DNA compaction in the early part of the SOS response is dependent on RecN and RecA

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The nucleoids of undamaged Escherichia coli cells have a characteristic shape and number, which is dependent on the growth medium. Upon induction of the SOS response by a low dose of UV irradiation an extensive reorganization of the nucleoids occurred. Two distinct phases were observed by fluorescence microscopy. First, the nucleoids were found to change shape and fuse into compact structures at midcell. The compaction of the nucleoids lasted for 10–20 min and was followed by a phase where the DNA was dispersed throughout the cells. This second phase lasted for ~1 h. The compaction was found to be dependent on the recombination proteins RecA, RecO and RecR as well as the SOS-inducible, SMC (structural maintenance of chromosomes)-like protein RecN. RecN protein is produced in high amounts during the first part of the SOS response. It is possible that the RecN-mediated ‘compact DNA’ stage at the beginning of the SOS response serves to stabilize damaged DNA prior to recombination and repair.

INTRODUCTION

Faithful duplication of the genome is necessary to ensure genomic integrity through generations. During DNA replication the replication fork can encounter many different lesions in the DNA that make the fork stall or collapse. When the replication fork is compromised there is great risk of mutations and chromosome rearrangements, which lead to genome instability (Kuzminov, 1995). Several pathways exist that are involved in the restart of stalled replication forks (Heller & Marians, 2006; Michel et al., 2004). Mechanisms to protect and stabilize the replication fork in order to preserve genomic integrity are also found in eukaryotes (Branzei & Foiani, 2010). In prokaryotes, the pathways for fork reactivation involve homologous recombination performed by RecA protein (Michel et al., 2004). RecA protein is a multifunctional enzyme that carries out both homologous recombinational repair and regulation of the SOS response (McGrew & Knight, 2003). Upon DNA damage, RecA protein binds to ssDNA and multimerizes into a helical nucleoprotein filament that stimulates autoproteolysis of the LexA repressor, which leads to derepression of more than 50 LexA-regulated genes (Courcelle et al., 2001; Fernández De Henestrosa et al., 2000; Friedberg et al., 2006). These genes encode proteins involved in repair, recombination, replication and inhibition of cell division as well as proteins of unknown function. During recombinational repair, the RecA nucleoprotein filament searches for an intact homologous template and catalyses the exchange of strands between the two DNA molecules (Kuzminov, 1999).

In eukaryotes, the newly replicated sister chromatids are held together by cohesins, thereby facilitating homologous recombinational repair of DNA double-strand breaks (DSBs) during S phase (Peters & Nishiyama, 2012; Watrin & Peters, 2006). Cohesins have also been found to be recruited to DSB during G2 phase of the cell cycle where homologous recombination is the predominant repair pathway (Ström et al., 2004). The cohesins belong to the SMC (structural maintenance of chromosomes) protein family together with the condensins. These proteins are essential for chromosome segregation, compaction and DNA repair (Hirano, 2005; Watrin & Peters, 2006). Bacteria contain several different SMC-like proteins with condensing-like functions (Graumann & Knust, 2009). So far, no cohesin-like protein has been identified in Escherichia coli. The SOS-inducible RecN protein has structural similarities to proteins belonging to the SMC protein family, and is found to have a role in both the RecFOR and the RecBCD recombinational repair pathways (Grove et al., 2009; Lloyd et al., 1983; Picksley et al., 1984). When DNA breakage is induced at two or more distantly located sites, the presence of RecN protein is crucial (Meddows et al., 2005). Biochemical studies of purified RecN protein showed that the protein can stimulate intermolecular DNA interactions and has a weak ATPase activity (Grove et al., 2009; Reyes et al., 2010). Thus, it seems that RecN protein could possibly function as a cohesin during homologous recombinational repair.

When cells are exposed to UV light, pyrimidine dimers are formed in the DNA that have to be removed by the...
nucleotide excision repair system. There are different models that try to explain what happens when the replication fork collides into a pyrimidine dimer. The replication fork could halt and DNA synthesis could resume downstream from the lesion, forming daughter strand gaps that are repaired by the RecFOR pathway (Rupp & Howard-Flanders, 1968; Sedgwick, 1975). Another model suggests that replication fork reversal is required for reactivation of the blocked fork (McGlynn et al., 2001; McGlynn & Lloyd, 2002). Ongoing DNA replication is found to stop for at least 15–20 min in UV-damaged cells (Rudolph et al., 2007). This observation does not fit with the idea that replication continues immediately downstream from a lesion, but rather suggests that replication is delayed to allow restart of the replication fork (Rudolph et al., 2007).

In eukaryotes, the process of DNA repair depends not only on DNA–protein interactions and enzymatic activities, but also on a higher-order organization through the formation of highly dynamic DNA repair foci consisting of many repair proteins that accumulate around the site of damage (Lukas et al., 2005). It has also become clear that the higher-order chromatin structure and the nuclear architecture are important for efficient repair and genome maintenance (Misteli & Soutoglou, 2009). The concept of DNA repair hyperstructures may also be true for prokaryotes (Norris et al., 2007). Visualization of DNA DSB repair in live Bacillus subtilis cells revealed that RecN protein is recruited to the break followed by RecO and RecF proteins (Kidane et al., 2004). Together, the proteins localized as a discrete focus on the nucleoid and increasing the number of DSBs did not lead to comparable increases in the number of DNA repair foci (Kidane et al., 2004).

In E. coli, little is known about the organization of DNA repair. One study showed that a coaggregate of RecA protein and DNA was formed after induction of the SOS response when cells were treated with nalidixic acid or UV light (Levin-Zaidman et al., 2000). RecA-dependent condensation of the nucleoid has also been observed in UV-damaged B. subtilis cells (Smith et al., 2002). Recently, a report showed stress-induced, transient compaction of E. coli nucleoids in the absence of RecA protein, questioning the existence of an extensive homology search prior to DSB repair and suggesting that homologous sister molecules have an intrinsic ability to co-condense (Sneath et al., 2013). This report also showed that RecA protein was required for prolonged nucleoid compaction. We have shown previously that E. coli nucleoids fuse into compact structures at midcell when cells are exposed to UV light (Odsbu et al., 2009; Weel-Sneve et al., 2013). Here, we further characterize this process. By studying various repair-deficient mutants, we found that compaction of the nucleoid in UV-damaged cells depends on the recombination proteins RecA, RecO and RecR. Also, the SMC-like protein RecN, that is strictly SOS-regulated, was found to be required.

**METHODS**

**Bacterial strains and growth conditions.** All strains used were derived from E. coli K-12 and are listed in Table 1. Strains IO18 and IO20 were constructed by P1 transduction. Cells were grown at 37 °C in AB minimal medium (Clark & Maaløe, 1967) supplemented with 1 g thiamine ml⁻¹, 0.2 % glucose and 0.5 % Casamino acids (glu-CAA medium). The appropriate antibiotics were added in the following concentrations: 50 µg kanamycin ml⁻¹ and 10 µg tetracycline ml⁻¹. Mass growth was monitored by measuring the OD₄₅₀ with a spectrophotometer.

**UV irradiation and survival.** Exponentially growing cells (OD₄₅₀ 0.15) were UV irradiated (254 nm) at 3 J m⁻² in liquid culture. After irradiation, the cells were put back in the water bath to continue growth and samples were taken at various time points from that time. It took ~1.5 min to irradiate the cells and put them back in the water bath. An aliquot of 1.5 ml cell culture was harvested, washed once and resuspended in 100 µl ice-cold, filtered TE buffer. Then, 1 ml ice-cold, filtered 77 % ethanol was added for fixation. To determine survival after UV irradiation, irradiated and mock-treated cells were diluted and plated on LB agar (three parallels of each culture).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source/reference*</th>
</tr>
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<tbody>
<tr>
<td>AB1157</td>
<td>WT</td>
<td>Dewitt &amp; Adelberg (1962)</td>
</tr>
<tr>
<td>BW25113</td>
<td>WT, rph-1</td>
<td>CGSC 7636; Baba et al. (2006)</td>
</tr>
<tr>
<td>IO18</td>
<td>AB1157 ΔrecN772::kan</td>
<td>AB1157 × P1 JW5416-1 (this work)</td>
</tr>
<tr>
<td>IO20</td>
<td>AB1157 lexA3 zja-505::Tn10</td>
<td>AB1157 × P1 PK2099 (this work)</td>
</tr>
<tr>
<td>JC4728</td>
<td>AB1157 recA142</td>
<td>Skarstad &amp; Boye (1988)</td>
</tr>
<tr>
<td>JC9924</td>
<td>AB1157 recA430</td>
<td>Skarstad &amp; Boye (1988)</td>
</tr>
<tr>
<td>JW5416-1</td>
<td>BW25113 ΔrecN772::kan</td>
<td>CGSC 11670; Baba et al. (2006)</td>
</tr>
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<td>BW25113 ΔrecF735::kan</td>
<td>CGSC 11687; Baba et al. (2006)</td>
</tr>
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<td>BW25113 ΔrecO737::kan</td>
<td>CGSC 10028; Baba et al. (2006)</td>
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<tr>
<td>JW0461-2</td>
<td>BW25113 ΔrecR776::kan</td>
<td>CGSC 8615; Baba et al. (2006)</td>
</tr>
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<td>PK2099</td>
<td>lexA3 zja-505::Tn10</td>
<td>Hendricks et al. (2000)</td>
</tr>
<tr>
<td>SF128</td>
<td>AB1157 seqA–YFP</td>
<td>S. Fossum-Raunehaug (unpublished)</td>
</tr>
</tbody>
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*CGSC, Coli Genetic Stock Center.
Colonies were counted after 18–20 h incubation at 37 °C. The percentage of cells that survived was determined from the number of colonies formed by irradiated cells compared with the number of colonies formed by mock-treated cells. The experiment was repeated three times.

**Fluorescence microscopy.** Fixed cells were mounted on a poly-l-lysine-coated microscope slide and DNA was stained with Hoechst 33258 (5 μg ml⁻¹) in mounting medium (40% glycerol in PBS, pH 7.5). Live cells were stained with DAPI (1 μg ml⁻¹) and immobilized on an agarose pad (1% agarose in PBS, pH 7.5). Fluorescence, phase-contrast and differential interference contrast (DIC) images were acquired with a Leica DM6000B microscope. Narrow band-pass filter sets were used for fluorescence imaging. Pictures were taken using a Leica DFC350 FX digital camera that was connected to a computerized image analysis system (LAS AF software, version 2.0.0; Leica). Images were processed and analysed by Image J and Adobe Photoshop CS2 software.

**Assigning cells to nucleoid shape categories.** The nucleoids of cells grown in glu-CAA medium, fixed in 70% ethanol and stained with Hoechst 33258 have characteristic shapes. Normal cells and elongated cells with normal-looking nucleoids were placed in category 1 (see Fig. 1). Upon UV irradiation each irregular bilobed nucleoid condensed into a sphere. Cells with condensed nucleoids were placed in category 2. In order to be placed in category 2, the nucleoid must have occupied less than one-third of the cell volume, be spherical and be situated at midcell (in small cells) or at the quarter positions (in large cells). Cells with diffuse, extended nucleoids were placed in category 3. Nucleoids were classified as extended (category 3) as long as they were continuous. When the diffuse, continuous nucleoids no longer were continuous they transformed to the more irregular, normal form. All experiments were performed by the same researcher (and overseen by the same supervisor) to ensure that assignment to category was as similar as possible in all experiments.

**Immunoblotting with LexA antibody.** Exponentially growing cells (OD₄₅₀ 0.15) were UV irradiated at 10 J m⁻² in liquid culture as described above and cell extracts were prepared as described previously (Torheim et al., 2000). The same amount of total protein from each strain was subjected to 15% SDS-PAGE and proteins were transferred to a PVDF membrane by semi-dry blotting. The membrane was probed with LexA polyclonal antibody (Abcam; ab50953). Detection was performed with the ECF Western blotting kit (GE Healthcare) and the membrane was scanned on a ChemiGenius instrument (Syngene). Quantification of fluorescent bands was carried out with ImageQuant software (Molecular Dynamics).

**RESULTS**

**Reorganization of the nucleoid into a compact structure in UV-damaged cells**

The nucleoid of undamaged cells has a characteristic doublet shape (Zimmerman, 2006) and the number of nucleoids in each cell depends on the growth medium. Rapidly growing cells (glu-CAA medium) contain two or four fully or partially...
separated nucleoids (Fig. 1a, untreated cells). Exponentially growing AB1157 WT cells were exposed to a sublethal UV dose (3 J m⁻²) and samples were taken at the indicated time points after irradiation. The cells were fixed, mounted on microscope slides and DNA stained with Hoechst 33258. The shape of the nucleoid was found to change over time. At 3 min after irradiation, ~13% of the cells contained one compact nucleoid in the middle of the cell, i.e. each bilobed nucleoid present in the undamaged cell had fused into one compact structure (or two bilobed nucleoids had fused into two compact structures in the largest cells) (Fig. 1a, b). The number of cells containing such compact structure(s) increased at 6 and 9 min, and reached a maximum at 12–15 min after UV irradiation (~60% of the cells). Whilst the number of cells with normal nucleoids decreased gradually until no such cells were found at 30 min, cells containing a more diffuse, extended nucleoid appeared at 9 min and almost all cells contained extended nucleoids at 30–60 min. These cells were also longer than normal as cell division was inhibited due to induction of the SOS response after UV irradiation. At 90 min, ~60% of the cells had divided and contained normal nucleoids, whilst the rest of the cells (the long ones) contained an extended nucleoid or several normal nucleoids. Taken together, these results indicate that the nucleoid is reorganized in response to UV damage. All the cells in the population seemed to show the same nucleoid compaction and decompaction dynamics. First, the two (or four) nucleoids fused into one (or two) compact structure(s) in the middle of the cell, then the compact structure was stretched out to an extended, diffuse appearance, and finally the extended nucleoid split and reformed into several normal nucleoids, and the cell divided. This sequence of events seems to be independent of UV dose. Cell cultures irradiated with UV light at 10, 25 or 50 J m⁻² all reached a maximum of ~60% cells with compact nucleoid(s) by ~15 min after irradiation (data not shown). A dose-dependence was observed in that the compact nucleoid persisted for a longer time period when the cells were exposed to higher UV doses. Also, the extended nucleoid stage seemed to last longer because all cells were found to be long and contain diffuse and extended nucleoids at the 90 min time point (after irradiation at 25 and 50 J m⁻²).

Reorganization depends on a functional RecA protein and SOS-inducible proteins

Two key proteins are involved in the induction of the SOS response upon DNA damage. These are the LexA repressor and RecA protein (Friedberg et al., 2006). Binding of LexA protein to the LexA-binding site(s) present in the promoter region of SOS-inducible genes represses their transcription under normal growth conditions. When the DNA is damaged, RecA protein binds to ssDNA and forms a nucleoprotein filament that is involved in the autoproteolysis of LexA protein (SOS induction) and in homologous recombination. We investigated DNA compaction in UV-irradiated lexA3 and recA mutant cells. In lexA3 mutant cells, the LexA repressor is not cleavable with RecA protease, i.e. genes regulated by the LexA repressor are not transcribed in response to DNA damage (Mašek et al., 1989). Figs 2(a) and 3(a) show that lexA3 mutant cells did not compact the nucleoid or exhibit a diffuse, extended nucleoid after UV irradiation. This suggests that SOS-regulated proteins are required for nucleoid compaction and decompaction to occur when the DNA is damaged. To investigate the role of RecA protein and to distinguish between the two enzymatic activities of RecA protein, we studied two different mutants, recA430 and recA142. The recA430 mutant is recombination-proficient and -deficient in some of the co-protease activities (Cazaux et al., 1991; Devoret et al., 1983; Roberts & Roberts, 1981). Only a slight SOS induction is observed, presumably due to the low capacity of the RecA430 mutant protein to promote LexA self-cleavage compared with WT RecA protein (Cazaux et al., 1991; Devoret et al., 1983). UV mutagenesis is hardly detectable, indicating that the recA430 mutant is unable to derepress the SOS-inducible genes and elicit a SOS response (Waleh & Stocker, 1979). The recA142 mutant is recombination-deficient with partly intact protease-stimulating functions (Clark, 1973; Dutreix et al., 1985; Kowalczykowski et al., 1989; Kowalczykowski & Krupp, 1989; Roberts & Roberts, 1981). The mutant displays UV-induced mutagenesis at a frequency lower than that observed for the WT (Blanco et al., 1982). Mutations in the recA gene that lead to recombination deficiency seem to be accompanied by alterations in protease function, i.e. it might not be possible to fully separate the two RecA functions (Tessman & Peterson, 1985). As shown in Fig. 2(b, c), as well as in Fig. 3(b, c), both the recA430 and the recA142 mutants were unable to compact the DNA after exposure to a low dose of UV light (3 J m⁻²). The shape of the nucleoids in both mutants changed directly from the normal shape to the extended, diffuse shape without entering the compact stage in between as in WT cells (Fig. 3b, c). These results indicate that both RecA filament formation and SOS induction are required to initiate a DNA compaction process in UV-damaged cells. We also investigated nucleoid compaction after RecA overproduction in the SOS-deficient cells (lexA3 mutant cells with production of extra RecA from a plasmid) and found that irradiated cells were unable to compact the DNA when RecA protein was present at a level well above what was found in UV-irradiated WT cells (data not shown). Thus, formation of the RecA filament alone is not sufficient for DNA compaction to occur.

RecN protein is required for reorganization of the nucleoid after UV irradiation

The SMC-like protein RecN is the second most induced protein in the SOS response and is found to have a role in DNA DSB repair (Courcelle et al., 2001; Picksley et al., 1984). In vitro, purified RecN protein has been found to have a weak ATPase activity and DNA-bridging activity (Grove et al., 2009; Reyes et al., 2010). We found that cells lacking RecN protein did not compact or decompact the nucleoid after irradiation with a UV dose of 3 J m⁻².
The UV-irradiated recN-deficient cells seemed to maintain the nucleoid morphology of untreated cells throughout the SOS response (Fig. 3d). This means that the diffuse and extended nucleoid stage was also missing in cells lacking RecN. We confirmed that recN-deficient cells mounted a normal SOS response by measurement of the extent of LexA repressor cleavage after irradiation (Fig. 4). The results showed that the SOS-inducible RecN protein was required for execution of the observed DNA compaction and decompaction program during the SOS response. Lack of degradation of RecN protein by ClpXP protease does not lead to prolonged compaction of the nucleoid

It has previously been shown that RecN protein is a substrate for the ClpXP protease (Nagashima et al., 2006; Neher et al., 2006). Controlled degradation of many SOS-regulated proteins has been shown to be important in adjusting protein levels during the SOS response (Pruteanu & Baker, 2009). Since RecN protein is rapidly degraded \( t_{1/2} = 10 \text{ min} \) after induction of the SOS response, it could be that RecN degradation is required to execute the decompaction process (Fig. 1b, diffuse, extended nucleoid). If so, the compact nucleoid may persist for a longer time in cells lacking the ClpXP protease due to a slower degradation of RecN protein. We investigated DNA compaction and decompaction in UV-irradiated clpX- and clpP-deficient cells, respectively, and found that they had the same phenotype as UV-irradiated WT cells (data not shown). Hence, lack of RecN degradation did not seem to affect the kinetics of the decompaction process.

Formation of a compact nucleoid is delayed in a recF-deficient mutant, and absent in recO- and recR-deficient mutants

The RecFOR complex, together with RecA protein, carries out recombinational repair of daughter strand gaps that are formed in the chromosomes of UV-irradiated cells (Kuzminov, 1999). The ssDNA-binding proteins that are bound to the ssDNA are displaced by the RecA filament with the aid of the RecFOR complex, thereby accelerating DNA strand exchange (Morimatsu & Kowalczykowski, 2003). As RecFOR proteins are involved in the repair of UV damage we investigated whether nucleoid compaction was impaired in recF, recO and recR deletion mutants, respectively. The nucleoids of BW25113 WT cells irradiated with 3 J m\(^{-2}\) UV light showed the same compaction/decompaction pattern as in AB1157 WT cells (compare Figs 5a and 1b). The same pattern was also observed in MG1655 WT cells (data not shown). This indicates that the reorganization of the nucleoid in response to DNA damage is independent of WT background. The recF-deficient cells

(Fig. 2d). The UV-irradiated recN-deficient cells seemed to maintain the nucleoid morphology of untreated cells throughout the SOS response (Fig. 3d). This means that the diffuse and extended nucleoid stage was also missing in cells lacking RecN. We confirmed that recN-deficient cells mounted a normal SOS response by measurement of the extent of LexA repressor cleavage after irradiation (Fig. 4). The results showed that the SOS-inducible RecN protein was required for execution of the observed DNA compaction and decompaction program during the SOS response.

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were found to be less efficient in nucleoid compaction compared with WT cells (Fig. 5b). Approximately 30% of the cells contained a compact nucleoid at 15 min after irradiation. In comparison, ~70% of the WT cells contained a compact nucleoid at the same time point (Fig. 5a). At 30 min, ~20% of the recF-deficient cells contained a compact nucleoid, whereas the nucleoids of most WT cells were found to be in a diffuse, extended state at that time point. Also, the number of cells containing normal nucleoids persisted for a longer time period in the recF-deficient cells. At 90 min, all cells still contained diffuse, extended nucleoids. These results indicate that the compaction/decompaction process is delayed when RecF protein is not present. In cells lacking RecO or RecR protein, formation of a compact nucleoid was not observed (Fig. 5c, d). Also, it was found that cells contained normal nucleoids for

Fig. 3. Nucleoid compaction in UV-irradiated lexA, recA and recN mutant cells. Exponentially growing (a) lexA3, (b) recA430, (c) recA142 and (d) recN mutant cells were irradiated with UV light at 3 J m⁻². The cells were fixed in 70% ethanol at the indicated time points, mounted on microscope slides and DNA stained with Hoechst 33258. The percentage of cells belonging to a specific category (based on the nucleoid shape) is plotted against the time point after UV irradiation. The categories are cells with normal nucleoids (○), compact nucleoids (□) and diffuse, extended nucleoid (Δ) (as in Fig. 1b). For the recN mutant cells, the mean ± SD of three experiments is shown; for the lexA3, recA430 and recA142 mutant cells, the mean ± SD of two experiments is shown. In total, 200–600 cells were counted for each time point.

Fig. 4. Cleavage of LexA protein in UV-irradiated WT and recN mutant cells. (a) Exponentially growing WT AB1157 cells and recN mutant cells were irradiated with UV light at 10 J m⁻². Samples for SDS-PAGE were prepared at indicated time points after UV irradiation and analysed by 15% SDS-PAGE. LexA protein was detected by immunoblotting with anti-LexA antibody. (b) The relative amount of LexA protein (normalized to the amount at time 0) was plotted against the time after UV irradiation (△, AB1157; ■, ΔrecN). The mean ± SD of three experiments is shown.
a longer time period after irradiation and all cells contained diffuse, extended nucleoids at 90 min.

**Active replication forks must be present in order to induce the SOS response**

Cells containing a compact nucleoid at midcell appeared at 3 min after UV irradiation and the total number of cells containing a compact nucleoid reached a maximum at 12–15 min (Fig. 1b). The response was not immediate in all cells. Some cells reacted a few minutes after irradiation, whilst other cells reacted later. The variation in the onset of DNA compaction could be explained by the observation that the SOS response is induced when cells attempt to replicate UV-damaged DNA, which leads to the generation of ssDNA and RecA filament formation (Sassanfar & Roberts, 1990). As replication forks will collide with the damaged DNA at different time points in different cells, the SOS response will be induced at various time points depending on when a replication fork encounters a pyrimidine dimer in a particular cell. We found that the initial stage of the DNA compaction process was largely the same for the four UV doses tested here. This finding supports the idea that it is the first encounter of a replication fork with a DNA lesion rather than the total number of DNA lesions present in the chromosome that decides if and when the SOS response is induced. To further investigate whether the presence of active replication forks are required to induce the SOS response, we studied DNA compaction in slowly growing WT cells (data not shown). When *E. coli* is grown in a medium containing acetate as the carbon source it has a cell cycle that is similar to the eukaryotic cell cycle, i.e. the smallest cells contain one chromosome and no replication forks (B phase), and initiation of DNA replication occurs later in the cell cycle (C phase) when the cells are bigger in size (Stokke et al., 2012). We observed that the smallest cells in the population remained small and contained normal nucleoids after irradiation at 50 J m$^{-2}$, whilst the bigger cells became larger and contained compact DNA. Thus, it seemed that the SOS response was not induced in the smallest cells containing no replication forks. This finding further supported the idea that replication is required for induction of the SOS response (Sassanfar & Roberts, 1990).

**Localization of replication forks is changed during compaction**

The SeqA protein binds to newly replicated, hemimethylated DNA behind the replication fork and forms foci that can be visualized by immunostaining with SeqA antibody.
UV dose of 3 J m⁻² tagged SeqA protein in live cells 15 min after exposure to a studied the localization of yellow fluorescent protein (YFP)-tagged SeqA protein in live cells 15 min after exposure to a (Molina & Skarstad, 2004; Morigen et al., 2009). Here, we studied the localization of yellow fluorescent protein (YFP)-tagged SeqA protein in live cells 15 min after exposure to a UV dose of 3 J m⁻² (Fig. 6). The cells were grown in glu-CAAmedium at 28 °C to obtain a situation where the cells contained four replication forks through most of the cell cycle and one to four SeqA foci (S. Fossum-Raunehaug and K. Skarstad, unpublished). As shown in Fig. 6(a), exponentially growing cells contained one to four SeqA foci as expected. In cells containing one SeqA focus, the proteins were localized typically at midcell, whilst in cells with more than one focus, the SeqA proteins were localized typically at the quarter positions of the cell in accordance with earlier results (Morigen et al., 2009). The DAPI-stained DNA in exponentially growing cells appeared diffuse and extended. DAPI staining is not ideal for visualization of the DNA organization in living cells and was simply included here to verify that compaction of the DNA had occurred after DNA damage. The different appearance from Hoechst-stained DNA of ethanol-fixed cells (Fig. 1a) could be due to differences in the state of the nucleoid in live and fixed cells (the appearance of nucleoids will necessarily change depending on the fixation technique used). After exposure to UV light, the DNA was reorganized into a more compact structure as observed in fixed cells (Figs 6b and 1a). Also, the SeqA foci were found in the middle of the cell bound to the compact DNA. In some cells, all the SeqA protein was seen as one large spot which seemed rather irregular in shape, i.e. it probably consisted of several SeqA foci positioned next to each other or on top of each other. Imaging of several layers (z-stack) of irradiated, fixed cells immunostained with SeqA antibody revealed that the large fluorescent spot observed at 15 min after irradiation must consist of several SeqA foci (data not shown). These results showed that SeqA focus formation is not lost during the DNA compaction process. The findings indicated that hemimethylated DNA was present during the DNA compaction part of the SOS response. The results are also in accordance with the finding of Shechter et al. (2013) of co-condensation of sister replicohores after stress induction.

**DISCUSSION**

**RecN protein has an important role in SOS-induced DNA compaction**

We report here that in WT cells irradiated with a sublethal dose of UV light, a massive and rapid compaction of the DNA occurred. Formation of the RecA nucleoprotein filament was found to be necessary for the DNA compaction. Further, it was found that the SOS-inducible RecN protein must be present. Little is so far known about the biological role of RecN protein in *E. coli* except that it has cohesin-like properties. The *recN* gene is induced rapidly after SOS induction and it has been suggested that RecN protein might be recruited to DNA DSBs in order to tether the DNA around the damaged sites (Meddows et al., 2005). The results presented here may indicate that RecN protein also has a more general role in DNA organization during the early part of the SOS response. Cells lacking RecN function are sensitive to mitomycin C and ionizing radiation, but not to UV light (Picksley et al., 1984). This observation might indicate that the RecN-mediated compaction of DNA is especially important when DSB repair is required.

The DNA compaction which requires the concerted action of the RecA filament and RecN protein may also involve several other proteins. Studies of *recF*, *recO* and *recR*-deficient cells revealed that DNA compaction occurred to a lesser extent in the *recF* mutant, and did not occur at all in the *recO* and *recR* mutants. In all mutants, the shift from cells containing normal nucleoids to cells containing

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**Fig. 6.** Localization of SeqA–YFP in live cells before and after exposure to UV light. Exponentially growing cells carrying YFP fused to the *seqA* gene were irradiated with UV light at 3 J m⁻². At 15 min after irradiation the cells were stained with DAPI and immobilized on an agarose pad (b). A portion of the cell culture was not exposed to UV light (a). The fluorescence images are shown merged with the DIC image. The experiment was repeated three times.
diffuse, extended nucleoids seemed to be delayed compared with WT cells. Also, cells containing diffuse, extended nucleoids persisted for a longer time. The observed delay in the reorganization of the nucleoid is consistent with the finding that the induction of the SOS response is delayed in UV-irradiated recF, recO and recR mutant cells (Hegade et al., 1995; Thoms & Wackernagel, 1987; Whitby & Lloyd, 1995). It has also been found that RecO and RecR, in the absence of RecF, are capable of binding to ssDNA-binding-protein-coated ssDNA and aid in loading of the RecA protein onto the ssDNA (Umezu et al., 1993; Umezu & Kolodner, 1994). The fact that nucleoid compaction occurred in the absence of RecF protein, but not in the absence of RecO or RecR proteins, further supports the idea that RecA filament formation is required in order to compact the nucleoid in response to DNA damage.

Diffuse and extended nucleoids

About 20 min after compaction of the DNA, the chromosome morphology changed again, allowing the DNA to unfold from the compact state and disperse, filling the cell volume. As described above, cells unable to turn on the SOS response or deficient in RecN, RecA, RecO or RecR function did not perform compaction of nucleoids upon UV irradiation. The cells deficient in RecA, RecO or RecR function did, however, after ~60–90 min, yield a phenotype very much like the ‘diffuse and extended nucleoid’ phenotype seen in WT cells. In lexA3 and recN mutant cells this did not happen. These cells had WT-looking nucleoids throughout the 90 min period that was studied. These results show that in the SOS-induction-deficient cells and the cells without RecN function, the launch of the compaction/decompaction process does not occur. In contrast, cells that were deficient in aspects of formation of the RecA filament eventually formed diffuse and extended nucleoids.

Transition from the ‘compact DNA’ stage to the ‘dispersed DNA’ stage

We found that failure to degrade the RecN protein did not lead to a prolonged duration of the compact DNA stage. Thus, it seems that the end of this stage is not dependent on a disappearance of RecN protein. Instead, it may be that the DNA compaction activity by RecN protein ceases upon reception of certain signals, at the same time also causing a transition in RecA protein activity. We found previously that overexpression of the SOS-inducible DinQ transmembrane peptide caused a prolongation of the compact DNA stage of the SOS response (Weel-Sneve et al., 2013). Thus, membrane-mediated signals may be required for nucleoids to cease being in a compact state and progress to the dispersed DNA stage.

DNA compaction may represent a stabilization stage prior to DNA repair

The SOS gene expression response was found to last 60–90 min after low-dose UV irradiation. This is a very long time, longer than it takes to replicate the entire genome. It could thus be that the first part of the response, i.e. the compaction stage, occurs in order to stabilize the situation in order to deal with critical damage, e.g. stabilize sister chromosomes prior to DSB repair. Less critical repair may be mainly performed during the diffuse and extended nucleoid phase of the SOS response. The observations by Shechter et al. (2013) and Levin-Zaidman et al. (2000) of DNA condensation and formation of an ordered RecA–DNA coaggregate in DNA-damaged cells support the idea of an early SOS response stage involving stabilization of the DNA.

In replicating cells, SeqA protein forms complexes with the new DNA behind replication forks in a dynamic fashion, i.e. continually binding the newest DNA and apparently allowing Dam methylase to methylate the DNA that is released from the complexes (Waldminghaus et al., 2012; Waldminghaus & Skarstad, 2009). Here, we observed that upon UV irradiation SeqA–hemimethylated DNA complexes were situated in the centre of the compact DNA and persisted throughout the compact DNA stage. This indicates that SeqA structures were transported with the bulk of the DNA to the cell centre during the compaction and that replication forks may have been stabilized during the compact DNA stage by the pre-existing SeqA–new DNA complexes. Our results are in accordance with the observation by Shechter et al. (2013) that compaction of the nucleoid after stress induction involves specifically co-condensation of sister replisomes. As induction of the SOS response results in replication inhibition and synthesis of the UmuC and UmuD proteins, it is possible that the UmuD2C complex, which antagonizes the activity of the replicative polymerase, causes inhibition of replication fork progression (Courecelle et al., 2005; Opperman et al., 1999). If so, SeqA complexes will necessarily cease to be dynamic (due to lack of DNA synthesis) and possibly contribute to the stabilization of forks destined to undergo replication fork reversal and recombination, possibly also contributing to a limitation of the extent of fork reversal.

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