Supplementary Materials and Methods

Reagents

Etoposide, camptothecin, hydroxyurea, protease inhibitor cocktail, phosphatase inhibitor cocktails I and II, cycloheximide and actinomycin D were purchased from Sigma (St. Louis, MO). Antibodies were purchased from the following commercial sources: anti-tubulin, anti-β-actin, anti-Flag and FITC-conjugated goat anti-rabbit secondary antibody from Sigma, anti-Bim and anti-Puma from Calbiochem (San Diego, CA), anti-Mcl-1 from Rockland Immunochemicals (Gilbertsville, PA), anti-PARP and anti-cleaved caspase-3 from Cell Signaling (Danvers, MA), anti-Bcl-2 from BD Pharmingen (Cat#554218; San Diego, CA), anti-RPA from Oncogene (Cambridge, MA), anti-Bak from Upstate (Lake Placid, NY), anti-Bax and goat anti-rabbit IgG-horseradish peroxidase from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-Rad9 and anti-Bcl-xL polyclonal rabbit antisera have been previously described (Komatsu *et al.*, 2000). Protein G agarose beads, trypan blue and culture medium were purchased from Invitrogen (Carlsbad, CA). The Nucleofector machine, as well as all Nucleofector solutions, was purchased from Amaxa Biosystems (Gaithersburg, MD).

Subcellular fractionation

Briefly, the cells were washed with PBS then resuspended in hypotonic lysis buffer (5 mM Tris HCl, pH 7.5, 5 mM NaCl, 1.5 mM $MgCl_2$, 0. 1 mM EGTA, 1 mM Na_3VO_4, 1 mM PMSF, 1 mM DTT, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 10 μg/ml pepstatin A). After incubation on ice for 30 min, the cells were homogenized

using a Dounce homogenizer. Samples were spun down at 510 x g for 5 min at 4 °C. The supernatant (cytosolic fraction) was cleared by centrifugation at 720 x g for 5 min at 4 °C, while the pellet (nuclear fraction) was washed with hypotonic lysis buffer, centrifuged at 720 x g for 5 min at 4 °C, then lysed in RIPA buffer.

Chromatin fractionation

Briefly, the cells were washed twice with PBS and resuspended in buffer A (10 mM HEPES, pH 7, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT) containing protease inhibitors. Triton X-100 was added to a final concentration of 0.1% and the cells were incubated for 5 min on ice. The nuclei were collected by low-speed centrifugation (4 min, 1,500 x g, 4 ˚C). The supernatant was clarified by high-speed centrifugation (15 min, 12,500 x g, 4 ˚C) to remove insoluble aggregates and the resulting lysate was designated the soluble fraction. The nuclei were washed once in buffer A and then lysed in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT) with protease inhibitors for 10 min on ice. Insoluble chromatin was collected by centrifugation (4 min, 2,000 x g, 4 ˚C) and washed once in buffer B. The final pellet was resuspended in 2X Laemmli buffer, boiled for 10 min and used as the chromatin fraction.

Komatsu K, Miyashita T, Hang H, Hopkins KM, Zheng W, Cuddeback S *et al* (2000). Human homologue of S. pombe Rad9 interacts with BCL-2/BCL-xL and promotes apoptosis. *Nat Cell Biol* **2:** 1-6.