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Research paper

RecJ nuclease is required for SOS induction after introduction of a double-strand break in a RecA loading deficient *recB* mutant of *Escherichia coli*

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Abstract

The SOS response is an important mechanism which allows *Escherichia coli* cells to maintain genome integrity. Two key proteins in SOS regulation are LexA (repressor) and RecA (coprotease). The signal for SOS induction is generated at the level of a RecA filament. Depending on the type of DNA damage, a RecA filament is produced by specific activities (helicase, nuclease and RecA loading) of either RecBCD, RecF or a hybrid recombination pathway. It was recently demonstrated that RecA loading activity is essential for the induction of the SOS response after UV-irradiation. In this paper we studied the genetic requirements for SOS induction after introduction of a double-strand break (DSB) by the I-*Sce*I endonuclease in a RecA loading deficient *recB* mutant (*recB1080*). We monitored SOS induction by assaying β-galactosidase activity and compared induction of the response between strains having one or more inactivated mechanisms of RecA loading and their derivatives. We found that simultaneous inactivation of both RecA loading functions (in *recB1080 recO* double mutant) partially impairs SOS induction after introduction of a DSB. However, we found that the RecJ nuclease is essential for SOS induction after the introduction of a DSB in the *recB1080* mutant. This result indicates that RecJ is needed to prepare ssDNA for subsequent loading of RecA protein. It implies that an additional type of RecA loading could exist in the cell.

Keywords: SOS response; Double-strand break (DSB); RecA loading; RecBCD; *E. coli*

1. Introduction

An essential requirement for all living organisms is the maintenance of genome integrity. Homologous recombination and several additional mechanisms of DNA repair evolved to perform this task. Bacterial cells often experience various environmental conditions which cause an increase in DNA damage. To increase the capacity for DNA repair the expression of more than 40 genes is elevated in a coordinated manner in response to DNA damage [1,2]. This important inducible DNA repair system is called the SOS response [3–5]. In addition to increased DNA repair, the elevated expression of SOS genes increases damage tolerance, DNA replication and mutagenesis [5]. Two key proteins in SOS regulation are LexA and RecA. The LexA protein is a repressor of the SOS regulon which binds to regulatory elements, called SOS boxes, located upstream of the SOS genes [6–8]. The RecA protein plays a role in the self-cleavage of the LexA repressor. To perform this role RecA protein must be activated, and this occurs at the level of a RecA-single-strand DNA (ssDNA) filament; for review see Ref. [5].

The RecA-ssDNA filament is produced by coordinated action of three enzymatic activities: helicase, nuclease and RecA

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loading on ssDNA. These activities are provided by the proteins of two recombination pathways in *Escherichia coli*: RecBCD and RecF [9]. The RecBCD enzyme exhibits all of these activities whereas in the RecF recombination pathway different proteins provide the different activities: RecQ (helicase), RecJ (nuclease) and RecFOR (RecA loading) [10]. RecJ is a 63 kDa protein with important biological functions in recombination and in the excision step of methyl-directed mismatch repair [11,12]. The RecJ protein is a processive 5′–3′ ssDNA exonuclease (degrades ∼ 1000 nucleotides after a single binding event) [13] which leaves the 3′-ssDNA tail coated with SSB protein. The RecA-ssDNA filament is generated by the replacement of SSB protein with RecA protein by RecFOR function. RecBCD-dependent SOS induction occurs after treatment with DNA-damaging agents (γ-irradiation, bleomycin, nalidixic acid, etc.) which introduce double-strand DNA breaks (DSBs) [5,14,15]. RecF-dependent SOS induction takes place when cells are treated with agents such as UV-irradiation which introduce intrastrand cross-links including pyrimidine dimers [5]. In *wt* cells RecF-dependent SOS induction is a result of the processing of single-strand gaps (SSGs) which occur in daughter strands after reinitiation of DNA replication downstream from a noncoding lesion [9,10,16]. The proteins of the RecF recombination pathway can induce the SOS response after the processing of a DSB in a multiple mutant *recBC sbcBC(D)* with inactivated RecBCD enzyme and SbcCD nuclease, and altered function of Exonuclease I [9,17].

It was recently shown that RecA loading activity is important for SOS induction after UV-irradiation [18]. This was done by testing for SOS induction after UV-irradiation in a *recB1080* mutant, a strain with deficient RecA loading, and its derivatives [19,20]. The point mutation in this allele is located in the nuclease domain of the RecBCD enzyme ($RecB^{nuc}$), which is also involved in RecA binding [21]. Consequently, the RecB1080CD form of the enzyme is nuclease deficient and is unable to load RecA protein onto ssDNA, but it has a functional helicase activity [19,22,23]. SOS induction after UV-irradiation in a *recB1080* mutant is independent of the RecQ helicase and is partially dependent on the RecJ nuclease [18]. A specific phenotype of the *recB1080* mutant is strong constitutive expression of the SOS regulon which is dependent on *recJ* and *recD* mutations, but independent of *recFOR* mutations [18]. Constitutive or basal SOS expression is caused by endogenous DNA damage. It was shown that in *wt* cells constitutive SOS expression is caused predominantely (in 62% of cases) by DSBs [24].

In this paper we studied the genetic requirements for SOS induction after the introduction of a double-strand break (DSB) *in vivo* by the I-*Sce*I endonuclease [25,26]. We compared the levels of SOS induction in strains in which one or both mechanisms of RecA loading were inactivated in the absence or presence of external DNA damage (DSB). We found that the RecA loading function of RecBCD and RecFOR proteins is partially required for SOS induction after introduction of a DSB (external DNA damage). RecFOR-mediated RecA loading and RecQ helicase are not important for constitutive SOS expression (endogenous DNA damage) in a *recB1080* genetic background [18]. On the other hand, we found that the RecJ nuclease is required for both, constitutive SOS expression, and SOS induction after the introduction of a DSB. This result indicates that RecJ is needed to prepare ssDNA for subsequent loading of RecA protein. Moderate SOS induction when both known mechanisms of RecA loading are inactivated (in *recB1080 recO* double mutant) may indicate the existence of an additional type of RecA loading which requires the RecJ nuclease function. Based on a comparison of the SOS response due to endogenous DNA lesions and due to induced DSB, we suggest that similar to *wt* cells [24], the majority of endogenous DNA lesions in a *recB1080* mutant are DSB, i.e. collapsed replication forks.

2. Materials and methods

2.1. Bacterial strains and bacteriophages

The bacterial strains used in this study are presented in Table 1. The N5071, TRM452 and TRM387 bacterial strains were kindly provided by R.G. Lloyd, University of Nottingham, England [26]. Transductions were carried out according to Miller [29].

2.2. Media and growth conditions

Bacteria were grown in high salt Luria broth (LB) medium at 37 °C with aeration to the early-log phase (OD_{600} ∼ 0.2), and then used for induction of I-*Sce*I expression and β-galactosidase assay.

2.3. DSB induction and measurement of β-galactosidase activity

The strains used in this study had a chromosomal deletion of the *lac* operon and a *lacZ* insertion downstream of the regulatory region of *sfiA*, a gene which belongs to the SOS regulon. The levels of SOS induction were assayed by monitoring β-galactosidase activity (expressed in Miller units) [29] which is proportional to the level of SOS induction. After reaching early-log phase, L-arabinose was added to a final concentration of 0.2 % (w/v) to induce the expression of I-*Sce*I endonuclease. The position of the I-*Sce*I endonuclease recognition sequence is within the *argE* gene which is linked to an antibiotic resistance marker. The I-*Sce*I cassette is driven by P_{BAD} (the *araBAD* promoter) [25]. Therefore, the addition of L-arabinose causes DNA breakage and the induction of the SOS response. In control cells, glucose was added to the same final concentration of 0.2 % (w/v) to prevent I-*Sce*I expression. To test if there is a possible leaky expression of the *araBAD* promoter as documented previously [30], we constructed a mutant strain carring the I-*Sce*I cassette without the I-*Sce*I cleavage site (strain IV408, see Table 1). The levels of β-galactosidase activity in this strain present ''uncut control'', i.e. basal expression from the *sfiA* promoter. To further show that the *araBAD* promoter is specifically induced by addition

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Table 1
E. coli K-12 strains used in this study

Bacterial strain	Relevant genotype	Source of reference
Bacterial strains related to AB1157		
AB1157	F *thr-1 leuB6* Δ(*gpt-proA)62 hisG4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 supE44 rpsL31 kdgK51 rfbD1 mgl-51* λ^{cs} *rac*$^-$	[27]
AM208	+*recR256*::Tn*5*	R.G. Lloyd
N5170	+*thr*$^+$*leu*$^+$Δ(*pro-lac*) *sfiA*::*Mud* (*Ap lac MuB*::Tn*9*)	R.G. Lloyd
N3071	+*recB268*::Tn*10*	R.G. Lloyd
RIK174	+*recB1080*	[20]
RIK144	+*recD1903*::Tn*10d(Tet)*	[20]
IRB103	+*recO1504*::Tn*5*	[28]
IIB360	+*recB1080 argA*::Tn*10*	P1.N5071 × RIK174
IIB290	+ *recB1080 recD1903*::Tn*10d(Tet)*	P1.RIK144 × RIK174
IIB294	+*recF400*::Tn*5*	P1.WA576 × AB1157
LMM1032	+*recJ2052*::Tn*10kan*	D. Zahradka
LMM1215	+Δ*recQ*::*kan*	D. Zahradka
WA576	+*recF400*::Tn*5*	W. Wackernagel
Bacterial strains related to MG1655		
MG1655	F$^-$*rec*$^+$(*wt*)	[27]
N5071	+*argA*::Tn*10*	R.G. Lloyd
TRM452	+Δ*lac* Δ*attB*::P_{BAD}I-*Sce*I	R.G. Lloyd
TRM387	+Δ*argE*::I-*Sce*I_{cs}::*cat* Δ*attB*::P_{BAD}I-*Sce*I	[26]
IV408	+Δ*lac* Δ*attB*::P_{BAD}I-*Sce*I *sfiA*::*Mud* (*Ap lac MuB*::Tn*9*)	P1.N5170 × TRM452
IIB385	+Δ*lac* Δ*attB*::P_{BAD}I-*Sce*I Δ*argE*::I-*Sce*I_{cs}::*cat*	P1.TRM387 × TRM452
IIB386	+Δ*lac* Δ*attB*::P_{BAD}I-*Sce*I Δ*argE*::I-*Sce*I_{cs}::*cat sfiA*::*Mud* (*Ap lac MuB*::Tn*9*)	P1.N5170 × IIB385
IIB388	as IIB386 + *recB1080 argA*::Tn*10*	P1.IIB360 × IIB386
IIB390	as IIB386 + *recB1080 recD1903*::Tn*10d(Tet)*	P1.IIB290 × IIB386
IIB392	as IIB386 + *recO1504*::Tn*5*	P1.IRB103 × IIB386
IIB393	as IIB386 + *recB1080 recD1903*::Tn*10d(Tet) recO1504*::Tn*5*	P1.IRB103 × IIB390
IIB395	as IIB386 + *recD1903*::Tn*10d(Tet)*	P1.RIK144 × IIB386
IIB401	as IIB386 + *recB1080 argA*::Tn*10 recO1504*::Tn*5*	P1.IRB103 × IIB388
IIB407	as IIB386 + Δ*recQ*::*kan*	P1.LMM1215 × IIB386
IIB408	as IIB386 + *recB1080 argA*::Tn*10* Δ*recQ*::*kan*	P1.LMM1215 × IIB388
IIB409	as IIB386 + *recJ2052*::Tn*10kan*	P1.LMM1032 × IIB386
IIB410	as IIB386 + *recB1080 argA*::Tn*10 recJ2052*::Tn*10kan*	P1.LMM1032 × IIB388
IIB420	as IIB386 + *recB268*::Tn*10*	P1.N3071 × IIB386
IIB570	as IIB386 + *recR256*::Tn*5*	P1.AM208 × IIB386
IIB571	as IIB386 + *recF400*::Tn*5*	P1.IIB294 × IIB386
IIB572	as IIB386 + *recB1080 argA*::Tn*10 recR256*::Tn*5*	P1.AM208 × IIB388
IIB573	as IIB386 + *recB1080 argA*::Tn*10 recF400*::Tn*5*	P1.IIB294 × IIB388
IIB574	as IIB386 + *recB1080 recD1903*::Tn*10d(Tet) recR256*::Tn*5*	P1.AM208 × IIB390
IIB575	as IIB386 + *recB1080 recD1903*::Tn*10d(Tet) recF400*::Tn*5*	P1.IIB294 × IIB390

of L-arabinose, *wt* (IIB386) and *recB1080* (IIB388) mutant strains were incubated in the presence of D-arabinose at the same final concentration. We detected comparable levels of β-galactosidase in the presence of glucose or D-arabinose (data not shown).

2.4. Survival test

To quantitatively determine the effect of the short and long presence of L-arabinose on survival of mutants, we measured the number of viable cells. The cultures were diluted 100-fold and incubated to early-log phase of growth (OD_{600} ∼ 0.2), after which they were split into two. L-arabinose was added to one half and glucose to the other, using the same final concentration of 0.2 % (w/v). The cultures were further incubated and samples were removed after 30 and 150 min of growth, serially diluted in phosphate buffer, and plated on LB plates. Plates were incubated overnight at 37 °C and scored for viable colonies. The cell survival was calculated as the ratio of colony forming units (cfu) after 30 or 150 min growth period in presence of the 0.2 % L-arabinose with cfu in the presence of 0.2% glucose.

3. Results

3.1. Cell survival in the presence of L-arabinose

In order to introduce DSBs we used a simple genetic system which consists of the I-*Sce*I endonuclease recognition sequence within the *argE* gene linked to an antibiotic resistance marker, and the I-*Sce*I cassette driven by P_{BAD} (the *araBAD* promoter) [25,26]. The expression of I-*Sce*I endonuclease can be induced by L-arabinose or repressed by glucose. When cells are grown in medium with L-arabinose, the I-*Sce*I endonuclease is produced and a DSB is introduced within the target sequence (*argE*). In our experimental system L-arabinose was present throughout the incubation time (3 h). A DSB formed within the target

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sequence (*argE*) can be repaired when at least one of the sister chromosomes is intact. The SOS response is induced when a RecA-ssDNA filament is formed during the process of recombinational repair. This means that only repairable DSBs can induce the SOS response [24]. We expect that longer incubation in L-arabinose will decrease the chance of finding the intact sister chromosomes, and consequently will decrease DNA repair and cell survival. To exclude the possibility that low SOS induction is a result of a low cell survival, we measured the cell survival in all strains in which SOS induction was studied. As expected, cell survival decreased with longer incubation in L-arabinose (compare the survival after 30 min in L-arabinose and after 150 min in L-arabinose) (Table 2). Cell survival in a *recB268* (*recB* null) mutant after 30 min in L-arabinose (Table 2) was not so strongly reduced as was reported previously [26]. After 150 min cell survival was most reduced in *recB268*, *recB1080 recO* and *recB1080 recJ* mutants (Table 2). There was no correlation between SOS induction and cell survival. As an example, the *recB1080* mutant had a smaller survival than *wt* and *recJ* mutant strains but had higher SOS induction than these two strains (Table 2). Also, the *recJ* mutant had similar survival as the *wt* strain but had smaller SOS induction than the *wt* strain (Table 2). Finally, the *recJ* mutant had the same survival after 150 min in L-arabinose as a *recO* mutant, but had smaller SOS induction. One can conclude that the level of SOS induction observed in a particular strain reflects the role of the specific gene in the formation of SOS signal rather than an effect on strain survival. It was recently demonstrated that about 65 % of SOS expressing *wt* cells are non-colony formers, but are alive (analogous to human senescent cells) [24]. According to this, we can conclude that our experimental system can be used for the comparison of SOS induction in different bacterial strains.

3.2. Effect of RecA loading inactivation on SOS induction after introduction of DSB

To test if RecA loading activity is required for SOS induction after introduction of a DSB, we used strains with separately inactivated RecBCD-mediated and RecFOR-mediated RecA loading, as well as strains in which both mechanisms of RecA loading were inactivated.

Fig. 1 shows the levels of SOS expression in a wild type (*wt*) strain, *recB268*, *recB1080* and *recO* mutants in Miller units (β-galactosidase units). Curves in Fig. 1A represent real experimental data which include both SOS expression due to endogenous DNA damage and SOS induction due to external DNA damage (DSB). On the other hand, Fig. 1B shows SOS induction exclusively due to external DNA damage (DSB). Such data represent differences between the values obtained in the presence of I-*Sce*I endonuclease and the values obtained in its absence. The highest level of SOS expression after introduction of a DSB was observed in the *recB1080* mutant, and the maximal value was ∼3100 β-galactosidase units reached after 180 min of incubation (Fig. 1A). However, the level of SOS expression in *wt* and *recO* cells was lower (maximal value ∼2000 and ∼1800 units, respectively) and had different kinetics (the maximum was reached after 120 min. of incubation and remained constant) (Fig. 1A). Similar results were obtained for *recR* and *recF* mutants (data not shown). These results indicate that SOS induction can be divided into two stages, early (up to 120 min) and late (after 120 min). The reason for higher levels of SOS expression in the *recB1080* mutant could be due to longer persistance of the DSB (less efficient DNA repair) and due to higher level of basal SOS expression in comparison to *wt* cells. As

Table 2
Induction of the SOS response measured as β-galactosidase units of mutant strains and their survival

Bacterial strain	Relevant genotype	βgal_0[a]	$\beta gal_{150,glc}$[b]	$\beta gal_{150,ara}$[c]	$\beta gal_{150,ara\text{-}glc}$[d]	Cell survival[e]	Cell survival[f]
IIB386	*wt*	64 ± 18	237 ± 9	1914 ± 261	1661	0.89 ± 0.16	0.2 ± 0.07
IIB388	*recB1080*	267 ± 63	1022 ± 132	2782 ± 336	1760	0.31 ± 0.13	0.034 ± 0.02
IIB395	*recD*	74 ± 13	262 ± 5	2037 ± 225	1792	0.48 ± 0.028	0.12 ± 0.028
IIB390	*recB1080 recD*	91 ± 14	302 ± 39	1860 ± 165	1559	0.48 ± 0.028	0.12 ± 0.028
IIB392	*recO*	97 ± 8	272 ± 13	1699 ± 13	1427	0.56 ± 0.45	0.31 ± 0.13
IIB401	*recB1080 recO*	204 ± 22	861 ± 89	1663 ± 315	802	0.11 ± 0.078	0.0063 ± 0.0035
IIB393	*recB1080recDrecO*	81 ± 13	355 ± 47	1888 ± 230	1533	0.40 ± 0.21	0.041 ± 0.0012
IIB407	*recQ*	60 ± 25	148 ± 3	1654 ± 435	1506	0.67 ± 0.16	0.16 ± 0.035
IIB408	*recB1080 recQ*	384 ± 18	1056 ± 83	1934 ± 420	879	0.50 ± 0.042	0.26 ± 0.035
IIB409	*recJ*	80 ± 23	182 ± 30	1086 ± 144	904	0.95 ± 0.071	0.31 ± 0.078
IIB410	*recB1080 recJ*	87 ± 15	335 ± 39	653 ± 203	318	0.24 ± 0.057	0.0098 ± 0.0045
IIB420	*recB268*	31 ± 20	105 ± 6	76 ± 28	0	0.075 ± 0.021	0.0026 ± 0.00021
IV408	*I-SceI*$^{+}$ *I-SceI*$^{-}_{cs}$	27 ± 4	135 ± 16	117 ± 7	0	N.D.	N.D.

[a] β-Galactosidase units in mutants cells at early log-phase (OD_{600} ∼ 0.2). Errors shown are the standard deviation from the mean.
[b] β-Galactosidase units after 150 min growth period in 0.2% glucose. Errors shown are the standard deviation from the mean.
[c] β-Galactosidase units after 150 min growth period in 0.2% L-arabinose. Errors shown are the standard deviation from the mean.
[d] β-Galactosidase units calculated as the subtraction between β-galactosidase units (150, ara)[c] and β-galactosidase units (150, glc)[b] $^{(d=c-b)}$.
[e] Cell survival as determined after a 30 min growth period in the presence of 0.2% L-arabinose compared with an identical growth period in the presence of 0.2% glucose. Errors shown are the standard deviation from the mean. Cell survival was not determined (N.D.) in the IV408 mutant strain which carries the I-SceI cassette without the cutting site.
[f] Cell survival as determined after a 150 min growth period in the presence of 0.2% L-arabinose compared with an identical growth period in the presence of 0.2% glucose. Errors shown are the standard deviation from the mean. Cell survival was not determined (N.D.) in the IV408 mutant strain which carries I-SceI cassette without the cutting site.

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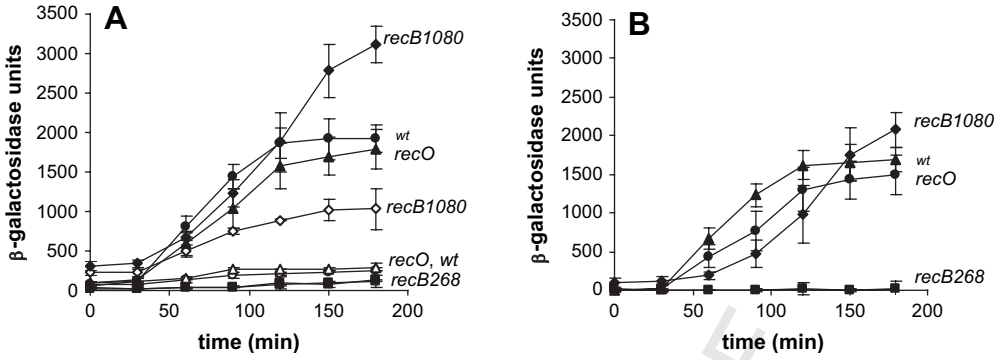

Fig. 1. (A) Induced levels of SOS expression shown as β-galactosidase units after introduction of a DSB for the *wt* strain (strain IIB386) (●), *recO* (strain IIB392) (▲), *recB1080* (strain IIB388) (◆) and *recB268* (strain IIB420) (■) mutants during 180 min incubation and basal levels of SOS expression shown as the β-galactosidase units for the *wt* strain (strain IIB386) (○), *recO* (strain IIB392) (△), *recB1080* (strain IIB388) (◇) and *recB268* (strain IIB420) (□) mutants during 180 min incubation. (B) Levels of SOS expression shown as β-galactosidase units calculated as the difference between the values obtained in the presence of I-*Sce*I endonuclease and the values obtained in its absence for the *wt* strain (strain IIB386) (▲), *recO* (strain IIB392) (●), *recB1080* (strain IIB388) (◆) and *recB268* (strain IIB420) (■) mutants during 180 min incubation. The symbols indicate means of at least two independent experiments, and the error bars indicate standard deviations.

expected, the level of SOS expression in the *recB268* (*recB* null) mutant was negligible (~100 units) (Fig. 1A) which is in agreement with the requirement for the RecBCD enzyme in the induction of an SOS response after introduction of DSBs [15,24]. When the values of basal SOS expression were subtracted from the values of SOS expression obtained in the presence of I-*Sce*I endonuclease, there was a smaller difference between the SOS induction in *wt* (~1700 units), *recB1080* (~2000 units), and *recO* (~1500 units) mutant (Fig. 1B). On the other hand, there was no induction of the SOS response due to DSB in a *recB* null mutant (Fig. 1B). The main conclusion from Fig. 1 is that the level of SOS expression in β-galactosidase units after introduction of DSBs is comparable to the *wt* level when one mechanism of RecA loading is functional. Consequently, either RecBCD-mediated (in *wt* strain and *recO* mutant) or RecFOR-mediated RecA loading (in *recB1080* mutant) is sufficient to induce the SOS response after introduction of DSBs.

Fig. 2 shows the induction of an SOS response when both mechanisms of RecA loading were eliminated (*recB1080 recO* double mutant), and when RecA loading of the RecBCD enzyme was partially restored (*recB1080 recD recO* triple mutant). The level of SOS expression after introduction of a DSB in the *recB1080 recO* double mutant (~2100 units) was lower than in the *recB1080* single mutant (~3100 units), but still higher than the basal level of SOS expression in the *recB1080* single mutant (~1100 units) and the *recB1080 recO* double mutant (~900 units) (Fig. 2A). When the results were expressed at the level of specific effect of external DSB (difference between values in the presence and in the absence of I-*Sce*I endonuclease), the SOS induction of *recB1080 recO* double mutant was ~1100 units (Fig. 2C). This means that the *recB1080 recO* strain is able to induce the SOS response although less efficiently than the *recB1080* strain (~2200 units) (Fig. 2C). Contrary to this, the *recO* mutation had no significant effect on constitutive SOS expression in the *recB1080* background (Fig. 2A) [18]. The inhibitory effect of *recO* mutation in the *recB1080* background was restricted to the early stage of SOS induction (up to 120 min). Similar results were obtained for the *recB1080 recR* and *recB1080 recF* double mutants (data not shown). We also investigated SOS induction in a *recB1080 recD recO* triple mutant (as well as *recB1080 recD recR* and *recB1080 recD recF*, data not shown) which partially restores RecA loading activity. It is known that RecB1080C(D^-), an enzyme produced by *recB1080 recD* cells, possesses RecA loading activity due to inactivation of the RecD subunit, which is an inhibitor of RecA loading [31]. The level of SOS expression in a *recB1080 recD recO* triple mutant after introduction of DSB was ~1900 β-galactosidase units (Fig. 2B) which is similar to the SOS expression in *recB1080 recD* (Fig. 2B) and *recB1080 recO* double mutants (Fig. 2A). However, the *recB1080 recD recO* triple mutant and *recB1080 recD* double mutant showed higher SOS induction due to external DSB (~1500 units) than the *recB1080 recO* double mutant (~1100 units) (Fig. 2C). From Fig. 2 we can conclude that inactivation of both known RecA loading functions decreases, but still renders induction of the SOS response after introduction of DSB.

3.3. Effects of inactivation of RecQ helicase and RecJ nuclease on SOS induction after introduction of a DSB in recB1080 and wt background

We wanted to test further whether the RecQ helicase and the RecJ nuclease play a role in the SOS signaling mechanism after introduction of a DSB in a *recB1080* mutant. For this purpose, we compared SOS induction after introduction of a DSB in the *recB1080* single mutant with SOS induction in *recB1080 recQ* and *recB1080 recJ* double mutants. The results are presented in Fig. 3. The *recQ* mutation had a moderate effect on SOS induction in a *recB1080* genetic background. The level of SOS expression in the *recB1080 recQ* double mutant after introduction of a DSB was ~2200 β-galactosidase units, and was lower than the SOS expression in *recB1080* single mutant (~3100 units). The basal level of SOS expression in the *recB1080* single mutant and the *recB1080 recQ* double mutant

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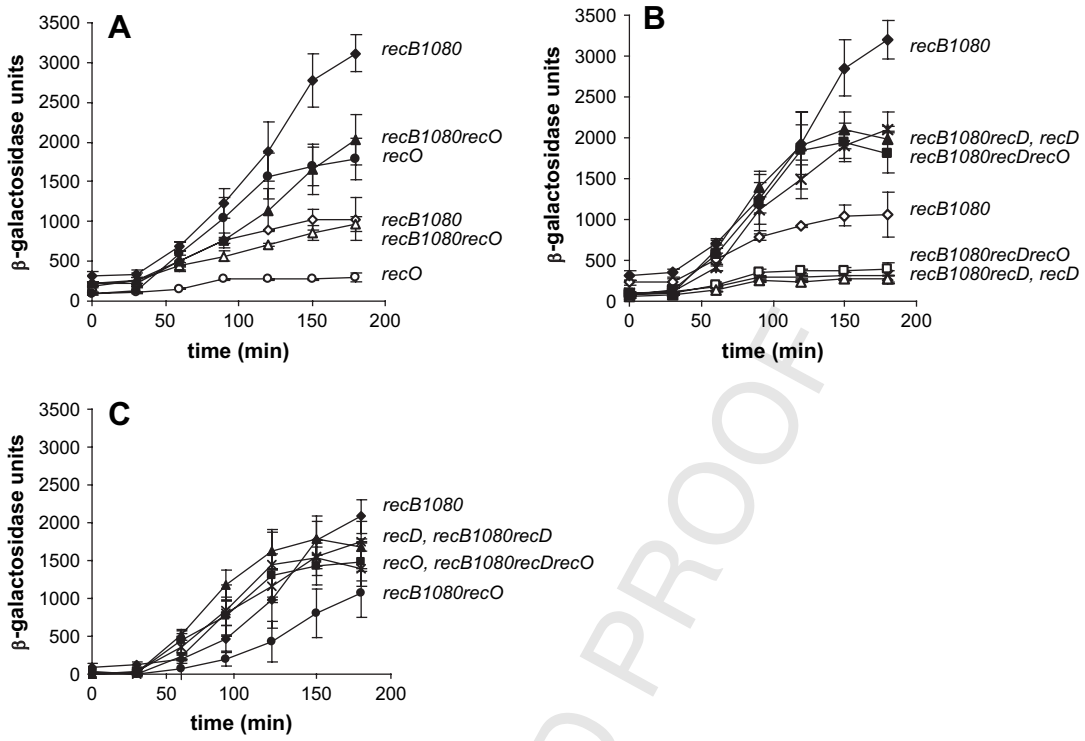

Fig. 2. (A) Effect of RecA loading inactivation on SOS induction after introduction of a DSB. Induced levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (◆), *recO* (strain IIB392) (●) and *recB1080 recO* (strain IIB401) (▲) mutants after introduction of a DSB during 180 min incubation period and basal levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (◇), *recO* (strain IIB392) (○) and *recB1080 recO* (strain IIB401) (△) mutants during 180 min incubation. (B) Induced levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (◆), *recD* (strain IIB395) (▲), *recB1080 recD* (strain IIB390) (*) and *recB1080 recD recO* (strain IIB393) (■) mutants after introduction of a DSB during 180 min incubation and basal levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (◇), *recD* (strain IIB395) (△), *recB1080 recD* (strain IIB390) (×) and *recB1080 recD recO* (strain IIB393) (□) during 180 min incubation. (C) Levels of SOS expression shown as β-galactosidase units calculated as the difference between the values obtained in the presence of I-*Sce*I endonuclease and the values obtained in its absence for *recB1080* (strain IIB388) (◆), *recO* (strain IIB392) (■), *recB1080 recO* (strain IIB401) (●), *recD* (strain IIB395) (▲), *recB1080 recD* (strain IIB390) (×) and *recB1080 recD recO* (strain IIB393) (*) mutants during 180 min incubation. The symbols indicate means of at least two independent experiments, and the error bars indicate standard deviations.

(Fig. 3A) remained the same as shown previously [18], indicating that RecQ was not required for constitutive SOS expression. The maximal level of SOS induction due to external DSB of the *recB1080 recQ* double mutant was ∼1100 units which was smaller than for the *recB1080* single mutant (∼2200 units) (Fig. 3C). Contrary to the effect of *recFOR* mutations, the effect of *recQ* mutation in a *recB1080* background was restricted to the late stage of SOS induction (after 120 min).

The effect of *recJ* mutation on SOS expression after introduction of a DSB in the *recB1080* background was much stronger than the effect of *recQ* and *recFOR* mutations. The level of SOS expression in the *recB1080 recJ* double mutant after introduction of a DSB was ∼760 β-galactosidase units (Fig. 3B). However, the induction of SOS response due to external DSB for the *recB1080 recJ* double mutant was ∼300 units (Fig. 3C). Also, the *recJ* mutation decreased the basal level of SOS expression (Fig. 2B) [18]. Taken together, this means that *recJ* mutation had an effect on both levels of SOS expression (induced by external DSB and basal). In addition, the effect of *recJ* mutation in a *recB1080* background was observable at both stages of SOS induction (early and late).

We also studied the effect of *recQ* and *recJ* mutation on SOS induction after introduction of a DSB in a *wt* background. *recQ* inactivation did not affect SOS expression, but interestingly *recJ* mutation reduced SOS expression to ∼1200 units (Fig. 3B) indicating a general role for RecJ nuclease in induction of the SOS response after introduction of a DSB. This result is in agreement with the previous observation that the RecJ nuclease is required for SOS induction after γ-irradiation in a *wt* background [32]. Taken together, we conclude that induction of the SOS response after introduction of a DSB was dependent on the RecJ exonuclease in both *wt* and *recB1080* background. The RecQ helicase showed a moderate effect in *recB1080*, but had no effect in a *wt* background. The SOS responses in all studied bacterial strains are also presented in Table 2. These data correspond to the SOS response measured after 150 min of incubation in appropriate media (glucose and L-arabinose), and can be used for the systematic comparison of SOS response in any strain.

4. Discussion

In this paper we studied the effect of inactivation of RecA loading (either of the RecBCD enzyme or RecFOR system), RecJ nuclease and RecQ helicase functions on the SOS signaling mechanism after the introduction of a DSB by the I-*Sce*I

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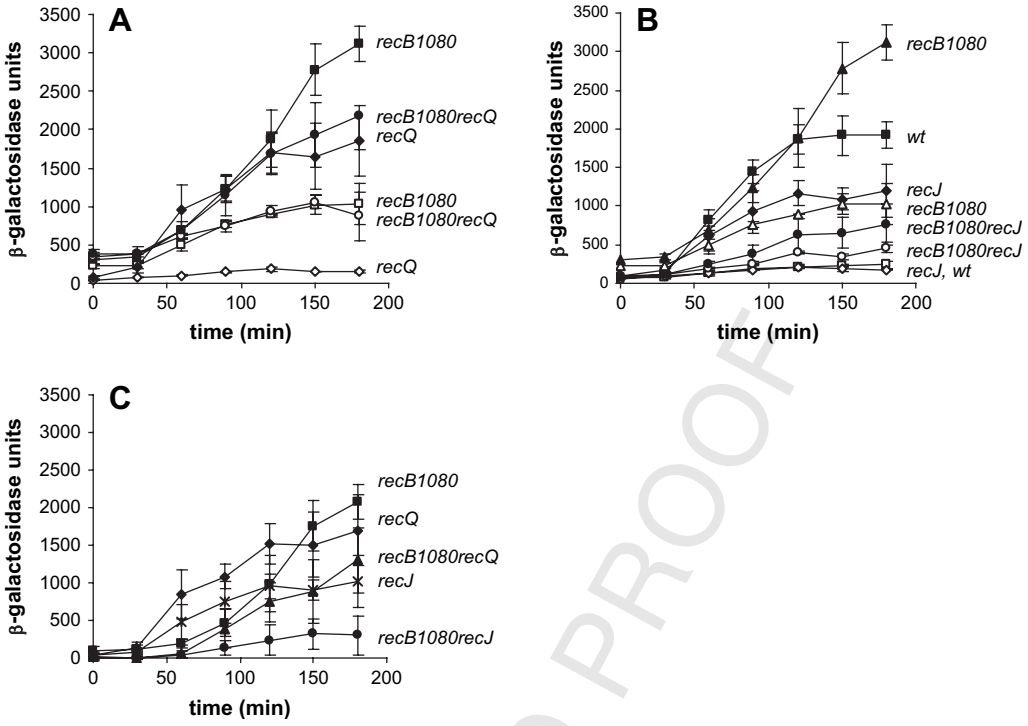

Fig. 3. (A) Effects of inactivation of RecQ helicase and RecJ nuclease on SOS induction after introduction of a DSB. Induced levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (■), *recQ* (strain IIB407) (◆) and *recB1080 recQ* (strain IIB408) (●) mutants after introduction of a DSB during 180 min incubation and basal levels of SOS expression shown as β-galactosidase units for *recB1080* (strain IIB388) (□), *recQ* (strain IIB407) (◇) and *recB1080 recQ* (strain IIB408) (○) mutants during 180 min incubation. (B) Induced levels of SOS expression shown as β-galactosidase units for the *wt* strain (strain IIB386) (■), *recJ* (strain IIB409) (◆), *recB1080* (strain IIB388) (▲) and *recB1080 recJ* (strain IIB410) (●) mutants after introduction of a DSB during 180 min incubation and basal levels of SOS expression shown as β-galactosidase units for the *wt* strain (strain IIB386) (□), *recJ* (strain IIB409) (◇), *recB1080* (strain IIB388) (△) and *recB1080 recJ* (strain IIB410) (○) mutants during 180 min incubation. (C) Levels of SOS expression shown as β-galactosidase units calculated as the difference between the values obtained in the presence of I-*Sce*I endonuclease and the values obtained in its absence for *recB1080* (strain IIB388) (■), *recQ* (strain IIB407) (◆), *recB1080 recQ* (strain IIB408) (▲), *recJ* (strain IIB409) (*) and *recB1080 recJ* (strain IIB410) (●) mutants during 180 min incubation. The symbols indicate means of at least two independent experiments, and the error bars indicate standard deviations.

endonuclease. When RecA loading and nuclease functions of RecBCD enzyme are inactivated (*recB1080* mutant) there is high constitutive (basal) level of SOS induction caused by endogenous DNA damage [18]. The effect of any mutation on SOS induction, measured in β-galactosidase units, is the consequence of action at the level of external DNA damage (introduction of DSB) or at the level of basal SOS expression due to endogenous DNA damage. To distinguish between these two possibilities, one can compare the basal (constitutive) level of SOS expression (in the absence of I-*Sce*I endonuclease) between appropriate strains. This can tell us whether a particular mutation has an effect at the level of basal SOS expression. In order to check the effect of a particular mutation at the level of external DSB, the experimental data should be expressed as the difference between the values obtained in the presence of I-*Sce*I endonuclease and the values obtained in the absence of I-*Sce*I endonuclease. From our results, it is clear that the effects of *recFOR* mutations in a *recB1080* genetic background are at the level of SOS induction due to an external agent (introduction of DSB) (Fig. 2C) since the *recB1080* single mutant and *recB1080 recO* (*F,R*) double mutants show similar increased levels of constitutive SOS expression (Fig. 2A). With respect to time course, the *recFOR* gene functions are required in the early stage of SOS induction in a *recB1080* background (slopes of *recB1080* and *recB1080 recO* are different before 120 min but similar after 120 min; Fig. 2A and C). In this study we show that simultaneous inactivation of both mechanisms of RecA-ssDNA filament formation causes a reduction in SOS induction after introduction of a DSB. Since the SOS induction due to external DSB of a *recB1080 recO* double mutant (∼1100 units) is smaller than the values for *recB1080* (∼2200 units), *recO* (∼1500 units) and *recB1080 recD recO* (∼1500 units), we concluded that SOS induction after introduction of a DSB is partially dependent on the RecA loading functions of both RecBCD and RecFOR proteins (Fig. 2C, Table 2). These results support the view that active RecA loading on ssDNA is part of the SOS signaling mechanism caused by the introduction of a DSB by I-*Sce*I endonuclease. The above results also suggest that an additional RecA loading mechanism could exist in the cell [18]. This additional RecA loading requires RecJ nuclease function (Fig. 3B and C) during the process of DNA unwinding. SOS induction after UV-irradiation is completely abolished (at the level of external damage) when both RecBCD and RecFOR-mediated RecA loading are eliminated (in *recB1080 recO*, *recB1080 recR* and *recB1080 recF* double mutants) [18]. Since the level of SOS induction after introduction of a DSB (external damage) is only partially affected by inactivation

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of both types of RecA loading (RecBCD and RecFOR), it could be that this additional type of RecA loading required for SOS induction is more specific for the processing of DSBs than other types of DNA lesions (Fig. 2) [18].

In the *recB1080* mutant, recombination and dsDNA break repair are performed by a hybrid recombination pathway in which helicase activity is provided by the RecB1080CD enzyme, 5′ → 3′ exonuclease by the RecJ nuclease, and RecA loading by the RecFOR proteins [33]. It is likely that similar interaction between the two recombination pathways exists in the *recB1080* mutant during the induction of SOS response after introduction of a DSB, and that RecFOR-mediated RecA-ssDNA filament formation participates in the SOS signaling mechanism in the *recB1080* mutant. On the other hand, the *recB* null mutant (*recB268*) completely abolishes SOS induction after introduction of a DSB, because in this strain interaction between the two recombination pathways is not possible [33]. Our results also show that inactivation of the RecQ helicase has a moderate effect on SOS induction in a *recB1080* background (Fig. 3A and C and Table 2). Since basal expression of the SOS response in a *recB1080* single mutant is similar to the basal expression of SOS response in a *recB1080 recQ* double mutant (Fig. 3A), one can conclude that the effect of *recQ* mutation is at the level of the introduction of external DSB (Fig. 3C). Also, the effect of *recQ* mutation is restricted to the late stage of SOS induction (Fig. 3A and C). On the other hand, inactivation of the RecJ nuclease in a *recB1080* background strongly decreases the induction of the SOS response after introduction of a DSB (from ∼3100 units to ∼760 units) (Fig. 3B). However, the RecJ nuclease is required for both constitutive SOS expression (Fig. 3B) [18] and for SOS induction due to external DSB (Fig. 3C). According to the time course, the RecJ nuclease is essential for both early and late stages in SOS induction after introduction of DSB in *recB1080* background (Fig. 3B and C). In addition, the inactivation of RecJ nuclease partially decreases the SOS induction in *wt* background, and it is required in the early stage of SOS induction (Fig. 3B).

Constitutive SOS expression is caused by endogenous DNA damage which can lead to the formation of ssDNA, a signal for SOS induction, and replication forks collapse. As shown recently, spontaneous collapsed replication forks are the main endogenous DNA damage that can induce the SOS response [24,34], and are equivalent to DSBs since they have dsDNA ends. Only repairable DSBs can induce the SOS response [24]. According to our results, RecJ-mediated nuclease activity could be important for the initial processing of these forks. The role of the RecJ nuclease could be to produce ssDNA which is crucial for the formation of RecA-ssDNA filament by RecFOR proteins and by an additional unknown mechanism of RecA loading required for SOS induction.

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