May 6th, 12:00 AM

Identification of the E3 Ligase that Directs the Degradation of Proteins that Control Cell Fate Decisions in Yeast

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Identification of the E3 ligase that directs the degradation of proteins that control cell fate decisions in yeast

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Abstract

The ubiquitin–proteasome system (UPS) and autophagy pathways are distinct, highly conserved proteolytic systems that play important roles in maintaining cellular homeostasis in response to environmental cues [1]. The goal of this project is to identify the E3 ligase that mediates the degradation of cyclin C following nitrogen starvation in yeast using quantitative Western blot analysis of cyclin C-myc following nitrogen starvation in mutants of known Ubc4/5 interacting E3 ligases. No potential E3 ligases were identified as stable after 4 hours of nitrogen starvation suggesting redundancy in function.

Table 1. Partial list of E3 ligases

E3 Ligase Plate					
Plate	Row	Column	ORF	Gene		Group
1	A	1	YAL002W	VPS8	GTPase binding protei	7
1	A	2	YLR024C	UBR2	May be incorrect	5
1	A	3	YLR097C	HRT3	F Box	2
1	A	4	YLR108C		No Fuction	7
1	A	5	YMR026C	PEX12		2
1	A	6	YML088W	UFO1	F BOX	2
1	A	7	YMR247C	RKR1		2
1	A	8	YMR231W	PEP5		2
1	A	9	YNL311C	SKP2	F BOX	5
1	B	1	YMR258C	ROY1	F Box Like	7
1	B	2	YOR080W	DIA2	F Box	1
1	B	3	YOL013C	HRD1		2
1	B	4	YOL054W	PSH1		2
1	B	5	YBR203W	COS111	Unkonw Function	7
1	B	6	YDR143C	SAN1		0
1	B	7	YDR103W	STE5	MAPK scaffold protein	7
1	B	8	YDR132C		Unkown Function	7
1	B	9	YER068W	MOT2/NOT4		0
1	C	1	YGR184C	UBR1	N Rule E3	3
1	C	2	YHL010C	ETP1	Unkown Function	7
1	C	3	YLR182W	SWI6	Transcription	7
1	C	4	YHR115C	DMA1		3
1	C	5	YKL034W	TUL1		1
1	C	6	YKL010C	UFD4		0
1	C	7	YLR224W	UCC1	F Box	3
1	C	8	YOR191W	ULS1	SUMO Ligase	3
1	C	9	YJL157C	FAR1	CDK inhibitor	8
1	D	1	YJL210W	PEX2		3
1	D	2	YJL149W	DAS1	F Box	3
1	D	3	YJL204C	RCY1	F Box	8
1	D	4	YLR352W	LUG1	F Box like	8
1	D	5	YLR247C	IRC20		6
1	D	6	YLR368W	MDM30	F BOX	4
1	D	7	YDR219C	MFB1	mito F Box	8
1	D	8	YNL230C	ELA1	F Box	5
1	D	9	YDR255C	RMD5		5
1	E	1	YKR017C	HEL1		4
1	E	2	YDR265W	PEX10		4

Introduction

Following nutrient depletion, autophagy in *S. cerevisiae* is predominantly upregulated and cells enter a quiescent state until nutrients become available again. However, autophagy is also upregulated following oxidative stress which evokes a cell death response. It remains unknown how cells translate these different environmental cues into cell fate decisions but work from mammalian systems has revealed that mitochondrial morphology is likely to play a key role. Here starvation induces mitochondria to become hyperfused and this promotes survival [2, 3]. In contrast, following oxidative stress, the mitochondria fragment and this is dependent upon the nuclear translocation of cyclin C, which is a member of the conserved Cdk8 kinase module (CKM) of the mediator complex [4]. Cell death ensues which is promoted by cyclin C dependent mitochondrial fragmentation [4]. Cyclin C is destroyed by a novel mechanism that sequentially utilizes the ubiquitin proteasome system (UPS) and the macro-autophagy machinery. In short, cyclin C is initially delivered to nuclear proteasomes but once the proteasomes are themselves targeted by proteaphagy [5-8] cyclin C co-translocates out of the nucleus captured within targeted proteasomes in autophagosomes. This promotes cell survival by preventing the translocation of cyclin C translocation to the mitochondria, thus circumnavigating the aberrant activation of a cell death response to a survival signal [9].

Methods and Materials

A literature search was performed to identify possible E3 ligases, which could degrade cyclin following nitrogen starvation. 111 possible proteins were identified and they were split into testing groups (Table 1). In short, mutants with known E3 ligase, E2 interactivity, or a RING motif were designated high priority whereas putative E3 ligases were tested designated low priority. To test if the ligases were responsible for the degradation of cyclin C null mutants of these ligases (from the Res. Gen collection) and a wild type control were transformed with a functional cyclin C-myc construct. Cells were grown in replete media (T=0) to mid –log, washed and then starved for nitrogen for 4 h. Protein extracts were made and cyclin C myc visualized using quantitative Western blot analysis. The blots were stripped and reprobed for Pgk1 as a total protein control.

Figure 1. Roles of cyclin C in cell fate decisions related to stress.

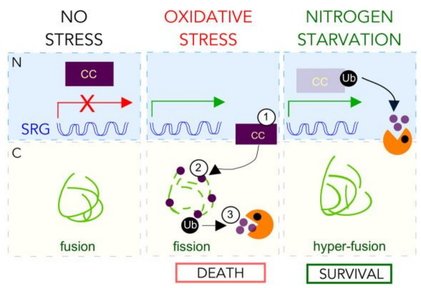

Figure 2. Representative Western blot analysis of cyclin C-myc degradation observed in the putative E3 ligase mutants.

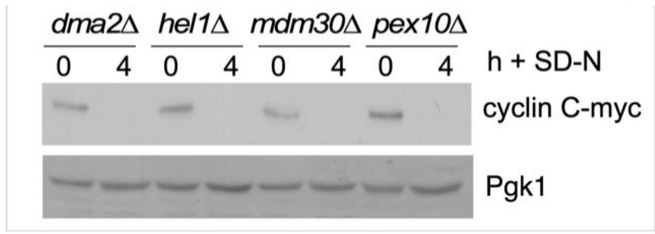

Table 2. List of E3 ligases cont.

1	E	3 YDR313C	PIB1	4
1	E	4 YDR281C	PHM6	Unkown function	8
1	E	5 YDR306C		F Box	8
1	E	6 YIL001W		Unkown Function	8
1	E	7 YLR032W	RAD5		1
1	E	8 YOL138C	RTC1		5
1	E	9 YMR119W-A		Dubious ORF	8
1	F	1 YPR093C	ASR1		0
1	F	2 YLR427W	MAG2	Unkown function	9
1	F	3 YML068W	ITT1	Unkown function	9
1	F	4 YCR066W	RAD18		4
1	F	5 YDL190C	UFD2		4
1	F	6 YJR036C	HUL4		9
1	F	7 YJR052W	RAD7		9
1	F	8 YNL008C	ASI3		9
1	F	9 YNL023C	FAP1		9
1	G	1 YNL116W	DMA2		4
1	G	2 YBR062C		Unkown function	9
1	G	3 YBR280C	SAF1	F Box	9
1	G	4 YIL030C	SSM4		1
1	G	5 YBR114W	RAD16		10
1	G	6 YDL013W	SLX5		1
1	G	7 YDR457W	TOM1		0
1	G	8 YER116C	SLX8		1
1	G	9 YGL131C	SNT2		6
1	H	1 YGL141W	HUB1		0
1	H	2 YDL074C	BRE1		1

Results

After testing all 111 potential E3 ligases, no known potential E3 ligases were found to be stable after 4 hours of nitrogen starvation. All samples were degraded after nitrogen starvation. One example is shown in Figure 1.

Discussion

This suggests a redundancy in that the E3 function may not be fulfilled by a single ligase, rather there are multiple ligases responsible for the degradation of cyclin C in nitrogen starvation.

Future Directions

Dr. Cooper's group will continue to map out the pathway of cyclin C degradation.

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