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JNK activation induced by ribotoxic stress is initiated from 80S monosomes but not polysomes

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Running Title: JNK activation by ribotoxin in 80S monosome

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SUPPLEMENTARY MATERIAL

Reagents

Deoxynivalenol (DON) and ATP were purchased from Sigma-Aldrich (USA). Anisomycin, emetine, and NSC119889 were purchased from Merck Millipore (USA).

Cell culture

Human fibrosarcoma HT1080 cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. These cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

siRNA and transfection

We purchased siRNA, including scramble and rpS6, from Bioneer (Korea). RACK1, rpL13, and rpL30 siRNA oligos were purchased from Santa Cruz Biotechnology (USA), and rpS3 siRNA was obtained from HAEL Lab (Korea). Each siRNAs transfection was performed with Lipofectamine™ RNAiMAX reagent (Invitrogen, USA) as instructed by the manufacturer. DNA transfection was also performed using Lipofectamine™ 2000 (Invitrogen, USA).

Antibodies and immunoblotting

Antibodies against JNK, p38, RACK1, rpL13, and rpL30 were purchased from Santa Cruz Biotechnology (USA). Antibodies against pJNK, pp38, and rpS6 were obtained from Cell Signaling Technology (USA) and anti-α-tubulin from Calbiochem (USA). Anti-rpS3 antibody
was obtained from HAEL Lab (Korea). The cells were lysed with lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 0.05% sodium deoxycholate, 50 mM NaF, 200 μM Na₃VO₄, 2 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) for 30 min on ice. The supernatants were collected by centrifugation at 12,000 × g for 10 min at 4 °C, and protein concentrations were determined using the Bradford protein assay. The lysates were boiled in SDS-PAGE sample buffer and separated with SDS-PAGE gel, transferred to PVDF membranes, probed with the antibodies, and illuminated with the enhanced chemiluminescence (ECL) system (Roche, Switzerland).

**Protein precipitation**

Proteins in the ribosomal fractions were precipitated with TCA/acetone. Briefly, 100% TCA solution was added to adjust to 20% final concentration in each fraction, and these samples were incubated for 1 h on ice. After centrifugation, the pellets were washed five times with ice-cold acetone and then dried in a heat block for 5 min to remove the residual acetone.

**Immunoprecipitation**

The HT1080 cells were lysed with cold lysis buffer for 30 min on ice. After centrifugation at 12,000 × g for 10 min at 4 °C, the supernatant was collected, pre-cleared by adding protein A agarose, and then incubated at 4 °C for 2 h with specific antibodies. Then, 30 µl of protein A agarose was added, and the solution was incubated for 16 h at 4 °C. After extensive washing, the immunoprecipitates were ready for use in other experiments.
JNK kinase assay

The cell lysates were subjected to immunoprecipitation with the anti-JNK antibody. The immunoprecipitates were washed three times with lysis buffer, twice with tyrosine kinase buffer (50 mM HEPES, pH 7.4, 10 mM MnCl$_2$, 10 mM MgCl$_2$, 2 mM DTT, 0.1 mM sodium vanadate), and then resuspended in 20 µl of kinase buffer containing 2 µCi of [$\gamma$-$^{32}$P] ATP and GST-cJun protein. The mixture was incubated for 30 min at 30 °C, and the reaction mixture was resolved using 11% SDS-PAGE and analyzed with autoradiography.

Statistical analysis

All the experiments were performed at least three times independently. The quantitative data are presented as mean ± standard error of the mean (SEM) values. $P$ values were calculated with the unpaired two-tailed $t$-test from the mean data of each group.