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Supplemental Information

tRNA Binds to Cytochrome *c* and Inhibits Caspase Activation

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Supplemental Figure Legends

Figure S1. The intrinsic apoptosis pathway (related to Figures 1, 2, 3, 5, 6, and 7)

Various intracellular apoptotic stimuli provoke the release of cytochrome *c* (cyt. *c*) from mitochondria (a). In the cytosol, cytochrome *c* binds to Apaf-1, promoting the hydrolysis of Apaf-1-bound dATP to dADP. This is followed by the release of dADP in exchange for dATP and the assembly of Apaf-1 into a heptameric complex known as the apoptosome (b, only two molecules of Apaf-1 are shown). The apoptosome recruits and oligomerizes procaspase-9 (pro-C9) (c), leading to the auto-proteolytic processing of procaspase-9 to the mature caspase-9 (d). Mature caspase-9 then converts procaspase-3 (pro-C3), which pre-exists as a dimer, to the active form (e).

Figure S2. Cytochrome *c* induces caspase-9 and caspase-3 activation in Jurkat S100 extract (related to Figure 1)

Jurkat S100 extracts were incubated with cytochrome *c* (cyt. *c*) (20 μg/ml) at 37 °C for the indicated time periods. The activation of caspase-9 and -3 in the extracts was analyzed by Western blots. The amount of actin in the extracts is shown for equivalent sample loading. Molecular weight standards (in kDa) are marked on the left.

Figure S3. Onconase treatment enhances cytochrome *c*-induced caspase-9 activation (related to Figure 7)

(A) HeLa S100 extracts were pre-incubated with indicated amounts of onconase (Onc) for 20 min at room temperature. Left: RNA was resolved on denaturing PAGE and stained with ethidium bromide. Right: extracts were further incubated with cytochrome *c* (20 μg/ml) at 37 °C for an additional 1 h, and analyzed for caspase-9 and -3 activation.

(B) tRNA degradation in onconase and doxorubincin-treated cells. Total RNA from the HeLa cells treated with onconase and/or doxorubicin (Figure 7C) was resolved on 8% urea-containing PAGE and visualized by ethidium bromide staining. Note that onconase-treated cells exhibited less apoptosis (Figure 7C), but had a more dramatic decrease in tRNA compared with doxorubicin-treated cells, suggesting that the degradation of tRNA in onconase-treated cells is not a secondary effect of apoptosis. The decrease of tRNA in doxorubicin-treated cells and the further decrease of tRNA in the cells treated with onconase plus doxorubicin were likely due to cell death.

Figure S1

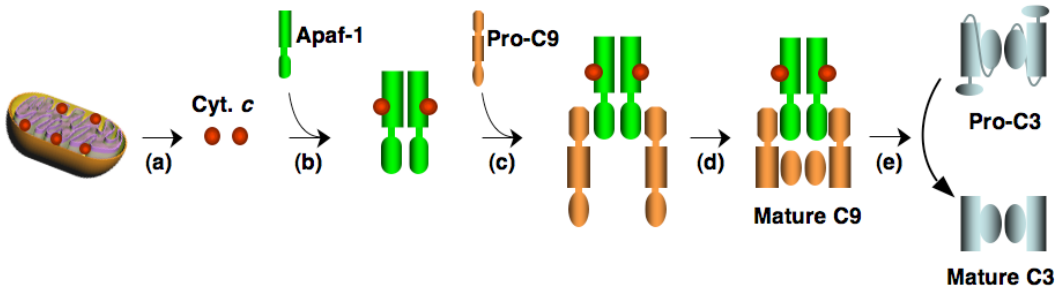


Figure S2

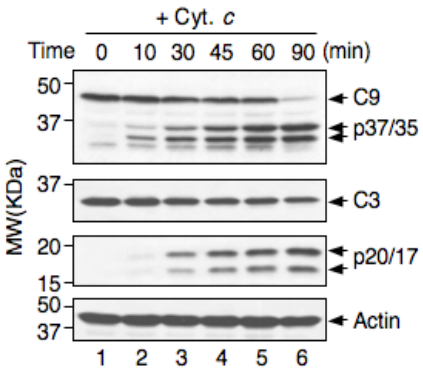
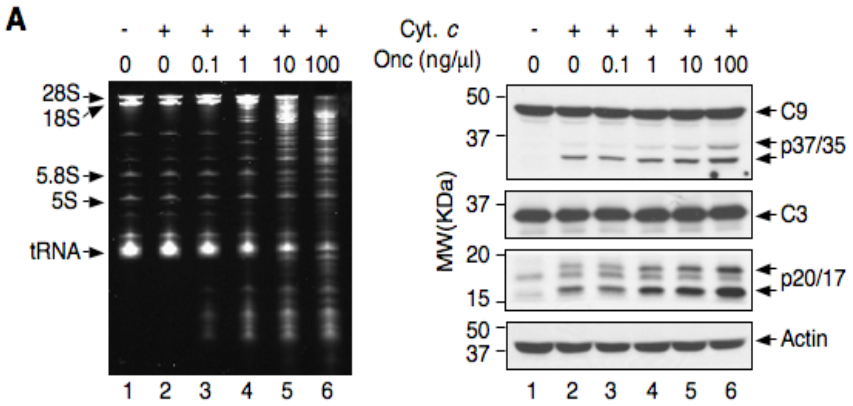
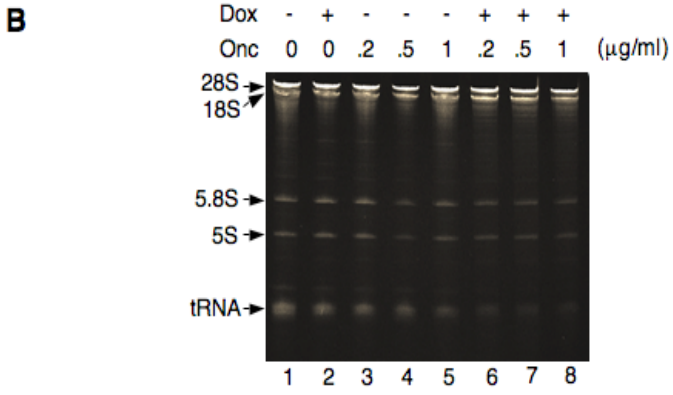

+ Cyt. c
Time 0 10 30 45 60 90 (min)
MW(KDa)
50
37
C9
p37/35
37
C3
20
15
p20/17
50
37
Actin
1 2 3 4 5 6

Figure S3


A
- + + + + +
0 0 0.1 1 10 100
28S
18S
5.8S
5S
tRNA
1 2 3 4 5 6
Cyt. c
Onc (ng/μl)
- + + + + +
0 0 0.1 1 10 100
MW(KDa)
50
37
C9
p37/35
37
C3
20
15
p20/17
50
37
Actin
1 2 3 4 5 6


B
Dox - + - - - + + +
Onc 0 0 .2 .5 1 .2 .5 1 (μg/ml)
28S
18S
5.8S
5S
tRNA
1 2 3 4 5 6 7 8