

Extra Views

Redundant Degrons Ensure the Rapid Destruction of Sic1 at the G₁/S Transition of the Budding Yeast Cell Cycle

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A fundamental feature of the eukaryotic cell cycle is the need to coordinate the activity of regulatory proteins with exquisite precision. In many cases, this depends on the regulation of protein stability through the activity of two ubiquitin ligases: SCF (Skp1, cullin, and F box) and APC (anaphase promoting complex).¹ Their ability to recognize specific proteins and catalyze their subsequent ubiquitination results in targeting these proteins for destruction by the 26S proteasome.

In budding yeast, SCF^{Cdc4} regulates the ubiquitination of Sic1, a stoichiometric inhibitor of the S-phase cyclin dependent kinase (S-CDK) complex.² Previous studies determined that multiple phosphorylation events are required for the recognition of Sic1 by Cdc4 of SCF^{Cdc4}, suggesting a switch-like mechanism for “turning on” S-CDK activity by rapidly turning over Sic1 once this threshold of phosphorylation is surpassed.^{3,4}

We sought to address the minimal signal required for the turnover of Sic1 by determining which lysines are the targets for ubiquitin conjugation.⁵ Unlike proteins that can be N-terminally ubiquitinated⁶ or may be degraded even without ubiquitination,^{7,8} Sic1 turnover in vivo and degradation in vitro requires multiubiquitin chain attachment on an internal lysine residue. While many of the 20 lysines scattered throughout Sic1 readily accept ubiquitin, only 6 lysines within the N-terminal 90 amino acids support Sic1 degradation. Surprisingly, Sic1 lacking these 6 lysines is extensively multiubiquitinated in vitro, but is not recognized by purified 26S proteasomes and is stable in vivo. We determined that these ubiquitin attachment sites are shielded by the assembly of Sic1 into a complex with S-CDK, indicating that physical occlusion by binding partners can restrict the exposed lysines to only those that comprise competent degradation signals (degrons). The opposite may also be true for some proteins—substrate ubiquitination and turnover may be regulated by binding proteins that shield critical destabilizing ubiquitin attachment sites.

Further dissection of the minimal Sic1 degron revealed that any of the six lysines within the N-terminal region is sufficient for Sic1 destabilization in vivo, suggesting that Sic1 contains redundant degrons. In vitro characterization of “single lysine” substrates revealed that while they are ubiquitinated to a similar extent, they are degraded by purified 26S proteasome at rates that differ by up to five fold. We do not know why presumably equivalent ubiquitin chains positioned at different sites are differentially active in sustaining turnover in vitro. The rate of turnover could be influenced by the local environment of the ubiquitin chain. Steps such as recognition by the multiubiquitin chain receptors, unfolding by the 19S ATPases,⁹ deubiquitination by Rpn11,^{10,11} or processing by the 20S peptidases could all be affected by structure or sequences of the substrate around the multiubiquitin chain.

So why does Sic1 contain redundant lysine degrons? These sites of ubiquitin attachment are juxtaposed near the CDK phosphorylation sites determined to be the most critical in regulating Sic1 stability.⁴ Further, it has been proposed that the recognition of Sic1 by SCF^{Cdc4} is directed through a single one of its low-affinity phosphorylation sites and that the requirement for phosphorylation on six sites reflects “kinetic trapping” of phosphorylated Sic1 on the F box substrate receptor Cdc4.¹² We propose that phosphorylated Sic1 assumes many different orientations on Cdc4, but can nevertheless be rapidly ubiquitinated because one of these six lysine residues is optimally positioned relative to the incoming ubiquitin molecule.

Support for this idea is provided by a recent analysis of the substrate specificity of SCF^{β-TrCP}.¹³ Model peptide substrates based on β-catenin that contain a single lysine residue at various positions upstream of the pSDXXGpS recognition motif are ubiquitinated by SCF^{β-TrCP} at different rates—with a lysine positioned 9 or 10 residues upstream being most optimal—even though all variant peptides tested associate with SCF with similar affinities. This number agrees with the spacing found in Sic1—the three pairs of lysines in

Table 1 POSITION OF LYSINE RESIDUES RELATIVE TO CDK PHOSPHORYLATION SITES

Rank ^a	CDK site	Amino Acid Sequence	K Position ^b	Distance	Optimal Position ^c
1	T45	KPSQNLVPT P STTKSFKNA	K32	-13	+
			K36	-9	+
			K50	+5	+
			K53	+8	+
2	S76	MTSPFNGLT S PQRSPFPKSS	K50	-26	
			K53	-23	
			K84	+8	+
			K88	+12	+
3	T5	MTP S T P RRSRGTRYL K32	K32	+27	
			K36	+31	
4	T33	SSALMQGQ K T P QKPSQNLVP	K32	-1	+/-
			K36	+3	+/-
			K50	+17	
			K53	+20	
5	S80	FNGLTSPQR S PFKSSVKRT	K84	+4	+/-
			K88	+8	+
5	S191	ELAKNWN N NS P KNDARSQES	K193	+2	+/-
			K185	-6	
6	T173	KKIHKDVP G T P SDKVITFEL	K177	+4	+/-
			K168	-5	+
			K165	-8	+
			K164	-9	+
7	S69	PPNSNMGMT S PFNGLTSPQR	K50	-19	
			K53	-16	
			K84	+15	
			K88	+19	
8	T2	M T P S T P RRSRGT	K32	+30	
			K36	+34	

^aRank of CDK site is based on in vivo stability experiments.⁴ The higher the rank, the greater the stabilization that is achieved by mutating the indicated CDK site.^bOnly K32, K36, K50, K53, K84, and K88 are necessary and individually sufficient for Sic1 destabilization.⁵ ^cOptimal Position scores are based on in vitro experiments using synthetic β -catenin peptides and SCF ^{β -TrCP}.¹³ A score of "+" represents lysine residues between 5 and 13 residues N-terminal (-) or C-terminal (+) of the phosphorylation site. Note that utilization of a lysine residue C-terminal of a phosphorylation site has not been reported, but for this analysis we focus only on relative distance and do not consider the potential effect of relative direction from the phosphorylation site.

the N-terminus are located within 5 to 13 residues from the key phosphorylation sites at T33, T45, and S76 (see Table 1). Thus, Sic1 tethered to Cdc4 by any of these phosphorylation sites may present an optimally positioned lysine to the ubiquitination machinery. Other SCF substrates such as p27 and I κ B α likewise contain lysine residues near critical phosphorylation sites.¹⁴⁻¹⁶ This proposal can now be tested by examining Sic1 derivatives carrying a single phosphorylation site with high affinity for Cdc4.⁴ The expectation is that lysines in the vicinity of this site will be preferentially ubiquitinated.

A corollary of our analysis of these single lysine substrates is the observation that SCF^{Cdc4} appears to favor assembly of chains containing ~6 to 15 ubiquitin protomers onto a targeted lysine. It would seem that SCF^{Cdc4} can somehow achieve an impressive gymnastic feat—bridging the proposed 59 Å gap between the substrate docking site and ubiquitin-loaded E2¹⁷—and generate a single chain that contains up to 15 ubiquitin molecules. It will be of great interest to determine the mechanism of substrate ubiquitination with respect to the recently solved SCF structures.

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