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Supplemental Information

Gemin5 Delivers snRNA Precursors to the SMN Complex for snRNP Biogenesis

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Supplemental Experimental Procedures

Materials

A library of about 50,000 pure bioactive chemicals that includes FDA-approved drugs, known inhibitors and activators of diverse enzymes and receptors, and pure natural products was assembled from commercial sources (Microsource Diversity, Tocris, Sigma/Aldrich and other suppliers). Cycloheximide, puromycin, thapsigargin, emetine, anisomycin and β-lapachone were purchased from Sigma Chemical Co. Cycloheximide-N-ethyl ethanoate and actinomycin D was from EMD Biosciences. All compounds were dissolved in DMSO.

High content screening

Processing of samples for immunofluorescence microscopy on cells cultured in 384-well plates was automated and performed with the aid of microplate washers (ELX405, Bio-Tek) in a similar manner to that previously used for individual samples on glass slides. The cells were also stained with DAPI to allow definition of the nucleus in each cell. Digital images were acquired with an automated microscopy and analysis system (IN Cell Analyzer 1000, GE Healthcare). Images were analyzed using automated algorithms with parameters set to calculate mean pixel intensity in each nucleus (defined

by DAPI staining and acquired in a separate channel), cytoplasm, and total cell, as well as the relevant calculated ratios of these values.

Preparation of radio-labeled probes and Northern blotting

Specific oligonucleotide sequences to probe for each RNAs are as follows; U2 probe (5'-TATATTGTCCTCGGATAGAGGACGTATCAGATATTAAACTGATAA GAACAGATACTACAC-3'), U4 probe (5'-TAGCAAAATCGCGCCTCGGATAAAC CTCATTGGCTACGATACTGCCACTGCGCAAAGCT-3'), pre-U2 probe (5'-ATCCC CGGAGGGGGTGCA-3'), U4atac probe (5'-GGGTGTGTTGTTCAGGCGTTAGCAG TACTG-3'), and 5S probe (5'-TGCTTAGCTTCCGAGATCAGACGAGATCGG-3'). For radio-labeling of probes for Northern blotting, 10 μM of oligonucloetides were incubated with T4 kinase for 30 min. in the presence of [γP^{32}]ATP.

Purification of native SMN complex

FLAG-SMN or HeLa tet-ON cells were grown in the presence of doxycycline (5 μg/ml). Total cell extracts prepared in RSB-100 buffer containing 0.1% NP-40 and protease inhibitors were incubated with anti-FLAG beads (Sigma) for 2 hours at 4°C. Supernatants were discarded and the beads were extensively washed with RSB-100 containing 0.02% NP-40. Three washes were performed with ten bead volumes of RSB-250 containing 0.02% NP-40 for 15 min at 4 °C. The bound proteins were either equilibrated with 10 bead volumes of RSB-100 containing 0.01% NP-40 for further experiments or eluted with 3X FLAG peptides (Sigma) at a final concentration of 0.5 mg/ml for 1 hr at 4 °C. Purified SMN complex was analyzed by 12.5% SDS-PAGE and silver staining.

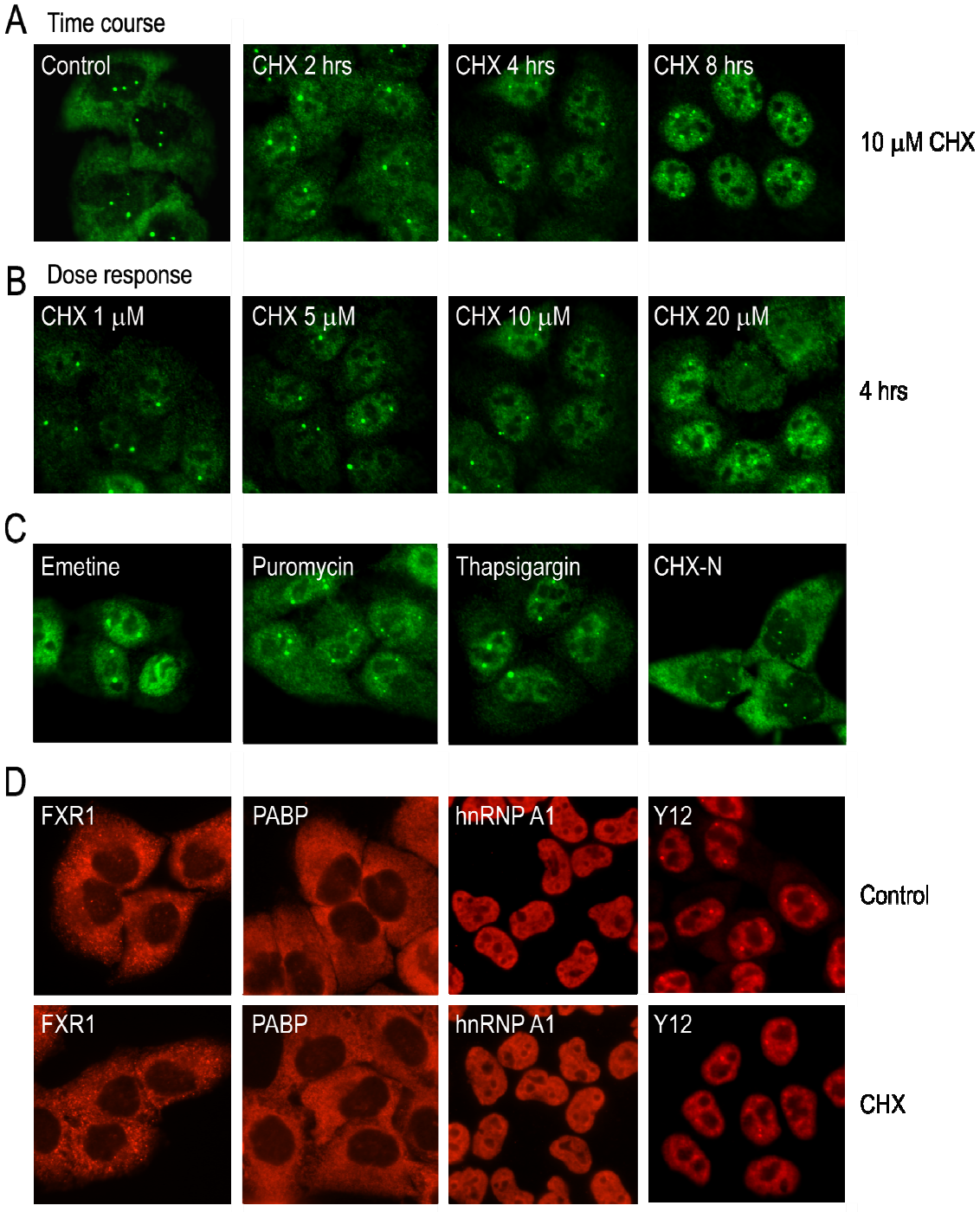
A
Time course
Control
CHX 2 hrs
CHX 4 hrs
CHX 8 hrs
10 μM CHX
B
Dose response
CHX 1 μM
CHX 5 μM
CHX 10 μM
CHX 20 μM
4 hrs
C
Emetine
Puromycin
Thapsigargin
CHX-N
D
FXR1
PABP
hnRNP A1
Y12
Control
FXR1
PABP
hnRNP A1
Y12
CHX

Figure S1

Figure S1, associated with Figure 1. Cycloheximide induces a specific nuclear accumulation of SMN.

(A) Time course of cycloheximide treatment on SMN localization in HeLa cells. Immunostaining of SMN in control HeLa PV cells and following treatment with 10 μM cycloheximide (CHX) for the indicated times. Control cells were treated with DMSO at the same final concentration as that used to dissolve CHX.

(B) Dose response of cycloheximide treatment on HeLa cells after 4 hours. The concentration of CHX used is indicated in the figure.

(C) HeLa cells were treated with emetine (10 μM), puromycin (10 μM), thapsigargin (10 μM) or cycloheximide-N-ethylethanoate (10 μM) for 6 hours. Cells were immunostained with the SMN antibody.

(D) Sub-cellular localization of cytoplasmic and nuclear proteins after CHX treatment. Immunostaining of HeLa cells for FXR1, PABP, hnRNP A1 and Sm proteins (snRNPs) in control cells (upper panel) and after 6 hrs of 10 μM CHX treatment (lower panel).

Table S1, associated with Figure2. Analysis of the SMN and Gemin5 complexes by LC-MS/MS mass spectrometry.

Flag-SMN

Inhibitors	CHX			ANS			DMSO		
	emPAI	No. of peptides	No. of spectra	emPAI	No. of peptides	No. of spectra	emPAI	No.of peptides	No. of spectra
SMN	1.75	8	65	1.20	7	46	2.44	9	93
Gemin2	0.25	2	6	0.12	1	3	0.25	2	13
Gemin3	0.43	10	19	0.37	8	16	1.47	21	97
Gemin4	0.13	4	11	0.00	0	0	0.63	14	53
Gemin5	0.04	4	5	0.04	2	5	0.57	20	73
Gemin6	0.75	2	13	0.45	2	8	0.75	2	19
Gemin7	0.61	2	5	0.27	1	2	0.61	2	3
Gemin8	0.13	2	5	0.28	2	4	0.28	2	16
unrip	0.20	2	5	0.32	3	3	0.45	4	17

Flag-Gemin5

Inhibitors	CHX			ANS			DMSO		
	emPAI	No. of peptides	No. of spectra	emPAI	No. of peptides	No. of spectra	emPAI	No. of peptides	No. of spectra
SMN	0.00	0	0	0.12	1	2	0.75	5	36
Gemin2	0.00	0	0	0.00	0	0	0.40	3	17
Gemin3	0.00	0	0	0.08	2	2	0.54	11	45
Gemin4	0.00	0	0	0.00	0	0	0.17	6	23
Gemin5	0.24	12	29	0.14	8	24	0.11	5	14
Gemin6	0.00	0	0	0.00	0	0	1.10	3	20
Gemin7	0.00	0	0	0.00	0	0	0.00	0	0
Gemin8	0.00	0	0	0.00	0	0	0.28	2	14
unrip	0.00	0	0	0.00	0	0	0.32	3	8

Table S2, associated with Figure 4. snRNA 3' extra precursor sequences and their loci on human chromosomes.

snRNA	Precursor sequence	Locus[a]
U1	AAUUUUUGUAAUGAAAAAAUAGACUC CCCUAUAAGGGUUAUUCUU	Chr1:145,977,583-145,977,791
U1	AUUUUUUGUAGUUUAAAGAAUAGUCU ACACAGCAAGGGUUACUUGUUUU	Chr1:147,490,633-147,490,845
U1	AAUUUU	Chr1:147,460,724-147,460,893
U2	CCUCCGGGGAU	17q12-q21[b]
U2	UCUCCCUUCGGGGAGAGAACA	Chr11:62,365,651-62,365,857
U4	AAUUUU	Chr12:119,215,274-119,215,423
U4	AAUUUUU	Chr12:119,213,939-119,214,089
U5	AAUGUUCUGUUACUAAAGAGAGACUG UGGGUGGGGUGUU	Chr1:44,960,004-44,960,159
U5	UAUGUGGUAAUCCAACAAUA	Chr15:63,375,442-63,375,578
U5	UAUAAA	Chr15:63,384,068-63,384,190
U5	UCAAC	Chr1:44,969,307-44,969,429
U5	AUUUGAGCGUUUUAAAAGUAGAUGCC CUUAGCCAUUACGACGGUUCGU	Chr1:11,892,402-11,892,571
U5	ACAUUUUUAAGUAU	Chr1:45,058,128-45,058,256
U5	AUUUUAA	Chr1:11,890,796-11,890,923
U11	UCUUUACUGUUAUAUGUUAGGCGAAA UAUUACGCGUUUGGAGUAAGUGGUGC UUUUUGUAACUGAAAAGAGAU	Chr1:28,847,698-28,847,905
U12	ACCUUAUUCACGCCUAAAAAGUAGAC UGACUGUGGGGUGGUCGUGUUUUUUG UUUCUUGUUGGUAGGUGGUGAAUGC	Chr22:41,341,194-41,341,420
U4atac	GAAAACCUGUUUUCAUAGACUUAUCA GUUCAAACAGCAGUAAUUC	Chr2:122,004,924-122,005,100

[a] Chromosomes and the loci numbers are based on the annotated sequence information on UCSC genome database (Human Mar. 2006 (NCBI36/hg18) Assembly).
[b] The cluster for this pre-U2 is designated since there are multiple copies in this locus.