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Target Identification for a Promising Anti-Lupus Drug

Target identification is one of the more difficult aspects of modern chemical genetics. In this issue of *Chemistry & Biology*, Johnson et al. provide a compelling case for going through the trouble [1]. As a byproduct of their identification of the cellular target of an anti-lupus compound, they have uncovered a powerful tool for the characterization of a fascinating molecular machine, the F1F0-ATP synthase.

The field of chemical biology/pharmacology is continuously searching for new "drug-like" compounds and their respective protein targets. The fruits of these labors are transformed into pharmacological tools in the research setting and/or therapeutics in the clinical realm. Based on the order in which the screening is performed, two major routes provide drugs: (1) the protein target is *validated* as a disease modifier prior to compound screening or (2) the target is *identified* only after a suitable drug is found. By the first method, a potential target is validated, usually via genetic means (i.e., RNA interference, transgenic animals, epidemiological studies, etc), and then diverse libraries of small molecules are screened for inhibitory potential and/or binding affinity. Although this approach is highly productive and expertly integrates elements of modern genetics, it can't claim to be the classical formula. The second route to drug discovery emphasizes uncovering small molecules based on their function- or disease-modifying activity. The moniker "forward chemical genetics" has been used in recent manifestations of this approach [2–6], but because the identity of the target is (initially) of little consequence, it could also be called the "shoot first, ask questions later" method. As a well-known example, the analgesic effects of aspirin were appreciated long before the physiological target was known. Importantly, however, the eventual identification of the target can lead to exciting new discoveries in cell biology, signaling, and pathology.

Because the path to discovery is open-ended, the "shoot first" approach doesn't require preexisting knowledge of the proteins involved in a process. In fact, previously under-appreciated functions of a protein target are often found. The high profile cancer target FRAP/mTor provides one illustrative example. The path to identification of FRAP/mTor begins with the natural product rapamycin, which was found to be a potent cell-cycle inhibitor in many cell types. The initial target of rapamycin in these cells was shown to be the cytosolic FK506 binding protein 12 (FKBP12) [7]. Binding of rapamycin to FKBP12, however, doesn't fully explain the phenotypic outcomes of drug treatment. In two landmark papers, the Schrieber and Snyder laboratories identified FRAP/mTor (and related yeast complexes) as the target that mediated rapamycin's interruption of G1 progression [8, 9]. An explosion of research aimed at treating cancer with FRAP/mTor inhibitors followed the identification of the drug's target. During the past year, a manuscript containing the keywords "rapamycin" and "cancer" appeared in citation databases every other day, on average. In this case, and in others, the drug came first, target identification followed, and important new understanding and excitement came along for the ride.

As methods to generate large, diverse libraries of small molecules have become more sophisticated and accessible, researchers have pioneered simultaneous improvements in target identification methods. Recent reviews (for examples, see references [10–14]) highlight some advances. In brief, one straightforward method to identify the target of a drug is to immobilize the small molecule on solid support and purify binding partners from cell lysates. Other methods include performing three-hybrid screens to find binding partners [15] and comparing the phenotype of drug-treated cells with that of cells treated with control compounds or RNA interference libraries. Regardless of the method, identifying the target of a drug can be complicated by the presence of multiple potential binding partners in complex cellular environments. Genetic methods (null alleles, conditional alleles, and/or RNA interference) are usually required to provide definitive characterization of drug specificity.

In this issue of *Chemistry & Biology*, the Glick and Opipari laboratories provide evidence that implicates the F1F0-ATPase as the primary target of their immunomodulatory drug Bz-423 [1]. This manuscript marks their latest efforts to isolate compounds that can be used to study and, eventually, treat systemic lupus erythematosus. In the context of this commentary, it is useful to review what these labs have already contributed to the characterization of this drug's activity; the story exemplifies what can be learned via the "shoot first" approach followed by careful target identification. Of particular interest are the insights into molecular motor function and immunobiology that are revealed as a byproduct of attempts to characterize the interaction of Bz-423 with the F1F0-ATPase.

Bz-423 was uncovered as part of an effort to screen a benzodiazepine library for modulators of autoimmunity. Bz-423 fit the initial functional criteria and was found to selectively induce apoptosis in autoreactive B and T lymphocytes by increasing intracellular reactive oxygen species (ROS) [16]. Subsequently, administration of Bz-423 was found to be effective in lupus mouse models. The target of Bz-423 was unknown but thought to be a mitochondrial protein based on subcellular fractionation results [16].

In this issue, Johnson et al report their use of phage display (and other methods) to implicate the oligomycin sensitivity conferring protein (OSCP) as the cellular target of Bz-423 [1]. OSCP is a subunit of the F1F0-ATPase and part of a regulatory hinge linking the ATP hydrolysis/synthesis F1 region with the F0 proton pump (for reviews on the mechanism and structure of the

F1F0-ATPase see: [17, 18]). The stalk region does not simply hold the two domains together but also transfers mechanical force generated by proton transport into chemical energy to drive ATP synthesis. Treatment of cells with the OSCP ligand, oligomycin, causes a severe drop in intracellular ATP levels and necrosis. Unlike cells treated with oligomycin, Bz-423-treated cells have stable ATP levels. The compound does, however, cause apoptosis via a dramatic rise in ROS. Thus, Bz-423 may be selectively toxic for activated lymphocytes because of the greater numbers of mitochondria and higher expression of F1F0-ATPase/OSCP in these cells. As Johnson et al. discuss, one mechanistic scenario is that endogenous control over reactive oxygen elimination is disrupted in those cells that contain elevated target levels and, thus, the potential for greater ROS production. This result adds to a growing body of evidence implicating energy homeostasis and mitochondrial function as good targets for immune therapeutics and specifically points to OSCP as a potential modifier.

The binding site of Bz-423 and the mechanism by which it inhibits ATPase function remind us of another strength of the "shoot first" approach. Little is known about the structure or function of OSCP. Thus, pharmacological modulators of activity, such as Bz-423, may yield important new insights into the regulation of ATP synthesis. It is particularly interesting that two OSCP binding compounds (oligomycin and Bz-423) induce distinct responses. This demonstrates that individual cycles of the ATPase motor can be trapped by small molecules that bind OSCP. Thus, not only is Bz-423 a potential therapeutic for lupus, but identification of its target has also provided hints at the importance of energy homeostasis for autoimmunity and a pharmacological tool for interrogating a fascinating molecular machine. Increased interplay between chemical-screening centers and basic biological research should only continue to provide such promising results.

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Selected Reading

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