between potential substrates (Fig. 1a, b). But enzymes that modify proteins and other macromolecules need to distinguish between similar (or even identical) sites within larger, dissimilar molecules. To do so, they must recognize the differences between substrates. That problem has been solved by diversifying the task of target recognition (Fig. 1c). Whereas the motif to be modified (one or a few amino acids in a protein, for instance) is recognized by the enzyme's active site, discrimination between different substrates bearing that motif is often accomplished through specific interactions between other sites on the enzyme and substrate.

Cyclin-dependent kinases have apparently broken down this process even further. Whereas responsibility for recognizing the target motif (a serine or threonine followed by a proline) is delegated to a catalytic subunit (the CDK), both genetic and biochemical studies suggest that exchangeable regulatory subunits (the cyclins) have a role in discriminating between distinct protein substrates (Fig. 1d). This is, perhaps, best illustrated by baker's yeast (*Saccharomyces cerevisiae*), where the cell-cycle-regulatory CDK, called Cdk1, can associate with nine distinct cyclins — three G1 cyclins (Cln1–3) and six B-type cyclins (Clb1–6). These cyclins, in addition to activating Cdk1, direct it towards distinct biological outcomes.

But although cyclins had been implicated in substrate recognition, Loog and Morgan's paper[1] describes the first comprehensive study to compare the substrate specificity of purified CDK complexes that differ only in their cyclin. Their findings show that Clb5–Cdk1 and Clb2–Cdk1 complexes phosphorylate most members of a group of 150 previously confirmed Cdk1 substrates[2] with roughly equal efficiency. However, 26 of those substrates are phosphorylated 2.5–800 times as efficiently by Clb5–Cdk1. In contrast, Clb2–Cdk1 does not preferentially phosphorylate any of the proteins.

The authors go on to extend previous studies[3–7] showing that a structural motif on the surface of some cyclins, referred to as the hydrophobic patch (HP), specifically interacts with a so-called RXL or Cy motif found on some CDK substrates and inhibitors. The HP motif is important for the biological activity of Clb5 (ref. 7). Loog and Morgan[1] now establish that this motif is essential for enhancing the activity of Clb5–Cdk1 towards its preferred substrates. Moreover, inactivating the Cy motif in the preferred Clb5–Cdk1 substrates eliminates their preferred status.

Strikingly, similar mutations in the Clb2 HP motif do not affect the efficiency with which Clb2–Cdk1 phosphorylates any of the substrates, regardless of the presence or absence of a Cy motif. That observation suggests that Clb2 does not use the HP motif for substrate recognition. In fact, Clb2 may not confer substrate specificity upon Cdk1. It may simply activate it and leave substrate recognition entirely to the active site. In keeping with that interpretation, Archambault *et al.*[8] have found that Cy-containing substrates depend upon the HP motif to interact with Clb5 in an *in vivo* assay, but that those lacking Cy motifs interact equally well with HP-deficient Clb5 and Clb2.

So what is the role of the HP motif in Clb2? Analysis of the relationship between the six yeast B-type cyclins reveals that, although Clb5 and Clb2 are closely related in terms of their overall sequence, their HP motifs appear to be significantly different[8]. Given the known structure of a complex between human cyclin A3 and a Cy-motif peptide[3], the Clb2 HP motif seems to be incompatible with binding to the Cy motif[8]. Nevertheless, it has been well conserved between different organisms, suggesting that it is still important to Clb2's function. One possibility is that it regulates a function of Clb2–Cdk1 other than its enzymatic activity. Indeed, mutation of the HP motif in Clb2 impairs the protein's export from the nucleus and its localization to at least one site in the cytoplasm[9]. Because Loog and Morgan's analysis was performed largely *in vitro*, using purified proteins, the importance of subcellular localization in substrate selection was not evaluated.

Loog and Morgan's study[1] underlines the importance of cyclins in recognizing appropriate CDK substrates. The extent to which similar mechanisms are exploited by other cyclins remains to be fully examined, but there is ample evidence that other properties of cyclins are also important in substrate selection. Subcellular localization, already mentioned in the context of Clb2, is a well-established determinant of the biological function of yeast G1 cyclins[10,11]. Of equal or even greater importance is the hallmark of the cyclin proteins — their periodic accumulation during the cell cycle. Clearly, for a substrate to be phosphorylated it must be present in the cell along with the specific form of CDK that phosphorylates it.

So cyclins have a substantial role in directing CDKs to specific substrates. But there are numerous mechanisms for doing so, more than one of which may be used by a single cyclin. Ultimately, it is the combined action of these mechanisms that orchestrates the orderly progression of events leading to the faithful duplication of cells. ■

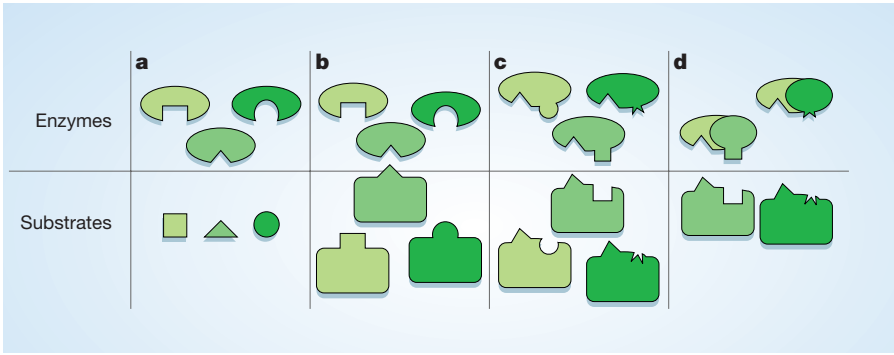

Figure 1 **How enzymes select their substrates.** a, b, **In general, enzymes recognize their targets through structural complementarity between the substrate and the enzyme's active site (indicated here by the shape of the 'pocket'). Small substrates** (a) **and relatively small modification sites on proteins** (b) **can be recognized by this mechanism.** c, **Some enzymes make additional, specific contacts with the substrate that enable them to distinguish between proteins that have identical or related sites of modification.** d, **Loog and Morgan[1] have compelling new evidence that cyclin-dependent protein kinases (CDKs) have relegated that function to the exchangeable cyclin subunit, enabling a single CDK catalytic subunit to exist in numerous forms with different specificities.**

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Correction

A misleading statement appeared in the News and Views article "Cardiology: Solace for the broken-hearted?" by Christine L. Mummery (*Nature* **433,** 585–587; 2005). The cardiac arrhythmias reported in reference 9 (P. Menasche *et al.*, *J. Am. Coll. Cardiol.* **41,** 1078–1083; 2003) were not the cause of fatalities in patients who received their own skeletal-muscle progenitor cells as therapy for heart damage, as implied in the passage concerned.

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