we fully understand the mechanisms behind microbial control of carbon cycling. Given our current state of ignorance, any large-scale manipulation of the oceans, such as proposals to increase phytoplankton growth by iron fertilization, could have catastrophic consequences.3,14. There is an urgent need for oceanographers to embark on a bold exploration of the oceans — this time at the millimetre scale.

Faroq Azam and Richard A. Long are in the Marine Biology Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92032-0202, USA.

e-mail: fazam@ucsd.edu


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**Six steps to destruction**

James E. Ferrell Jr

Cell division relies on the properly timed activation and destruction of certain regulatory proteins. New work shows that many rounds of phosphorylation can help to establish the timing of protein destruction.

The process by which cells multiply consists of a strictly ordered series of events. A cell copies its genetic material, grows, and segregates the duplicated DNA into two new cells. Then, after a rest period, the process begins again. If cells are to progress smoothly from one phase of this 'cell cycle' to the next, key regulatory molecules must be turned on and off at just the right times. On page 514 of this issue, Nash and colleagues1 provide new insight into the molecular controls that allow budding yeast cells to move from the G1 phase (the rest period) to the S phase (when DNA is copied). Their work builds on the fact that the Sic1 protein needs to be enzymatically modified with phosphate groups (phosphorylated) over and over again before cells can progress from G1 into the S phase. Sic1 provides a splendid example of how cells can use cumulative, multistep modifications to produce a strict, switch-like transition.

The job of the Sic1 protein is to inhibit the protein complex that drives budding yeast cells from the G1 phase into the S phase.4 This protein complex comprises an enzyme from a family known as the cyclin-dependent kinases (this enzyme is Cdc28 in yeast, and Cdk1 in mammals) and its regulatory subunit, a cyclin protein (from the Clb family in yeast). During the G1 phase, cells have high levels of Sic1; this ensures that Cdc28–Cln complexes are inactive, so DNA replication is suppressed. At the end of the G1 phase, Sic1 is degraded, allowing Cdc28–Clb to drive cells into S phase. In yeast strains that lack S phase is blocked by the protein Sic1, which inhibits a key enzymatic complex. Nash et al.1 have discovered that Sic1 must be phosphorylated at least six times by the Cdc28–Cln complex before it can bind to the Cdc4 protein, be tagged with ubiquitin groups, and destroyed. The multistep phosphorylation of Sic1 may function as a sort of primordial timing mechanism, ensuring that Sic1 destruction is precisely timed so that the S phase does not happen too early.

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They found that Sic1 with two, three or five phosphorylation sites in vivo. This raises the question of why Sic1 has so many phosphorylation sites and is phosphorylated so many times.

Nash and colleagues approached this question by mutating different numbers of phosphorylation sites to find out how many are needed for proper degradation of Sic1. They found that Sic1 with two, three or even five sites could not bind Cdc4 in vitro, but Sic1 with six, seven or nine sites could. Likewise, Sic1 with just two, three or five phosphorylation sites prevented yeast strains from multiplying — presumably because they arrested in the G1 phase — but strains containing Sic1 with six, seven or nine sites divided normally.

Why isn’t one phosphorylation (or even five) sufficient? One possibility is that Cdc4 has six phosphate-binding pockets. But quantitative binding studies and mutational analysis indicate that it has only one1. An alternative is suggested by the consensus amino-acid sequence within phosphorylated peptides that allows them to bind optimally to Cdc4 (ref. 1). The mammalian cyclin E protein, another SCF substrate, has a single phosphorylation site that targets it for degradation; the sequence around this site fits the Cdc4-binding consensus and binds Cdc4 with high affinity. But it turns out that none of the phosphorylation sites in Sic1 matches this consensus very well. This implies that Sic1 needs to be phosphorylated many times because none of its phosphorylation sites is all that good at binding Cdc4.

But is it important that Sic1’s destruction is driven by the combined effects of six phosphorylation sites? Or would a single high-affinity site work just as well? To find out, Nash et al. replaced seven of Sic1’s nine phosphorylation sites with a single high-affinity site derived from cyclin E. The resulting protein inhibited Cdc28–Cln in vitro, but failed to restrain DNA replication properly in vivo. Apparently it really does matter how Sic1 comes to be destroyed.

The fact that Sic1 does not appreciably bind Cdc4 and begin to be destroyed until the sixth phosphate is added has several kinetic implications. It means that there should be a discrete lag time between the activation of Cdc28–Clns and the destruction of Sic1 (Fig. 1). Those inefficient first five phosphorylations need to be accomplished before the critical sixth one can occur. So the time required for the first five phosphorylations acts as a temporal threshold for Cdc28–Cln activation. In one sense, the first five phosphorylations accomplish nothing — but sometimes doing nothing is of the utmost importance.

The requirement for six phosphates also means that the destruction of Sic1 could be a highly nonlinear function of the amount of Cdc28–Cln activity in a cell. If so, the regulation of the G1/S transition would have a noise filter built into it — low levels of Cdc28–Cln activity would result in very little Sic1 being destroyed. Sic1 would then respond to higher levels of Cdc28–Cln activity in a decisive, switch-like fashion; doubling Cdc28–Cln activity could increase the destruction of Sic1 by a factor of 2^6 (64-fold)9.

The six phosphorylations may also help to ensure the specificity of Sic1 destruction. Suppose that some kinase other than Cdc28–Cln can inappropriately phosphorylate Sic1 at a low frequency, ε. The frequency of inappropriate destruction of Sic1 would then be as low as ε^2. This property has been termed ‘kinetic proof-reading’10 (P. Swain and E. Siggia, personal communication), and is another potential advantage of the six-phosphate mechanism.

Not all substrates of the SCF complex need multiple phosphorylations for destruction; cyclin E is a good example of this. So the timing and abruptness of destruction seems to be regulated by the properties of the enzymes that catalyse phosphorylation and destruction, and of their substrates. SCF substrates apparently have a primordial timing mechanism built into them, on top of which other controls can be built.

Studies of budding yeast have a long history of yielding valuable insights into cell biology. Nash and co-workers have shown that such studies can also shed light on the biochemical logic of cell regulatory systems — with a lot of hard work, a bit of quantitative reasoning, and the ability to count to six.

James E. Ferrell Jr is in the Departments of Molecular Pharmacology and Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5174, USA.

References