EXTENDED DISCUSSION

State of UvrA in pre-TCRC and TCRC

The UvrA "tight dimer" has been described for a DNA-bound *activated* UvrA in complex with a nucleotide cofactor when it adopts a more stable dimer conformation[1]. In other settings, UvrA-UvrA appears to be in a transient state[2,3]. At low, more physiological, concentrations and without a cofactor, UvrA is predominantly *monomeric* in solution[4]. Notably, the available UvrA crystal structures were obtained using highly concentrated preps of UvrA bound to ADP (PDB ID: 2R6F)[5] and damaged DNA (PDB ID: 3PIH)[1]. Our XLMS analysis uncovers two separate RNAP-UvrA interfaces: (i) RNAP-UvrA1 interface near the β' jaw and C-terminus clamp arm, captured by XLMS *in vitro* and *in vivo* under normal growth conditions and (ii) RNAP-UvrA2 near the secondary channel, captured predominantly after genotoxic stress, which supports a second UvrA (UvrA2) bound to RNAP, as well as the UvrA1-UvrA2 dimer. The majority of RNAP-UvrA2 crosslinks were obtained *after* genotoxic stress. Although we cannot rule out a possibility that a fraction of pre-TCRC containing UvrA1-UvrA2 forms before genotoxic stress (it can certainly be the case), XLMS data in combination with the results of Fig.1c and Extended Data Fig. 2 support a model where most of UvrA dimerization *in vivo* occurs upon the transition from a pre-TCRC to a TCRC.

Effect of rifampicin on NER

It has been shown previously that Rif can inhibit NER, although the extent of inhibition varied depending of the experimental conditions[6,7] . It is likely that Crowley and Hanawalt[6] failed to detect a near absolute inhibition of NER by Rif, as reported by Lin et al.[7] and by us here and in Martinez et al.[8] due to a low amount of the drug used (50 μg/ml). This concentration is enough to stop *E. coli* growth, but not enough to stop all transcription. Moreover, the authors pre-incubated cells with Rif for only 10 min, which is not sufficient to saturate them with Rif. We used 750 μg/ml of Rif for at least 40 min of preincubation. To illustrate the difference between "high" (750 μg/ml) and "low" (50 μg/ml) Rif on transcription and NER, we performed the experiments shown in **Extended Data Fig. 9.** Using RT-qPCR we show that there is an approximately 100-fold difference in "residual" transcription at a representative highly active gene (IPTG-induced *lacZ*) between cells treated with high and low Rif (**Extended Data Fig. 9a)**. Accordingly, quantitative CPD immunostaining shows that in contrast to high Rif, which prevented virtually any repair during 40 min of recovery from UV, low Rif still allowed much of the repair to occur (**Extended Data Fig. 9b**). These results explain the only partial NER inhibition by Rif in Crowley and Hanawalt[6]. It is hard to achieve a high intracellular concentration of Rif in Gram-negative bacteria, which is the reason why Rif is bacteriostatic (it is bactericidal in Gram-positive). We therefore used a very high dose of Rif to almost completely stop ongoing transcription, which resulted in nearly complete abolishing of NER. And because Lin *et al* used *ex vivo* nucleoid fraction, not intact bacteria, even a relatively low amount of Rif was sufficient to completely abolish repair[7].

Pervasive TCR in *E. coli*

In the accompanied paper, Martinez *et al*[8] have adopted a NGS assay, "CPD-seq", to monitor genome-wide NER in *E. coli* at a single nucleotide resolution. In accord with the present results (**Fig. 5**), Martinez *et al* demonstrated that the high doses of Rif severely suppressed NER in *both* the template strand (TS) and non-template strand (NTS). The effect of Rif was comparable to that

of *uvrD* and *uvrA* deletions. The intracellular levels of UvrABCD were not reduced during Rif treatment and the SOS response has a relatively minor effect on NER. These results mean that TCR occurs *everywhere* in the *E. coli* genome, including NTS (antisense) parts and intergenic regions, i.e. NER and TCR are essentially the same process, whereas GGR must be very inefficient, if it exists at all in *E. coli*. Thus, the traditional definition of TCR as the ratio between repair in TS vs NTS is *fundamentally* flawed, as both strands must be transcribed for NER to occur. It is the rate of local transcription that determine NER efficiency.

Martinez *et al* also explain *how* "NTS" and intergenic regions become accessible for transcription to permit NER in those genomic areas by uncovering a hitherto unknown mechanism of antitermination that becomes activated in response to UV stress and enables *global* transcriptional readthrough.

Role of backtracking in TCR

UvrD-deficient cells are a million times more sensitive to UV than *mfd* cells (e.g. **Extended Data Fig. 1a**), yet they are somewhat less UV sensitive and less deficient in NER than *uvrA* cells (Fig. 4c)[8], suggesting that UvrD-mediated backtracking is critical, but not absolutely required for NER. The fact that the deletion of anti-backtracking *greAB* and *mfd* partially suppresses *uvrD* phenotype[9,10], argues that spontaneous RNAP backtracking occurs at UV lesions *in vivo*, partially alleviating the necessity for UvrD. Furthermore, such spontaneous backtracking is promoted by ppGpp and DksA[9]. The former is strongly induced upon genotoxic stress[9] whereas the latter competes with GreAB for the same binding site on RNAP (e.g. Molodtsov et al)[11]. These and other pro-backtracking factors (including NusA) are likely to contribute to UvrD-independent, but backtracking-dependent NER. It is also possible that with some probability the TCRC recognizes lesions and initiates NER on the spot, i.e., before any backtracking could occur. In this case, UvrD would be dispensable, but the elongating RNAP is still essential, serving as a scaffold for the NER complex assembly and delivery to the lesion site.

Role of Mfd in NER: a unifying model

The traditional model of TCR postulates that (i) Mfd recruits UvrA to the lesions sites after terminating stalled RNAP; (ii) Mfd is necessary and sufficient for TCR in bacteria[12]. Although our results do not formally rule out the first postulate of the Mfd-centric model, they do demonstrate that Mfd-mediated TCR must be a minor auxiliary pathway, which is independent of the TCRC mechanism proposed in this study (**Extended Data Fig. 14; Movie 2**). The TCRC pathway appears to be responsible for most NER events in *E. coli*. To explain the effects of Mfd on NER, we propose an alternative model that accommodates and reconciles existing biochemical and *in vivo* evidence connecting Mfd to NER. In this model, UvrA of the pre-TCRC facilitates Mfd recruitment to DNA *in vivo*. Mfd then acts as a *cleanup* factor that removes obstructive RNAP molecules stalled in front of the pre-TCRC/TCRC, thereby facilitating the access of pre-TCRC/TCRC to damage sites (**Extended Data Figs. 15, 16; Movie 3**). This model explains the strand bias of Mfd observed *in vivo* and *in vitro* and the importance of Mfd interaction with UvrA[8,13-15]. It also explains why the stimulating effect of Mfd on NER is noticeable early during the recovery phase with the most highly expressed genes, but not with mid- or low-expressed genes (**Fig. 5c,d**; [8,12]), even though the repair of all those genes is still fully depended on ongoing local transcription (**Fig. 5**; [8]).

UvrA-mediated recruitment of Mfd

The function of Mfd as a TCR factor capable of recruiting UvrA to DNA was postulated based on *in vitro* observations[15,16]. Those experiments, however, did not demonstrate Mfd-mediated recruitment of UvrAB. Instead, they relied on excess of Mfd to "clean" DNA from pre-bound proteins, such as stalled RNAP, thereby providing UvrAB with more accessible DNA. However, the number of Mfd molecules per *E. coli* cell is at least 10 times smaller than the number of RNAP molecules engaged in elongation at any given moment (**Extended Data Fig. 2**;[17,18]. Thus, Mfd must be always limited *in vivo*. Yet, the overexpression of Mfd renders cells more sensitive to UV and genotoxic agents[9] and drastically *inhibits* NER (**Extended Data Fig. 8b)**[8]. Furthermore, *mfd* deletion *suppresses uvrD/pcrA* phenotypes[9,10] and *reduces* mutagenesis[19-23]. These phenotypes are difficult to rationalize if Mfd were the TCR factor. Moreover, as Mfd interacts with elongating RNAPs indiscriminately and regardless of DNA damage[17,24-26], the chances it can be rapidly recruited to a small fraction of RNAPs stalled exactly at the site of damage *in vivo* are negligible.

Our *in vivo* results show that it is UvrA of the TCRC that helps in recruiting Mfd (**Fig. 1d; Extended Data Figs. 15, 16**), not the other way around[16]. Indeed, it is the initial binding to DNA that unmasks the UvrA-binding domain of Mfd[27,28]. Interaction between Mfd and UvrA of the pre-TCRC may activate Mfd processive conformation[27]. Mfd would then translocate to the next stalled RNAP downstream of the TCRC (**Extended Data Figs. 16; Movie 3)**[24]. By pushing/terminating multiple queuing RNAPs, even a relatively small number of Mfd molecules could "cleanup" DNA ahead of the TCRCs to facilitate NER (**Extended Data Fig. 16; Movie 3**)[24,25]. This model explains why Mfd contribution is noticeable only at the most highly transcribed genes, where the congestion on DNA is the highest (**Fig. 5d) (Martinez et al., co-submitted)**[29]. It also explains why Mfd overexpression inhibits NER (**Extended Data Fig. 8b**) – too many Mfd molecules would inadvertently push/terminate pre-TCRCs. Mfd is also expected to facilitate post-repair transcription recovery by eventually pushing backtracked TCRCs forward after repair has been completed (**Extended Data Fig. 16; Movie 3**)[9]. Indeed, the role of Mfd in transcription recovery from UV stress has been demonstrated *in vivo*[30]. Note, that TCRCs are likely to be protected from Mfd by UvrD2, which interacts with the same β pincer domain of RNAP as Mfd does[26]. Once UvrD2 has been displaced, possibly by UvrB that also binds UvrD-CTD[31] (**Extended Data Figs. 5 and 14; Movie 2)**, and the lesion is repaired, the complex becomes accessible to Mfd for a prompt recovery from its backtracked state (**Extended Data Fig. 16; Movie 3**).

Mfd as a "DNA cleaning" factor

There is a difference in the rate of repair at the very early time points between Δ*mfd* and wt (**Fig. 5**), which can be readily explained in terms of our model of Mfd, in which it functions as a DNA cleaning factor, not as a TCR factor *per se*. Note, the difference is *only* noticeable at the *highest* transcriptional output from pTet promoter (≥200 ng of the inducer; **Fig. 5c,d**). Already at the 100 ng of the inducer, the NER difference between Δ*mfd* and wt disappears, even though in both wt and Δ*mfd* cells NER remained *fully* dependent on local transcription, as no repair can be detected without the inducer (**Fig. 5c,d**). These results show that Mfd contributes to NER only when a target gene is most highly transcribed, but not when transcription is less efficient. These results are consistent with our genome-wide results[8] where we show that Mfd accelerates NER of the most highly transcribed genes, but has no effect on the majority of genes that are less efficiently transcribed, and can even be detrimental to NER for many of those genes. Therefore, the net effect of Mfd on repair is very *modest* (matching its marginal NER phenotype), whereas most of TCR occurs independently of Mfd, as all those genes still *require* active transcription for NER to occur[8].

If Mfd were to bring UvrA to the site of damage after terminating RNAP (the traditional model), it is unclear why Mfd would only contribute to repair of the most highly transcribed genes. As UvrA of the pre-TCRC/TCRC helps to recruit Mfd (**Fig. 1**), we propose a model where Mfd acts not as a TCR factor *per se*, but as a *cleanup* factor that terminates multiple RNAP in front of TCRC giving the latter an unimpeded access to the lesion sites (**Extended Data Figs 15 and 16**; **Movie 3**). This model appears to reconcile all the available findings and explains why Mfd acts early to accelerate repair of the most highly transcribed genes, where DNA clogging with multiple queuing RNAPs is most pronounced.

Of note, the mutations in Rho that compromise its transcription termination activity behave very similar to Δ*mfd*: they compromise repair at the most highly transcribed genes, but improve repair of poorly transcribed genomic regions[8]. Rho has not been considered as a TCR factor, yet it behaves similarly to Mfd with respect to NER.

Although we cannot completely rule out a traditional model of Mfd as a parallel minor TCR pathway, in light of all the above-mentioned findings this traditional model seems highly unlikely. NER-related Mfd phenotypes (strand specificity and stimulation of repair at the most highly transcribed genes) can be explained by Mfd acting as a cleanup/termination factor, similar to Rho.

Role of Mfd-RNAP interactions in NER

Mfd is a constitutively expressed protein that plays a housekeeping role as a general anti-backtracking factor. For example, it normally rescues transcription elongation complexes at hard-to-transcribe sequences[32,33]. UvrA is not needed for Mfd to act on backtracked or stalled RNAPs[25,34]. However, in case of pre-TCRC/TCRC, UvrA ensures Mfd to be recruited faster and to act immediately downstream of the pre-TCRC/TCRC, thereby establishing directionality and strand-specificity (**Extended Data Figs. 15 and 16)**. In our model, TCRC itself is protected from Mfd- (or Rho) mediated termination by UvrD2. Once recruited by pre-TCRC/TCRC, Mfd then interacts with multiple stalled RNAPs in front of TCRC, terminating them one by one, hence the UvrA-mediated enrichment of Mfd in the RNAP pulldowns post-UV (**Fig. 1d**). Note, that without UV damage, RNAPs won't be a subject to Mfd-mediated *termination*, as they are free to move to their natural termination sites unimpeded by CPDs. In other words, Mfd would not be able to catch up and interact with multiple unblocked elongating RNAPs, which explains why UvrA-mediated Mfd enrichment occurs only after UV stress (**Fig. 1d**).

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Supplementary Table 1. List of *in vivo* and *in vitro* crosslinks used for XLMS-guided docking

Crosslink #	Group	Protein1	Protein2	Residue1	Residue2	Condition	Crosslinker
1	UvrA-RNAP	RpoB	UvrA	161	769	*In vitro*	DSS
2		RpoB	UvrA	163	769	*In vitro*	DSS
3		RpoB	UvrA	203	769	***In vivo***	DSS
4		RpoB	UvrA	203	779	***in vivo*+4NQO**	DSS
5		RpoB	UvrA	236	1	***in vivo*+4NQO**	DSS
6		RpoB	UvrA	278	156	***in vivo*+4NQO**	EDC
7		RpoB	UvrA	430	188	***in vivo*+4NQO**	EDC
8		RpoC	UvrA	650	146	***in vivo***	DSS
9		RpoC	UvrA	681	159	**in vivo+4NQO**	DSS
10		RpoC	UvrA	972	454	*in vitro*	DSS
11		RpoC	UvrA	1072	1	**in vivo+4NQO**	DSS
12		RpoC	UvrA	1072	3	***in vivo***	DSS
13		RpoC	UvrA	1151	769	***in vivo***	DSS
14		RpoC	UvrA	1167	769	*in vitro*	DSS
15		RpoC	UvrA	1297	809	*in vitro*	DSS
16	UvrA-UvrA	UvrA	UvrA	1	458	*in vitro*	DSS
17		UvrA	UvrA	1	458	***in vivo***	DSS
18		UvrA	UvrA	3	25	*in vitro*	DSS
19		UvrA	UvrA	3	25	***in vivo***	DSS
20		UvrA	UvrA	3	458	*in vitro*	DSS
21		UvrA	UvrA	3	458	***in vivo***	DSS
22		UvrA	UvrA	3	937	*in vitro*	DSS
23		UvrA	UvrA	15	458	*in vitro*	DSS
24		UvrA	UvrA	70	458	*in vitro*	DSS
25		UvrA	UvrA	70	458	***in vivo***	DSS
26		UvrA	UvrA	70	461	*in vitro*	DSS
27		UvrA	UvrA	70	461	***in vivo***	DSS
28		UvrA	UvrA	159	779	***in vivo***	DSS
29		UvrA	UvrA	159	783	*in vitro*	DSS
30		UvrA	UvrA	454	767	*in vitro*	DSS
31		UvrA	UvrA	454	767	***in vivo***	DSS
32		UvrA	UvrA	461	769	*in vitro*	DSS
33		UvrA	UvrA	461	769	***in vivo***	DSS
34	UvrA-UvrB	UvrA	UvrB	15	246	*in vitro*	DSS
35		UvrA	UvrB	159	591	*in vitro*	DSS
36		UvrA	UvrB	329	613	***in vivo*+4NQO**	DSS
37		UvrA	UvrB	597	272	*in vitro*	DSS
38		UvrA	UvrB	611	272	*in vitro*	DSS
39		UvrA	UvrB	611	279	*in vitro*	DSS

40		UvrA	UvrB	809	279	*in vitro*	DSS
41	UvrB-UvrB	UvrB	UvrB	12	591	*in vitro*	DSS
42		UvrB	UvrB	12	592	*in vitro*	DSS
43		UvrB	UvrB	67	605	*in vitro*	DSS
44		UvrB	UvrB	111	246	*in vitro*	DSS
45		UvrB	UvrB	264	272	*in vitro*	DSS
46		UvrB	UvrB	264	279	*in vitro*	DSS
47		UvrB	UvrB	358	378	*in vitro*	DSS
48		UvrB	UvrB	525	556	*in vitro*	DSS
49		UvrB	UvrB	549	591	*in vitro*	DSS
50		UvrB	UvrB	549	592	*in vitro*	DSS
51		UvrB	UvrB	549	605	*in vitro*	DSS
52		UvrB	UvrB	591	605	*in vitro*	DSS
53		UvrB	UvrB	591	613	*in vitro*	DSS
54		UvrB	UvrB	630	635	*in vitro*	DSS
55	UvrD-RNAP	RpoA	UvrD	271	448	*in vitro*	DSS
56		RpoA	UvrD	298	448	*in vitro*	DSS
57		RpoB	UvrD	161	623	*in vitro*	DSS
58		RpoB	UvrD	324	680	*in vitro*	DSS
59		RpoB	UvrD	496	448	***in vivo***	DSS
60		RpoB	UvrD	844	448	*in vitro*	DSS
61		RpoB	UvrD	844	498	*in vitro*	DSS
62		RpoB	UvrD	844	501	*in vitro*	DSS
63		RpoB	UvrD	844	501	***in vivo***	DSS
64		RpoB	UvrD	890	448	*in vitro*	DSS
65		RpoB	UvrD	890	501	***in vivo***	DSS
66		RpoB	UvrD	900	501	*in vitro*	DSS
67		RpoB	UvrD	908	338	***in vivo*+4NQO**	EDC
68		RpoB	UvrD	914	448	*in vitro*	DSS
69		RpoB	UvrD	915	135	***in vivo*+4NQO**	EDC
70		RpoB	UvrD	941	151	***in vivo***	EDC
71		RpoB	UvrD	943	448	*in vitro*	DSS
72		RpoB	UvrD	958	623	***in vivo*+4NQO**	DSS
73		RpoB	UvrD	958	671	***in vivo***	DSS
74		RpoB	UvrD	1022	124	***in vivo***	DSS
75		RpoB	UvrD	1022	623	***in vivo***	DSS
76		RpoB	UvrD	1032	501	***in vivo***	DSS
77		RpoB	UvrD	1262	448	*in vitro*	DSS
78		RpoC	UvrD	40	203	*in vitro*	DSS
79		RpoC	UvrD	40	242	*in vitro*	DSS
80		RpoC	UvrD	40	486	*in vitro*	DSS
81		RpoC	UvrD	40	501	*in vitro*	DSS

82		RpoC	UvrD	50	203	*in vitro*	DSS
83		RpoC	UvrD	50	498	***in vivo***	DSS
84		RpoC	UvrD	50	501	*in vitro*	DSS
85		RpoC	UvrD	87	448	*in vitro*	DSS
86		RpoC	UvrD	296	135	***in vivo***	DSS
87		RpoC	UvrD	321	389	*in vitro*	DSS
88		RpoC	UvrD	321	501	*in vitro*	DSS
89		RpoC	UvrD	334	623	*in vitro*	DSS
90		RpoC	UvrD	371	389	***in vivo***	DSS
91		RpoC	UvrD	371	501	***in vivo*+4NQO**	DSS
92		RpoC	UvrD	371	623	***in vivo***	DSS
93	UvrD-UvrD	UvrD	UvrD	128	448	*in vitro*	DSS
94		UvrD	UvrD	128	501	***in vivo***	DSS
95		UvrD	UvrD	135	389	*in vitro*	DSS
96		UvrD	UvrD	203	448	*in vitro*	DSS
97		UvrD	UvrD	389	448	*in vitro*	DSS
98		UvrD	UvrD	448	486	*in vitro*	DSS
99		UvrD	UvrD	498	124	*in vitro*	DSS
100		UvrD	UvrD	498	448	*in vitro*	DSS
101		UvrD	UvrD	498	486	*in vitro*	DSS
102		UvrD	UvrD	501	486	*in vitro*	DSS
103		UvrD	UvrD	623	448	*in vitro*	DSS
104		UvrD	UvrD	671	389	*in vitro*	DSS
105		UvrD	UvrD	671	448	*in vitro*	DSS
106		UvrD	UvrD	671	486	*in vitro*	DSS
107		UvrD	UvrD	671	623	*in vitro*	DSS
108		UvrD	UvrD	680	307	*in vitro*	DSS
109		UvrD	UvrD	680	389	*in vitro*	DSS
110		UvrD	UvrD	680	448	*in vitro*	DSS
111		UvrD	UvrD	680	486	*in vitro*	DSS
112		UvrD	UvrD	680	609	*in vitro*	DSS
113		UvrD	UvrD	708	448	*in vitro*	DSS
114	UvrD-NusA	UvrD	NusA	128	83	***in vivo***	EDC
115		UvrD	NusA	389	95	***in vivo***	EDC
116		UvrD	NusA	448	52	*in vitro*	DSS
117		UvrD	NusA	623	38	***in vivo*+4NQO**	DSS
118		UvrD	NusA	680	94	***in vivo***	EDC
119	NusA-RNAP	RpoA	NusA	200	38	***in vivo*+4NQO**	DSS
120		RpoA	NusA	298	37	*in vitro*	DSS
121		RpoA	NusA	298	38	*in vitro*	DSS
122		RpoA	NusA	298	38	***in vivo***	DSS
123		RpoB	NusA	890	37	*in vitro*	DSS

124		RpoB	NusA	890	37	***in vivo***	DSS
125		RpoB	NusA	890	37	***in vivo*+4NQO**	DSS
126		RpoB	NusA	890	111	*in vitro*	DSS
127		RpoB	NusA	890	111	***in vivo***	DSS
128		RpoB	NusA	890	111	***in vivo*+4NQO**	DSS
129		RpoB	NusA	890	159	***in vivo*+4NQO**	EDC
130		RpoB	NusA	900	1	*in vitro*	DSS
131		RpoB	NusA	900	1	***in vivo***	DSS
132		RpoB	NusA	900	1	***in vivo*+4NQO**	DSS
133		RpoB	NusA	900	3	*in vitro*	DSS
134		RpoB	NusA	900	3	***in vivo***	DSS
135		RpoB	NusA	900	3	***in vivo*+4NQO**	DSS
136		RpoB	NusA	900	16	*in vitro*	DSS
137		RpoB	NusA	900	16	***in vivo***	DSS
138		RpoB	NusA	900	16	***in vivo*+4NQO**	DSS
139		RpoB	NusA	900	22	*in vitro*	DSS
140		RpoB	NusA	900	111	***in vivo***	DSS
141		RpoB	NusA	900	111	***in vivo*+4NQO**	DSS
142		RpoB	NusA	908	3	***in vivo*+4NQO**	EDC
143		RpoB	NusA	909	16	***in vivo***	DSS
144		RpoB	NusA	909	16	***in vivo*+4NQO**	DSS
145		RpoB	NusA	909	37	*in vitro*	DSS
146		RpoB	NusA	909	37	***in vivo***	DSS
147		RpoB	NusA	909	37	***in vivo*+4NQO**	DSS
148		RpoB	NusA	909	38	***in vivo*+4NQO**	DSS
149		RpoB	NusA	909	111	***in vivo***	DSS
150		RpoB	NusA	909	111	***in vivo*+4NQO**	DSS
151		RpoB	NusA	914	111	*in vitro*	DSS
152		RpoB	NusA	914	111	***in vivo***	DSS
153		RpoB	NusA	914	111	***in vivo*+4NQO**	DSS
154		RpoC	NusA	50	37	*in vitro*	DSS
155		RpoC	NusA	66	111	***in vivo***	DSS
156		RpoC	NusA	66	111	***in vivo*+4NQO**	DSS
157		RpoC	NusA	395	37	***in vivo***	DSS
158		RpoC	NusA	395	38	***in vivo***	DSS
159		RpoC	NusA	395	111	***in vivo***	DSS
160		RpoC	NusA	395	111	***in vivo*+4NQO**	DSS

Supplementary Table 2. Bacterial strains used in this study

Strain	Genotype	Reference
BK1001	MG1655-UvrA-FLAG*::Km^r-rpoC-6X-His*	This study
BK1002	MG1655-UvrB-FLAG*::Km^r-rpoC-6X-His*	This study
BK1003	MG1655-UvrC-FLAG*::Km^r-rpoC-6X-His*	This study
BK1004	MG1655-UvrD-FLAG*::Km^r-rpoC-6X-His*	This study
BK1005	MG1655- Δ*uvrA::Cm^r*	Lab stock
BK1006	MG1655- Δ*uvrB::Cm^r*	Lab stock
BK1007	MG1655- Δ*uvrD::km^r*	Lab stock
BK1008	MG1655 *Δmfd::Cm^r*	Lab stock
BK1009	MG1655-Mfd-FLAG*::Km^r-rpoC-6X-His*	This study
BK1010	MG1655-Mfd-FLAG*::Km^r*Δ*uvrA::Cm^r-rpoC-*	This study
BK1011	MG1655-Mfd $^{mut\text{-}uvrA}$-FLAG*::Km^r*)- *rpoC-6X-*	This study
BK1012	MG1655-rpoC-6X-His	Lab stock
BK1013	MG1655 *lexA3:: Km^r*	Lab stock
BK1014	MG1655-*uvrD*Δ*CTD*	Lab stock
BK1015	MG1655-Δβi4	Lab stock
BK1016	MG1655-*uvrD*Δ*CTD*-Δ*greAB*	Lab stock
BK1017	MG1655-Δβi4-Δ*greAB*	Lab stock
N4849	*rpoB*35*	[113]
BK1018	*rpoB*35 uvrD*Δ*CTD*	This study
BK1019	pVector (pCA24N)	[74]
BK1020	pMfd-pCA24N	[74]
BK1021	MG1655-NusA-10X-His::*Km^r*	This study
BK1022	MG1655-rpoC-10X-His	Lab stock
MG1655	F$^-$, λ$^-$, rph-1	Lab stock
MG01	Derivative of MG1655 with intrinsic	This study
MG02	lacZ promoter deletion derivative of MG01	This study
MG03	Δ*mfd* derivative of MG01	This study
MG04	lacZ promoter deletion derivative of MG03	This study
MG05	Δ*uvrD* derivative of MG01	This study
MG06	lacZ promoter deletion derivative of MG05	This study
MG07	Derivative of MG1655 with intrinsic	This study
MG08	lacZ promoter deletion derivative of MG07	This study
MG09	Derivative of MG1655 with intrinsic	This study
MG10	lacZ promoter deletion derivative of MG09	This study
MG11	Flag tagged UvrA derivative of MG01	This study
MG12	Flag tagged UvrB derivative of MG01	This study
MG13	Flag tagged UvrA derivative of MG02	This study
MG14	Flag tagged UvrB derivative of MG02	This study
MG15	Flag tagged UvrA derivative of MG07	This study
MG16	Flag tagged UvrB derivative of MG07	This study
MG17	Flag tagged UvrA derivative of MG08	This study
MG18	Flag tagged UvrB derivative of MG08	This study
MG19	Flag tagged UvrA derivative of MG09	This study
MG20	Flag tagged UvrB derivative of MG09	This study

Supplementary Table 3. Plasmids and oligonucleotides used in this study

Construct	Description	Reference
pET28a-TEV-*Ec*UvrD	6xHis-tagged *Ec*UvrD	This work
pET28a-TEV-*Ec*UvrD (E690C)	*Ec*UvrD (E690C)	This work
pET28a-TEV-*Ec*UvrD-CTD	*Ec*UvrD-CTD (aa 654-720)	This work
pET28a-TEV-*Ec*RNAP-β2i4	*Ec*RNAP-β2i4 (aa 152-443)	This work
pRL706 (Δβi4)	*Ec*RNAP-β (Δ225-343)	This work
pRL706 (T306A)	*Ec*RNAP-β (T306A)	This work
pRL706 (E308A)	*Ec*RNAP-β (E308A)	This work
pRL706 (N357C)	*Ec*RNAP-β (N357C)	This work
pET51b-*Ec*UvrD	Strep II-tagged *Ec*UvrD	This work
pET51b-*Ec*UvrD (H678A)	Strep II-tagged *Ec*UvrD (H678A)	This work
pET51b-*Ec*UvrD (F681A)	Strep II-tagged *Ec*UvrD (F681A)	This work
pET51b-*Ec*UvrD (K708A)	Strep II-tagged *Ec*UvrD (K708A)	This work
pET51b-*Ec*UvrD (W709A)	Strep II-tagged *Ec*UvrD (W709A)	This work
pET51b-*Ec*UvrD (L710A)	Strep II-tagged *Ec*UvrD (L710A)	This work
pGADT7-*Ec*UvrD	GAL4-AD-*Ec*UvrD	This work
pGADT7-*Ec*UvrD-NTD	GAL4-AD-*Ec*UvrD (aa 1-653)	This work
pGADT7-*Ec*UvrD-CTD	GAL4-AD-*Ec*UvrD (aa 654-720)	This work
pGADT7-*Ec*UvrD (H678A)	GAL4-AD-*Ec*UvrD (H678A)	This work
pGADT7-*Ec*UvrD (K680A)	GAL4-AD-*Ec*UvrD (K608A)	This work
pGADT7-*Ec*UvrD (F681A)	GAL4-AD-*Ec*UvrD (F681A)	This work
pGADT7-*Ec*UvrD (E690A)	GAL4-AD-*Ec*UvrD (E690A)	This work
pGADT7-*Ec*UvrD (H695A)	GAL4-AD-*Ec*UvrD (H695A)	This work
pGADT7-*Ec*UvrD (R697A)	GAL4-AD-*Ec*UvrD (R697A)	This work
pGADT7-*Ec*UvrD (K708A)	GAL4-AD-*Ec*UvrD (K708A)	This work
pGADT7-*Ec*UvrD (W709A)	GAL4-AD-*Ec*UvrD (W709A)	This work
pGADT7-*Ec*UvrD (L710A)	GAL4-AD-*Ec*UvrD (L710A)	This work
pGADT7-*Ec*UvrD (V711A)	GAL4-AD-*Ec*UvrD (V711A)	This work
pGADT7-*Ec*UvrD (Y714A)	GAL4-AD-*Ec*UvrD (Y714A)	This work
pGBKT7-*Ec*RNAP-β2i4	GAL4-DBD-*Ec*RNAP-β2i4 (aa 143-500)	This work
pGBKT7-*Ec*RNAP-β2i4 (D300A)	GAL4-DBD-*Ec*RNAP-β2i4 (D300A)	This work
pGBKT7-*Ec*RNAP-β2i4 (I302A)	GAL4-DBD-*Ec*RNAP-β2i4 (I302A)	This work
pGBKT7-*Ec*RNAP-β2i4 (E304A)	GAL4-DBD-*Ec*RNAP-β2i4 (E304A)	This work
pGBKT7-*Ec*RNAP-β2i4 (T306A)	GAL4-DBD-*Ec*RNAP-β2i4 (T306A)	This work
pGBKT7-*Ec*RNAP-β2i4 (E308A)	GAL4-DBD-*Ec*RNAP-β2i4 (E308A)	This work
pGBKT7-*Ec*RNAP-β2i4 (L309A)	GAL4-DBD-*Ec*RNAP-β2i4 (L309A)	This work
pGBKT7-*Ec*RNAP-β2i4 (T216A)	GAL4-DBD-*Ec*RNAP-β2i4 (T216A)	This work
pGBKT7-*Ec*RNAP-β2i4 (Q219A)	GAL4-DBD-*Ec*RNAP-β2i4 (Q219A)	This work
pGBKT7-*Ec*RNAP-β2i4 (N357A)	GAL4-DBD-*Ec*RNAP-β2i4 (N357A)	This work
pGBKT7-*Ec*RNAP-β2i4 (D358A)	GAL4-BDD-*Ec*RNAP-β2i4 (D358A)	This work
pGBKT7-*Ec*UvrB	GAL4-BD-*Ec*UvrB	This work
pGBKT7-*Ec*UvrB-B1	GAL4-BD-*Ec*UvrB (aa 1-414)	This work
pGBKT7-*Ec*UvrB-B2	GAL4-BD-*Ec*UvrB (aa 415-618)	This work
pGBKT7-*Ec*UvrB-B1B2	GAL4-BD-*Ec*UvrB (aa 1-618)	This work
pGBKT7-*Ec*UvrB-B4	GAL4-BD-*Ec*UvrB (aa 619-673)	This work
lacZ1	GTGGAATTGTGAGCGGATAAC	RR Fwd
lacZ2	GCTGCAAGGCGATTAAGTTG	RR Rev
lacZ3	CCATGACCTGACCATGCAGAGGATG	SLR Rev
mCherry1	ATGGTGAGCAAGGGCGAGGAG	RR Fwd
mCherry2	TCTCGAACTCGTGGCCGTTC	RR Rev
mCherry3	GATGGTGTAGTCCTCGTTGTG	SLR Rev

Supplementary Table 4. Statistics of X-ray crystal structures

	***Ec*UvrD-CTD/RNAP-β2i4**	***Tt*UvrD-CTD/UvrB-NTD**
Data collection		
Space group	$P2_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	60.1, 42.2, 87.8	92.3, 114.6, 125.4
α, β, γ (°)	90.0, 105.2, 90.0	90.0, 90.0, 90.0
Resolution (Å)	40.00-1.70 (1.76-1.70)*	50.00-2.60 (2.64-2.60)
R_{sym} or R_{merge}	0.053(0.371)	0.118 (0.118)
$I/\sigma I$	11.0 (2.1)	18.1 (1.3)
Completeness (%)	0.968 (0.984)	0.986 (0.909)
Redundancy	4.8 (3.9)	6.1 (4.8)
CC1/2 in highest shell	0.751	0.704
Refinement		
Resolution (Å)	40.00-1.65	50.00-2.58
No. reflections	45693	41517
$R_{work/}$ R_{free}	0.203/0.234	0.215/0.249
No. atoms		
Protein	2705	7234
Ligand/ion	6	0
Water	255	63
B-factors (Å^2)		
Protein	38.5	62.60
Ligand/ion	49.6	/
Water	40.1	60.61
R.m.s deviations		
Bond lengths (Å)	0.008	0.005
Bond angles (º)	1.06	0.78
Ramachandran plot		
Favored (%)	96.25	98.56
Allowed (%)	3.75	1.44
Disallowed (%)	0	0
PDB code	7EGS	7EGT

* Highest resolution shell is shown in parenthesis.