

The CCR4-NOT deadenylase complex mediates tubulin autoregulation via specific adapters CNOT10 and CNOT11

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Abstract

Tubulin autoregulation maintains cellular microtubule homeostasis by triggering rapid degradation of tubulin mRNAs in response to an increase in soluble α - and β -tubulin levels. Through siRNA knock-down of several RNA decay pathways coupled with Roadblock-qPCR kinetic measurements, we independently validate and extend prior work by identifying the CCR4-NOT deadenylase complex components CNOT1, CNOT10, and CNOT11 as central effectors both in tubulin autoregulation and basal tubulin mRNA stability. In contrast, depletion of ribosome quality control and other decay factors has little effect. These findings corroborate CCR4-NOT adaptors as essential effectors of tubulin autoregulation and provide molecular entry points to dissect microtubule homeostasis.

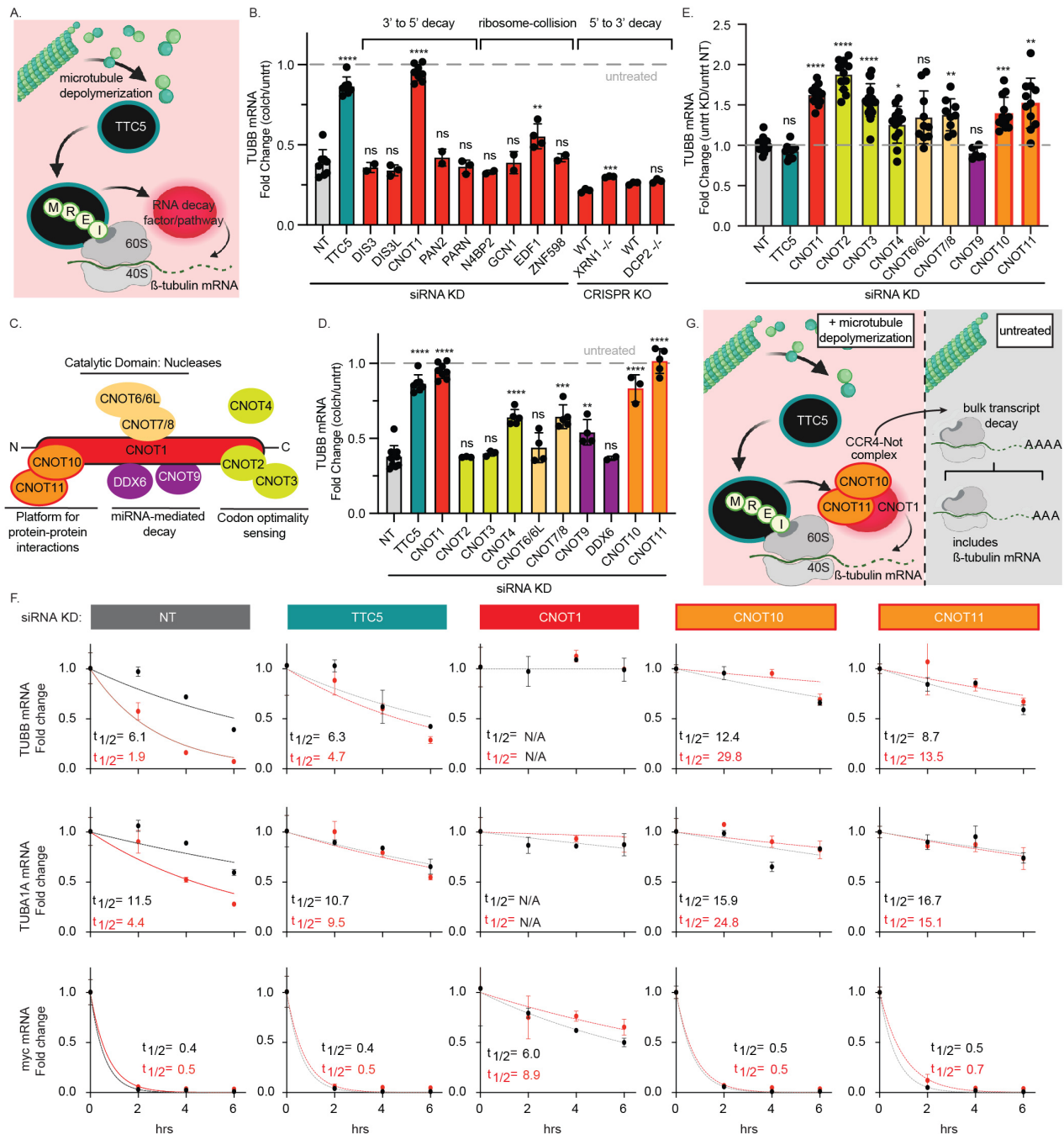


Figure 1. Tubulin autoregulation is mediated by CCR4-NOT complex and its subunits CNOT10 and 11:

(A) Graphical depiction of tubulin autoregulation—an increase in soluble tubulin due to microtubule depolymerization (such as upon colchicine treatment) results in co-translational degradation of tubulin transcripts *via* TTC5. (B) RT-qPCR analysis of TUBB mRNA expression upon colchicine treatment (10 μ M; 4hrs) of either CRISPR gene-edited lines knocked-out for the indicated gene (XRN1^{-/-} in A549, DCP2^{-/-} in HEK-293) or HEK-293 cells first transfected for 72 hrs with the panel of siRNAs to induce knock-down of the indicated gene. Data are graphed as the fold change in response to colchicine relative to the untreated condition for each knock-out or siRNA KD (preceded by normalization to housekeeping gene Glyceraldehyde 3-Phosphate Dehydrogenase [GAPDH]). For the CRISPR KO lines, each point on the graph reflects three biological replicates. For the siRNA KDs, each point represents an independent experiment comprised of two biological replicates. Error bars indicate mean $\pm$ SD and significance calculated by Student's two-tailed t-test to non-targeting (NT) control. (C) Graphical depiction of the CCR4-NOT deadenylase complex with associated factors and functions. (D) RT-qPCR analysis of TUBB mRNA expression upon colchicine treatment (10 μ M, 4 hrs) following transfection of HEK-293 cells for 72 hrs with the panel of indicated CCR4-NOT-centric siRNAs. Fold change and significance calculated as in (B). (E) Normalization of RT-qPCR data to the NT untreated condition to assess baseline changes in TUBB mRNA levels upon siRNA knock-down of the indicated genes—each point represents an independent replicate across a minimum of 3 independent experiments. Error bars indicate mean $\pm$ SD and significance calculated by Dunnett's One-way ANOVA to the NT control. (F) Measurement of mRNA decay rates in untreated and colchicine-

treated cells by Roadblock-qPCR upon knock-down of tubulin autoregulation-implicated factors. HEK-293 cells were transfected with indicated siRNAs for 72 hrs followed by simultaneous addition of 4sU +/- 10 μ M colchicine for collection at 2-, 4- and 6-hours post-treatment alongside a -4sU control. All fold changes were calculated relative to the -4sU untreated control for each siRNA KD condition (following normalization to GAPDH control). mRNA half-lives in untreated (black) versus colchicine-treated cells (red) were calculated using a single-phase exponential decay model (confidence intervals provided in Table 1 beneath Roadblock-qPCR methods); $n = 3$ biological replicates. Error bars indicate mean $\pm$ -SD. (G) Graphical summary of CCR4-NOT complex regulation of tubulin transcript levels constitutively, and upon increase in soluble tubulin levels. Graphics generated using BioRender.

Description

Microtubules, cytoskeletal components underlying intracellular transport, motility and morphology, are non-covalent polymers that self-assemble from $\alpha\beta$ -tubulin heterodimers. Microtubule growth rate and abundance are directly dependent on the concentration of these heterodimers (Mitchison & Kirschner, 1984). Cells possess a widely conserved feedback mechanism to monitor and maintain tubulin levels termed tubulin autoregulation. When cells sense an influx of tubulin subunits, such as upon drug-mediated microtubule depolymerization, deflagellation or changes in shape (Baker, Schloss, & Rosenbaum, 1984; Ben-Ze'ev, Farmer, & Penman, 1979; Cleveland, Lopata, Sherline, & Kirschner, 1981; Mooney, Hansen, Langer, Vacanti, and Ingber, 1994; Pachter, Yen, & Cleveland, 1987), they induce co-translational tubulin mRNA degradation. The process requires the first four amino acids of β -tubulin, MREI, which are common to all vertebrate β -tubulin isoforms (David A. Gay, Yen, Lau, & Cleveland, 1987; Pachter et al., 1987; Yen, Gay, Pachter, & Cleveland, 1988), allowing their tandem regulation. Increased soluble tubulin also coordinately triggers the degradation of α -tubulin transcripts, which begin with amino acids MREC (Bachurski, Theodorakis, Coulson, & Cleveland, 1994). While this unique feedback mechanism was first characterized over four decades ago, the molecular players mediating this process remained unknown. Recent identification of the tetratricopeptide repeat domain 5 (TTC5) protein marked the first molecular inroad into the pathway (Lin et al., 2020). Upon microtubule depolymerization, TTC5 binds both the ribosome and the MREI motif as it emerges from the ribosome exit channel (Fig 1A). While this finding resolved how tubulin-translating ribosomes could be specifically recognized, it highlighted the requirement for additional downstream decay effectors.

Tubulin autoregulation necessitates the involvement of cellular RNA decay factors to carry out the final step of tubulin transcript destruction (Fig 1A). Transcript turnover in cells is complex and involves interconnected and regulated processes that act on various features within the mRNA. mRNA turnover may proceed due to activities of specialized enzymes at the 5' or 3' end of the transcript *via* deadenylation or decapping, respectively. Decay intermediates can be further processed from 5' to 3' by Xrn1 or from 3' to 5' by the exosome (Houseley & Tollervey, 2009). In addition to determining bulk mRNA decay, certain pathways or individual factors within these pathways can be targeted to specific transcripts by *cis* (sequence or structure-specific features) or *trans* (through interaction with adaptor proteins) regulatory factors, or through activation of mRNA surveillance pathways. To assess which, if any, of these decay pathways is responsible for the targeted decay of tubulin transcripts during the tubulin autoregulatory response, we selected a panel of genes representing discrete categories of RNA turnover: key genes associated with 3' to 5' decay through deadenylation (poly(A) specific ribonuclease subunit PAN2 [PAN2], poly(A)-specific ribonuclease [PARN], CCR4-NOT transcription complex subunit 1 [CNOT1]) as well as the exosome complex (DIS3 exosome endoribonuclease and 3'-5' exoribonuclease [DIS3], DIS3 like exosome 3'-5' exoribonuclease [DIS3L]) (Passmore & Collier, 2022; Tomecki et al., 2010) and 5' to 3' decay factors such as decapping mRNA 2 [DCP2] and 5'-3' exoribonuclease 1 [XRN1] (Li, Song, & Kiledjian, 2011; Liu & Moss, 2016). We also prioritized factors related to ribosome stalling and collision-sensing (NEDD4 Binding Protein 2 [N4BP2], General Control Non-depressible 1 [GCN1], Endothelial Differentiation Related Factor 1 [EDF1], Zinc Finger Protein 598 [ZNF598]) (D'Orazio et al., 2019; Sinha et al., 2020; Veltri, D'Orazio, & Green, 2020) as early studies demonstrated the reliance of tubulin autoregulation on active translation and reported enhanced tubulin transcript degradation at low levels of cycloheximide and emetine (D. A. Gay, Sisodia, & Cleveland, 1989) known now to trigger ribosome collisions (Juszkiewicz et al., 2018). We knocked down each gene by siRNA transfection (for validation *via* RT-qPCR see Extended Figure) or utilized previously characterized knock-out lines and measured β -tubulin (TUBB) transcript levels upon colchicine-induced microtubule depolymerization (Fig. 1B).

While knock-down of most factors has no significant effect on colchicine-induced tubulin autoregulation (which reduces TUBB transcripts to ~35% of the level in untreated cells), CNOT1 knock-down results in complete loss of tubulin decay, comparable to that upon knock-down of known pathway effector TTC5 (Fig 1B). CNOT1 is the central scaffold of the CCR4-NOT (Carbon Catabolite Repression 4-Negative On TATA-less) complex which is responsible for both global deadenylation functions as well as targeted degradation of transcripts through specific adaptor proteins (Hagkarim & Grand, 2020; Passmore & Collier, 2022; Shah et al., 2024; Shirai, Suzuki, Morita, Takahashi, & Yamamoto, 2014). The complex is organized into three main functional modules: an N-terminal domain comprised of subcomplex CNOT10 and 11, a central catalytic domain comprised of CCR4 (CNOT6 or 6L) and Caf1 (CNOT7 or 8) which possess deadenylase activity with differing specificities (Raisch et al., 2019; Yi et al., 2018) and a C-terminal or "Not" module composed of

CNOT2, CNOT3 and the transiently associated CNOT4 subunit (an E3 ubiquitin ligase), which function together in the detection of stalled or slowed ribosomes (Absmeier et al., 2022; Allen et al., 2021; Buschauer et al., 2020; Zhu, Cruz, Zhang, Erzberger, & Mendell, 2024) (Fig 1C). Direct interactions between CNOT1 and DDX6, and CNOT9 and TNRC6 can alternatively link CCR4-NOT complex activity to miRNA-dependent repression (Chen et al., 2014; Mathys et al., 2014). To gain further insight into which subfunctions of the complex are required for its role in tubulin autoregulation, we performed siRNA knock-down of each component in the complex (with combinatorial knock-down of potentially redundant catalytic components CNOT6/6L and CNOT7/8) and assayed for tubulin autoregulation upon colchicine-induced microtubule depolymerization. While we observed partial loss of tubulin autoregulation upon knock-down of several CCR4-NOT components, complete loss of tubulin decay upon CNOT1 knock-down was recapitulated only upon reduction of the factors comprising the N-terminal module, CNOT10 and CNOT11 (Fig 1D). These subunits have recently emerged as a key interaction platform for the targeted recruitment of cellular factors (Mauxion et al., 2022; Mauxion, Prève, & Séraphin, 2013) supporting a model in which this subcomplex serves as an adaptor module for direct or indirect recruitment to TTC5-associated tubulin-translating ribosomes (Fig 1G). We also note that knock-down of most CNOT1 complex components (with exception of CNOT6/6L and CNOT9) results in a baseline increase in tubulin transcript levels relative to the non-targeting control, even in the absence of microtubule depolymerization and even for subunits that had no impact on the autoregulation readout (Fig 1E). This is consistent with the canonical contribution of the CCR4-NOT complex to bulk mRNA turnover (Gillen et al., 2021; Passmore & Collier, 2022) and suggests that the CCR4-NOT complex in addition to its targeted role in tubulin autoregulation also acts constitutively in tubulin transcript turnover (Fig 1G).

While best known for its role in mRNA decay, the CCR4-NOT complex has also been shown to mediate transcriptional repression. We reasoned it could therefore have both direct and/or indirect effects on tubulin transcript levels (Collart & Struhl, 1994; Kruk, Dutta, Fu, Gilmour, & Reese, 2011; Winkler, Mulder, Bardwell, Kalkhoven, & Timmers, 2006). To distinguish between these possibilities and obtain finer resolution into the kinetics of tubulin decay in both untreated and colchicine-treated cells, we utilized Roadblock-qPCR (Watson, Park, & Thoreen, 2021; Watson & Thoreen, 2022). This method uses uridine-analog (4SU) incorporation and adduct addition to distinguish between newly transcribed and existing RNA pools for subsequent mRNA half-life determination while avoiding the general toxicity associated with the more traditional actinomycin D (“transcriptional shutoff”) approach for determining transcript turnover kinetics. As expected, microtubule depolymerization in control cells (NT) resulted in dramatic enhancement of tubulin transcript decay kinetics with TUBB and TUBA1A mRNA half-lives decreasing from ~ 6.1 to ~ 1.9 hrs and ~ 11.5 to ~ 4.4 hrs, respectively (Fig 1F) indicative of a robust tubulin autoregulation response. The untreated half-lives are consistent with previously-reported values (Bachurski et al., 1994). In contrast, in TTC5 KD cells, this autoregulation-dependent reduction in TUBB and TUBA1A half-lives was lost. Knock-down of CNOT1, 10 and 11 all similarly abolished tubulin transcript decay in response to colchicine treatment, however their knock-down also extended the half-lives of tubulin even in untreated cells. This baseline increase in tubulin transcript abundance was consistent with the increase in steady-state tubulin levels we observed upon knock-down of each of these factors using conventional qPCR (Fig. 1E). Our results also align with a recent imaging-based screen in HeLa cells in which CRISPR knock-out of CNOT1 and more specifically CNOT10 and 11 strongly increased cellular tubulin intensity even in the absence of microtubule depolymerization (Funk et al., 2022).

In addition to characterizing the decay kinetics of TUBB and TUBA1A, we also assessed the turnover of myc mRNA, a transcript serving as a positive control for rapid turnover (Jones & Cole, 1987). Interestingly, myc half-life increases dramatically upon knock-down of CNOT1 but not upon knock-down of CNOT10 or CNOT11, suggesting it is a target of the CCR4-NOT complex but independent of CNOT10 and 11 (Fig 1F). This observation highlights the modular nature of the CCR4-NOT complex (Ogami, Hosoda, Funakoshi, & Hoshino, 2014) and a specialized role for CNOT10 and 11 in targeting specific transcripts for degradation.

During preparation of this work, the Hegde group similarly identified the CCR4-NOT complex and factors CNOT10 and 11 as the mediators of tubulin autoregulation through an iterative proximity-labeling approach (Höpfler et al., 2023). The protein SCAPER was also uncovered as the molecular bridge between tubulin-translating ribosome recognition and decay by binding to both TTC5 and CNOT11. Our work is a valuable corroboration of these findings using an entirely independent and complementary approach of RNA-decay pathway siRNA knock-down screening while also providing evidence that other canonical decay pathways are dispensable for this process. Together, these studies demonstrate how a general RNA processing complex can be specialized to specific substrates in a context-dependent manner and identify additional molecular handles for exploring the role of tubulin and microtubule homeostasis in cells and tissues.

Methods

Mammalian cell line culture and generation: All HEK-293 cells were cultured in DMEM+Glutamax (ThermoFisher 10564029) supplemented with 10% FBS (Life Technologies A5209402) at 37 °C and 5% CO₂. DCP2^{-/-} KO and WT HEK-293 derivative lines were generated and characterized as previously described and obtained courtesy of Mergerditch Kiledjian (Mauer et al., 2017). XRN1^{-/-} KO and WT A549 lines were generated and characterized as previously described

(Liu & Moss, 2016) obtained courtesy of Bernard Moss and maintained in RPMI media (ThermoFisher 11875093) supplemented with 10% FBS.

Drug treatments: Microtubule depolymerization was performed by administration of colchicine (Sigma C9754) resuspended in water as a 0.1 mM stock and diluted to a final concentration of 10 μ M.

siRNA knock-down in HEK-293: For siRNA knock-downs, 3.75×10^4 HEK-293 cells were reverse-transfected with Silencer Select siRNAs (reconstituted in water and stored at 20 μ M stock solution) using Lipofectamine RNAiMAX (ThermoFisher 13778150) to a final concentration of 40 nM according to manufacturer instructions. Where double-knockdowns (ex. CNOT6/6L, and CNOT7/8) or multiple siRNAs targeting the same gene (ex. PAN2, PARN) were employed, both siRNAs were included at a final concentration of 40 nM. For tubulin autoregulation experiments, at approximately 72 hours post-transfection, cells were either left untreated or treated with colchicine (10 μ M) by spiking in an additional 100 μ l media and collected at four hours post-treatment for subsequent RNA extraction and quantification. Four hour colchicine treatment was selected based on a timecourse of tubulin autoregulation in HEK-293s and to minimize toxicity in addition to siRNA gene-specific knock-down effects. Silencer select siRNAs (ThermoFisher) used in this study include Negative Control No. 1 (4390843), TTC5 (s40806), CNOT1 (s22842), CNOT10 (s24720), CNOT11 (s30995), CNOT2 (s226689), CNOT3 (s9628), CNOT4 (s9631), CNOT6 (s33101), CNOT6L (s48341), CNOT7 (s26637), CNOT8 (s225112), CNOT9 (s17424), N4BP2 (s31353), DDX6 (s4010), GCN1 (s21626), ZNF598 (s56951), EDF1 (s16609), PAN2 (s19252 and s19253), PARN (s10048 and s10047), DIS3 (s22607), DIS3L (s41866).

RNA extraction, cDNA preparation and RT-qPCR: All RNA extractions were carried out using the Zymo Direct-zol RNA Mini- (R2053) or MicroPrep (R2063) Kits according to manufacturer's instructions and quantified by nanodrop for generation of cDNA from 500-1000 ng of RNA input using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies 4368814). RT-qPCR was performed using Powerup Sybrgreen MasterMix (ThermoFisher A25777) with the primer pairs listed below (either previously described (Lin et al., 2020) or designed using NCBI PrimerBlast) and run on a QuantStudio 6 Flex Real-time PCR System according to manufacturer's instructions. Each sample was assayed in technical duplicate. For analysis, cDNA input was normalized (dCt) to GAPDH, and expression fold changes were calculated using the ddCt method to a normalizing control (either the untreated or non-targeting condition).

Roadblock-qPCR for transcript kinetics measurements: To assay mRNA transcript turnover kinetics, Roadblock-qPCR was performed as previously described (Watson et al., 2021; Watson & Thoreen, 2022). Specifically, 300,000 HEK-293 cells were reverse transfected with indicated Silencer Select siRNAs (as described above). At 72 hours post-transfection, cells were treated with 400 μ M 4-thiouridine (4sU) (Cayman Chemical 16373) with or without commensurate addition of colchicine (10 μ M) and cells were harvested at intervals of 2 hrs after addition over a time course of 6 hrs. Cells incubated for the duration of the time course +/- colchicine but without the addition of 4sU were harvested as the normalizing control. RNA was extracted using the Zymo miniprep kit as above, treated with N-ethylmaleimide (NEM) (Sigma E3876) and reisolated using RNAClean XP beads (Beckman Coulter) according to manufacturer instructions. Throughout, steps involving 4sU prior to reaction with NEM were performed in the dark to minimize light exposure. The purified RNA was then converted to cDNA using an oligod(T)18 primer (NEB S1316) and Protoscript II reverse transcriptase (NEB M0368) and quantified by RT-qPCR as above, with 5'-biased gene-specific primers including those for GAPDH and myc as previously described (Watson et al., 2021) and noted in primer table using Powerup Sybrgreen MasterMix according to manufacturer's instructions. All fold changes were calculated using the ddCt method relative to the -4sU untreated control for each siRNA KD condition (following normalization to GAPDH control). mRNA half-lives were calculated using a single-phase exponential decay model in Graphpad Prism (Table 1).

		siRNA KD:				
		NT	TTC5	CNOT1	CNOT10	CNOT11
TUBB mRNA	untrt	6.1 [4.1-10.1]	6.3 [4.0-11.6]	N/A [N/A]	12.4 [9.2-18.2]	8.7 [6.9-11.3]
	+colch	1.9 [1.5-2.4]	4.7 [3.2-7.3]	N/A [N/A]	29.8 [7.3-N/A]	13.5 [8.1-30.7]
TUBA1A mRNA	untrt	11.5 [6.9-26.4]	10.7 [7.7-16.4]	N/A [N/A]	15.9 [9.6-37.1]	16.7 [11.7-28.9]
	+colch	4.4 [3.3-5.9]	9.5 [6.6-15.3]	N/A [N/A]	24.8[14.5-69.2]	15.1 [10.9-23.5]
myc mRNA	untrt	0.4[N/A-0.6]	0.4 [N/A-0.7]	6.0 [3.6-11.4]	0.5 [0.4-0.6]	0.5 [0.4-0.6]
	+colch	0.5 [N/A-0.7]	0.5 [N/A-0.8]	8.9 [5.1-21.2]	0.5 [0.4-0.7]	0.7 [0.5-0.8]

Table 1. Half-lives with confidence intervals [CI] for TUBB, TUBA1A and myc mRNA upon indicated siRNA knock-down.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism 9. Single, double, triple and quadruple asterisks indicate $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$ respectively, ns=non-significant for $p > 0.05$.

Reagents

RT-qPCR Primer Pairs:

Gene	Forward (5'→3')	Reverse (5'→3')
TTC5	GGCTTCACCGAATTCAGCAC	TGAGGCTTCCCATTACCAC
GAPDH	AACATCATCCCTGCCTCTACTGG	GTTTTCTAGACGGCAGGTCAGG
DIS3	TTGCATCATACAGCGAGTGG	GCCAAGTCTTTGGCTGAGTC
DIS3L	AGAAGGTGCTGCTGCTGAG	ACTTCCAGTCTGGGATCACG
N4BP2	CCGCTCCAGAAGCAGTAAGAA	ATCTTGCACAATCCAGCAGT
GCN1	AGCAAAGTCAAGCCTCCGAA	CCAGCCAGTCCTTTCACGAT
EDF1	AAGGTGATCCAGCAAGGTCG	CCTTCTCGATGGGCTTTCCA
ZNF598	TGCTCTACCAAGATGCGGG	CCTTGCGGAGTACCACTT
PARN	CAAGCTCAGTGTGCGAAATC	TTTACAGCTCCCAGCACAG
PAN2	TCTTCCCGTCCAGGAATCAG	AGGATGAGTAGCGCTCCAAG
DDX6	TAAAACAGCATGAGCACGGC	GTGGAGGTCACATCCGAAGTT
CNOT1	CCAGTAGGTGGTCTTGGCAC	CTGAGACAAGTCCGAAGGTTTC

CNOT2	TAGCCCCAGGGAAACGGTAG	GTCGTGTCACAGGAAGGACC
CNOT3	CCGGGGCGCGAGAAAAAG	TGAGGCAGCGATCAATCTCA
CNOT4	ACAGCAAACCCACCTCAAA	GCCAGTGTGGAGGATTGTCA
CNOT6	ACAGAACAACCACCTCCAAGG	TGTAGTCCCAGTTTAGCGCC
CNOT6L	TAGGACCGAGAGTGTGGGAA	ATGTACTTAGGCTCCGCACTC
CNOT7	CTGGGCGAGAGGTGTCTATG	AGATGCCAAGCATCAAAATGTTA
CNOT8	ATCCGGGGTAGAGGAAAAGA	GTTTTACAGTGCAAGCCAGACA
CNOT9	GTCCGGCTGTGGAAGAGAG	GACTGGTGTGCTGTCAAGGT
CNOT10	CGTCTGCCCACTCCTCTAGC	ACTGACCTGTGCCTTCATGTT
CNOT11	TTCAAAAAGACGCCTCGCCA	GAGGCGGTGGACGAATAAAC
TUBB	GAAGCCACAGGTGGCAAATA	CGTACCACATCCAGGACAGA
TUBA1A	CCACAGTCA TTGA TGAAGTTCG	GCTGTGGAAAACCAAGAAGC
MYC	CGTCCTCGGATTCTCTGCTC	GCTGCGTAGTTGTGCTGATG
GAPDH (5')	TTCTTTTGCGTCGCCAGCCGA	ACCAGGCGCCAATACGACCA

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Extended Data

Description: Validation of siRNA knock-down efficiency for RNA decay and CCR4-NOT complex factors. RT-qPCR for each gene-of-interest to assess degree of knock-down relative to a NT control siRNA at time of colchicine treatment, 72 hrs post-transfection. Data are graphed as the fold change relative to the NT control condition for each siRNA KD (preceded by normalization to housekeeping gene GAPDH). . Resource Type: Image. File: [250928_ExtendedFig.jpg](#). DOI: [10.22002/2j04v-t2c81](#)

References

- Absmeier E, Chandrasekaran V, O Reilly FJ, Stowell JAW, Rappsilber J, Passmore LA. 2022. Specific recognition and ubiquitination of slow-moving ribosomes by human CCR4-NOT. *bioRxiv*: 2022.07.24.501325. 26. DOI: [10.1101/2022.07.24.501325](#)
- Allen GE, Panasenkov OO, Villanyi Z, Zagatti M, Weiss B, Pagliazzo L, et al., Collart MA. 2021. Not4 and Not5 modulate translation elongation by Rps7A ubiquitination, Rli1 moonlighting, and condensates that exclude eIF5A. *Cell Reports*. 36: 109633. 27. DOI: [10.1016/j.celrep.2021.109633](#) PMID - 34469733
- Bachurski CJ, Theodorakis NG, Coulson RM, Cleveland DW. 1994. An amino-terminal tetrapeptide specifies cotranslational degradation of beta-tubulin but not alpha-tubulin mRNAs. *Molecular and Cellular Biology*. 14: 4076–4086. 9. DOI: [10.1128/mcb.14.6.4076](#) PMID - 8196646
- Baker EJ, Schloss JA, Rosenbaum JL. 1984. Rapid changes in tubulin RNA synthesis and stability induced by deflagellation in *Chlamydomonas*. *The Journal of cell biology*. 99: 2074–2081. 2. DOI: [10.1083/jcb.99.6.2074](#) PMID - 6209288
- Ben Zeev A, Farmer SR, Penman S. 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. *Cell*. 17: 319–325. 3. DOI: [10.1016/0092-8674\(79\)90157-0](#) PMID - 455467

- Buschauer R, Matsuo Y, Sugiyama T, Chen YH, Alhusaini N, Sweet T, et al., Beckmann R. 2020. The Ccr4-Not complex monitors the translating ribosome for codon optimality. *Science*. 368 28. DOI: [10.1126/science.aay6912](https://doi.org/10.1126/science.aay6912) PMID - [32299921](https://pubmed.ncbi.nlm.nih.gov/32299921/)
- Chen Y, Boland A, Kuzuoglu Ozturk D, Bawankar P, Loh B, Chang CT, Weichenrieder O, Izaurrealde E. 2014. A DDX6-CNOT1 Complex and W-Binding Pockets in CNOT9 Reveal Direct Links between miRNA Target Recognition and Silencing. *Molecular Cell*. 54: 737–750. 30. DOI: [10.1016/j.molcel.2014.03.034](https://doi.org/10.1016/j.molcel.2014.03.034) PMID - [24768540](https://pubmed.ncbi.nlm.nih.gov/24768540/)
- Cleveland DW, Lopata MA, Sherline P, Kirschner MW. 1981. Unpolymerized tubulin modulates the level of tubulin mRNAs. *Cell*. 25: 537–546. 4. DOI: [10.1016/0092-8674\(81\)90072-6](https://doi.org/10.1016/0092-8674(81)90072-6) PMID - [6116546](https://pubmed.ncbi.nlm.nih.gov/6116546/)
- Collart MA, Struhl K. 1994. NOT1(CDC39), NOT2(CDC36), NOT3, and NOT4 encode a global-negative regulator of transcription that differentially affects TATA-element utilization. *Genes & Development*. 8: 525–537. 35. DOI: [10.1101/gad.8.5.525](https://doi.org/10.1101/gad.8.5.525) PMID - [7926748](https://pubmed.ncbi.nlm.nih.gov/7926748/)
- D Orazio KN, Wu CCC, Sinha N, Loll Krippleber R, Brown GW, Green R. 2019. The endonuclease Cue2 cleaves mRNAs at stalled ribosomes during No Go Decay. *eLife*. 8: e49117. 16. DOI: [10.7554/elife.49117](https://doi.org/10.7554/elife.49117) PMID - [31219035](https://pubmed.ncbi.nlm.nih.gov/31219035/)
- Funk L, Su KC, Ly J, Feldman D, Singh A, Moodie B, Blainey PC, Cheeseman IM. 2022. The phenotypic landscape of essential human genes. *Cell*. 185: 4634–4653.e22. 40. DOI: [10.1016/j.cell.2022.10.017](https://doi.org/10.1016/j.cell.2022.10.017) PMID - [36347254](https://pubmed.ncbi.nlm.nih.gov/36347254/)
- Gay DA, Sisodia SS, Cleveland DW. 1989. Autoregulatory control of beta-tubulin mRNA stability is linked to translation elongation. *Proceedings of the National Academy of Sciences*. 86: 5763–5767. 19. DOI: [10.1073/pnas.86.15.5763](https://doi.org/10.1073/pnas.86.15.5763) PMID - [2762294](https://pubmed.ncbi.nlm.nih.gov/2762294/)
- Gay DA, Yen TJ, Lau JTY, Cleveland DW. 1987. Sequences that confer β -tubulin autoregulation through modulated mRNA stability reside within exon 1 of a β -tubulin mRNA. *Cell*. 50: 671–679. 8. DOI: [10.1016/0092-8674\(87\)90325-4](https://doi.org/10.1016/0092-8674(87)90325-4) PMID - [3621343](https://pubmed.ncbi.nlm.nih.gov/3621343/)
- Gillen SL, Giacomelli C, Hodge K, Zanivan S, Bushell M, Wilczynska A. 2021. Differential regulation of mRNA fate by the human Ccr4-Not complex is driven by coding sequence composition and mRNA localization. *Genome Biology*. 22: 284. 34. DOI: [10.1186/s13059-021-02494-w](https://doi.org/10.1186/s13059-021-02494-w) PMID - [34615539](https://pubmed.ncbi.nlm.nih.gov/34615539/)
- Hagkarim NC, Grand RJ. 2020. The Regulatory Properties of the Ccr4–Not Complex. *Cells*. 9: 2379. 21. DOI: [10.3390/cells9112379](https://doi.org/10.3390/cells9112379) PMID - [33138308](https://pubmed.ncbi.nlm.nih.gov/33138308/)
- Hopfler M, Absmeier E, Peak Chew SY, Vartholomaïou E, Passmore LA, Gasic I, Hegde RS. 2023. Mechanism of ribosome-associated mRNA degradation during tubulin autoregulation. *Molecular Cell*. 83: 2290–2302.e13. 43. DOI: [10.1016/j.molcel.2023.05.020](https://doi.org/10.1016/j.molcel.2023.05.020) PMID - [37295431](https://pubmed.ncbi.nlm.nih.gov/37295431/)
- Houseley J, Tollervey D. 2009. The Many Pathways of RNA Degradation. *Cell*. 136: 763–776. 11. DOI: [10.1016/j.cell.2009.01.019](https://doi.org/10.1016/j.cell.2009.01.019) PMID - [19239894](https://pubmed.ncbi.nlm.nih.gov/19239894/)
- Jones TR, Cole MD. 1987. Rapid Cytoplasmic Turnover of c-myc mRNA: Requirement of the 3' Untranslated Sequences. *Molecular and Cellular Biology*. 7: 4513–4521. 41. DOI: [10.1128/mcb.7.12.4513-4521.1987](https://doi.org/10.1128/mcb.7.12.4513-4521.1987) PMID - [3325826](https://pubmed.ncbi.nlm.nih.gov/3325826/)
- Juszkiewicz S, Chandrasekaran V, Lin Z, Kraatz S, Ramakrishnan V, Hegde RS. 2018. ZNF598 Is a Quality Control Sensor of Collided Ribosomes. *Molecular Cell*. 72: 469–481.e7. 20. DOI: [10.1016/j.molcel.2018.08.037](https://doi.org/10.1016/j.molcel.2018.08.037) PMID - [30293783](https://pubmed.ncbi.nlm.nih.gov/30293783/)
- Kruk JA, Dutta A, Fu J, Gilmour DS, Reese JC. 2011. The multifunctional Ccr4–Not complex directly promotes transcription elongation. *Genes & Development*. 25: 581–593. 36. DOI: [10.1101/gad.2020911](https://doi.org/10.1101/gad.2020911) PMID - [21406554](https://pubmed.ncbi.nlm.nih.gov/21406554/)
- Li Y, Song M, Kiledjian M. 2011. Differential utilization of decapping enzymes in mammalian mRNA decay pathways. *RNA*. 17: 419–428. 14. DOI: [10.1261/rna.2439811](https://doi.org/10.1261/rna.2439811) PMID - [21224379](https://pubmed.ncbi.nlm.nih.gov/21224379/)
- Lin Z, Gasic I, Chandrasekaran V, Peters N, Shao S, Mitchison TJ, Hegde RS. 2020. TTC5 mediates autoregulation of tubulin via mRNA degradation. *Science*. 367: 100–104. 10. DOI: [10.1126/science.aaz4352](https://doi.org/10.1126/science.aaz4352) PMID - [31727855](https://pubmed.ncbi.nlm.nih.gov/31727855/)
- Liu R, Moss B. 2016. Opposing Roles of Double-Stranded RNA Effector Pathways and Viral Defense Proteins Revealed with CRISPR-Cas9 Knockout Cell Lines and Vaccinia Virus Mutants. *Journal of Virology*. 90: 7864–7879. 15. DOI: [10.1128/jvi.00869-16](https://doi.org/10.1128/jvi.00869-16) PMID - [27334583](https://pubmed.ncbi.nlm.nih.gov/27334583/)
- Mathys H, Basquin J, Ozgur S, Czarnocki Cieciora M, Bonneau F, Aartse A, et al., Filipowicz W. 2014. Structural and Biochemical Insights to the Role of the CCR4–NOT Complex and DDX6 ATPase in MicroRNA Repression. *Molecular Cell*. 54: 751–765. 31. DOI: [10.1016/j.molcel.2014.03.036](https://doi.org/10.1016/j.molcel.2014.03.036) PMID - [24768538](https://pubmed.ncbi.nlm.nih.gov/24768538/)
- Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al., Jaffrey SR. 2017. Reversible methylation of m6Am in the 5' cap controls mRNA stability. *Nature*. 541: 371–375. 44. DOI: [10.1038/nature21022](https://doi.org/10.1038/nature21022) PMID - [28002401](https://pubmed.ncbi.nlm.nih.gov/28002401/)

- Mauxion F, Basquin J, Ozgur S, Rame M, Albrecht J, Schafer I, Seraphin B, Conti E. 2022. The human CNOT1-CNOT10-CNOT11 complex forms a structural platform for protein-protein interactions. *Cell Reports*. 111902. 32. DOI: [10.1016/j.celrep.2022.111902](https://doi.org/10.1016/j.celrep.2022.111902) PMID - 36586408
- Mauxion F, Preve B, Seraphin B. 2013. C2ORF29/CNOT11 and CNOT10 form a new module of the CCR4-NOT complex. *RNA Biology*. 10: 267–276. 33. DOI: [10.4161/rna.23065](https://doi.org/10.4161/rna.23065) PMID - 23232451
- Mitchison T, Kirschner M. 1984. Dynamic instability of microtubule growth. *Nature*. 312: 237–242. 1. DOI: [10.1038/312237a0](https://doi.org/10.1038/312237a0) PMID - 6504138
- Mooney DJ, Hansen LK, Langer R, Vacanti JP, Ingber DE. 1994. Extracellular matrix controls tubulin monomer levels in hepatocytes by regulating protein turnover. *Molecular Biology of the Cell*. 5: 1281–1288. 5. DOI: [10.1091/mbc.5.12.1281](https://doi.org/10.1091/mbc.5.12.1281) PMID - 7696710
- Ogami K, Hosoda N, Funakoshi Y, Hoshino S. 2014. Antiproliferative protein Tob directly regulates c-myc proto-oncogene expression through cytoplasmic polyadenylation element-binding protein CPEB. *Oncogene*. 33: 55–64. 42. DOI: [10.1038/onc.2012.548](https://doi.org/10.1038/onc.2012.548) PMID - 23178487
- Pachter JS, Yen TJ, Cleveland DW. 1987. Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. *Cell*. 51: 283–292. 6. DOI: [10.1016/0092-8674\(87\)90155-3](https://doi.org/10.1016/0092-8674(87)90155-3) PMID - 2444342
- Passmore LA, Collier J. 2022. Roles of mRNA poly(A) tails in regulation of eukaryotic gene expression. *Nature Reviews Molecular Cell Biology*. 23: 93–106. 12. DOI: [10.1038/s41580-021-00417-y](https://doi.org/10.1038/s41580-021-00417-y) PMID - 34594027
- Raisch T, Chang CT, Leviansky Y, Muthukumar S, Raunser S, Valkov E. 2019. Reconstitution of recombinant human CCR4-NOT reveals molecular insights into regulated deadenylation. *Nature Communications*. 10: 3173. 24. DOI: [10.1038/s41467-019-11094-z](https://doi.org/10.1038/s41467-019-11094-z) PMID - 31320642
- Shah K, He S, Turner DJ, Corbo J, Rebbani K, Dominguez D, et al., Murn J. 2024. Regulation by the RNA-binding protein Unkempt at its effector interface. *Nat Commun*. 15: 3159. 23. PubMed ID: [38605040](https://pubmed.ncbi.nlm.nih.gov/38605040/)
- Shirai YT, Suzuki T, Morita M, Takahashi A, Yamamoto T. 2014. Multifunctional roles of the mammalian CCR4-NOT complex in physiological phenomena. *Frontiers in Genetics*. 5: 286. 22. DOI: [10.3389/fgene.2014.00286](https://doi.org/10.3389/fgene.2014.00286) PMID - 25191340
- Sinha NK, Ordureau A, Best K, Saba JA, Zinshteyn B, Sundaramoorthy E, et al., Green R. 2020. EDF1 coordinates cellular responses to ribosome collisions. *eLife*. 9: e58828. 17. DOI: [10.7554/elife.58828](https://doi.org/10.7554/elife.58828) PMID - 32744497
- Tomecki R, Kristiansen MS, Lykke Andersen S, Chlebowski A, Larsen KM, Szczesny RJ, et al., Jensen TH. 2010. The human core exosome interacts with differentially localized processive RNases: hDIS3 and hDIS3L. *The EMBO Journal*. 29: 2342–2357. 13. DOI: [10.1038/emboj.2010.121](https://doi.org/10.1038/emboj.2010.121) PMID - 20531386
- Veltri AJ, D Orazio KN, Green R. 2020. Make or break: the ribosome as a regulator of mRNA decay. *Cell Research*. 30: 195–196. 18. DOI: [10.1038/s41422-019-0271-3](https://doi.org/10.1038/s41422-019-0271-3) PMID - 31913358
- Watson MJ, Park Y, Thoreen CC. 2021. Roadblock-qPCR: a simple and inexpensive strategy for targeted measurements of mRNA stability. *RNA*. 27: 335–342. 38. DOI: [10.1261/ma.076885.120](https://doi.org/10.1261/ma.076885.120) PMID - 33288682
- Watson MJ, Thoreen CC. 2022. Measuring mRNA Decay with Roadblock-qPCR. *Current Protocols*. 2: e344. 39. DOI: [10.1002/cpz1.344](https://doi.org/10.1002/cpz1.344) PMID - 35041257
- Winkler GS, Mulder KW, Bardwell VJ, Kalkhoven E, Timmers HTM. 2006. Human Ccr4-Not complex is a ligand-dependent repressor of nuclear receptor-mediated transcription. *The EMBO Journal*. 25: 3089–3099. 37. DOI: [10.1038/sj.emboj.7601194](https://doi.org/10.1038/sj.emboj.7601194) PMID - 16778766
- Yen TJ, Gay DA, Pachter JS, Cleveland DW. 1988. Autoregulated changes in stability of polyribosome-bound beta-tubulin mRNAs are specified by the first 13 translated nucleotides. *Molecular and Cellular Biology*. 8: 1224–1235. 7. DOI: [10.1128/mcb.8.3.1224](https://doi.org/10.1128/mcb.8.3.1224) PMID - 2835666
- Yi H, Park J, Ha M, Lim J, Chang H, Kim VN. 2018. PABP Cooperates with the CCR4-NOT Complex to Promote mRNA Deadenylation and Block Precocious Decay. *Molecular Cell*. 70: 1081–1088.e5. 25. DOI: [10.1016/j.molcel.2018.05.009](https://doi.org/10.1016/j.molcel.2018.05.009) PMID - 29932901
- Zhu X, Cruz VE, Zhang H, Erzberger JP, Mendell JT. 2024. Specific tRNAs promote mRNA decay by recruiting the CCR4-NOT complex to translating ribosomes. *Science*. 386: eadq8587. 29. PubMed ID: [39571015](https://pubmed.ncbi.nlm.nih.gov/39571015/)

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