SUPPLEMENTARY MATERIALS AND METHODS

Protein purification

For purification of ^{His}RAD18-^{FLAG}RAD18 hetero complexes with RAD6A, BL21 (DE3) (1) harboring both pET-RAD6A/hisRAD18 and pAC-RAD6A/flagRAD18 was grown in 1 liters of Terrific broth (2) supplemented with ampicillin (250 μg/ml) and chloramphenicol (30 μg/ml) at 15ºC with aeration until the culture reached an A_{600} value of 1.0. Isopropyl β-$_D$-thiogalactopyranoside (IPTG) was added to 0.2 mM, and the incubation was continued for 16 hr. The resultant cell paste (6 g) was resuspended in 12 ml of buffer I (50 mM HEPES-NaOH, pH 7.5/500 mM NaCl/0.1 mM EDTA/10 mM β-mercaptoethanol) and frozen in liquid nitrogen. After thawing in ice water, 2 ml of buffer I containing 100 mM spermidine, 4 mg/ml lysozyme and 1 mM phenylmethylsulfonyl fluoride was introduced. The cells were then incubated on ice for 30 min, heated in a 37°C water bath for 1 min, and further incubated on ice for 30 min. Cell lysates were clarified by centrifugation twice at 85,000 x *g* for 30 min at 4ºC and subsequent column chromatography was carried out at 4ºC, using an FPLC system (GE Healthcare Life Science). After adding imidazole to 50 mM, the lysate was applied at 0.2 ml/min to a 1-ml HiTrap chelating HP column (GE Healthcare Life Science), which had been treated with 0.1 M $NiSO_4$ and then equilibrated with buffer A (50 mM HEPES-NaOH, pH 7.5/10% glycerol/10 mM β-mercaptoethanol) containing 500 mM NaCl and 50 mM imidazole. The column was washed with 10 ml of equilibration buffer, and then ^{His}RAD18-^{FLAG}RAD18 hetero complexes were eluted at 100 mM imidazole, while ^{His}RAD18-^{His}RAD18 homo complexes were eluted at 300 mM imidazole. Fractions containing ^{His}RAD18-^{FLAG}RAD18 hetero complexes were pooled and diluted with the same volume of buffer A, and then applied at 0.2 ml/min to a 1-ml HiTrap Heparin HP column (GE Healthcare Life Science), equilibrated with buffer A containing 280 mM NaCl. The column was washed with 10 ml of equilibration buffer, and proteins were eluted with 10 ml of a linear gradient of 280-800 mM NaCl in buffer A at 0.1 ml/min. Fractions containing ^{His}RAD18-^{FLAG}RAD18 hetero complexes were pooled, and applied at 0.1 ml/min onto a Superdex 200 10/300 GL column (GE Healthcare Life Science) equilibrated with buffer A containing 300 mM NaCl. The peak fraction containing ^{His}RAD18-^{FLAG}RAD18 hetero complexes was frozen in liquid nitrogen and stored at –80ºC. To verify the presence of ^{FLAG}RAD18, a

fraction eluted from the Superdex column was loaded onto a 0.8-ml column of ANTI-FLAG M2 Affinity Gel (Sigma, A2220), equilibrated with buffer A containing 300 mM NaCl. The column was washed with 10 ml of equilibration buffer, and protein was eluted with buffer A containing 300 mM NaCl and 100 μg/ml of FLAG peptide (Sigma, F3290).

For purification of RAD18$^{\Delta C3}$, a N-terminal fragment of RAD18 consisting of 1-115 amino acid residues, ^{His}RAD18$^{\Delta C3}$ and ^{FLAG}RAD18$^{\Delta C3}$ were coproduced with RAD6A in a same *E. coli* cells as described above. When the lysate was applied to a HiTrap chelating HP column as described above, ^{FLAG}RAD18$^{\Delta C3}$, but no RAD6A, was co-purified with ^{His}RAD18$^{\Delta C3}$. Fractions containing ^{His}RAD18$^{\Delta C3}$ and ^{FLAG}RAD18$^{\Delta C3}$ were pooled and diluted with buffer A to 100 mM NaCl, and then applied at 0.2 ml/min to a 1-ml HiTrap Q HP column (GE Healthcare Life Science), equilibrated with buffer A containing 100 mM NaCl. The flow-through fraction was applied at 0.2 ml/min to a 1-ml HiTrap Heparin HP column (GE Healthcare Life Science), equilibrated with buffer A containing 100 mM NaCl. Again the flow-through fraction was collected and concentrated by a 1-ml HiTrap chelating HP column and analyzed with a Superdex 200 10/300 GL column as described above.

For purification of RAD18$^{\Delta N1}$, an N-terminal deletion mutant of RAD18 consisting of 113-495 amino acid residues, in a complex with RAD6A, ^{His}RAD18$^{\Delta N1}$ and ^{FLAG}RAD18 were co-produced with RAD6A in the same *E. coli* cells as described above. When the lysate was applied to a HiTrap chelating HP column as described above, RAD6A, but no ^{FLAG}RAD18, was co-purified with ^{His}RAD18$^{\Delta N1}$. Fractions containing RAD6A and ^{His}RAD18$^{\Delta N1}$ were pooled and diluted with buffer A to 150 mM NaCl, and then applied at 0.2 ml/min to a 1-ml HiTrap Heparin HP column (GE Healthcare Life Science), equilibrated with buffer A containing 150 mM NaCl. The column was washed with 10 ml of equilibration buffer, and proteins were eluted with 10 ml of a linear gradient of 150-650 mM NaCl in buffer A at 0.1 ml/min. Fractions containing RAD6A and ^{His}RAD18$^{\Delta N1}$ were pooled, and further purified using a Superdex 200 10/300 GL column as described above.

For purification of ^{His}RAD18-^{FLAG}RAD18 hetero complexes with ^{FLAG}RAD6A, BL21 (DE3) (1) harboring both pET-flagRAD6A/hisRAD18 and pAC-flagRAD6A/flagRAD18 was grown and a cell lysate was prepared as described above. After adding imidazole to 20 mM, the lysate was applied at 0.2 ml/min to a

1-ml HiTrap chelating HP column (GE Healthcare Life Science), which had been treated with 0.1 M $NiSO_4$ and then equilibrated with buffer A containing 500 mM NaCl and 20 mM imidazole. The column was washed with 10 ml of equilibration buffer, and then ^{His}RAD18-^{FLAG}RAD18 hetero complexes were eluted at 50 mM imidazole. Fractions containing ^{His}RAD18-^{FLAG}RAD18 hetero complexes were further purified as described above.

For RAD6A, BL21 (DE3) (1) harboring pET-RAD6A was grown in 4 liters of LB supplemented with ampicillin (250 μg/ml) at 15ºC with aeration until the culture reached an A_{600} value of 1.0. Isopropyl β-$_D$-thiogalactopyranoside (IPTG) was added to 0.2 mM, and the incubation was continued for 44 hr. The resultant cell paste (13 g) was resuspended in 26 ml of buffer II (50 mM HEPES-NaOH, pH 7.5/10% sucrose /10 mM β-mercaptoethanol), and frozen in liquid nitrogen. The cells were thawed in ice water, and mixed with 276 μl of 5 M NaCl, 400 μl of 1 M spermidine and 40 μl of 0.1 M phenylmethylsulfonyl fluoride, then disrupted using a Multi-Bead Shocker (MB601U(S), YASUI KIKAI, Osaka, Japan) with 40 g of glass beads (0.1 mm diameter) at 0ºC. Cell lysates were clarified by centrifugation twice at 85,000 x *g* for 30 min at 4ºC and applied at 0.5 ml/min to a 5-ml HiTrap Q FF column (GE Healthcare Life Science) equilibrated with buffer A containing 100 mM NaCl. The column was washed with 50 ml of equilibration buffer, and eluted with 50 ml of a linear gradient of 100-500 mM NaCl in buffer A. Fractions containing RAD6A were pooled and applied at 0.5 ml/min to a 5-ml HiTrap Benzamidine FF column (GE Healthcare Life Science) equilibrated with buffer A containing 250 mM NaCl. The column was washed with 50 ml of equilibration buffer, and eluted with 50 ml of a linear gradient of 250-1500 mM NaCl in buffer A. Fractions containing RAD6A were pooled, diluted with buffer A to 400 mM of NaCl, and applied at 0.5 ml/min to a 5-ml HiTrap Capto adhere column (GE Healthcare Life Science) equilibrated with buffer A containing 400 mM NaCl. The column was washed with 50 ml of equilibration buffer, and eluted with 50 ml of a linear gradient of 400-2000 mM NaCl in buffer A. Fractions containing RAD6A were pooled and after the concentration of $(NH_4)_2SO_4$, was adjusted to 1.5 M by introduction of 4 M $(NH_4)_2SO_4$ then applied at 0.2 ml/min to a 1-ml HiTrap Phenyl HP (GE Healthcare Life Science) equilibrated with buffer A containing 1.5 M $(NH_4)_2SO_4$. The column was washed with 10 ml of equilibration buffer, and eluted with 10 ml of a linear gradient of 1.5-0 M $(NH_4)_2SO_4$ in buffer A. Fractions containing RAD6A were pooled

and applied at 0.1 ml/min to a Superdex 200 10/300 GL column (GE Healthcare Life Science) equilibrated with buffer A containing 100 mM NaCl. RAD6A was eluted in 16.97 ml, corresponding to an apparent molecular mass of 17 kDa. The peak fraction of RAD6A was frozen in liquid nitrogen and stored at –80ºC.

To purify ^{HA}RAD6A-^{FLAG}RAD18 complexes from human cells, a crude lysate from 293FT cells expressing ^{HA}*RAD6A* and ^{FLAG}*RAD18* was prepared following the manufacturer's instructions for the ANTI-FLAG M2 Affinity Gel (Sigma, A2220) except that the NaCl concentration was 300 mM. The lysate was applied to an ANTI-FLAG M2 Affinity Gel (Sigma, A2220) as described above. The eluted fraction was further purified using affinity to agarose conjugated Anti-HA-Tag (MBL, 561-8) following the manufacturer's instructions with buffer A containing 300 mM NaCl. For partial purification of untagged RAD6A-RAD18 complexes, a crude lysate from 293FT cells expressing *RAD6A* and *RAD18* was prepared similarly. The lysate was diluted 10 fold with buffer A containing 280 mM NaCl and purified through a 1-ml HiTrap Heparin HP column (GE Healthcare Life Science), and then a Superdex 200 10/300 GL column (GE Healthcare Life Science) following an established method to purify recombinant RAD6A-RAD18 complexes from *E. coli* cells (3).

References

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