Supporting Online Material

Materials and methods

**Cell culture.** HeLa cells were cultured as monolayers in Dulbecco’s Minimal Essential Medium (Gibco BRL, Invitrogen Corporation, Carlsbad, CA, USA), supplemented with 10% fetal calf serum. Cells were incubated in a 37°C incubator in an atmosphere of 10% CO₂ in air. VH25 and C5RO normal human fibroblasts (kindly provided by Dr. L. Mullenders, Leiden University, The Netherlands and Dr. N. G. J. Jaspers, Erasmus MC, respectively) were cultured as monolayers in Dulbecco’s Minimal Essential Medium, supplemented with 15% fetal calf serum. The cells were incubated in a 37°C incubator in an atmosphere of 5% CO₂ in air. CHO cells expressing PCNA-GFP were grown as monolayers in a mixture of Dulbecco’s Minimal Essential Medium and Ham’s F10 medium (1:1) supplemented with 10% fetal calf serum and incubated in a 37°C incubator in an atmosphere of 5% CO₂ in air. Other primary fibroblasts used in this study were derived from ataxia telangiectasia-like disorder (ATLD) patients that carry mutations in Mre11 (S1). Two independent cell strains, ATLD2 and ATLD4, were used. The DNA repair deficient CHO cell lines used in this study were homologous recombination deficient irs1SF cells, defective in XRCC3, and non-homologous DNA end joining deficient XR-C1 cells (kindly provided by Dr. M. Zdzienicka, Leiden University) defective in the catalytic subunit of DNA-dependent protein kinase (S2).
**Irradiation of cells.** For irradiation the cells were cultured on ultra-thin Mylar film in custom-made culture dishes. The 1.8 µm Mylar film (Birkelbach Kondensatortechnik, Emdtebruck, Germany) was glued to glass rings with a diameter of 50 mm using Silicone Rubber (General Electric Silicones, Waterford, NY, USA). The dishes were left to cure at room temperature for 24 hours and then incubated during 15 min at 120°C to stretch the film. The Mylar surface was coated with carbon to improve attachment and spreading of cells (S3). Cells were plated at a concentration of 300,000 to 400,000 per dish in 16 ml culture medium and incubated for 24 hours at 37°C before treatment. The cells were irradiated at 21°C with α-particles using an Americium (Am-241) source with an activity of 140 kBq. The source with a diameter of 1 mm was placed at a distance of about 3 mm underneath the Mylar film. On average the α-particles entered the culture dish at an angle of 30° from the horizontal plane. The exposure time was 1 min. Subsequently, the cells were incubated at 37°C, unless otherwise noted.

**Fixation and immunohistochemical staining of cells.** Cell cultures were fixed at different time intervals after irradiation. For γH2AX staining and for γH2AX/Rad51 and γH2AX/Mre11 double staining cells were washed three times in PBS. To detect avidly bound Mre11 only, cells were washed with CSK and CSS buffer for γH2AX/Mre11 double staining (S4). The cultures were then fixed by incubation in a 2% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA, USA) solution in PBS for 15 min at 21°C and washed three times with PBS. Prior to immunochemical staining the cells were incubated for 30 min in TNBS (PBS supplemented with 0.1% Triton-X 100 and 1% fetal calf serum) to improve their permeability. Cells were then
incubated for 90 min in PBS+ (PBS supplemented with 1% fetal calf serum) containing the primary antibodies. For γH2AX staining Mouse anti-phospho Histone H2A.X (ser139), clone JBW301 (Upstate Biotechnology, Waltham, MA, USA) was used at a dilution of 1:100. For γH2AX/Rad51 double staining JBW301, diluted 1:100 was used in combination with rabbit anti-Rad51 (S5), diluted 1:2500. For γH2AX/Mre11 double staining JBW301 at a dilution of 1:100 in combination with rabbit anti-Mre11 (S6), diluted 1:500. After washing twice with TNBS for 5 min, cells were incubated in PBS+ containing the secondary antibodies: Goat anti-mouse CY3 (Jackson ImmunoResearch Laboratories, West Grove, Pen, USA, nr 115-165-100) and Goat anti-rabbit FITC (Jackson, nr 111-095-144). After another wash with TNBS cells were stained with Hoechst 33342 in a final concentration of 5 µg/ml for 10 min. After washing again with TNBS a droplet of Vectashield (Molecular Probes, Eugene, OR, USA) was placed on top of the stained cells and the cells were covered with a coverslip. The piece of Mylar containing the stained cells was then cut out and was placed, together with the coverslip, on a slide. The piece of Mylar with the coverslip on top of it was glued to the slide using rubber cement.

Occasionally we detected nuclei with more than one track. In the vast majority of those cases all tracks within a particular nucleus were of the same type. We observed that during the first 30 min some of the γH2AX-CDs increased in size. After 60 min the fraction of nuclei with tracks had decreased three-fold, suggesting that DNA double-strand break (DSB) repair might have occurred in a fraction of the cells. However, we have not been able to unambiguously identify signatures of DSB repair in the α-particle...
tracks visualized by γH2AX staining. This is due in part to the unknown nature of dephosphorylation events on γ2AX in response to the repair of a DSB. The phosphatase mediating this reaction has not been identified (S7). If the phosphatase were to act globally, all signal in the track would disappear simultaneously. By contrast, if it were to act locally, remnants of α-particle track as detected by γH2AX staining might be observed.

Time-lapse recordings covering a period of 4 hours following exposure revealed no disintegration of the cells, consistent with the application of a low dose of irradiation. Based on the assumption that every γH2AX domain harbors a complex DNA damage site and that the total number of DSBs is of the same order, the number of DSBs per track is approximately 22. Assuming that 40 DSBs per nucleus corresponds to a dose of 1 Gy, we estimate the dose deposited in one α-particle track at approximately 0.5 Gy.

**Microscopic analysis.** Preparations were observed using a Leica (Wetzlar, Germany) fluorescence microscope (DM RA HC). Micrographs were recorded using a cooled CCD camera (KX1400, Apogee Instruments, CA, USA). Stacks of 40 images were collected at intervals of 200 nm in the Z direction. The stacks of images were reconstructed and rendered using Huygens software (Scientific Volume Imaging, Hilversum, The Netherlands) and maximum intensity projections were made using Image Pro Plus software (Mediacybernetics, Carlsbad, CA, USA). For the analysis of the different types of tracks at least 100 cells per time point were used.
**PCNA expression.** CHO cells were transfected with a construct encoding PCNA-GFP. Immunoblotting was used to identify clones that expressed the fusion protein at endogenous levels. Time-lapse imaging of the cell line showed that the GFP signal reported on the different phases of the cell cycle as previously established by immunodetection of PCNA (S8). Cells in early, mid and late S phase (distinctive focal PCNA patterns) were easily distinguished from cells in G1 (a small nucleus with a homogeneous PCNA distribution) and G2 (a large nucleus with a homogeneous PCNA distribution) phase of the cell cycle.
Table S1. Quantification of α-particle-induced γH2AX-CD track morphology and Rad51 co-localization in primary human fibroblasts as a function of time after irradiation.

<table>
<thead>
<tr>
<th>Time after irradiation (min)</th>
<th>Group I Tracks with γH2AX-CD clusters</th>
<th>Group II Tracks without γH2AX-CD clusters</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-group Ia: Clusters only</td>
<td>Sub-group Ib: Clusters and non-clusters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rad51 foci</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend to table S1

Table S1. Quantitation of $\alpha$-particle-induced $\gamma$H2AX-CD track morphology and Rad51 co-localization in primary human fibroblasts as a function of time after irradiation. Displayed are the percentages of tracks observed within the indicated categories. The details of the $\gamma$H2AX-CDs track morphologies are described in the text. Co-localization of the $\gamma$H2AX-CDs with Rad51 protein is indicated. Tracks in the category ‘Other’ could not be classified unambiguously as Group I or II. Per time point at least 100 tracks were analyzed.

References

S5. J. Essers et al., DNA Repair 1, 779, 2002.