Emerging molecular targets

'Ideal' targets, be they enzymes, receptors, transcription factors, structural proteins or other cellular components, usually exhibit certain common characteristics. The target must play a critical role in the molecular machinery or regulation of the therapeutic area of interest, and should be highly specific to the indication and the particular cell type or tissue. In reality, cellular constituents tend to play different roles in different cell types; a good understanding of the role the target plays in cellular physiology is therefore desirable and often provides clues to possible undesirable side effects. The human form of the target is therefore usually cloned or isolated before an extensive screening program is embarked upon.

It should be possible to adapt the corresponding assay to a high-throughput format; that is, the procedure should fulfil the following criteria:

- be simple and highly reproducible
- require small volumes of material
- require few additions and separations
- offer a simple colorimetric, fluorescent or radioactive readout

Nevertheless, bioactive compounds with unknown mechanisms of action have contributed enormously to our understanding of cellular physiology and in defining new molecular targets. The efficiency of a screening assay may be sacrificed if the target is of sufficient interest, and because screening can be performed so rapidly, it may be reasonable to proceed with a nonhuman molecular target to find an initial bioactive compound for proof-of-principle studies.

Calcineurin

The protein phosphatases have long been considered the definition of a poor molecular target for drug discovery. They have been considered to be nonselective in terms of cellular distribution and activity, and of secondary importance to the protein kinases. This situation has changed in recent years.

Calcineurin is a serine/threonine phosphoprotein phosphatase that plays an essential role in T-cell activation. The immunosuppressants, cyclosporin and FK506, when bound to their respective immunophilin-binding proteins, form ternary complexes with calcineurin and inhibit its phosphoprotein phosphatase activity. The evidence indicates that inhibition of calcineurin phosphatase activity is the mechanism by which cyclosporin and FK506 exert their immunosuppressant activity.

Griffith and coworkers have reported the X-ray structure of the calcineurin–immunophilin–FK506 complex [*Cell* (1995) 82, 507–522]. The structure confirmed that the immunophilin–immunosuppressant complex does not bind to the active site of calcineurin. Instead, it binds to a surface on calcineurin that is more than 10 Å from the active site. An allosteric mechanism appears responsible for its inhibition of calcineurin activity.

Calcineurin is an example of an enzyme that might not be considered a 'good' molecular target according to the above criteria. It can be found in all cells and tissues, and until the discovery of the role in T-cell activation, little was known about its physiological function. The distribution studies suggest that a calcineurin antagonist would possess severe side effects.

However, the immunosuppressants, cyclosporin and FK506, have proved to be immensely valuable drugs, in spite of a potential problems with renal toxicity. Perhaps the availability of the molecular structure for calcineurin will provide a rationale for a new class of immunosuppressant drugs or suggest ways the multiple isoforms of calcineurin may be exploited to reduce the kidney toxicity problems.

CDC25 *phosphatase isozymes*

The *CDC25* phosphatase isozymes are another group of phosphatase enzymes emerging as important drug discovery targets. The three *CDC25* phosphatase isozymes dephosphorylate phosphotyrosine and phosphothreonine residues and play a key regulatory role in cell cycle progression.

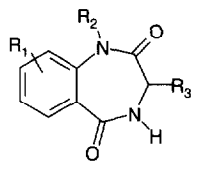
In a recent report [*Science* (1995) 269, 1575–1577], Galaktionov and coworkers provide evidence that when either of two of the *CDC25* isozymes are overexpressed in cell lines along with the oncogene Ha-*ras*, the cells are transformed and capable of producing tumors in nude mice. Transformation does not occur when the Ha-*ras* oncogene or *CDC25* alone are transfected into the cells. They also evaluated archived tumor tissues and found high levels of *CDC25* in many of the samples. The report suggest that some cancers may be caused by an overexpression of *CDC25* along with the Ha-*ras* oncogene and that *CDC25* antagonists might ultimately provide useful anticancer drugs.

Robert W. Wallace

Solid-phase synthesis in the development of small-molecule libraries

The need to identify novel lead structures rapidly has led to the preparation and screening of combinatorial libraries with primary emphasis on the solid-phase synthesis of natural and unnatural biopolymers. Recent interest has focused on the development of libraries of small nonpolymeric compounds. 1,4-Benzodiazapine-2,5-diones have been shown to be effective as anticonvulsants, RGD tripeptide mimetics and as lead structures for the development of benzodiazepine receptor antagonists. Boojamra and coworkers [*J. Chem. Soc.* (1995) 60, 5742–5743] have investigated the use of solid-phase synthesis to produce libraries of these compounds. Their approach uses three commercially available components (anthranilic acids, alkylating agents and α-amino esters) to produce molecular diversity at R_1, R_2 and R_3 respectively (**1**).

The synthetic strategy used lactamization to effect ring closure and therefore required the acyclic precursor to contain a tertiary rather than a secondary amide. This was achieved using the synthetic route outlined in Scheme I. The approach uses a modified Merrifield resin with a resin–aldehyde to which α-amino esters are coupled by reductive amination. The resultant secondary amine **2** is then reacted with an appropriate anthranilic acid using a carbodiimide procedure to yield **3**. This undergoes base



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