SUPPLEMENTARY INFORMATION FILE

Regulation of human Dicer by the resident ER membrane protein CLIMP-63

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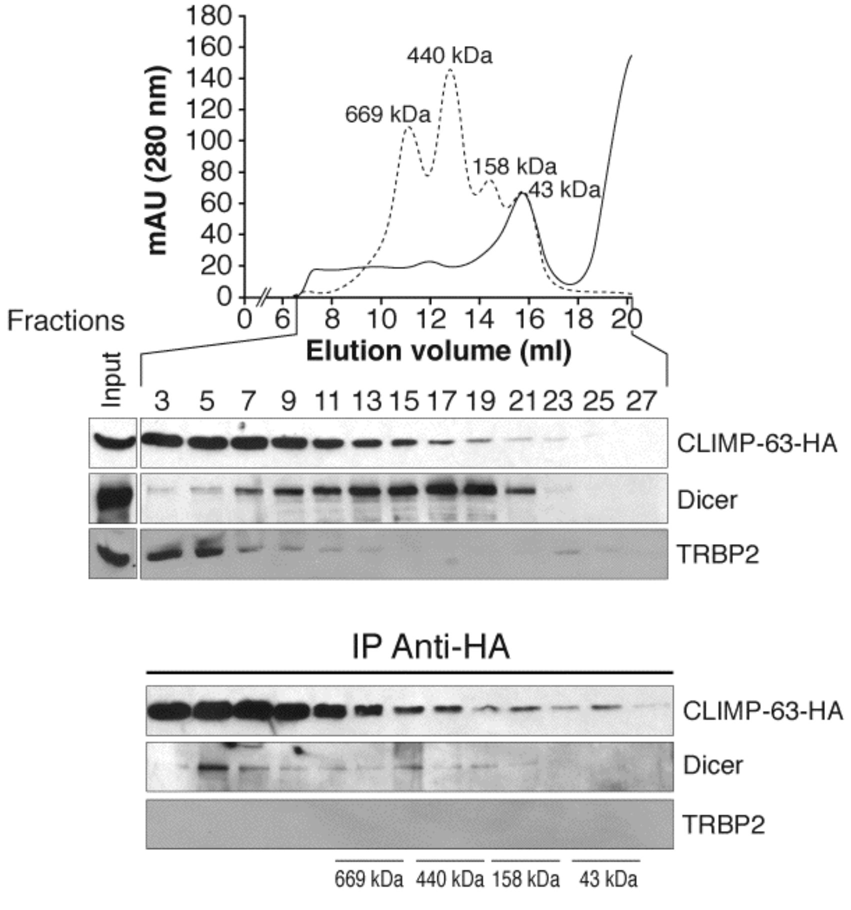
1. SUPPLEMENTARY FIGURES AND LEGENDS



Supplementary Figure S1. TRBP2 could not be detected in CLIMP-63 immune complexes. Protein extracts from HEK 293 cells transiently expressing Flag-Dicer and CLIMP-63-HA proteins were separated by gel filtration chromatography, and the odd fractions collected were analyzed by Western blot (upper panels) or subjected to anti-HA immunoprecipitation (IP) followed by Western blot analysis of CLIMP-63-HA (G1/296 Alexis Biochemicals), Dicer (6,22) and TRBP2 (22) proteins (lower panels). mAU, milliabsorbance unit.

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ACCESSION: Q9UPY3               DICER_HUMAN               LENGTH: 1922 AA

MKSPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNTIVCLNTGSGKTFIAVLLTKE   80
LSYQIRGDFSRNGKRTVFLVNSANQVAQQVSAVRTHSDLKVGEYSNLEVNASWTKERWNQEFTKHQVLIMTCYVALNVLK  160
NGYLSLSDINLLVFDECHLAILDHPYREIMKLCENCPSCPRILGLTASILNGKCDPEELEEKIQKLEKILKSNAETATDL  240
VVLDRYTSQPCEIVVDCGPFTDRSGLYERLLMELEEALNFINDCNISVHSKERDSTLISKQILSDCRAVLVVLGPWCADK  320
VAGMMVRELQKYIKHEQEELHRKFLLFTDTFLRKIHALCEEHFSPASLDLKFVTPKVIKLLEILRKYKPYERQQFESVEW  400
YNNRNQDNYVSWSDSEDDDEDEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT  480
GHGIGKNQPRNKQMEAEFRKQEEVLRKFRAHETNLLIATSIVEEGVDIPKCNLVVRFDLPTEYRSYVQSKGRARAPISNY  560
IMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSVDTGETDIDPVMDDDDVFPPYVLRPDDGGPRVTINTAIGHINRYCAR  640
LPSDPFTHLAPKCRTRELPDGTFYSTLYLPINSPLRASIVGPPMSCVRLAERVVALICCEKLHKIGELDDHLMPVGKETV  720
KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRDSYPRPDQPCYLYVIGMVLTTPLPDELNFRRRKLYPPEDTTR  800
CFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFMLSLQMLELITRLHQYIFSHILRLEKPALEFKPTDADSAYCVLP  880
LNVVNDSSTLDIDFKFMEDIEKSEARIGIPSTKYTKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF  960
PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKRKAKWESLQNKQILVPELCAI 1040
HPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGVGVRSLPADFRYPNLDFGWKKSIDSKSFISISNSSSAENDN 1120
YCKHSTIVPENAAHQGANRTSSLENHDQMSVNCRTLLSESPGKLHVEVSADLTAINGLSYNQNLANGSYDLANRDFCQGN 1200
QLNYYKQEIPVQPTTSYSIQNLYSYENQPQPSDECTLLSNKYLDGNANKSTSDGSPVMAVMPGTTDTIQVLKGRMDSEQS 1280
PSIGYSSRTLGPNPGLILQALTLSNASDGFNLERLEMLGDSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLG 1360
KKKGLPSRMVVSIFDPPVNWLPPGYVVNQDKSNTDKWEKDEMTKDCMLANGKLDEDYEEEDEEEESLMWRAPKEEADYED 1440
DFLEYDQEHIRFIDNMLMGSGAFVKKISLSPFSTTDSAYEWKMPKKSSLGSMPFSSDFEDFDYSSWDAMCYLDPSKAVEE 1520
DDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSIADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVIKRTDRE 1600
KALCPTRENFNSQQKNLSVSCAAASVASSRSSVLKDSEYGCLKIPPRCMFDHPDADKTLNHLISGFENFEKKINYRFKNK 1680
AYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSALVNNTIFASLAVKYDYHKYFKA 1760
VSPELFHVIDDFVQFQLEKNEMQGMDSELRRSEEDEEKEEDIEVPKAMGDIFESLAGAIYMDSGMSLETVWQVYYPMMRP 1840
LIEKFSANVPRSPVRELLEMEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPQVP 1920
NS
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Supplementary Figure S2. Human Dicer amino acid sequence harbors several putative glycosylation sites. Bioinformatic prediction of N-glycosylation and O-glycosylation sites in the amino acid sequence of human Dicer using the NetNGlyc 1.0 server interface at http://www.cbs.dtu.dk/services/NetNGlyc/ and the NetOGlyc 3.1 server interface at http://www.cbs.dtu.dk/services/NetOGlyc/, respectively. Asn-Xaa-Ser/Thr sequons are highlighted in blue, whereas Asparagines (N) predicted to be N-glycosylated are highlighted in red. Tryptophan (T) residues predicted to be O-glycosylated are highlighted in red and underlined.

		Cleavage site	
Human growth hormone	M A T G S **R** T S ***L L L A F G L L C L P W L*** Q E G S A		F P T
Human proinsulin	M A L W M **R** ***L L P L L A L L A L W*** G P D P A A A		F V N
Bovine proalbumin	M **K** W V T F I S ***L L L F*** S S A Y S		R G V
Mouse antibody H chain	M **K** V L S ***L L Y L L*** T A I P H I M S		D V Q
Chicken lysozyme	M **R** S ***L L I L V L C F L*** P K ***L A A L*** G		K V F
Bee promellitin	M **K** F L V N ***V A L V F M V V Y I*** S Y I Y A		A P E
Drosophila glue protein	M **K** ***L L V V A V I A C M L I G F A*** D P A S G		C K D
Zea maize protein	M A A **K** ***I F C L I M L L G L*** S A S A A T A		S I F
Yeast invertase	***M L L*** O ***A F L F L L A*** G ***F A A*** **K** ***I*** S ***A***		S M T
Human influenza virus A	***M*** **K** ***A*** **K** ***L L V L L Y A F V A*** G		D Q I
Human Dicer	***M*** **K** S ***P A L*** Q ***P L*** S ***M A G L*** Q ***L M*** T ***P A*** S S ***P M G P F F G L P W*** Q Q E ***A I***		H D N

Supplementary Figure S3. Presence of a putative signal peptide at the N-terminal extremity of human Dicer that could mediate its translocation across the ER membrane. Alignment of the amino acid sequence of human Dicer (amino acids 1 to 40; Acc. No. Q9UPY3) with some eukaryotic secreted and membrane proteins harboring signal peptide sequences, which are generally localized at their N-terminal extremity (52). These signal sequences commonly (i) range from 13 to 36 amino acids in length, (ii) contain a highly hydrophobic core (residues in bold italic) typically 10 to 15 amino acids long that forms the center of the signal sequence (A, L, I, M and F residues are common in this region), and (iii) usually harbor a residue, on the N-terminal side of the cleavage site for signal peptidase (vertical bar, in red), with a small neutral side chain (A is most common). As compared to most of the signal peptide sequences shown above, human Dicer only lacks the positively charged residue (in bold, larger font) (K and R are the most common) usually present in the N-terminal part of the signal. Adapted from Stryer (1995) (60).

2. SUPPLEMENTARY REFERENCES

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60. Stryer L. 1995. *Biochemistry*: W. H. Freeman and Company, New York.