The human β–globin as a model to study quality control of gene expression in the nucleus

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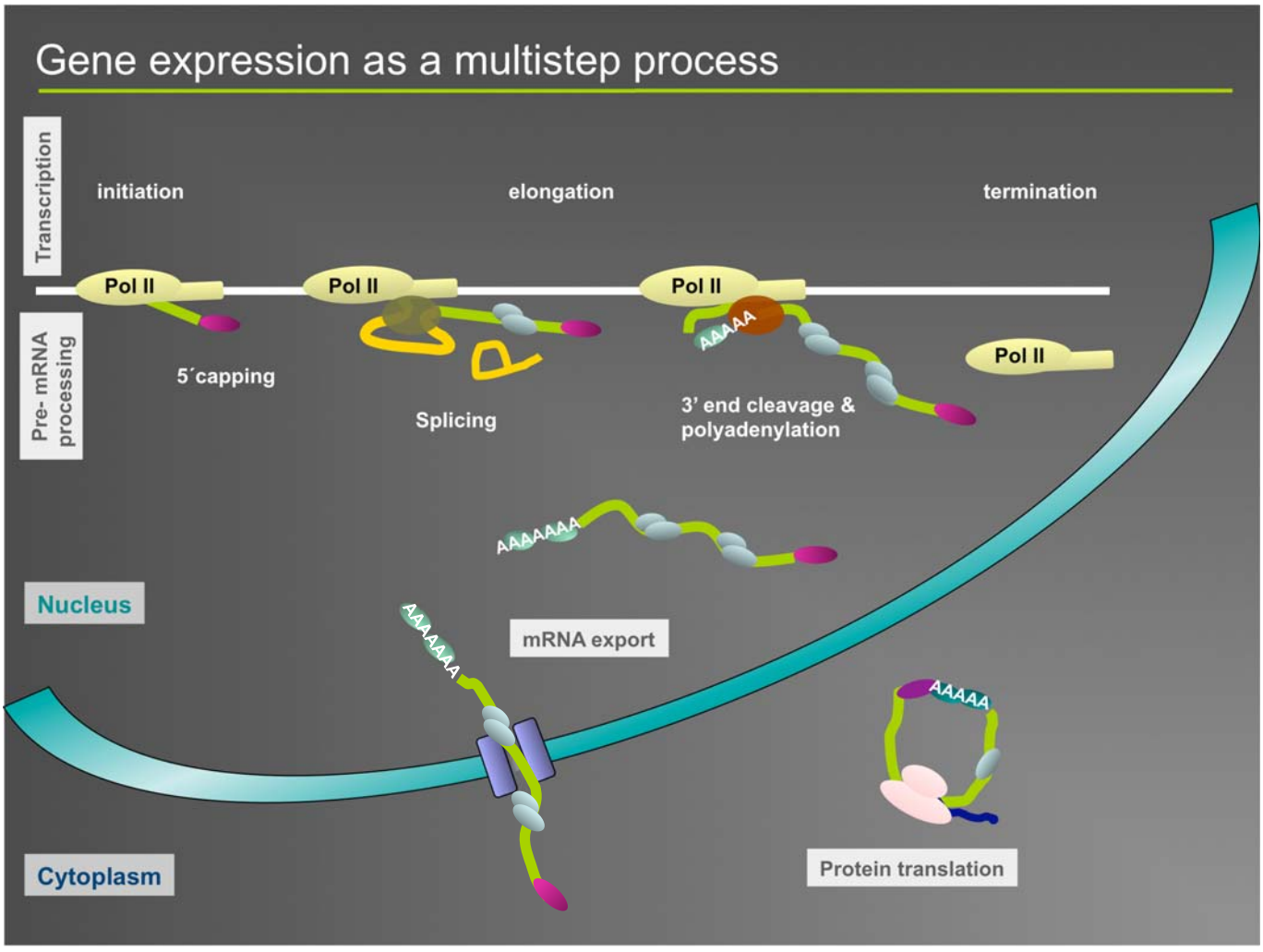
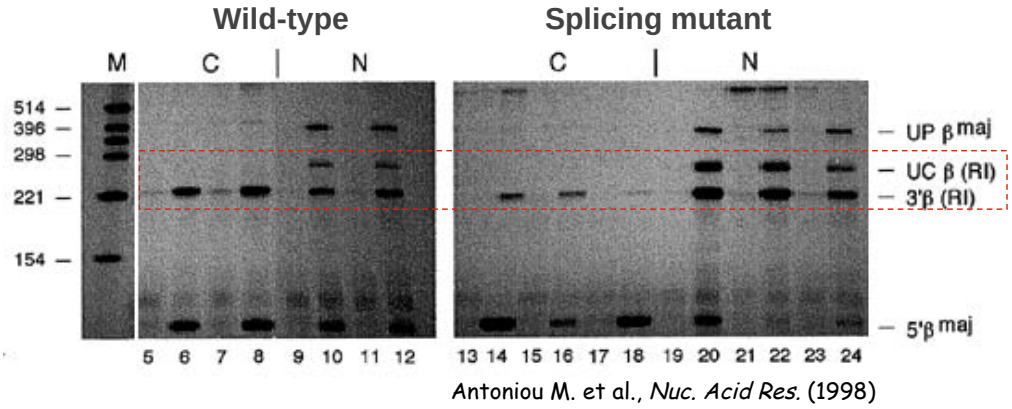


Thalassemia like human β-globin transcripts are not exported to the cytoplasm



Antoniou M. et al., *Nuc. Acid Res.* (1998)

Retention in the nucleus of incorrectly processed transcripts

Working Hypothesis: A quality control mechanism must exist in the nucleus to retain the incorrectly processed transcripts

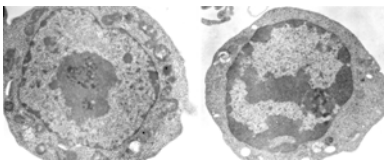
Goals of the work: Elucitate this quality control mechanism

1) Determine the intranuclear localisation of the retained transcripts

2) Identify the molecular players responsible for the retention

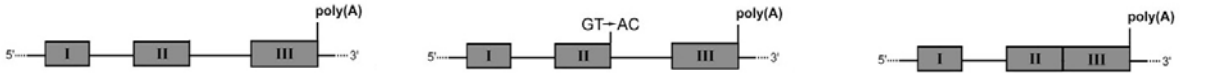
Model system: MEL cells stable transfected with the human β-globin gene

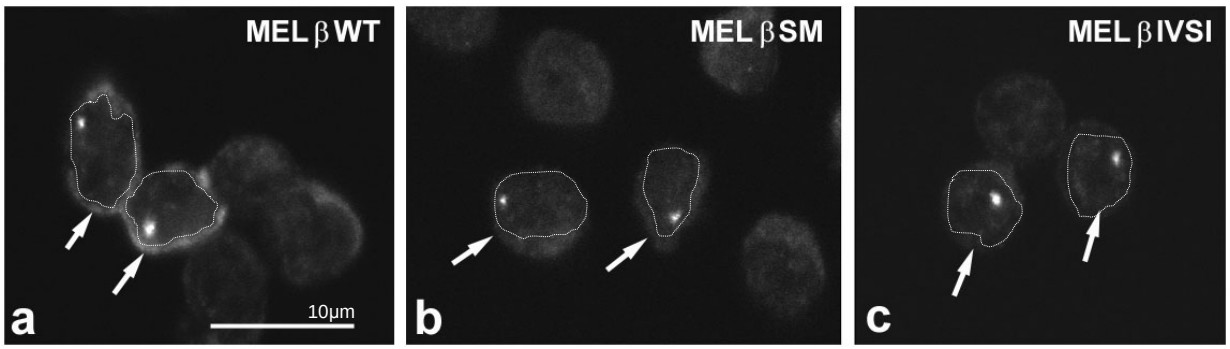
Murine erythroleukemia (MEL) cells



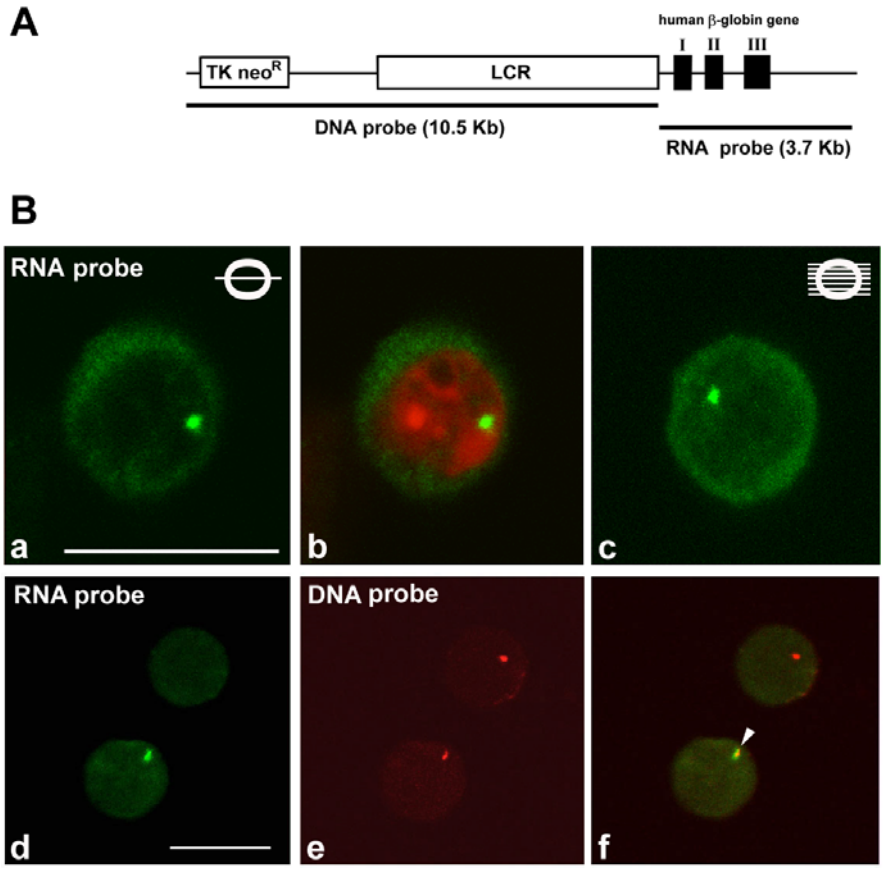
Uninduced 4-day induced

Wild-type **mRNA processing mutants**

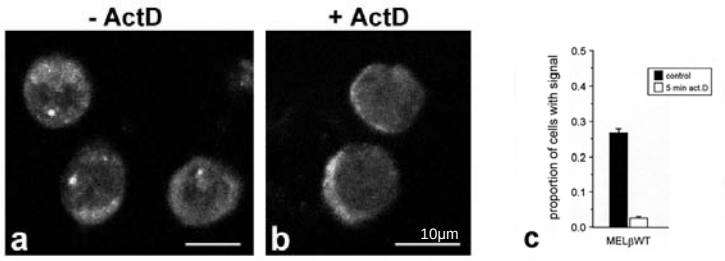




Human β-globin RNA is detected at the transcription site

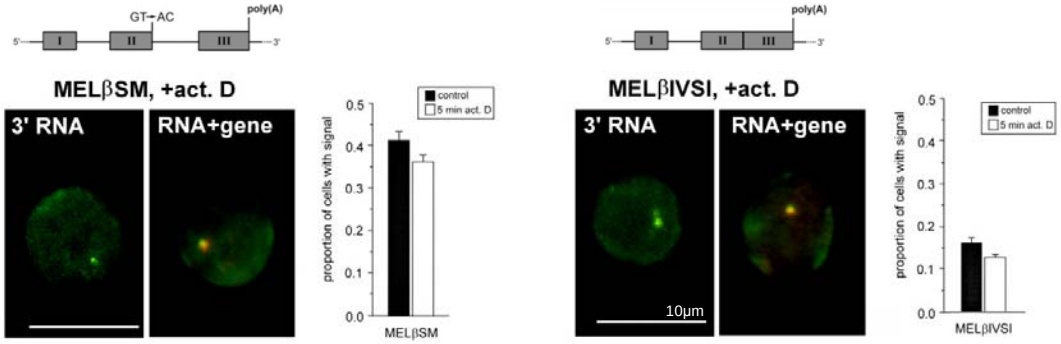


Inefficient processing impairs release of RNA from the site of transcription



Wild-type β-globin transcripts are no longer detected in nuclear foci after actinomycin D treatment

β-globin RNA processing mutants remain at the transcription site in cells treated with act.D



The quality control mechanism responsible for the nuclear retention of incorrectly processed transcripts operates at the site of transcription

Custódio N. *et al.*, EMBO J (1999)

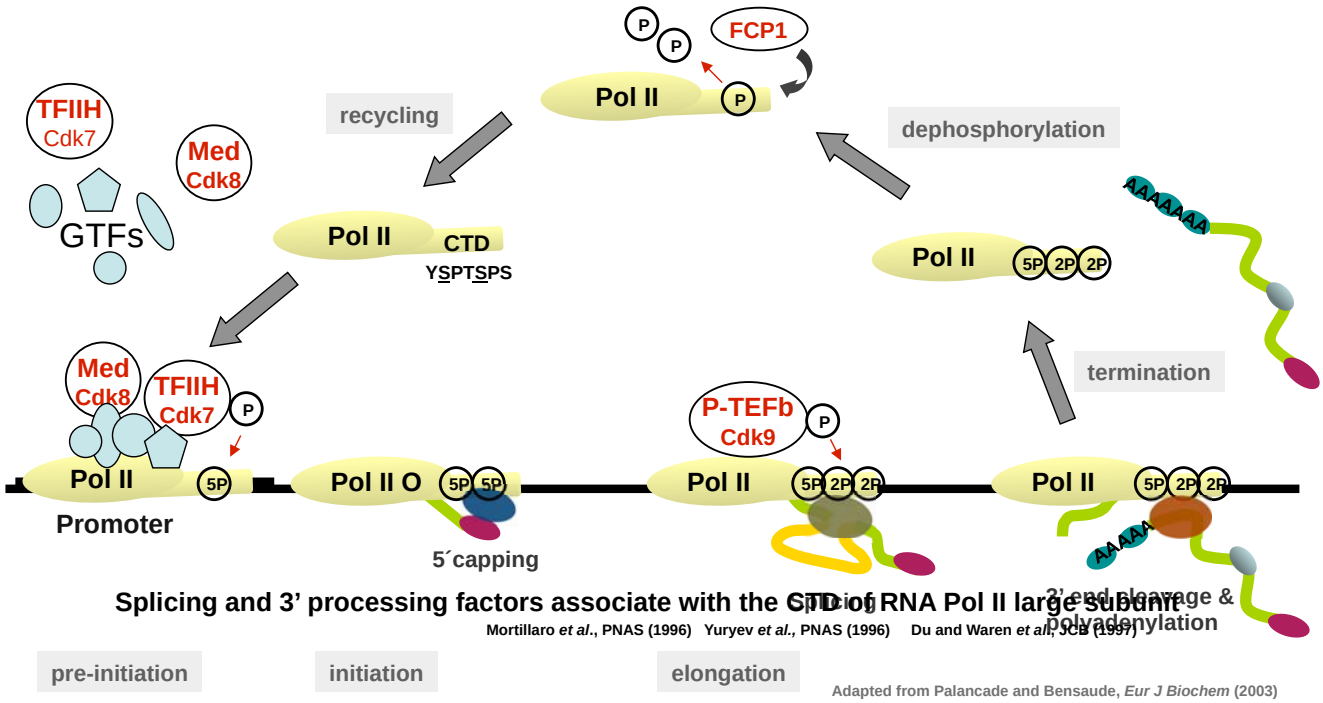
What are the molecular players involved in the quality control mechanism that operates at the transcription site?

Working Hypothesis:

1. Proteins essential for the release and/or transport of the transcripts from the gene locus are not recruited to mutant transcripts

2. The retention is mediated by proteins that are bound to the nascent transcripts and become stalled due to incomplete processing

Transcription cycle and pre-mRNA processing



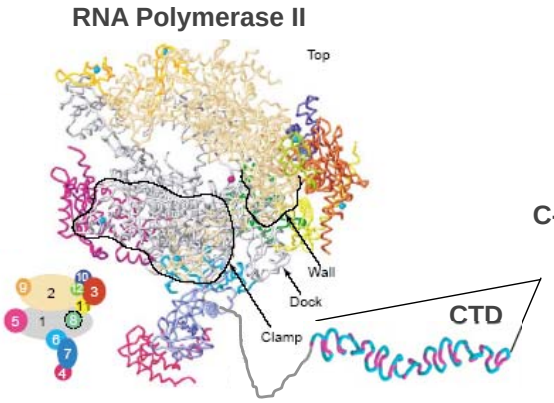
Splicing and 3' processing factors associate with the CTD of RNA Pol II large subunit

Mortillaro *et al*., PNAS (1996) Yuryev *et al.*, PNAS (1996) Du and Waren *et al*, JCB (1997)

Adapted from Palancade and Bensaude, *Eur J Biochem* (2003)

The processing mutants analyzed are able to assemble at least partially the processing machinery

Transcription cycle and pre-mRNA processing



Adapted from Cramer, P. *Curr Opin Gen & Dev.* (2004)

C-terminal domain of RNA Pol II LS

Tyr-Ser-Pro-Thr-Ser-Pro-Ser

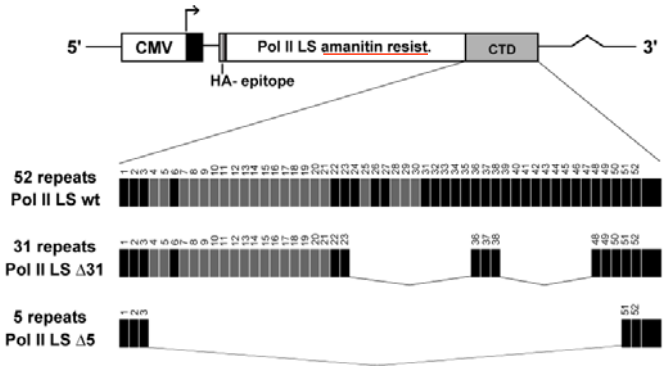
52 x in mammals
26 x in yeast

Splicing and 3' processing factors associate with the CTD of RNA Pol II large subunit

Mortillaro *et al.*, PNAS (1996) Yuryev *et al.*, PNAS (1996) Du and Waren *et al.*, JCB (1997)

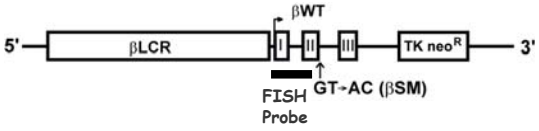
The release of the mutant transcripts from the transcription site could be blocked by the stalled or abnormal processing machinery associated with the CTD

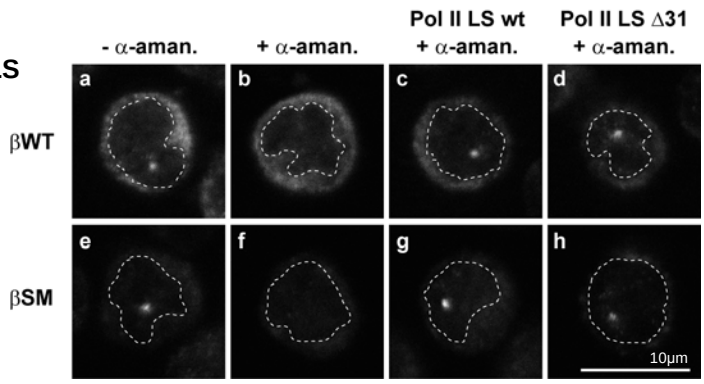
Establishment of MEL cell clones expressing α-amanitin resistant RNA Pol II LS with mutated versions of the CTD



RNA Pol II Δ5 fails to support LCR-dependent transcription

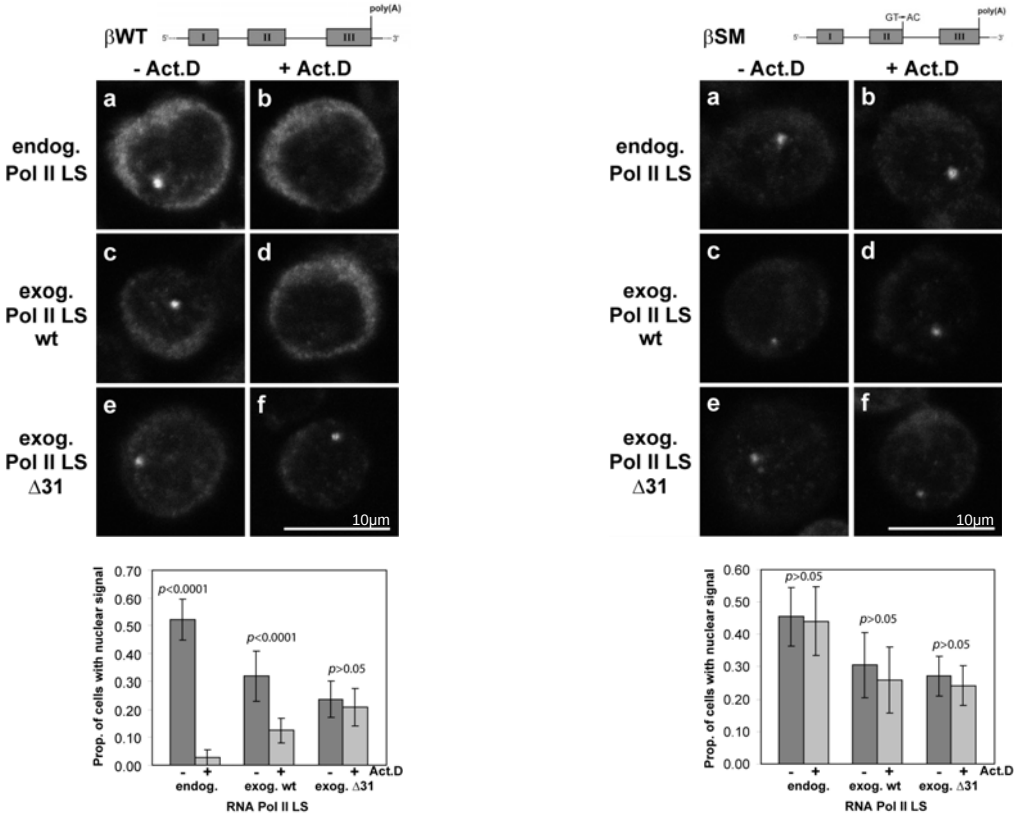
Transcription of human β-globin gene by exogenous α-amanitin resistant RNA Pol II LS





Custódio N. *et al.*, JCB (2007)

Δ31 CTD truncation impairs release of βWT RNA from the transcription site but has no effect on the retention of the splicing-defective RNA



Custódio N. *et al.*, JCB (2007)

Why are transcripts synthesized by RNA Pol II Δ31 retained at the site of transcription?

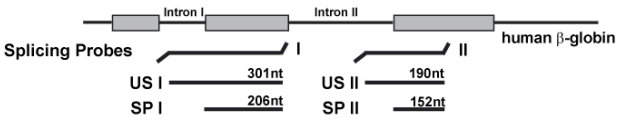
Truncation of the CTD reduces the efficiency of capping, splicing and 3' end cleavage.

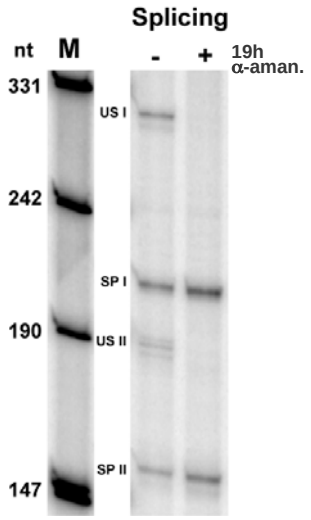
McCracken *et al*, Nature (1997)
McCracken *et al*, G&D (1997)
Fong & Bentley G&D (2001)

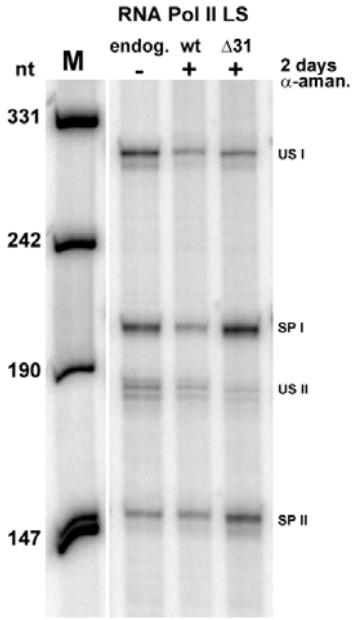
Are the RNA Pol II Δ31 transcripts correcly processed?

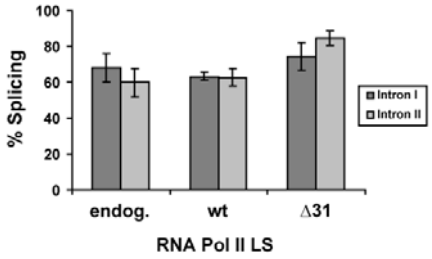
RNA transcribed by RNA Pol II LS Δ31 is spliced

RNase protection assay





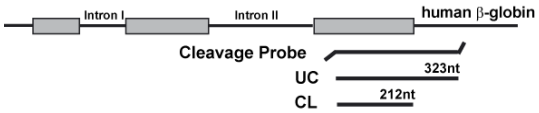


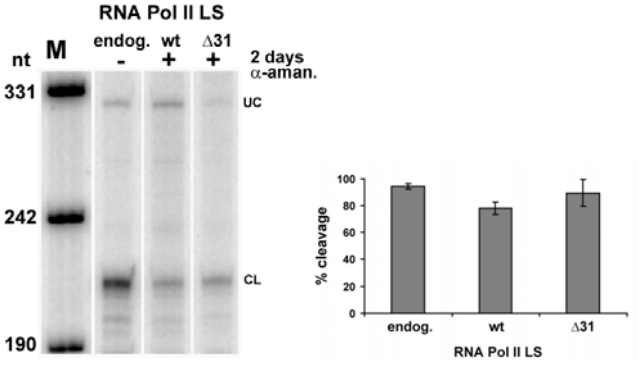


Custódio N. *et al.*, JCB (2007)

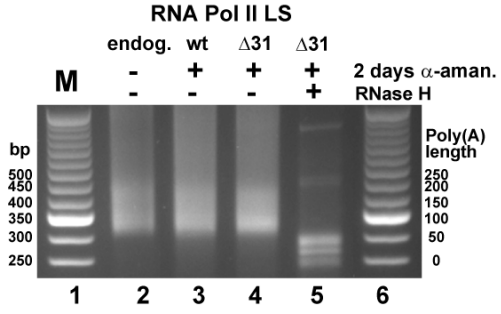
RNA transcribed by RNA Pol II LS Δ31 is 3' end cleaved and polyadenylated

RNase protection assay





Poly(A) tail length analysis (LM-PAT assay)



The CTD repeats missing in the Δ31 mutant are required for transcription site release but not for pre-mRNA processing

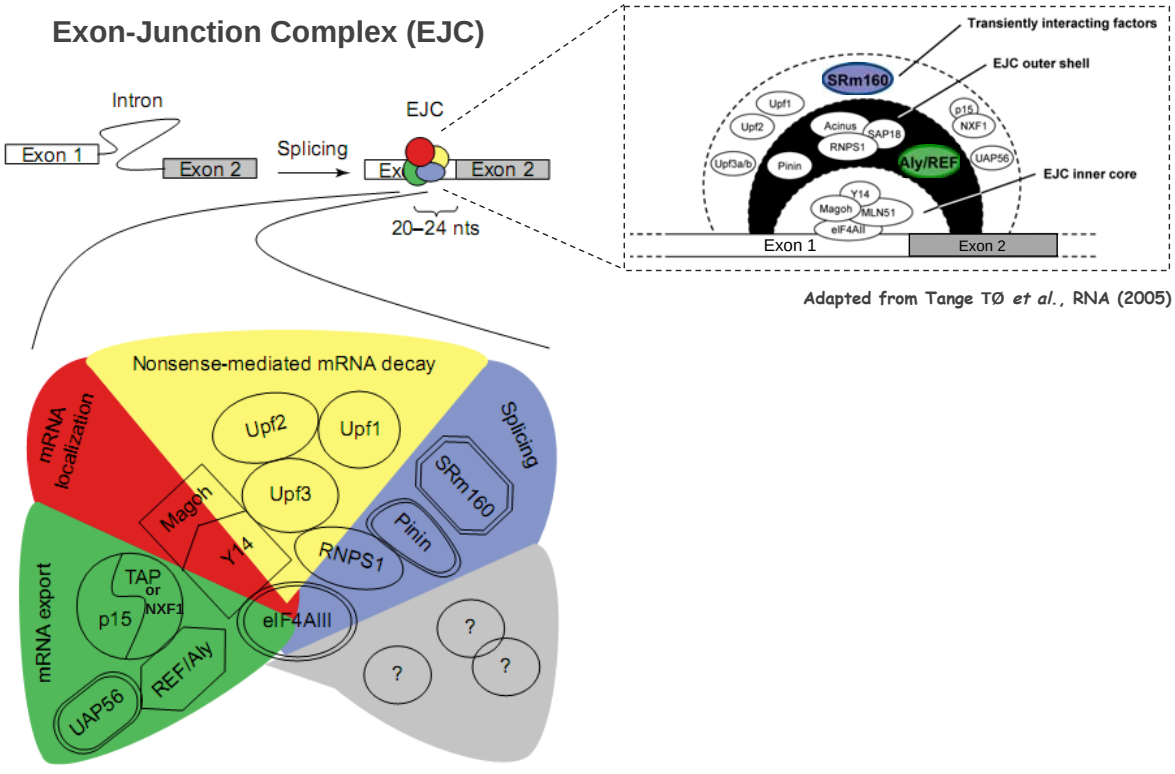
Custódio N. *et al.*, JCB (2007)

The CTD is necessary for recruitment of splicing factors to sites of transcription in vivo.

Misteli and Spector, *Mol Cell* (1999)

Are the missing repeats in CTD Δ31 preventing the recruitment of proteins required for release of the transcripts?

In vivo recruitment of exon-junction complex proteins to transcription sites in the nucleus

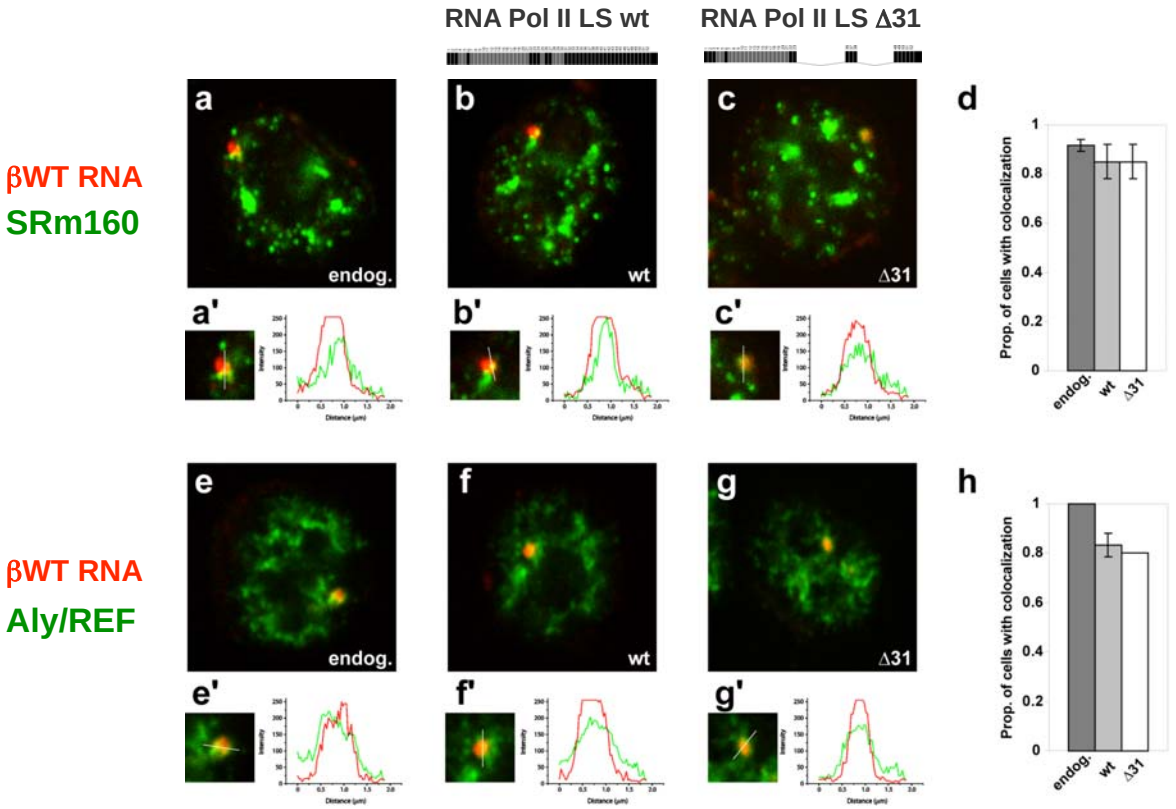


Adapted from Tange TØ *et al.*, RNA (2005)

Current Opinion in Cell Biology

Tange TØ *et al* Curr Opin Cell Biol., (2004)

EJC proteins are recruited to nascent transcripts synthesized by RNA Pol. II LS Δ31



Custódio N. *et al.*, JCB (2007)

In yeast, transcription site retention of abnormally processed transcripts requires the nuclear exosome subunit Rrp6.

Hilleren *et al.*, Nature (2001)

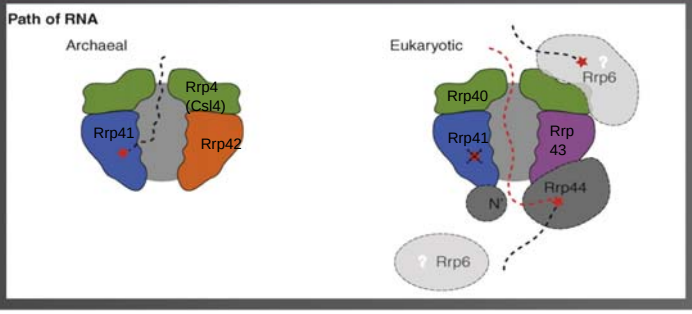
Libri *et al.*, MCB (2002)

The exosome is a complex of 3' to 5' exoribonucleases

Initially identified as an activity required for the 3'-end processing of rRNA precursors
Components named Rrp (rRNA-processing) proteins

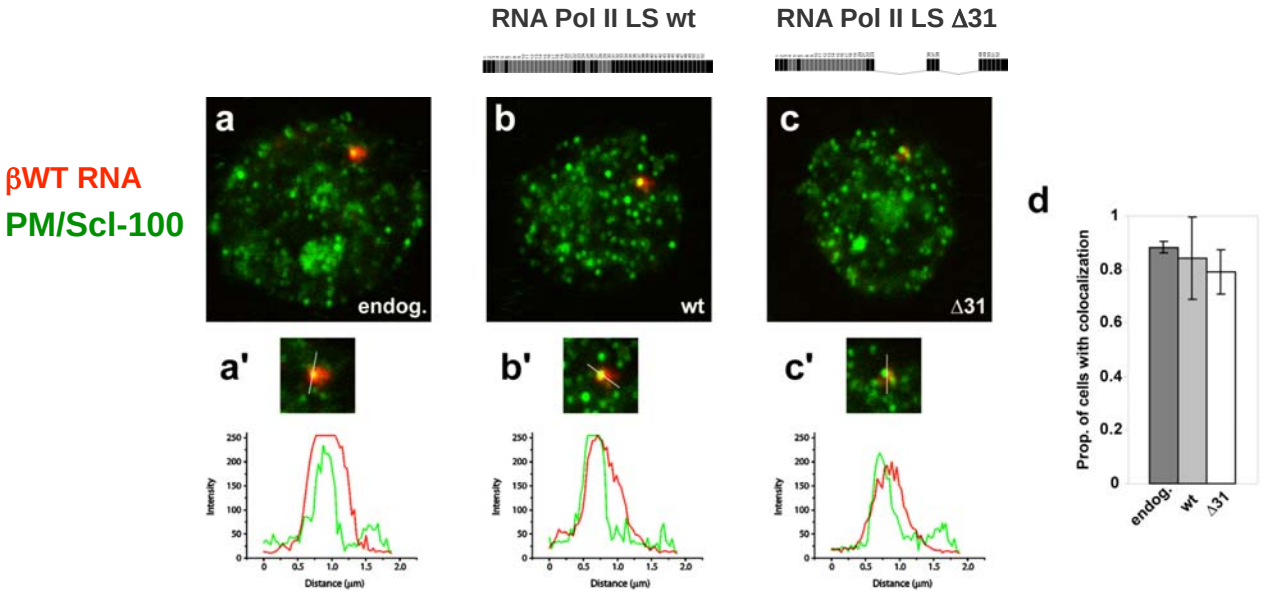
Participates in most nuclear and cytoplasmic 3' to 5' RNA -degradation and -processing pathways

	Domains	Archaea[b]	*S. cerevisiae*[c]	Loc[d]	Human[c]	Loc[d]
Exosome core	RNasePH	Rrp41	Rrp41p (Ski6p)	n+c	hRrp41 (hSki6; EXOS4)	n+c
		Rrp42	Rrp42p	n+c	hRrp42 (EXOS7)	n+c
		(Rrp41)	Rrp46p	n+c	hRrp46 (EXOS5)	n+c
		(Rrp42)	Rrp43p	n+c	hRrp43 (OIP2; EXOS8)	n+c
		(Rrp41)	Mtr3p	n+c	hMtr3 (EXOS6)	n+c
		(Rrp42)	Rrp45p	n+c	hRrp45 (Prn/Scl-75; EXOS9)	n+c
	S1 and KH domains	Rrp4	Rrp4p	n+c	hRrp4 (EXOS2)	n+c
		(Rrp4)	Rrp40p	n+c	hRrp40 (EXOS3)	n+c
		Csl4	Csl4p	n+c	hCsl4 (EXOS1)	n+c
Exonuclease	RNase II	–	Rrp44p (Dis3p)	n+c	hRrp44 (hDis3)	n+c
	RNase D	–	Rrp6p	n	hRrp6 (PM/Scl-100; EXOS10)	n+c



Schmid & Torben, TBS (2008)

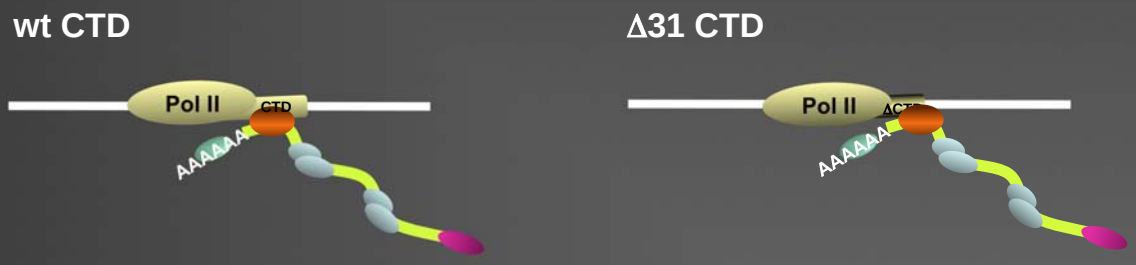
PM/Scl-100 (hRrp6) exosome subunit is recruited to nascent transcripts synthesized by RNA Pol. II LS Δ31



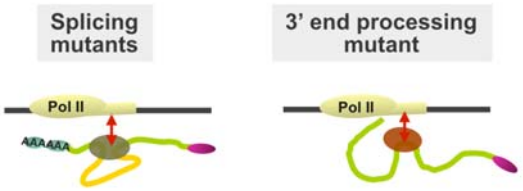
Recruitment of neither EJC proteins nor nuclear exosome Rrp6 class of proteins to nascent mRNA is sufficient for its release from the site of transcription

Working hypothesis

The Δ31 CTD truncation mutant may be impaired to recruit protein factors required to complete the maturation of spliced and 3' end processed mRNA into export-competent mRNPs.

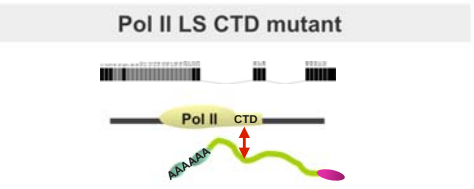


Conclusions



Retention near the transcription site

Custódio N et al *EMBO J* (1999)



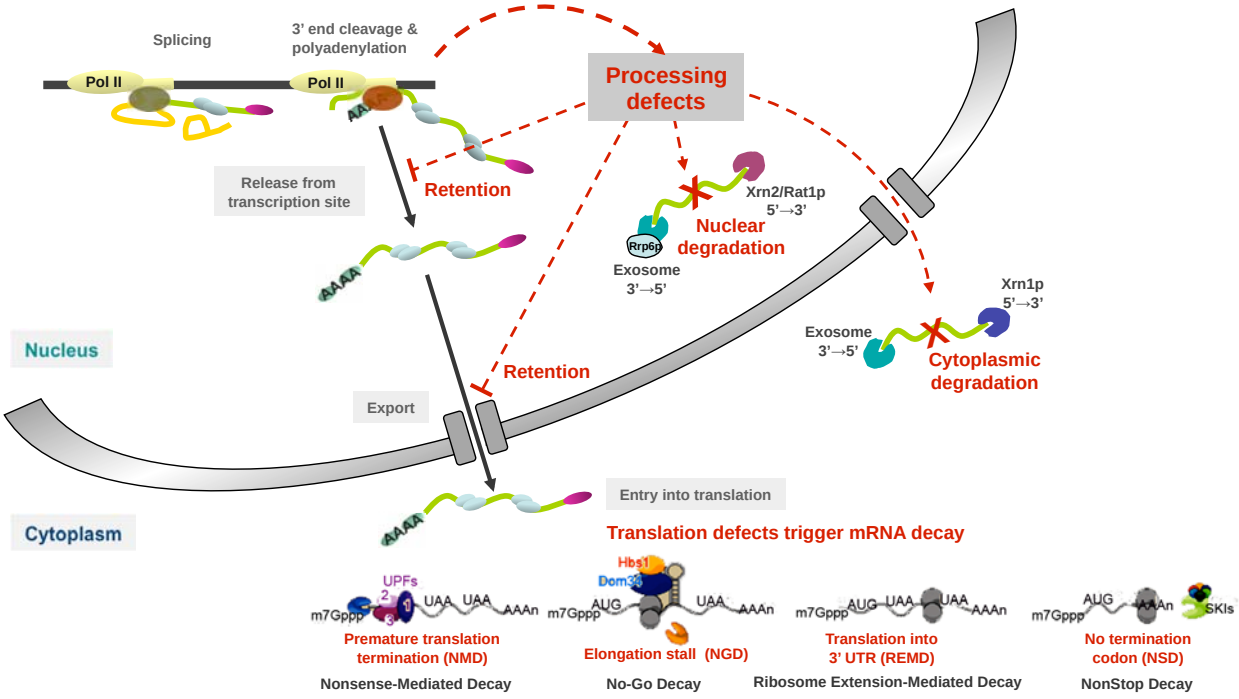
Retention near the transcription site of fully processed mRNA

Custódio N et al *JCB* (2007)

mRNA release from the site of transcription is an important step in mRNA biogenesis and an important checkpoint for mRNA integrity

The CTD is involved in processes that control the release of the mRNA from the site of transcription by a mechanism independent of pre-mRNA processing

Quality Control of mRNA biogenesis



Adapted from Doma, M and Parker R *Cell* (2007)

Checkpoints are operating in the nucleus to ensure the quality of the mRNA that cross the NPC and reach the translation machinery in the cytoplasm

What are the molecular mechanisms for this checkpoints?

Acknowledgements

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