Meeting report

Chemical approaches to understanding biological mechanisms

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A report on work with small-molecule inhibitors of cellular processes, presented at the 39th annual meeting of the American Society for Cell Biology, Washington DC, December 11–15, 1999.

How can we identify and study factors involved in complicated biological phenomena? A few research groups have returned to a tried and true method – that of identifying specific small-molecule inhibitors and using these to study biological functions and identifying important factors in these processes. The talks in the session entitled 'Drug targets and chemical approaches to biological mechanisms' at the 39th annual meeting of the American Society for Cell Biology focused on the development of novel small molecule inhibitors of cellular processes. Most discussed the use of libraries of small molecules to identify potential lead compounds; a variety of small-molecule libraries are now available and more are being made all the time.

Thomas Mayer (Harvard Medical School) identified three novel mitotic inhibitors by screening for inhibitors that increased the level of a mitotic phosphorylation event in cultured cells (indicative of a mitotic arrest) but that did not affect microtubule dynamics. One of the three causes the formation of monopolar spindles and has thus been dubbed monastrol. The target of monastrol appears to be Eg5, a tetrameric kinesin-like microtubule motor required for the maintenance and formation of the mitotic spindle. Two other inhibitors were also described, one that caused the formation of tripolar spindles and one that causes the formation of poorly organized spindles. The specificity of these effects suggests that they are interacting with single components, so the reagents should be powerful tools for further understanding the formation of the mitotic spindle. A second presentation (Sarah Vignall, University of California Berkeley) discussed the use of a library of analogues of a known inhibitor of cyclin-dependent kinases to screen for regulators of microtubule dynamics. To date, 34 compounds have been identified that affect bipolar spindle formation *in vitro* and cause microtubules to shorten in both interphase and mitotic cells. One compound that has been targeted for further analysis does not depolymerize microtubules assembled *in vitro* from pure tubulin. As of the date of the meeting, the target(s) of this compound had not yet been identified.

Chemical inhibitors have classically been used to dissect complex pathways. Kevan Shokat (University of California San Francisco) has used a new variant of this approach to assess the functions of individual kinases and to identify substrates of protein kinases *in vivo*. The strategy involves making a mutation in the back of the enzyme's ATP-binding site that does not affect the activity of the enzyme – a 'silent' mutation – but does open a 'space' that can be filled by an ATP analogue bearing an accessory group. The mutant enzyme is now uniquely inhibited by the ATP analog, while all other unmutated (wild-type) kinases are unaffected. The effect of inhibiting individual kinases either in living cells or transgenic animals can then be assayed. This is useful, not only to study the functions of individual protein kinases, but also to provide a powerful approach that helps validate potential chemotherapeutic targets. A transgenic animal carrying a kinase with a silent active-site mutation can be treated with an inhibitor and the effects of removing kinase function can be assessed *in vivo*.

But how silent is a 'silent' mutation? Shokat presented evidence that these mutations have little effect, at least in *Saccharomyces cerevisiae*. By making the appropriate mutation in *cdc28*, the gene that codes for the cyclin-dependent kinase involved in cell cycle control, the status of cells bearing the mutant kinase could be compared with that of wild-type cells. The method Shokat used to compare cellular status was analysis of gene expression levels, by hybridization of cDNAs to DNAs presented on microarrays. In the whole *S. cerevisiae* genome, the transcription levels of only 11 genes

were changed, and no systematic changes were detected. This striking control experiment helps validate Shokat's approach for analysing protein kinase function. Furthermore, the combination of designed inhibitors and appropriately mutated targets with gene-array screening may be a useful approach for many other signaling pathways.

Finally, how should we design new inhibitor libraries? One strategy is to use a known inhibitor to design a backbone and then add a variety of functional groups at reactive sites situated around the exterior of the molecule. An alternative is to design a scaffold that allows an exterior functional group to sample as much volume as possible (Dave Austin, Yale). This, of course, depends on the choice of functional groups. Austin presented a scaffold that, when supplemented with functional groups derived from the side chains of naturally occurring amino acids, maximizes the number of rotamer positions the amino acid groups can sample. This is fundamentally different from using peptide based inhibitors, as there are significant barriers to rotation in peptides. An isopropyl group attached to the core can sample a whole hemisphere. The result is an extremely compact library of molecules that have the flexibility to find the correct conformation to bind to active sites. Austin's approach promises to lead to additional libraries of small molecules that can be used to probe important cellular processes.