Cdc42 and the Ste20-like kinase Don3 act independently in triggering cytokinesis in *Ustilago maydis*

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Summary

In the dimorphic fungus *Ustilago maydis* the Rho-family GTP-binding protein Cdc42 and the Ste20-like kinase Don3 are both essential for triggering cell separation during cytokinesis. Since Don3 does not contain a Cdc42/Rac interaction and binding domain (CRIB), it is unclear how Cdc42 and Don3 cooperate in the regulation of cytokinesis. To analyse the regulatory network we generated an analogue-sensitive Don3 variant (Don3-as) that allows specific inhibition in vivo. The engineered kinase Don3M157A is fully active in vivo and can be specifically inhibited by low concentrations of the ATP-analogue NA-PP1. Inhibition of the Don3-as kinase activity immediately blocked cell separation resulting in the formation of clusters of non-separated cells. Covalent labelling of cell wall proteins showed that, upon release of inhibition, cytokinesis was resumed instantaneously in all cells. By sequentially activating Don3 and Cdc42 we were able to demonstrate that both proteins act independently of each other and that Don3 activity precedes that of Cdc42. We provide evidence that Don3 and Cdc42 are crucial for the assembly of a contractile actomyosin ring, which is a prerequisite for secondary septum formation. We propose, that Don3 is involved in establishing a landmark, at which the Cdc42-dependent actomyosin ring formation will occur.

Introduction

Cytokinesis in yeast-like growing fungi involves septin-ring assembly, septum formation and cell separation. In the ascomycete yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* complex regulatory signalling modules trigger cytokinesis. The mitotic exit network (MEN) and the septation initiation network (SIN), respectively, coordinate the onset of septation and cytokinesis with the exit from the mitotic cell cycle (Krapp et al., 2004; Simanis, 2003; Walther and Wendland, 2003). In the dimorphic basidiomycete *Ustilago maydis*, the GTP-binding protein Cdc42 together with its activator Don1 and the Ste20-like kinase Don3 are essential for the formation of a secondary septum, which is required for proper cytokinesis (Mahlert et al., 2006; Weinzierl et al., 2002). Deletion of either of *don1*, *cdc42* or *don3* interferes with cell separation resulting in the formation of large clusters of cells that are connected by their primary septum (Sandrock et al., 2006; Weinzierl et al., 2002).

Don3 belongs to the germinal centre kinase (GCK) subfamily of Ste20-like kinases. Although many GCKs have been described in a variety of organisms so far, only little is known about their functions in cellular signalling (Dan et al., 2001). Don3 is related to Sid1p kinase, an essential component of the *S. pombe* SIN pathway (Sandrock et al., 2006). Interestingly, in *U. maydis* the other components of the SIN pathway are not involved in cytokinesis but were shown to regulate nuclear envelope breakdown (NEBD) during mitosis (Sandrock et al., 2006; Straube et al., 2005). Up to now only Don3 plays a dual role and is required to trigger both NEBD and cell separation (Sandrock et al., 2006). Whereas the function of Don3 in the regulation of NEBD appears to be homologous to that of the SIN-pathway kinase Sid1p, the question how Don3 triggers cell separation and how it interacts with the Cdc42 signalling cascade has not been resolved.

To elucidate the function of Don3 during cytokinesis and to investigate the crosstalk with Cdc42 we used chemical genetics. This approach was developed by the Shokat group (Shah et al., 1997) and enables functional characterization of protein kinases in vivo. It has been used for many protein kinases in the yeasts *S. cerevisiae* and *S. pombe* as well as in mammalian cells (Bishop et al., 2001; Burkard et al., 2007; Knight and Shokat, 2007). Chemical-genetic analysis of serine/threonine kinases involves specific inhibition of engineered kinases, whose ATP-binding pockets were modified to accommodate bulky kinase-inhibitor analogues, such as NA-PP1, that were specifically designed for such modified kinases (Bishop et al., 1998).

We created an analogue-sensitive Don3 kinase (Don3-as), which was fully active in vivo and could specifically inhibited by low concentrations of NA-PP1. We used this modified kinase to demonstrate that Don3 and Cdc42 act independently from each other during cytokinesis and that Don3 activity precedes that of Cdc42. In addition, we demonstrate that Don3 and Cdc42 are required for assembly of a contractile actomyosin ring as monitored by Cdc15-GFP accumulation. We propose that the GC-kinase Don3 phosphorylates a yet-unknown landmark protein, whose
phosphorylation is prerequisite for Cdc42 triggered actomyosin-ring formation.

**Results and Discussion**

To study the molecular function of the Ste20-like kinase Don3 during cytokinesis, we used chemical genetics (Shah et al., 1997). On the basis of sequence alignments of Don3 with other kinases, we identified the amino acid methionine at position 157 (M157) in the catalytic domain as the putative ‘gatekeeper’ of the ATP-binding pocket (Fig. 1A) (Knight and Shokat, 2007; Zhang et al., 2005). We substituted this amino acid for either glycine (M157G) or alanine (M157A) by site-directed mutagenesis. The resulting kinases Don3M157G and Don3M157A, respectively, were tested for complementation of the cell separation defect of U. maydis don3 mutant strains. To this end, the corresponding open reading frames were expressed in Don3 mutants under control of the arabinose-crg-promoter (Fig. 1B, Pcrg) (Bottin et al., 1996). Upon induction, only wild type Don3 and the modified kinase Don3M157A were able to trigger cell separation in these cells (Fig. 1C). Thus, substitution of methionine with the small amino acid glycine obviously interfered with kinase activity because Don3M157G was unable to complement the Don3− phenotype (Fig. 1C). Similar results have been obtained also for other kinases, such as the yeast Ste20-like kinase Cla4p, for which only the exchange of M with A was compatible with kinase activity (Weiss et al., 2000).

To test whether the analogue-sensitive kinase Don3M157A is active at normal protein levels we introduced the corresponding mutation into the endogenous don3 locus to generate the analogue-sensitive don3-as allele. Cells expressing don3-as from its own promoter grew as wild-type cells, indicating that the modified kinase is fully active (Fig. 2A, left). Next we analysed whether don3-as cells are sensitive to PP1-derivatives. To this end don3-as cells were incubated with increasing amounts of NA-PP1. Already at 1 μM NA-PP1 cell separation was blocked completely and budding cells started to form cell clusters (Fig. 2A). By comparison with other organisms, in which modified kinases have been analysed in vivo, this minimal inhibitory concentration of 1 μM is remarkably low (Ventura et al., 2006; Weiss et al., 2000). The phenotype of don3-as cells inhibited by NA-PP1 was indistinguishable from that of don3 mutant cells (Fig. 1C, left panel). The inhibitor had no effect on either the growth rate, morphology or cytokinesis in wild type cells even if NA-PP1 was added in higher concentration (10 μM) (data not shown). Addition of NA-PP1 resulted in long-lasting inhibition, because cell separation was blocked even after overnight incubation (data not shown). Cells treated with NA-PP1 overnight were still viable (see supplementary material Fig. S1), indicating that the inhibitor is not toxic for cells expressing don3-as and NA-PP1 is quite stable at least under these medium conditions. Together, these data demonstrate that strains expressing the analogue-sensitive kinase Don3M157A can be used to mimic the phenotype of Don3 loss-of-function mutants.

In fungi, septum initiation is tightly controlled and occurs in a spatially and temporally well-defined manner (Bähler, 2005; Dobbelrae and Barral, 2004), suggesting that Don3 activity is also coupled to cell cycle progress. During the budding process Don3 triggers the formation of the secondary septum in newly formed daughter cells immediately after completion of the primary septum. This raises the question whether don3-as cells, in which secondary septum formation was blocked by NA-PP1, are able to resume cytokinesis if inhibition is released. To follow the fate of individual cells during subsequent generations, we labelled cells by covalent biotinylation of cell wall proteins using the membrane-permeant cross-linking reagent NHS-LC-biotin. Biotinylated cells could be detected even after several rounds of mitosis by staining with fluorescent TRITC-conjugated avidine (Fig. 2B). To find out whether don3-as cells can resume cytokinesis upon release of NA-PP1 inhibition, we labelled single budding cells with biotin. Then, Don3-as kinase activity was blocked for 5 hours by addition

![Fig. 1.](image-url) The analogue-sensitive Don3M157A kinase is active in vivo. (A) Alignment of the ATP-binding pocket of the U. maydis (Um) Don3 kinase domain with corresponding sequences of other yeast (Sc) and human (Hs) kinases, which have been used to create analogue-sensitive kinases. The gatekeeper amino acids (positions 140 and 157) are bold and the position of the amino acid exchange is indicated. (B) Pcrg::don3 and Pcrg::don3M157G/A expression cassettes. The kinase domain (KD) is indicated, and the site of the amino acid exchange is marked by an asterisk. (C) don3 cells expressing Don3, Don3M157G and Don3M157A under control of the inducible Pcrg-promoter. DIC-images illustrate the repressed state (glucose) and the induced state (arabinose). Scale bars, 10 μm.
of NA-PP1. This resulted in the formation of cell clusters consisting of 4-8 cells, in which only the mother cell was stained (Fig. 2B). The labelling pattern showed that the biotinylated cell wall proteins are restricted to the mother cells and do not spread to daughter cells. After 5 hours inhibition of Don3-as kinase activity was released by transferring don3-as cells to inhibitor-free medium. Within 30 minutes after release, most cell clusters disintegrated into single cells indicating the abundance of single budding cells (1-2 cells) and cell clusters consisting of three or more cells in percent (n=200) (>3 cells).

Fig. 2. Inhibition of don3-as cells with NA-PP1 results in a reversible cytokinesis arrest. (A) In the presence of 1 µM NA-PP1 don3-as cells form large cell clusters. Scale bar, 10 µm. (B) don3-as cells were labelled with NHS-LS-biotin and the activity of Don3-as was inhibited with 1 µM NA-PP1. After 5 hours cells were washed and further incubated without inhibitor. Fluorescence and DIC images were taken at the time-points indicated. Scale bars, 10 µm. Diagrams indicate the abundance of single budding cells (1-2 cells) and cell clusters consisting of three or more cells in percent (n=200) (>3 cells).

separation defect indistinguishable from that of cdc42 mutant strains (Mahlert et al., 2006). To study this potential functional interaction between Don3 and Cdc42, we introduced the analogue-sensitive Don3-as kinase into the conditional Pcerg::cdc42 mutant strain, in which the promoter of the cdc42 gene had been replaced by the arabinose-inducible Pcerg-promoter (Mahlert et al., 2006), resulting in strain CB46. In this strain, both the kinase activity of Don3-as and the expression levels of Cdc42 can be regulated independently from each other. CB46 cells were grown in glucose-containing medium and displayed a cell separation defect due to the absence of Cdc42 (Fig. 3A) (Mahlert et al., 2006). At time point 0 we labelled the cell clusters with NHS-LC-biotin. Immediately afterwards the kinase activity of Don3-as was blocked by addition of NA-PP1 (Fig. 3A).

Expression of Cdc42 was induced 90 minutes after inhibition of Don3-as by transferring cells into medium containing NA-PP1 and arabinose. This sequential inactivation of Don3 and activation of Cdc42 was performed to ensure that a functional interaction between these two proteins was impossible. Upon increasing expression of Cdc42, the cell clusters gradually disintegrated into single labelled cells. In these cells cytokinesis was obviously completed by Cdc42, although Don3 kinase activity was inhibited by NA-PP1. Intriguingly, the released cells again showed a cytokinesis defect and formed new clusters, in which only the ‘founder mother cells’ were labelled (Fig. 3A, 10 hours). This indicates that, after inhibition of Don3 activity, Cdc42 can trigger only a single round of cytokinesis. Interestingly, if this experiment was performed in reversed order, the release of Don3-as from NA-PP1 inhibition was not sufficient to restore cytokinesis in the absence of Cdc42 (data not shown).

Therefore, we assume that Don3 phosphorylates a yet-unknown target that may act as landmark protein. Such a putative landmark protein could then serve as a platform for Cdc42-triggered initiation of the secondary septum. Although we observe accumulation of fluorescently labelled Don1 protein at the site of septum formation (data not shown), it can be excluded that Don1 itself serves as landmark protein because the cell separation defect of don1 deletion mutants can be fully rescued by expressing a constitutively active variant of Cdc42 (Mahlert et al., 2006). This indicates that the presence of Don1 is not required for correct septation.

To find out whether Don3 kinase activity is required for targeting Cdc42 to the site of septum initiation we expressed a GFP-Cdc42 fusion protein in Δdon3 cells. In dividing wild-type cells GFP-Cdc42 shows membrane association and accumulates at the vacuolar membrane, and at both the primary and the secondary septum (Fig. 3B, upper panel). In Δdon3 and Δdon1 cells vacuolar membrane targeting was not affected, but GFP-Cdc42 is detectable only at the primary septum (Fig. 3B and magnification). Since don3 mutants cannot be rescued by expressing constitutive active Cdc42, we assume that recruitment or activation of potential Cdc42 targets, e.g. the septins to the site of septation, depends on the activity of Don3. This is in accordance with our observation that localisation of GFP-Don3 at the site of septum formation is independent of both Cdc42 and Don1 (Fig. 3B, lower panel).
In fungi, septation requires the assembly of a contractile actomyosin ring. To follow the dynamics of this ring formation we expressed a Cdc15-GFP fusion protein in don3-as cells. The *U. maydis* Cdc15 protein (MUMDB entry number um00168) is highly similar to the *S. pombe* septation protein Cdc15, which coordinates the assembly of the cytokinetic contractile ring in fission yeast (Aspenström et al., 2006). During division of *U. maydis* wild-type cells, Cdc15-GFP is visible in a ring-like structure at the neck of the mother-bud (Fig. 4A,K and 4B,L) and disappeared after completion of the first septum (Fig. 4C,H,M). Intriguingly, shortly afterwards Cdc15-GFP reappeared as a ring-like structure at the daughter side of the primary septum (Fig. 4D,I,N) and vanished again after completion of the secondary septum (Fig. 4E,J,O). Thus, cytokinesis in *U. maydis* involves the sequential formation of two independent cytokinetic contractile rings. In don3-as cells treated with NA-PP1, Cdc15-GFP was detectable only in emerging bud cells during assembly of the first septum (Fig. 4P; arrows). Upon inhibitor release, instantaneous accumulation of Cdc15-GFP-labelled actomyosin rings could be detected within 10 minutes in all cells of the cluster (Fig. 4Q,T; magnifications). This indicates that Don3 triggers the initiation of a cytokinetic actomyosin ring only during secondary septum formation but not during primary septum formation. Therefore, we propose that phosphorylation by Don3 renders a pre-assembled landmark protein active and, thus, provides the positional information at which formation of the contractile actomyosin ring is triggered by activated Cdc42.

To test this hypothesis, we studied the assembly of the Cdc15 ring depending on the presence or absence of Cdc42. To this aim we followed Cdc15-GFP localization in conditional *cdc42* mutant cells. Cells in which expression of *cdc42* is repressed by glucose, Cdc15-GFP was detected only during the assembly of the primary septum (not shown, but similar to results shown in Fig. 4P). Upon induction of *cdc42*, accumulation of Cdc15-GFP at the site of secondary septum formation was visible within 80 minutes (Fig. 4R,U; magnifications). That actomyosin ring formation is significantly delayed compared with inhibitor release, is probably owing to the fact that Cdc42 has first to be transcribed and translated. Together, these data indicate that both active Don3 kinase and Cdc42 are required to trigger Cdc15 assembly.

The don3-as strain has allowed us to dissect the dynamics of primary and secondary septation using chemical genetics and has led us to the hypothesis that a putative landmark protein is the target of Don3 kinase activity. This putative landmark protein would then serve as a starting point for secondary septum formation. A good candidate for this landmark would be the septin collar that assembles at the neck of the mother-bud during cytokinesis in *U. maydis* (Boyce et al., 2005). Septin proteins would also provide a link to the small GTP-binding protein Cdc42, which in *S. pombe* and *S. cerevisiae* is involved in septin ring assembly and actomyosin ring formation (Iwase et al., 2006; Narumiya and Yasuda, 2006; Versele and Thorner, 2004). In *S. cerevisiae*, septin assembly depends on direct phosphorylation by the Cdc42 effector protein kinase Cla4p (Versele and Thorner, 2004). Therefore, because our previous studies imply that Cla4p in septation formation, it is tempting to speculate that, in *U. maydis*, Cla4-triggered septin phosphorylation is responsible only for primary septum formation, whereas phosphorylation of septins by Don3 is crucial to trigger secondary septum formation (Leveleki et al., 2004).

**Fig. 3.** Don3 and Cdc42 act independently to trigger secondary septum initiation. (A) Strain CB46 (don3-as; *Perg::cdc42*) was grown in glucose-containing medium, in which the *Perg* promoter is inactive (*Cdc42, no activity*). The cell clusters were washed and labelled with biotin. At the same time (0 h) Don3 activity was blocked by adding 1 μM NA-PP1 to the medium (*Don3, no activity*). Cdc42 expression was induced after 90 minutes by switching the carbon source of the NA-PP1-containing medium from glucose to arabinose (*Cdc42, increasing activity*). The cell clusters were monitored at the given time-points using fluorescence and DIC microscopy. Scale bars, 10 μm. (B) GFP-Cdc42 (upper panel) and Don3 (lower panel) expressed from the constitutive *Etef*-promoter in wild type cells (left panel), in the respective complementation deletion strain (central panel) and in Δdon1 cells (right panel). Boxed areas are magnified, and arrowheads mark the localisation of GFP-Cdc42 at both septa in the wild-type or the primary septum in the mutant strains. Scale bars, 10 μm.
**Materials and Methods**

**Strains**
The *Escherichia coli* strain DH5α was used for cloning and amplification of plasmid DNA. The *U. maydis* strain Bub8 (Schulz et al., 1990) was used as wild-type background for all strains created in this manuscript. Bub8Δ*don3* has been described previously (Weinzierl et al., 2002).

**Transformation**
In general, transformation of *U. maydis* was performed as described previously (Schulz et al., 1990). For expression studies, constructs expressing Don3, Don3M157A and Don3M157G under control of the crg-promoter were integrated into the *cbx*-locus by homologous recombination (Loubradou et al., 2001).

**Mutagenesis**
The analogue-sensitive mutants Don3M157A and Don3M157G were generated by PCR using a two-step mutagenesis protocol. The ATG codon (Met) at nucleotide position 469-471 was replaced with GCT (Ala) or GGG (Gly) in Don3M157G under control of the *cbx*-promoter generated by homologous recombination (Kämper, 2004). Primer sequences will be supplied by the corresponding author (B.S.) on request.

**Generation of strains**
A bipartite resistance cassette (Sandrock et al., 2006) was used to generate *U. maydis* strains expressing Don3M157A under control of the native promoter. To this end, the entire ORF containing the corresponding M157A-mutations was amplified by PCR using primers that introduce a characteristic SfiI-site and used as one of the flanking regions for homologous recombination. Correct integration was tested by PCR and sequencing. For Cdc15-GFP expression from the endogenous *CDC42* promoter a linear fragment composed of the *cdc15* ORF (um00168), the GFP-NAT resistance cassette and the *cdc15* flanking region was used for homologous recombination in the *don3-as* strain following the protocol described previously (Brachmann et al., 2004). The strain CB46 was derived from the *don3-as* strain and contains additionally the ORF of the *crg*-promoter under the control of the *cbx*-promoter generated by homologous recombination using the PCR protocol of published previously (Kämper, 2004). Primer sequences will be supplied by the corresponding author (B.S.) on request.

**Specific inhibition of Don3 kinase activity**
The activity of the engineered kinase Don3M157A was inhibited by addition of the indicated amounts of NA-PP1 (Calbiochem). Inhibition of Don3M157A in logarithmically growing cells blocked cell separation immediately.

**Biotin labelling of cell surfaces**
For cell-surface biotinylation, 5×10⁶ cells from a logarithmically growing culture were collected and washed twice in six volumes of KP buffer (50 mM K₂PO₄, pH
8.0). Cells were then incubated for 10 minutes in 1 ml KP plus 1.0 mg/ml NHC-LS-biotin at RT. Cells were washed twice in six volumes of TM (50 mM Tris-HCl, pH 7.5 plus 50 mM MgCl₂) and once in six volumes KP buffer. Cells were either analysed by ExtrAvidin®-TRITC staining or further grown in fresh medium for the indicated time points.

ExtrAvidin®-TRITC staining
5×10⁶ cells were washed twice in six volumes H₂O, resuspended and incubated with 1 μl ExtrAvidin-TRITC (Sigma E3011) for 10 minutes. Cells were then washed twice in 1 ml H₂O and resuspended in fresh medium.

Microscopy
Ustilago maydis cells from logarithmically growing cultures were analysed. Cells were visualised by differential interference contrast (DIC) and epifluorescence microscopy using a Zeiss Axioshot microscope. Calcofluor White staining was performed as described previously (Pringle et al., 1989). TRITC fluorescence of biotin-labelled cells were analysed using a Zeiss Axioshot microscope. Image processing was carried out using Photoshop (Adobe).

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References
Aspenström, P., Fransson, A. and Richnau, N. (2006). Pomphe Cdc15 homology proteins: regulators of membrane dynamics and the actin cytoskeleton. Trends Biochem. Sci. 31, 670-679.

Bähler, J. (2005). A transcriptional pathway for cell separation in fission yeast. Cell Cycle 4, 39-41.

Bishop, A. C., Shah, K., Liu, Y., Wittucki, L., Kung, C. and Shokat, K. M. (1998). Design of allele-specific inhibitors to probe protein kinase signaling. Curr. Biol. 8, 257-266.

Boye, K. J., Chang, H., D’Souza, C. A. and Kronstad, J. W. (2006). Chemical genetic analysis of the time course of signal transduction by JNK. Mol. Microbiol. 60, 719-730.

Mahlert, M., Leveleki, L., Hlubek, A., Sandrock, B. and Bölk er, M. (2006). Rac1 and Cdc42 regulate epithelial growth and cytokinesis in the dimorphic fungus Ustilago maydis. Mol. Microbiol. 59, 567-578.

Narumiya, S. and Yasuda, S. (2006). Rho GTPases in animal cell mitosis. Curr. Opin. Cell Biol. 18, 199-205.

Pringle, J. R., Preston, R. A., Adams, A. E. M., Stearns, T. and Drubin, D. G. (1989). Fluorescence microscopy methods for yeast. Methods Cell Biol. 31, 357-435.

Sandrock, B., Böhmer, C. and Bölk er, M. (2006). Dual function of the germinal centre kinase Don3 during mitosis and cytokinesis in Ustilago maydis. Mol. Microbiol. 62, 655-666.

Schulz, B., Bannett, D., Dahl, M., Schlesinger, R., Schäfer, W., Martin, T., Herskovitz, I. and Kahmann, R. (1990). The b alleles of U. maydis, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell 60, 295-306.

Shah, K., Liu, Y., Deimengian, C. and Shokat, K. M. (1997). Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proc. Natl. Acad. Sci. USA 94, 3565-3570.

Simanis, V. (2003). The mitotic exit and septation initiation networks. J. Cell Sci. 116, 4261-4262.

Siraube, A., Weber, I. and Steinberg, G. (2005). A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. EMBO J. 24, 1674-1685.

Ventura, J. J., Hubner, A., Zhang, C., Flavell, R. A., Shokat, K. M. and Davis, R. J. (2006). Chemical genetic analysis of the time course of signal transduction by JNK. Mol. Cell 21, 701-710.

Versele, M. and Thorner, J. (2004). Septin collar formation in budding yeast requires GTP binding and direct phosphorylation by the PAK, Cla4. J. Cell Biol. 164, 701-715.

Wachtler, V., Huang, Y., Karagiannis, J. and Balasubramanian, M. K. (2006). Cell cycle-dependent roles for the FCH-domain protein Cdc15p in formation of the actomyosin ring in Schizosaccharomyces pombe. Mol. Biol. Cell 17, 3254-3266.

Walther, A. and Wendland, J. (2003). Septation and cytokinesis in fungi. Fungal Genet. Biol. 40, 187-196.

Weinzierl, G., Leveleki, L., Hassel, A., Kost, G., Wanner, G. and Bölk er, M. (2002). Regulation of cell separation in the dimorphic fungus Ustilago maydis. Mol. Microbiol. 45, 239-231.

Weiss, E. L., Bishop, A. C., Shokat, K. M. and Drubin, D. G. (2000). Chemical genetic analysis of the budding-yeast p21-activated kinase Cla4p. Nat. Cell Biol. 2, 677-685.

Zhang, C., Keskis, D. M., Paulson, J. L., Bonshtien, A., Sessa, G. and Cross, J. V., Templeton, D. J. and Shokat, K. M. (2005). A second-site suppressor strategy for chemical genetic analysis of diverse protein kinases. Nat. Methods 2, 435-441.