Supplemental Data

Sororin Is Required for Stable Binding of Cohesin to Chromatin and for Sister Chromatid Cohesion in Interphase

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Supplemental Experimental Procedures

Reagents

Rabbit sororin antibody was raised against peptide SPTKPLRRS QRKSGSELPS, corresponding to residues 30–49 of human sororin. All other antibodies have been described [S1]. The plasmid expressing wild-type sororin has been described [S2]. Fugene (Invitrogen) was used according to the manufacturer's instructions for the transfection of plasmids with serum-free medium (OptiMEM, Invitrogen).

Cell Extracts, Immunoblotting, Immunoprecipitation, and Mass Spectrometry Analysis

Cell extracts were prepared as described [S3]. For solubilization of chromatin-bound proteins, the chromatin pellet was extensively washed with extraction buffer containing protease inhibitors. After resuspension in extraction buffer, chromatin was sonicated on ice four times via ten pulses on a Branson Sonifier 250 (output 3, 50% duty cycle). Nonsolubilized chromatin was spun down (10,000 × g, 10 min). Supernatant was collected and solubilized chromatin was digested with 0.6 U/μl TurboDNase (Ambion) for 20 min at 37°C according to the manufacturer's instructions. Total digestion of DNA fragments was assessed on an agarose gel by ethidium bromide staining. Protein concentration was between 2 and 4 μg/μl. Extracts were frozen in liquid nitrogen. Immunoprecipitations, immunoblotting, and mass spectrometry analysis were performed as described [S1].

Cell Culture, Chromosome Spreads, RNAi, and FACS

HeLa, HeLa CENP-A-EGFP [S4], and HeLa Smc1-EGFP [S5] cells were cultured in DMEM supplemented with 10% FCS, 0.2 mM L-glutamine, and antibiotics (all Invitrogen). For synchronization, double thymidine arrest was performed as described [S6]. Nocodazole was used at 100 ng/ml, etoposide at 5 μM, and caffeine at 2 mM (all from Sigma).

Chromosome spreads were performed as described [S7]. Synthetic siRNA oligonucleotides were purchased from Ambion. The sense sequence of the sororin siRNA oligo was GCCUAGGUGUC CUUGAGCU. Sequences of the other siRNA oligos have been described [S1, S6]. Transfection of siRNAs was performed as described [S8]. Cell-cycle profiling by FACS was performed as described [S3].

Immunofluorescence Microscopy

Cells were pre-extracted with 0.1% Triton X-100 prior to fixation and processed for immunofluorescence as described [S9]. DNA was counterstained with 1 μg/ml Hoechst 33342 (Molecular Probes). Coverslips were mounted onto slides with ProLong Gold (Molecular Probes). Images were acquired on a Zeiss Axioplan 2 microscope with a 40× Plan-Apochromat objective lens (Carl Zeiss, Jena). Scc1 fluorescence intensities were quantified with Definies Developer/Cellenger (Definies). Hoechst staining was used to define interphase cells, and CENP-A-EGFP expression was used to distinguish untransfected from siRNA-transfected cells. Total background-corrected Scc1 fluorescence intensities were normalized to total background-corrected Hoechst staining intensities. Scc1 fluorescence intensities of siRNA-transfected cells were then normalized to untransfected cells. The significance of the differences in Scc1 fluorescence intensities was tested by Student's t test at an α-level of 0.01.

Fluorescence In Situ Hybridization

BAC clone RP11-113F1 corresponding to the human *tff1* gene was from Children's Hospital Oakland Research Institute (Oakland, CA). FISH probes were labeled with PrimeIt II Random primer labeling kit (Stratagene) and Cy3-dCTP (Amersham Biosciences). Human cot-1 DNA and salmon sperm DNA (both from Invitrogen) were added and probes were precipitated and resuspended in hybridization buffer (50% formamide, 10% dextrane sulfate, 2× SSC).

Cells were spun onto glass slides with a Cytospin centrifuge (Shandon brand, Thermo Electric). Prior to fixation in 4% PFA, cells were preextracted with 0.1% Triton X-100 as described [S9]. Fixed cells were then treated successively with 0.5% Triton X-100, 50 μg/ml RNase, 100 mM HCl, 0.5% Triton X-100, and 73°C heated 70% and 50% formamide in SSC. Cells were incubated with the probe in a humidified chamber at 42°C and subsequently treated with 50% formamide in SSC at 42°C. DNA was counterstained with 0.25 μg/ml Hoechst 33342 (Molecular Probes) and slides were mounted with ProLong Gold (Molecular Probes).

Images were acquired on an Olympus IX71 microscope integrated into a DeltaVision deconvolution system (Applied Precision) with a PlanApo 100× objective lens. Z-stacks were deconvolved and projected with maximum intensity. Distance measurements were performed in Image J (http://rsb.info.nih.gov/ij/). The significance of the differences in distance was tested by Student's t test at an α-level of 0.01. In control- and Sgo1-depleted cells, the distance between paired FISH signals was near the resolution limit of the microscope, and only about half of the paired signals were resolved clearly enough to allow accurate distance measurements. This likely resulted in an overestimation of the interchromatid distance in control- and Sgo1-depleted cells. However, in sororin- and Scc1-depleted cells, the vast majority of paired signals were clearly resolved and could be measured. Therefore, the actual difference in interchromatid difference between control-/Sgo1-depleted cells and Sor-/Scc1-depleted cells is likely to be larger than our analysis suggests.

Photobleaching Experiments

Photobleaching experiments were performed as described [S10] with slight modifications. Cells were grown in LabTek I chambered cover glasses (Nunc). 1 hr before imaging, medium was changed to CO_2-independent medium without phenol red (Invitrogen) supplemented with 20% FCS, 0.2 mM L-glutamine, antibiotics, and 1 mM Na-pyruvate (all Invitrogen). 0.15 μg/ml Hoechst 33342 (Molecular Probes) was used to stain DNA, and 1 μg/ml cycloheximide (Sigma) was added to avoid new synthesis of Smc1-EGFP.

Images were acquired on a Zeiss LSM5 Live Duo Scan microscope with a 40× EC PlanNeofluar objective lens and open pinhole. Images were captured automatically over time with Multitime macro (Zeiss). For photobleaching, a Diode 100 mW 488 nm laser was used. The whole cell except for a small nuclear region was bleached. Because soluble Smc1-EGFP present in the unbleached area diffused into the bleached regions during the repetitive photobleaching, the fluorescence intensity of the unbleached nuclear region was also reduced. The first postbleach frame was acquired 2 min after photobleaching to allow for complete equilibration of bleached soluble Smc1-EGFP across the nucleus.

Image analysis was performed as described [S10]. Nuclear movements were corrected by registration based on the Hoechst channel, with the TurboReg plugin for ImageJ. Background-corrected mean intensities were normalized to the initial fluorescence intensity distribution and to total fluorescence, and the fluorescence recovery was quantitated by the difference in mean intensity between bleached and unbleached nuclear regions.

To determine the residence times and fractions of dynamically and stably chromatin-bound Smc1-EGFP in G2 cells, we fitted a sum of two exponential functions (biexponential) to individual data sets with least-square optimization for $k_{off}1$, $k_{off}2$, and the fraction of each in GraphPadPrism 4 (GraphPad Software). Stably chromatin-bound fractions of Smc1-EGFP in control and sororin-depleted cells were determined by fitting a biexponential function to individual data sets with $k_{off}1$ and $k_{off}2$ as previously determined. Nuclear Hoechst intensities were measured as

background-corrected total intensities. The significance of the difference in stably chromatin-bound fractions was tested by Student's t test at an α-level of 0.01.

Supplemental References

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