Preventing protein secretion with chemical glue

Brent R Stockwell

A small-molecule inhibitor of protein secretion has been found that targets Cdc42, a regulator of the secretory pathway. This compound acts through a previously undescribed mechanism—by recruiting a protein inhibitor.

One approach to studying biological systems involves using small organic molecules to perturb cellular processes[1]. In one version of this approach, thousands, or even millions, of chemicals are tested for their ability to affect a specific cellular process; subsequently the target proteins and mechanisms of action are determined for active compounds derived from such a screen. Pelish *et al.* have used this approach to find chemical inhibitors of the process by which proteins are sent to the surface of cells[2]. One such inhibitor, which they named secramine, was found to prevent protein trafficking by inhibiting Cdc42, a known protein regulator of this process, through an unexpected mechanism.

Proteins destined to reside on the surface of cells are sent through a specialized secretory pathway involving the endoplasmic reticulum and the Golgi apparatus. Secramine was discovered in a screen for compounds that prevent trafficking from the Golgi apparatus to the plasma membrane, by imaging the location of a fluorescent cell-surface protein in cells treated with various compounds. Using an *in vitro* reconstituted model system, secramine was found to target the protein Cdc42.

Cdc42 is a known protein regulator of the secretory pathway that acts by stimulating actin polymerization. To perform its traffic-regulating function, Cdc42 needs to be activated and then localized to Golgi membranes. Secramine was found to prevent localization of Cdc42 to lipid membranes and thereby prevent its activation. This effect required the presence of RhoGDI, an inhibitor of Cdc42. Secramine thus appears to stabilize the interaction between Cdc42 and its inhibitor protein RhoGDI. By bridging the interaction between Cdc42 and RhoGDI, secramine thus prevents trafficking of proteins from the Golgi to the cell surface. However, the precise mechanism may be more complex, involving, for example, extraction of Cdc42 from membranes into solution in the presence of RhoGDI. Nonetheless, this study resulted in the discovery of a powerful chemical tool for inactivating Cdc42.

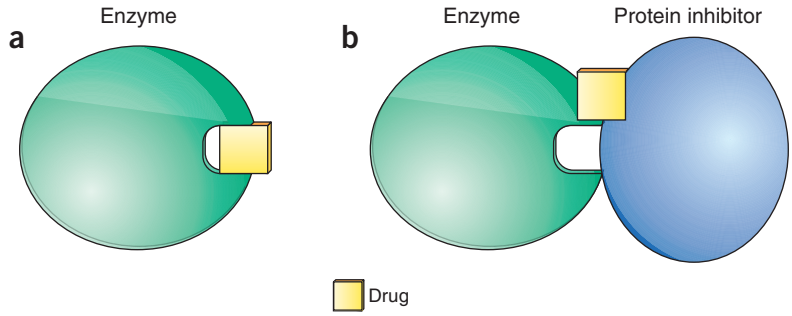

Figure 1 A newly discovered means of inhibiting Cdc42 activity and preventing trafficking of proteins from the Golgi to the cell surface. Pelish *et al.*[2] screened 2,500 natural-product-like compounds to discover secramine, which blocks activation of Cdc42. Most small-molecule inhibitors act by binding to the active site of an enzyme (left panel). Secramine's mechanism of action involves stabilizing the interaction of Cdc42 with an inhibitory protein RhoGDI (right panel). This represents a previously unknown means of inactivating small GTPases, such as Cdc42.

When researchers design drugs and other chemicals that alter the functions of proteins, they usually seek enzyme inhibitors—small molecules that bind to the active site of an enzyme and prevent it from catalyzing the reaction that it would normally accelerate (**Fig. 1**). Most known drugs act by this type of mechanism, and it is relatively straightforward to design drugs acting in such a way once the atomic resolution structure of an enzyme is known. This process has become the preferred drug discovery method of the pharmaceutical industry and is known as 'rational' or structure-based drug design[3].

However, Cdc42 and related proteins (known as GTPases) are not amenable to inhibition through such an approach. The enzymatic activity of these proteins accelerates the hydrolysis of a bound nucleotide. But the cellular function of such proteins is inactivated when they are bound to the hydrolyzed nucleotide. That is, the enzymatic activity of these proteins serves to turn *off* their cellular function. Thus, chemicals that turn off their enzymatic activity would *activate* their cellular functions. These proteins cannot be shut down with small molecules in the usual fashion[4].

The findings of Pelish *et al.* shed new light on this conundrum. These investigators found that it is indeed possible to inhibit Cdc42 with a small molecule. The unexpected mechanism appears to involve stabilizing the interaction of Cdc42 with another protein (RhoGDI) that inhibits Cdc42's cellular functions (**Fig. 1**). Thus, by stabilizing the interaction between an inhibitory protein and Cdc42, it is possible to turn off, rapidly and reversibly, Cdc42 function in cells. It may be possible to extend this strategy to related GTPases. For example, the RAS proteins are mutated in ~30% of human cancers but have been refractory to inhibition with small molecules for the reasons discussed above[5]. Perhaps a compound that

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stabilizes the interaction between a RAS inhibitory protein and RAS proteins would allow this previously intractable class of cancer-causing proteins to be inactivated. To pursue such ideas, it would be necessary to characterize the precise molecular mechanism of action for secramine, ideally using X-ray crystallography and direct-binding studies.

One might suspect that finding such interaction-stabilizing compounds would be difficult, as they should be rare. However, to find secramine, these researchers tested only 2,500 compounds. Of note is the fact that these compounds were synthetic analogs of a natural product, galanthamine[6]. It would be interesting to compare these galanthamine-related compounds to other compound collections to see if the finding of secramine-like activity is a common event or a rare event. If it is a rare event, it might suggest that natural products, which generally have complex structures, are particularly effective at stabilizing protein-protein interactions.

At the very least, this study provides a new chemical tool for rapidly and reversibly inactivating Cdc42 in cells. Secramine will likely be used in the future to reveal new roles for Cdc42 in other biological processes. In addition, Pelish *et al.* have demonstrated that systematic serendipity can teach us new ways of altering protein function. By screening for compounds that induce a desired effect in cells, we are often surprised at the mechanisms we ultimately uncover. To paraphrase William Shakespeare, there are more mechanisms *in vivo* and *in vitro*, drug designers, than are dreamt of in your philosophy.

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Chemical expansion of cofactor activity

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Methylation is a key regulatory event in several biological processes. The enzymatic transfer by methyltransferases of extended carbon chain analogs of *S*-adenosylmethionine to DNA opens up new avenues for investigating biological methylation.

Methyltransferases are enzymes that transfer a methyl group from *S*-adenosylmethionine (SAM) to a wide variety of biological molecules from proteins to DNA. Methylation of these molecules is a central biological regulatory mechanism for diverse cellular events including signal transduction and transcriptional regulation. Methyltransferases are generally believed to be limited to transferring methyl groups. In this month's issue, Weinhold, Klimasauskas and colleagues report the synthesis of SAM analogs that are used by DNA methyltransferases to transfer extended carbon chains to DNA, a process that promises to have a wide variety of applications[1].

Based on their limited composition of the twenty naturally occurring amino acids, enzymes are restricted in their chemical reactivity to primarily acid- and base-catalyzed mechanisms. To expand their reactive repertoire, enzymes use small organic molecules or metal ions called cofactors to open up the broad range of chemical reactivity necessary for metabolic processing. As expected, because nature commonly exploits useful tools in multiple ways, a single cofactor is often used by many different enzymes.

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Biological chemists have taken advantage of this fact to manipulate the reaction of a whole class of enzymes through the development of synthetic analogs. A variety of organic cofactor analogs have recently been used to expand cofactor-mediated pathways (**Fig. 1**). For instance, by expanding the ATP-binding pocket of kinases by mutagenesis, adenosine analogs (**Fig. 1a**) are used by the engineered kinases and can provide an exquisitely sensitive tool to regulate and study the activity of these enzymes within biological systems[2]. Similarly, reporter-linked analogs of coenzyme A may be used in post-translational modification of carrier proteins for site-specific reporter labeling *in vivo*, co-opting a pathway common to fatty acid, polyketide and nonribosomal peptide synthesis (**Fig. 1b**)[3]. The successful alteration of a common cofactor used by other metabolic enzymes indicates that such alteration may also be applicable to these other pathways, expanding the ability to visualize various metabolic occurrences. In the case of SAM analogs, these tools may also be applicable to studying both, for instance, transcriptional regulation and post-translational protein modification. In this regard, the results presented in the current issue provide a new twist on cofactor manipulation.

SAM is the second-most prevalent cofactor in cells after ATP. As a result, SAM participates in the majority of methyltransferase processes found in the metabolism, far surpassing folate, the other biological methyl donor, in this role. SAM-dependent methyltransferases are a broad class of enzymes containing at least five distinct structural motifs[4]. Although all of these enzymes contain a binding pocket for SAM, the structure of the binding site diverges significantly among these enzymes, and sequence homology can be quite low even within a particular class of methyltransferases. Evidently nature has evolved diverse mechanisms for promoting the same electrophilic SAM reactivity.

Perhaps the best-studied biological function of SAM is in the methylation of DNA. Several different positions along the DNA polymer are candidates for methylation; chief among them are the N6 position of adenine and the N4 and C5 positions of cytosine. All of these transformations are catalyzed by well-studied enzymes[5]. In addition to the specificity of the position of modification within the nucleotide, methylation is also specific to DNA sequences, with methyltransferases showing sequence specificity comparable to restriction nucleases. These factors allow for a broad range of specific methylation patterns that in turn have important roles in transcriptional regulation. For instance, entire regions of DNA may be blocked from transcription by methylation, or, alternatively, methylation may provide binding sites for transcription factors[6]. As methylation has such a prominent regulatory function, it is not surprising that