Phosphoglycerate kinase	ND
	DEF1	RNA polymerase II degradation factor	ND
	VMA4	Vacuolar ATPase	ND
	PEP1	Vacuolar sorting protein	ND
	ERG1	Squalene monooxygenase	ND

Confirmed, confirmation of screening results by coimmunoprecipitation. NAC, nascent polypeptide–associated complex; ND, not done.

experiments had shown that Uri1p cannot be used in a classical yeast two-hybrid assay owing to the presence of a long acidic region which autonomously activates transcription. Using a classical two-hybrid system, the only choice would be to divide the protein into subdomains or fragments and to screen those in isolation. However, this might result in a loss of potential interactors.

In contrast, the DUALhunter system allowed us to assay full-length Uri1p. We confirmed pairwise interactions between Uri1p and two of its known interaction partners, Pfd6p and Rbp5p (**Fig. 2**). We transformed yeast with bait and prey plasmids expressing either Uri1p and Pfd6p or Uri1p and Rbp5p, and assayed the interactions by growth on selective minimal medium. To demonstrate the specificity of the interaction, we used several unrelated control preys.

cDNA library screen to identify new interactors

We then screened the Uri1p bait against a *Saccharomyces cerevisiae* cDNA library (available from Dualsystems Biotech) to identify new interaction partners. We screened seven million independent clones and identified 63 putative interactors. After analyzing these clones in detail, we subgrouped the clones encoding the same protein. In total, we identified 21 different putative interactors of Uri1p in this screen. Of those, 15 clones interacted reproducibly with Uri1p in a bait-dependency test and did not interact with several unrelated control baits (**Table 1**). Several interactors were involved in protein translation, supporting a previously suggested role for Uri1p in this process. Coimmunoprecipitation assays confirmed the interactions between Uri1p and several of the newly identified proteins (data not shown).

We have also used several other transcriptionally active proteins in the DUALhunter assay, including p53, binding partners of p53 and several members of the NF-κB complex (data not shown). The DUALhunter system is very flexible and can be used to quickly identify interaction partners of proteins that are unsuitable for use in classical yeast two-hybrid systems.

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