Abstract

Mitotic destruction of cyclin A has remained enigmatic ever since it was discovered. Regulation of cyclin A proteolysis appears to be different from that of other mitotic cyclins in diverse species. Despite being substrates of the same destruction machinery - the E3 ligase called Anaphase Promoting Complex/Cyclosome (APC/C) - the proteolysis of cyclin A concludes before that of the B-type cyclins and other crucial substrates like securin. Moreover cyclin B and securin, but not cyclin A, gets stabilized upon activation of the spindle assembly checkpoint, which is a surveillance mechanism that inhibits the APC/C. Somehow, APC/C activity towards cyclin A escapes checkpoint control. Defining the cyclin A destruction signals is paramount for solving this riddle. Unfortunately, in spite of years of research, the cyclin A degradation signal remains ill-defined and poorly characterized. A single and simple destruction box (D-box) motif is responsible for cyclin B turnover. But the cyclin A degradation signal is much more complex. In *Drosophila* cyclin A (CycA), a putative D-box and another element called the KEN box have been implicated; but eliminating these do not cause stability. In order to characterize the elusive CycA destruction signals, an extensive analysis was carried out in this study. It was found that both the N- and C-terminal regions are involved in turnover, which implies a synergistic action by multiple parts of the molecule. A KEN box, a D-box and an aspartic acid at position 70 are required at the N-terminus and they make additive contributions to degradation when the checkpoint is active or inactive. From the C-terminal region, the cyclin box contributes. Single point mutations in these four elements totally abolish mitotic destruction. Very importantly, it was observed that the cyclin box mediates the spindle checkpoint bypass. The normal function of the cyclin box is to mediate Cdk1 binding, and previous studies had claimed that this interaction is essential for timely destruction. But it was found here that the cyclin box provides a function different from Cdk1 binding for turnover; most likely it presents an interaction motif for the APC/C. Furthermore, eight potential ubiquitin acceptor lysine residues surrounding the N-terminal signals were found to be preferentially used for proteolysis. Combining mutations in these lysines and the N-terminal signals caused full

stability leading to mitotic arrest phenotypes. But, mutating the lysines alone only prolonged the duration of mitosis. Thus, presumably, lysines elsewhere on the protein are used when the preferred ones are absent. This apparent shift in ubiquitination is mediated by the N-terminal signals. In conclusion, this study defines the CycA destruction signals and gives an explanation for how checkpoint destruction can be accomplished.