

# A proteomic analysis of p27<sup>kip1</sup>-binding proteins reveals a putative role in transcription regulation through RNA polymerase II interaction.



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## Introduction and Objectives

The protein p27<sup>kip1</sup> is a member of the Cip-Kip family of cyclin-dependent kinase (CDK) inhibitors.

In addition to its role in the cell cycle, p27 also behaves as a transcriptional regulator.

It directly interacts with the p130/E2F4 subunit of the DREAM transcriptional repressor complex through its carboxyl moiety.

To further identify new mechanisms of transcriptional regulation mediated by p27 we aimed to identify nuclear proteins that associates with its carboxyl terminal domain

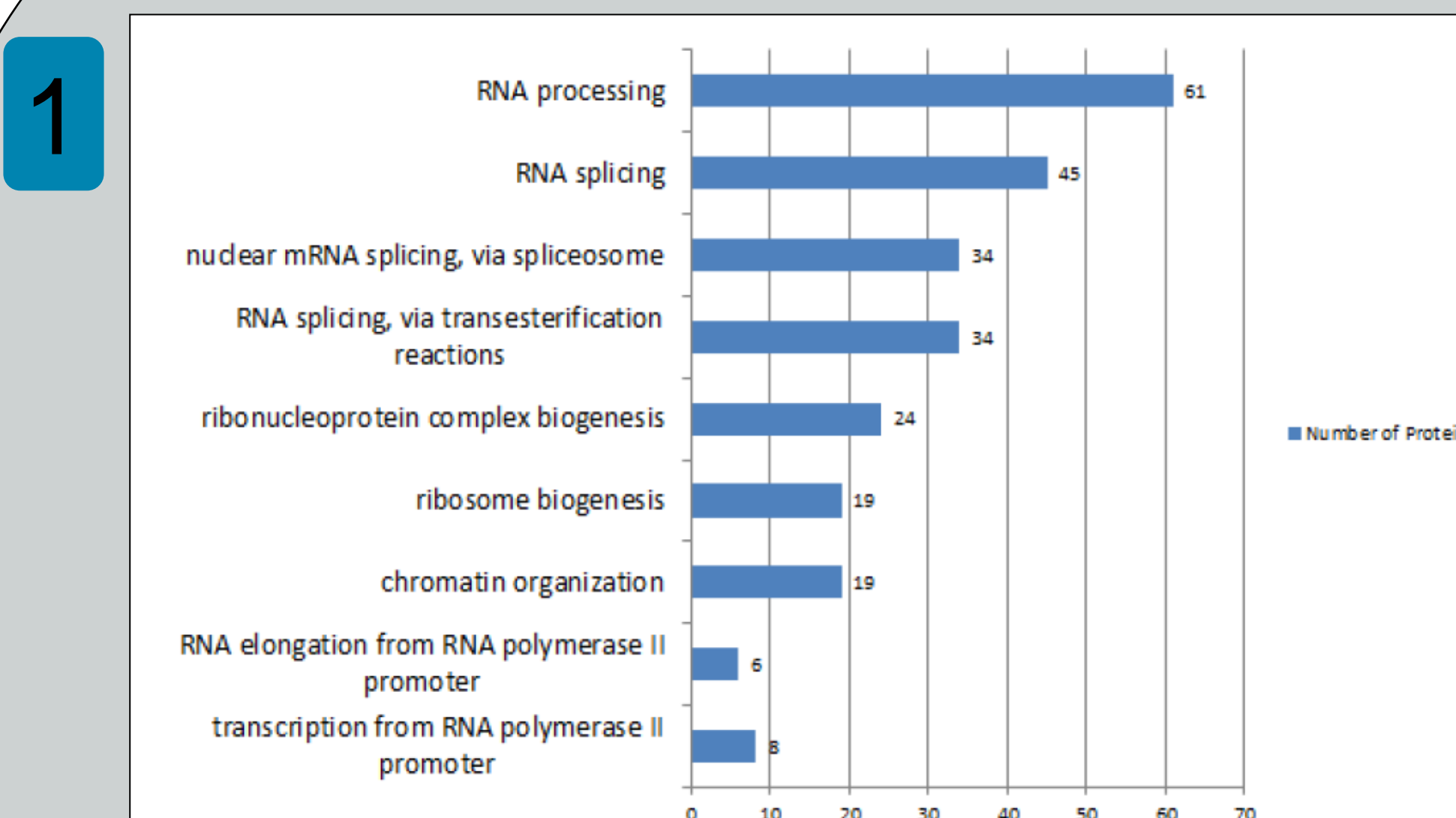
## Materials & Methods

Affinity chromatography columns of a p27 peptide (including aminoacids 161-198) bound to sepharose-beads were used to purify p27-binding proteins.

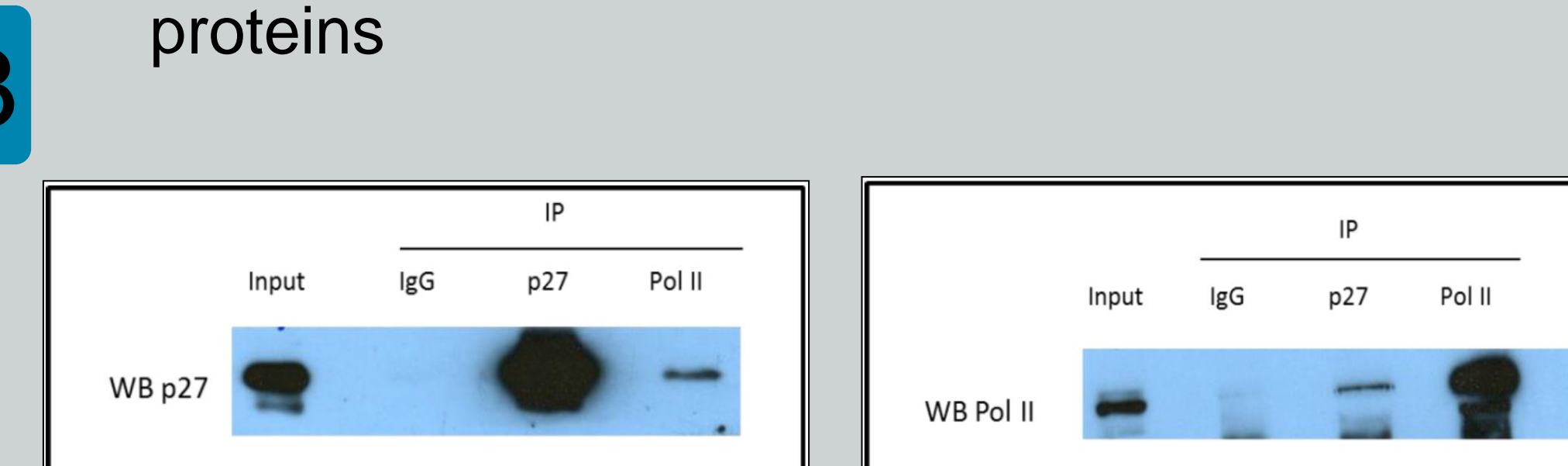
These proteins were subsequently identified by mass spectrometry (LC-MS/MS) and a Protoarray assay

*In vivo* interaction was tested by Immunoprecipitation assays

## Results



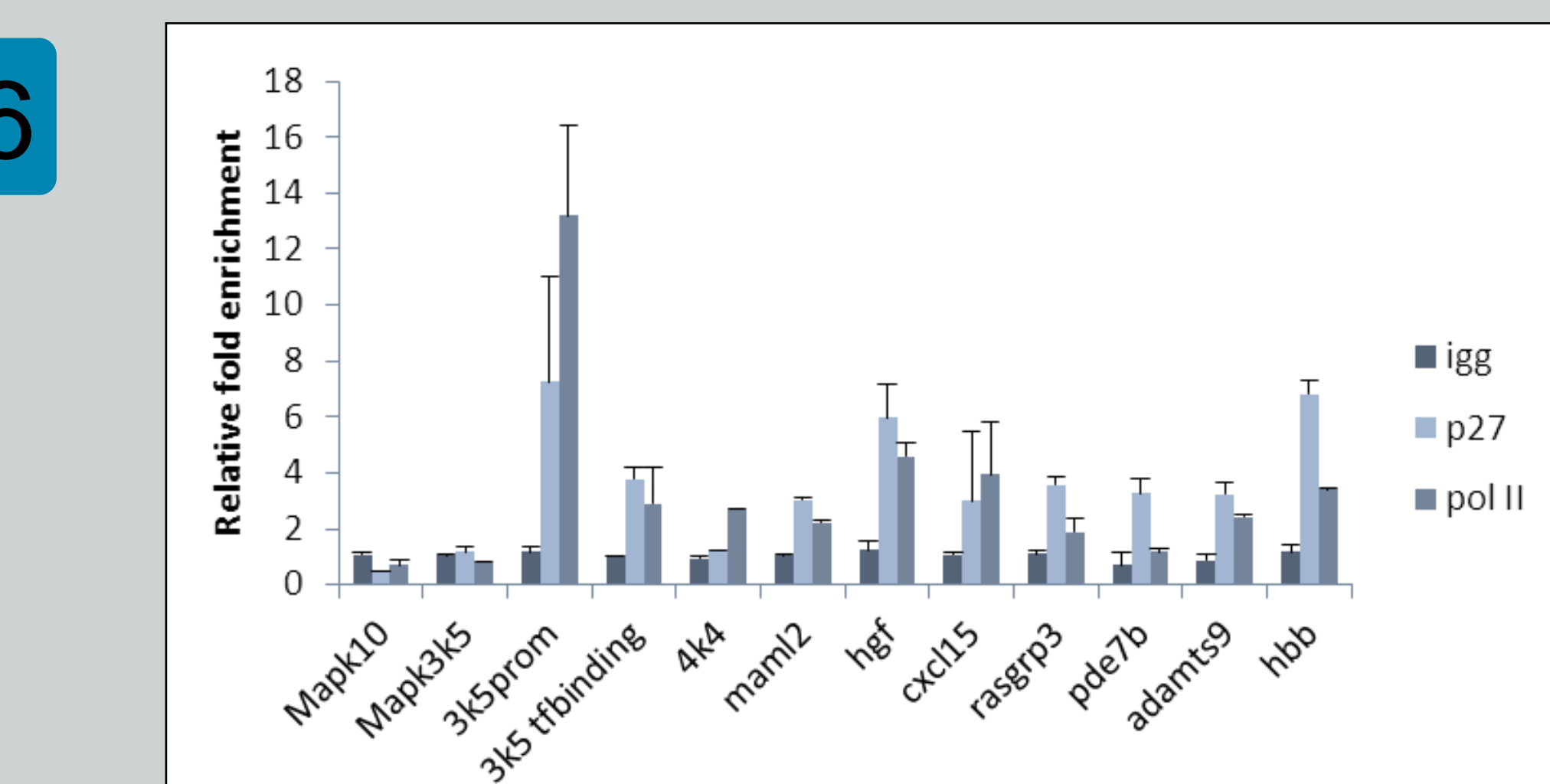
**Fig 1.** Gene Ontology classification of identified proteins



**Fig 3.** Co-IP assays carried out to validate the *in vivo* interaction between p27 and RNA polymerase II



**Fig 5.** Affinity Chromatography assays carried out to validate the direct interaction between p27 and the K subunit of RNA polymerase II



**Fig 6.** ChIP experiments show a correlation between the loading of RNA pol II on the promoters of specific target genes and the presence of p27 on these promoters.

Gene Name	Protein Name	Spectrums	Peptides	Single Peptides
Dhx9	DEAH(Asp-Glu-Ala-His) box polypeptide 9	121	45	45
Ascc3l1	Protein Ascc3l1	52	38	38
Polr2a	DNA-directed RNA polymerase	52	36	36
Ncl	Nucleolin	138	31	31
Sf3b1	Protein Sf3b1	70	30	30
LOC100911576	Heterogeneous nuclear ribonucleoprotein C	195	28	28
Lmna	Lamin A, isoform CRA_b	68	29	27
Acin1	Protein Acin1	87	27	27
Cps1	Carbamoyl-phosphate synthase [ammonia], mitochondrial	45	26	26
Eftud2	Protein Eftud2	30	20	20
Hsp90b1	Endoplasmic	25	19	19
Hp1bp3	Heterochromatin protein 1-binding protein 3	47	18	18
Thrap3	Thyroid hormone receptor-associated protein 3	75	19	18
Vcp	Transitional endoplasmic reticulum ATPase	30	17	17
Pnn	Protein Pnn	51	17	17
Polr2b	DNA-directed RNA polymerase	18	16	16
Srsf1	Protein Srsf1	192	17	16
Nol5a	Nucleolar protein 5A	31	15	15
Smc3	Structural maintenance of chromosomes protein 3	21	15	15
Eif4a3	Eukaryotic initiation factor 4A-III	29	14	14

**Fig 2.** The 20 most significant proteins identified. We can see highlighted in green different subunits of RNA polymerase II

Signal Used	Z-Factor	Z-Score	CI P-Value	Assay	Inter-assay CV	Neg Signal Used	Fold Increase Over Neg	Protein Description
65446	0,97	5,29	9,79E-05	50 ng/ul #1	21%	294	222,6	Transcription cofactor HES-6
65439	0,97	5,29	9,79E-05	50 ng/ul #1	32%	86	760,9	myogenic factor 6 (herculin)
65414	0,97	5,29	9,80E-05	50 ng/ul #1	19%	181	361,4	Forkhead box protein N3
65215	0,96	5,27	9,92E-05	50 ng/ul #1	15%	99	662,1	Ligand-dependent nuclear receptor corepressor-like protein
58293	0,87	4,56	1,30E-04	50 ng/ul #1	17%	60	971,6	nuclear receptor subfamily 4, group A, member 1
55887	0,62	5,33	1,03E-04	50 ng/ul #2	21%	196	285,9	Thyroid hormone receptor alpha
49299	0,94	3,65	1,75E-04	50 ng/ul #1	29%	101	488,1	REST corepressor 3 (RCOR3)
49103	0,84	3,63	1,86E-04	50 ng/ul #1	11%	53	935,3	estrogen-related receptor gamma
48879	0,88	4,49	1,19E-04	50 ng/ul #2	4%	52	949,1	vitamin D receptor (VDR)
47880	0,86	3,50	1,93E-04	50 ng/ul #1	17%	223	215,2	RNA polymerase II associated protein 2 (RPAP2)
47451	0,85	4,32	1,28E-04	50 ng/ul #2	13%	588	80,8	chromodomain helicase DNA binding protein 2 (CHD2)
47174	0,86	3,43	1,99E-04	50 ng/ul #1	14%	55	857,7	MLL3
46667	0,93	3,38	1,97E-04	50 ng/ul #1	27%	57	826,0	ETS1
43163	0,92	3,02	2,31E-04	50 ng/ul #1	18%	55	784,8	NF-kappa-B inhibitor zeta
42532	0,51	3,74	1,89E-04	50 ng/ul #2	8%	509	83,6	transcription elongation factor A (SII)-like 2 (TCEAL2)
42520	0,86	3,73	1,59E-04	50 ng/ul #2	28%	132	323,3	Homeobox protein Hox-B6
42803	0,89	3,53	1,70E-04	50 ng/ul #2	22%	301	135,6	SMYD3
37412	0,94	3,12	1,98E-04	50 ng/ul #2	9%	203	184,3	SMYD5
36928	0,84	3,07	2,12E-04	50 ng/ul #2	1%	49	753,6	polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa (POLR2K)
36632	0,94	3,03	2,06E-04	50 ng/ul #2	30%	212	173,2	ETS2
10980	0,89	3,39	2,29E-04	5 ng/ul #2	24%	60	184,5	NFKB1B
10963	0,88	3,38	2,30E-04	5 ng/ul #2	15%	24	456,8	Amino-terminal enhancer of split

**Fig 4.** Protoarray assay performed to identify proteins that directly interact with p27. Highlighted in green the K subunit of RNA polymerase II

## Conclusions

- p27 directly interacts with RNA polymerase II through the subunit K of this enzyme
- p27 might regulate transcription by loading RNA polymerase II on the promoters of specific target genes.
- Next Steps: p27 affects the functionality of RNA Polymerase II?

## Bibliography

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