

Periodic Ubiquitination of Ku70 subunit regulates the cell cycle related activity of Ku protein

Indranil Modak^{1,2}, Sanjima Pal¹ & Partha Saha^{1,2*}

¹Crystallography and Molecular Biology Division, Biophysical Sciences Group, Saha Institute of Nuclear Physics, Kolkata-700 064, West Bengal, India

²Homi Bhabha National Institute, Mumbai-400 094, Maharashtra, India

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Supplementary Data

Suppl. Table S1 — Predicted ubiquitination sites of Ku70 by various online Tools

Residue	Location*, Domain	Ubiquitination site predicted by, Probability		
K9	Dimerization			UbPred, Low
K31	Dimerization		UniProtKB	UbPred, Med
K46	Dimerization		UbiSite, High	
K92	Dimerization		UbiSite, High	UbPred, Low
K94	Dimerization		UniProtKB	
K100	Surface, Dimerization	CKSAAP, High	UniProtKB	
K114	Surface, Dimerization	CKSAAP, High	UniProtKB	
K123	Surface, DNA-Binding	CKSAAP, High	UniProtKB	
K129	DNA-Binding	CKSAAP, Low	UniProtKB	
K182	DNA-Binding		UniProtKB	UbPred, Med
K189	DNA-Binding		UniProtKB	
K206	Surface, DNA-Binding	CKSAAP, High	UniProtKB	
K207	DNA-Binding	CKSAAP, Low	UniProtKB	
K238	Surface, DNA-Binding	CKSAAP, High	UniProtKB	
K265	DNA-Binding		UniProtKB	
K279	DNA-Binding	CKSAAP, Low		
K282	DNA-Binding	CKSAAP, Low	UbiSite, High	UniProtKB
K287	DNA-Binding	CKSAAP, High	UbiSite, High	UniProtKB
K297	DNA-Binding		UniProtKB	
K299	DNA-Binding		UniProtKB	
K317	Surface, DNA-Binding	CKSAAP, High	UniProtKB	
K331	DNA-Binding		UniProtKB	UbPred, Low
K338	DNA-Binding		UniProtKB	
K351	DNA-Binding	CKSAAP, High	UniProtKB	
K357	DNA-Binding	CKSAAP, High	UniProtKB	
K358	DNA-Binding	CKSAAP, High	UniProtKB	
K388	DNA-Binding		UniProtKB	
K392	DNA-Binding		UniProtKB	
K424	DNA-Binding		UniProtKB	
K443	DNA-Binding		UbiSite, High	UniProtKB
K445	Surface, DNA-Binding	CKSAAP, High	UniProtKB	UbPred, Low
K451	Surface, DNA-Binding	CKSAAP, High	UniProtKB	UbPred, Low
K461	DNA-Binding		UniProtKB	
K463	DNA-Binding		UniProtKB	
K468	DNA-Binding		UniProtKB	

K510	SAP			UbiSite, High	
K516	SAP			UbiSite, High	UniProtKB
K526	SAP			UbiSite, High	UniProtKB
K539	SAP				UbPred, Low
K553	SAP				UniProtKB
K556	SAP			UbiSite, High	UniProtKB
K565	Surface, SAP		CKSAAP, High		UniProtKB
K570	Surface, SAP		CKSAAP, High		UniProtKB
K575	SAP		CKSAAP, High		UniProtKB
K582	Surface, SAP		CKSAAP, Low		UniProtKB
K591	SAP				UniProtKB
K595	SAP			UbiSite, High	UniProtKB
K596	SAP			UbiSite, High	UniProtKB
K605	Surface, SAP		CKSAAP, High		UniProtKB

*Surface localization of lysine residue was based on the structural model by PyMol

Suppl. Table S2 — Predicted ubiquitination sites of Ku70 by various online Tools. Determination of Ubiquitination sites on the wild type Ku70 by LC-ESI-MS analysis. K299 and K605 were found to be sites of Ubiquitination on wildtype Ku70

Experiment: KU_70_UB_INDRANIL

Accession	Peptides	Score	Anova (p)*	Fold	Tags	Description	Average Normalised Abundances
P12956	128 (128)	772.48	---	1.00		X-ray repair cross-complementing protein 6 OS=Homo sapiens OX=9606 GN=XRCC6 PE=1 SV=2	5.87e-006

Sequence	Peptide Ion	Score	Hits	Mass	Charge	Tags	Conflicts	Modifications	In quantitation	Average Normalised Abundances
AGDLRDTGIFLDMHLK	4853	7.69	1	1930.0275	4		0	[14] Oxidation M	yes	270.09
AIVEK	904	8.21	2	558.3383	1		0		yes	3636.30
ALKP	3410	0.00	1	409.2802	1		0		yes	582.48
ALKPPPIK	619	8.40	2	862.5852	2		0		yes	1.24e+004
ALTK	2040	0.00	2	431.2842	1		0		yes	2010.95
QELLEALTKHFQD	2365	6.85	1	1684.8007	3		0	[9] UBIQUITINATION_SIDE	yes	2222.37
QIILEK	1808	8.42	2	742.4856	2		0		yes	2014.52
QIILEKEETEELK	805	8.52	2	1600.8653	3		0		yes	1.40e+004
TFNTSTGGLLLPSDTKR	5	8.82	1	1806.6826	3		1		yes	1.48e-006
TKTRTFNTSTGGLLLPSDTK	4201	7.26	2	2365.0776	3		0	[2] UBIQUITINATION_SIDE [20] UBIQUITINATION_SIDE	yes	576.53
TNEDNPHGNSAK	3243	0.00	1	1397.5571	2		0		yes	631.47

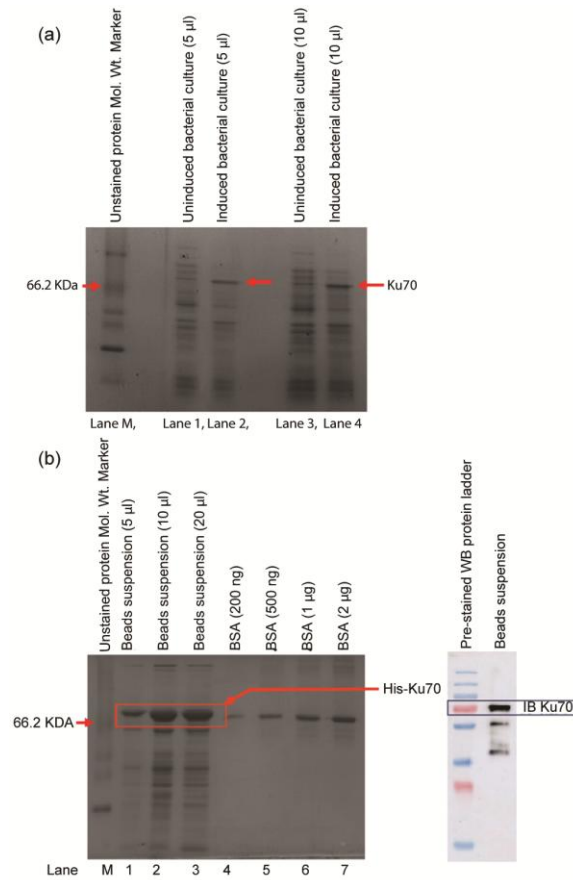
Suppl. Table S3 — Determination of ubiquitination sites on Ku70-K299R-K605R by LC-MS/MS analysis. K94, K461/463 and K543 were found to be the probable sites on the double mutant of Ku70

KU_70_MUT_INDRANIL

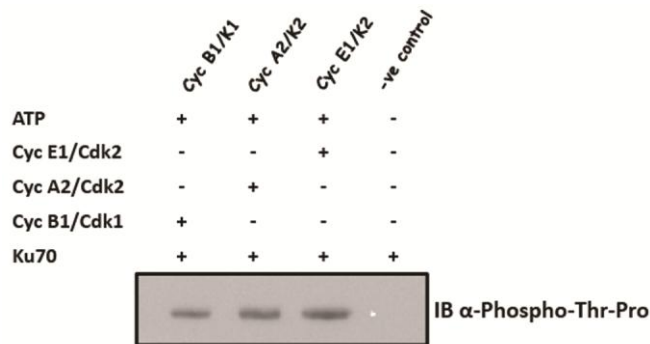
Experiment: KU_70_UB_INDRANIL
Report created: 2/8/2021 6:37:20 PM

Accession	Peptides	Score	Anova (p)*	Fold	Tags	Description	Average Normalised Abundances
P12956	143 (143)	851.13	---	1.00		X-ray repair cross-complementing protein 6 OS=Homo sapiens OX=9606 GN=XRCC6 PE=1 SV=2	1.77e-007

Sequence	Peptide Ion	Score	Hits	Mass	Charge	Tags	Conflicts	Modifications	In quantitation	Average Normalised Abundances
AALCR	3450	0.00	1	589.2991	1		0	[4] Carbamidomethyl C	yes	0.00
DKNSVNFK*	6660	---	---	1178.3628	1		0	[8] UBIQUITINATION_SIDE [C-term] UBIQUITINATION_C	yes	0.00
DKNSVNFK*	7611	6.57	1	1178.3666	2		0	[8] UBIQUITINATION_SIDE [C-term] UBIQUITINATION_C	yes	0.00
IMATPEQVGK	822	7.83	1	1088.5451	2		0	[2] Oxidation M	yes	5.53e-005
IMATPEQVGKMKAIVEK	8506	4.82	1	1986.0354	3		0	[17] UBIQUITINATION_SIDE	yes	4.25e-004
IMLFTNEDNPHGNSAK	404	8.40	2	1901.8384	3		0		yes	2.00e-006
KHDNEGSGSK*	91	6.12	1	1171.5073	3		0	[C-term] UBIQUITINATION_C	yes	0.00
KHDNEGSGSK*	57	---	---	1171.5093	2		0	[C-term] UBIQUITINATION_C	yes	0.00
KMPFTEK	988	8.56	2	879.4720	2		0		yes	0.00
KMPFTEK	3817	7.36	1	895.4693	2		0	[2] Oxidation M	yes	0.00



Suppl. Fig. S1 — A. Expression of Ku70 protein after IPTG induction - checked by SDS-PAGE of whole cell protein lysate followed by Coomassie blue staining. B. The presence of His-Ku70 was checked in Ni-agarose beads after affinity chromatography and the protein was estimated by comparing with the Coomassie blue stained bands of BSA of known concentrations. C. The affinity purified protein was confirmed to be Ku70 by Western blot analysis using anti-Ku70 antibody



Suppl. Fig. S2 — Ku70 is phosphorylated in vitro by Cyclin B1/Cdk1, Cyclin A2/Cdk2, Cyclin E1/Cdk2. The cyclin/Cdks were expressed in Sf9 insect cells and purified