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DNA-damage response control of E2F7 and E2F8

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Here, we report that the two recently identified E2F subunits, E2F7 and E2F8, are induced in cells treated with DNA-damaging agents where they have an important role in dictating the outcome of the DNA-damage response. The DNA-damage-dependent induction coincides with the binding of E2F7 and E2F8 to the promoters of certain E2F-responsive genes, most notably that of the *E2F1* gene, in which E2F7 and E2F8 coexist in a DNA-binding complex. As a consequence, E2F7 and E2F8 repress E2F target genes, such as *E2F1*, and reducing the level of each subunit results in an increase in E2F1 expression and activity. Importantly, depletion of either E2F7 or E2F8 prevents the cell-cycle effects that occur in response to DNA damage. Thus, E2F7 and E2F8 act upstream of E2F1, and influence the ability of cells to undergo a DNA-damage response. E2F7 and E2F8, therefore, underpin the DNA-damage response.

Keywords: E2F; cancer; DNA damage; apoptosis; transcription factor

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INTRODUCTION

In mammalian cells, most E2F DNA-binding is made up of an E2F–DP heterodimer (Stevens & La Thangue, 2003). So far, eight members of the E2F family and three DP genes have been characterized. Subunits E2F1 to E2F6 form heterodimers with DP proteins, whereas the two most recently identified family members, E2F7 and E2F8, bind to DNA in a DP-independent manner (de Bruin *et al*, 2003; Di Stefano *et al*, 2003; Logan *et al*, 2004, 2005; Maiti *et al*, 2005). Moreover, there is increasing evidence that E2F activity participates in other processes in addition to cell-cycle control, such as apoptosis, differentiation and senescence (Trimarchi & Lees, 2002; Stevens & La Thangue, 2004). Furthermore, E2F1 is stabilized during the DNA-damage response in which it becomes phosphorylated by DNA-damage-responsive protein kinases, such as Chk2 (Stevens & La Thangue, 2003). It is under these conditions that E2F1 might exert apoptotic activity as part of the checkpoint response to DNA damage.

Each of the subunits E2F7 and E2F8 has two distinct DNA-binding domains, and the integrity of both domains is required for DNA-binding activity (de Bruin *et al*, 2003; Logan *et al*, 2004, 2005). E2F7 and E2F8 repress E2F site-dependent transcription in a pRb-independent manner and delay cell-cycle progression, arguing that they might affect negative cell-cycle control through transcriptional repression. It is interesting to note that their general organization is similar to several plant E2F-like (E2L) proteins, such as E2F7 and E2F8, which have two separate DNA-binding domains (Kosugi & Ohashi, 2002). The function of the plant E2L proteins has been implicated in negative regulation of endo-replication, because inactivation of the *E2L* gene prompts several rounds of DNA replication (Vlieghe *et al*, 2005).

Here, we describe a new role for E2F7 and E2F8 during the DNA-damage response. We show that E2F7 and E2F8 are induced in cells treated with DNA-damaging agents, which coincide with binding to the promoters of certain target genes, such as *E2F1*. Both subunits coexist in a DNA-binding complex, and depletion of either E2F7 or E2F8 causes an induction of E2F target genes, including *E2F1*. Most importantly, E2F7 and E2F8 are required for the cell-cycle effects that occur in response to DNA damage. Together, these results indicate that E2F7 and E2F8 are intimately involved with the cellular response to DNA damage.

RESULTS

E2F7 and E2F8 during the DNA-damage response

When U2OS cells were treated with etoposide, there was a specific induction of E2F7 and E2F8 (Fig 1A); p53 and E2F1 were, as expected (Stevens *et al*, 2003), induced under these conditions (Fig 1A). A similar response was apparent in various other cell types, including MCF7, HeLa, T98G, HCT15 and HCT116 tumour cells and mouse embryonic fibroblasts, as a consequence of treating with different DNA-damaging agents, such as etoposide and bleomycin (Fig 1B,C). The increased protein level did not coincide with changes in E2F7 and E2F8 RNA (Fig 1D,E). A more detailed time course was made between the DNA-damage response of E2F7 and E2F8 with E2F1. E2F1 levels increased rapidly during the response in MCF7 cells (usually reaching maximum levels after 3 h), whereas, in case of E2F7, it reached peak levels at 5 h and for E2F8 by 3 h (Fig 1F,G); thus, E2F7 and E2F8 are DNA-damage-responsive proteins.

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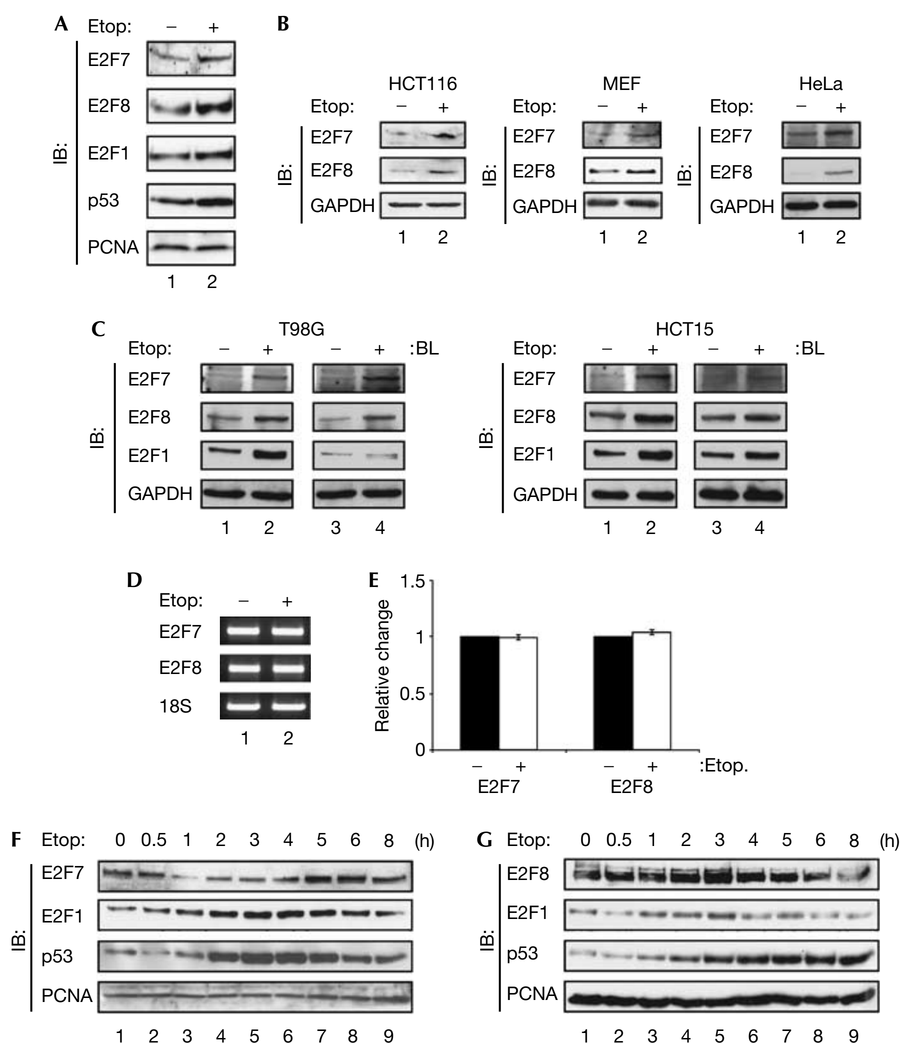

Fig 1 | Regulation of E2F7 and E2F8 during the DNA-damage response. (**A**) Immunoblot (IB) of E2F7 and E2F8 levels in U2OS cells treated with etoposide (+, 10 μM) for 3 h. For comparison, E2F1 and p53 are shown; PCNA provides the internal control. (**B**) Immunoblot of E2F7 and E2F8 levels in HCT116, MEF and HeLa cells treated with etoposide (+, 10 μM) for 16 h; GAPDH provides the internal control. (**C**) Levels of E2F7, E2F8 and E2F1 in T98G and HCT15 cells treated with either etoposide (10 μM) or bleomycin (5 μg/ml) for 16 h, as indicated; GAPDH provides the internal control. (**D**) RNA extracted from U2OS cells treated with etoposide (+, 10 μM) for 16 h was subjected to RT–PCR (as described) using primers for E2F7 and E2F8; 18S ribosomal RNA was incorporated as a control. (**E**) Quantitation of E2F7 and E2F8 RNA by quantitative RT–PCR. (**F**) Time-course analysis of E2F7, E2F1 and p53 in MCF7 cells treated with etoposide (10 μM); PCNA was used as a loading control. (**G**) Time-course analysis of E2F8 in MCF7 cells treated with etoposide (10 μM), as described in (**F**). BL, bleomycin; Etop, etoposide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MEF, mouse embryonic fibroblast; PCNA, proliferating-cell nuclear antigen; RT–PCR, reverse transcription PCR.

E2F7 and E2F8 locate to the E2F1 promoter

The increased levels of E2F7 and E2F8 might reflect their regulation of E2F target genes. To test this idea, we assessed whether E2F7 and E2F8 were present on the promoter of the *E2F1* gene, which is a well-characterized E2F target gene (Fig 2A; Neuman *et al*, 1994; Stevens & La Thangue, 2003). Chromatin immunoprecipitation (ChIP) showed that the binding of E2F7 to the E2F1 promoter increased in cells treated with DNA-damaging agents compared with untreated cells (Fig 2B). Moreover, when the primary anti-E2F7 ChIP immunocomplex was reimmunoprecipitated with anti-E2F8, both E2F7 and E2F8 were found to be present in the E2F1 promoter-bound DNA-binding complex, which was more apparent in DNA-damaged cells (Fig 2C).

E2F7 and E2F8 suppress E2F1 expression

As E2F7 and E2F8 target the E2F1 promoter, and as both subunits can repress transcription (de Bruin *et al*, 2003; Logan *et al*, 2004, 2005; Maiti *et al*, 2005), they might downregulate the *E2F1* gene. We used short interfering RNAs (siRNAs) that selectively deplete E2F7 and E2F8 (Fig 2D), which were effective on both ectopic and endogenous proteins (Fig 2D,E) and, as anticipated, reduced E2F7 and E2F8 RNA levels (supplementary Fig 1A online). The control

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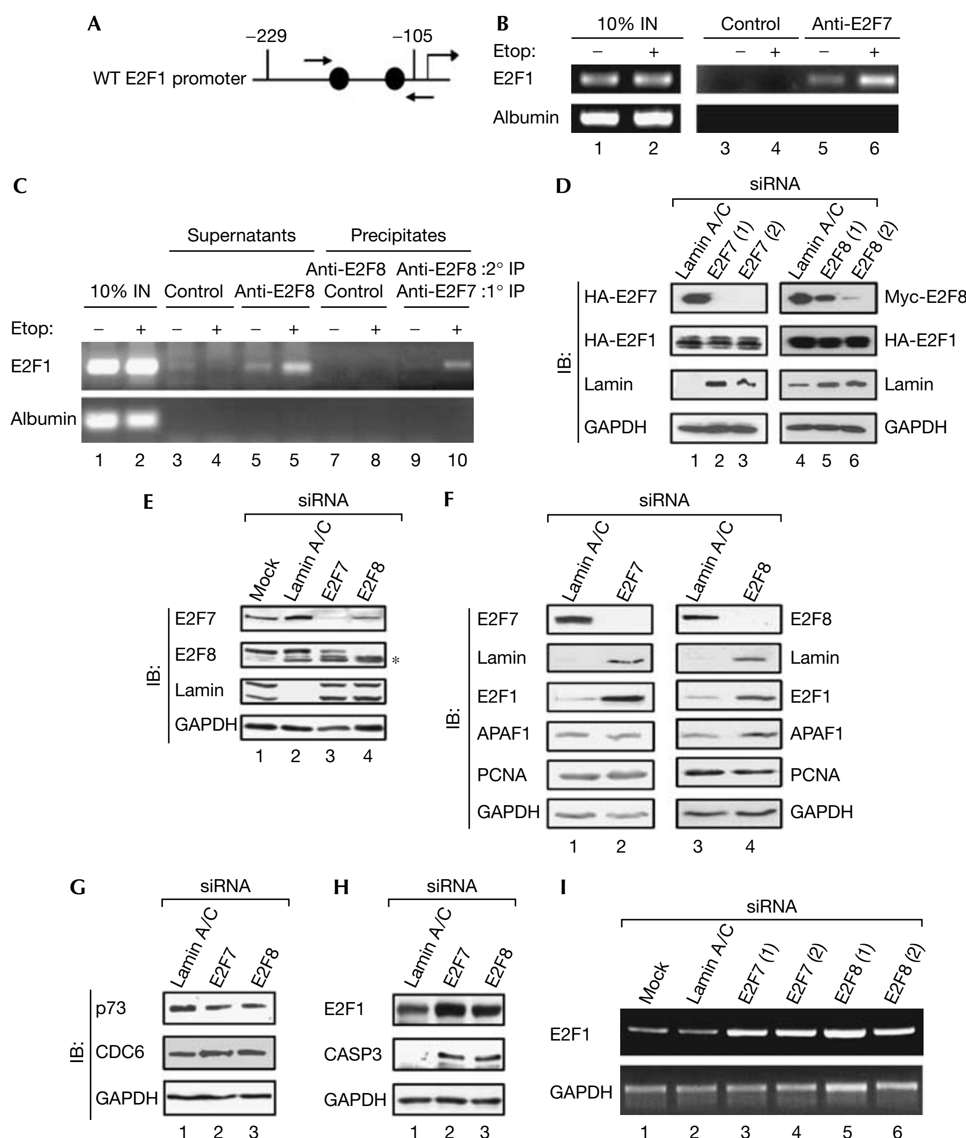

Fig 2 | E2F7 and E2F8 regulate E2F1 expression. (**A**) Summary of the E2F1 promoter: the circles indicate the location of the E2F sites and the arrows indicate the position of the primers used in the ChIP analysis. (**B**) ChIP analysis on extracts prepared from U2OS cells carried out using E2F7 or control antibody after treatment with etoposide (+, 10 μm) for 4 h. The input (IN) chromatin is indicated. (**C**) Double immunoprecipitation on U2OS cells was carried out as described in (**B**) using either the control antibody or anti-E2F8 to reimmunoprecipitate the chromatin collected in the E2F7 immunoprecipitate as indicated. The 'supernatants' show the material collected during the primary (1 ° IP) ChIP step, subsequently reimmunoprecipitated in the second (2 ° IP) ChIP step with anti-E2F8. The input chromatin is indicated. (**D**) U2OS cells were transfected with expression vectors encoding HA-E2F7, Myc-E2F8 or HA-E2F1, together with the appropriate siRNA as indicated (final concentration 50 nM and two different siRNAs, 1 and 2, for each of the subunits E2F7 and E2F8). Lamin A/C siRNA acted as an siRNA control. Cell extracts were prepared at 48 h post-transfection and immunoblotted (IB) with anti-HA or anti-Myc. GAPDH acted as a loading control. (**E**) U2OS cells were transfected with E2F7 or E2F8 siRNA as indicated. Lamin A/C siRNA acted as a control. Cell extracts were prepared at 72 h post-transfection and immunoblotted as indicated. GAPDH acted as a loading control. The asterisk in the anti-E2F8 immunoblot indicates a nonspecific polypeptide. (**F**) U2OS cells were transfected with the indicated siRNA. Cell extracts were prepared as previously described and immunoblotted with the indicated antibodies. GAPDH acted as a loading control. (**G**) U2OS cells were treated with E2F7 or E2F8 siRNA, as indicated, and the level of p73 and CDC6 measured by immunoblotting. GAPDH was used as a loading control. (**H**) U2OS cells were treated with E2F7 or E2F8 siRNA, as indicated, and the level of E2F1 and caspase 3 (CASP3) measured. GAPDH was used as a loading control. (**I**) U2OS cells were treated with E2F7 (1 and 2), E2F8 (1 and 2) or lamin A/C siRNA as indicated. RNA was prepared and reverse transcription–PCR was carried out using appropriate primer pairs as described previously. The relative level of induction of E2F1 RNA from lamin A/C control to E2F7 or E2F8 siRNA was about twofold, which was similar to the relative increase in the level of E2F1 protein (**F**). ChIP, chromatin immunoprecipitation; Etop, etoposide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HA, haemagglutinin; IP, immunoprecipitation; siRNA, short interfering RNA; WT, wild type.

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lamin A/C siRNA reduced lamin A/C but failed to affect E2F7 or E2F8 levels (Fig 2D). The effect of E2F7 siRNA on endogenous E2F8 protein, and vice versa (Fig 2E), might reflect the effect of the interaction between E2F7 and E2F8.

The effect of E2F7 and E2F8 depletion was monitored on a selection of E2F target genes. We not only focused our attention on *E2F1* but also considered that other genes regulated by the E2F pathway might be influenced by E2F7 and E2F8. Under the conditions of E2F7 depletion, there was an increase in the level of E2F1 (Fig 2F). Other E2F target genes induced after E2F7 depletion included caspase 3, whereas *APAF1, p73* and *CDC6* were not significantly affected (Fig 2F–H). Several control genes, including lamin A/C, proliferating-cell nuclear antigen (PCNA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were not altered by E2F7 depletion. A parallel experiment carried out using E2F8 siRNA yielded similar results (Fig 2F–H). Furthermore, the increase in E2F1 protein reflected increased levels of E2F1 RNA (Fig 2I), suggesting that E2F7 and E2F8 regulate the expression of the *E2F1* gene.

E2F7 and E2F8 interaction in cells

Both E2F7 and E2F8 show similarity across a domain that is responsible for dimerization between E2F subunits (Fig 3A; Logan *et al*, 2004, 2005). To assess whether E2F7 and E2F8 interact, we expressed epitope-tagged proteins in U2OS cells and carried out immunoprecipitation followed by immunoblotting. Flag-E2F7 was detected in a Myc-E2F8 immunoprecipitate, whereas Myc-E2F8 was detected in a HA-E2F7 (HA for haemagglutinin) immunoprecipitate (Fig 3B,C). The interaction between ectopic proteins reflected complex formation between the endogenous E2F7 and E2F8 proteins (Fig 3E).

We investigated the domains in E2F7 and E2F8, which are necessary for the interaction, by studying the ability of mutant derivatives (Fig 3A) to bind to each other. Mutation of each of the DNA-binding domains in E2F7 or E2F8 (the DBD mutants) did not affect the interaction with the opposite protein, which was equally efficient with the wild-type protein (Fig 3B,C). By contrast, mutating residues within the dimerization domain (the DD mutants) resulted in a decrease in the interaction with the other wild-type protein (Figure 3C,D). The dimerization-like domains are therefore involved in formation of the E2F7–E2F8 protein complex.

To assess the functional importance of the DNA-binding and dimerization domains, we measured the activity of the mutant proteins on the E2F1 promoter. Both E2F7 and E2F8 repressed E2F1 promoter activity (Fig 3F), as found in previous studies (Logan *et al*, 2004, 2005). However, repression was dependent on the integrity of the DNA-binding and dimerization domains as mutation in either domain resulted in proteins that failed to repress E2F1 promoter activity (Fig 3F).

E2F7 and E2F8 suppress the activity of E2F1

The introduction of either E2F7 or E2F8 siRNA into U2OS cells using flow cytometry caused a marked increase in sub-G1 apoptotic cells, which was in contrast to the effect of the control siRNA (Fig 4A). Similar results were obtained when annexin 5 was used to measure the level of apoptotic cells; depleting either E2F7 or E2F8 caused a marked increase in apoptosis (Fig 4B). As increased apoptosis owing to the E2F7 and E2F8 siRNA treatment might reflect the deregulation of E2F1 activity, we studied the level of apoptosis by repeating the treatment in the presence of E2F1 siRNA. Interestingly, there was a marked decrease in the level of apoptosis, determined by measuring either sub-G1 cells or annexin 5 staining (Fig 4B; data not shown). Also, annexin 5-stained cells reached 28% on E2F7 and E2F8 siRNA treatment, but decreased to 8% on co-treatment with E2F1 siRNA. In addition, most of the annexin 5-positive cells failed to take up propidium iodide, further supporting the presence of apoptotic cells (Fig 4B). The E2F1 siRNA reduced the level of endogenous E2F1, and its ability to deplete E2F1 protein was apparent in the presence of E2F7 or E2F8 siRNA (Fig 4C). These results indicate that E2F7 and E2F8 suppress E2F1 activity.

E2F7 and E2F8 regulate the response to DNA damage

As E2F7 and E2F8 are induced by DNA damage, and as they regulate E2F1 activity, E2F7 and E2F8 might have important roles in DNA-damaged cells. Therefore, we studied the effect of depleting E2F7 and E2F8 in U2OS cells treated with the DNA-damaging agent etoposide.

In the presence of etoposide, U2OS cells undergo a DNA-damage response in which, typically, there is a reduction in the G1 population and an increase in the number of cells in S and G2/M phases (Fig 5A). When DNA-damaged cells were treated with either E2F7 or E2F8 siRNA, the cell-cycle profile resembled the profile of untreated cells (Fig 5A). When the effect of E2F7 or E2F8 siRNA on sub-G1 cells in the presence of etoposide was monitored, the level of sub-G1 cells was enhanced (supplementary Fig 1A online), and thus reflects the effects observed in untreated cells (Fig 4). Bromodeoxyuridine incorporation showed similar effects in untreated and etoposide-treated cells, when E2F7 or E2F8 depletion was compared with the control treatment (supplementary Fig 1B online), indicating that DNA synthesis was not enhanced by the depletion of E2F7 or E2F8. These results therefore indicate that, in U2OS cells under these conditions, E2F7 and E2F8 are required for the cell-cycle response to DNA damage.

It was of interest to establish the role of E2F1 in DNA-damaged U2OS cells treated with E2F7 and E2F8 siRNA. DNA-damaged U2OS cells co-treated with E2F7, E2F8 and E2F1 siRNA showed a decrease in the G1 population, together with an increase in S- and G2/M-phase cells (Fig 5A), and therefore resembled the typical cell-cycle profile seen in U2OS cells treated with etoposide. Moreover, depleting E2F7 or E2F8 in conditions of etoposide treatment resulted in increased levels of E2F1 compared with the usual induction of E2F1 during the DNA-damage response (Fig 5B), which coincided with an increase in E2F1 RNA under E2F7 and E2F8 siRNA treatment conditions (supplementary Fig 1C online).

In summary, these results suggest that the cell-cycle response to E2F7 and E2F8 depletion requires E2F1 activity, and also that the cell-cycle effects apparent on DNA damage require E2F7 and E2F8 to modulate E2F1 activity.

DISCUSSION

E2F7 and E2F8 are induced by DNA damage

These results indicate that E2F7 and E2F8 have an important role in regulating the activity of E2F1 during the DNA-damage response. As E2F7 and E2F8 exist as a complex on the E2F1

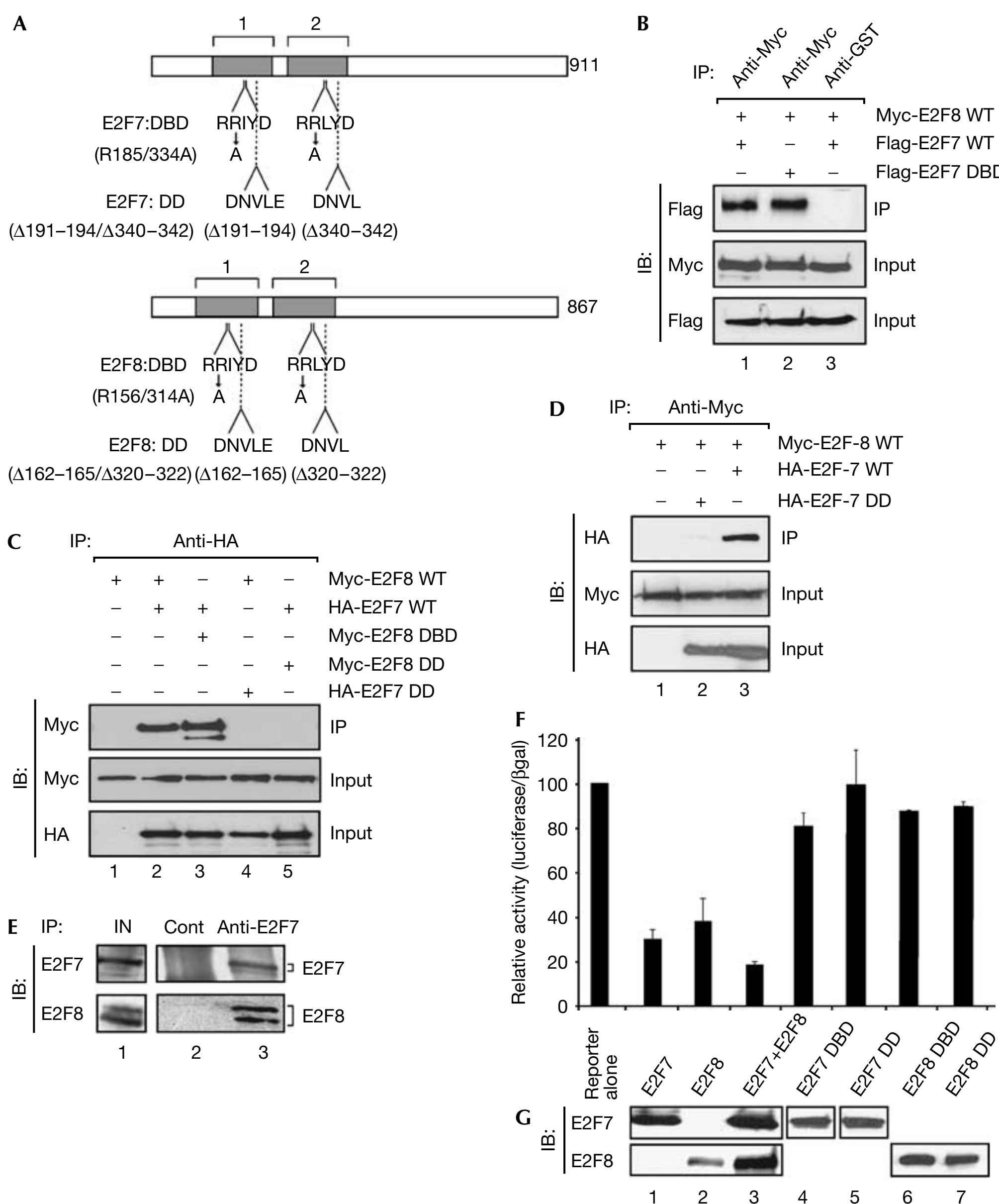

Fig 3 | E2F7 and E2F8 form a protein complex. (**A**) Arg185Ala/Arg334Ala (referred to as DBD) and Δ191–194/340–342 (referred to as DD) represent E2F7 mutants carrying mutations in the DNA-binding domain and dimerization domains, respectively, and Arg156Ala/Arg314Ala and Δ162–165/320–322 carry analogous mutations in E2F8. (**B**) U2OS cells were transfected with expression vectors (4 μg) encoding Myc-E2F8 and Flag-E2F7, or E2F7 DBD as indicated, and immunoprecipitated (IP) with either Myc or GST control antibody, followed by immunoblotting (IB) with anti-Flag. Inputs were immunoblotted with anti-Myc and anti-Flag as indicated. (**C,D**) U2OS cells were transfected with expression vectors (4 μg) encoding wild-type HA-E2F7 or DD derivatives, and wild-type Myc-E2F8, DBD or DD as indicated, followed by immunoprecipitation and immunoblotting as described previously. (**E**) Immunoprecipitation was carried out on endogenous HeLa cell proteins as previously described using E2F7 (lane 3) or control (lane 2) antibody, followed by immunoblotting with anti-E2F8 as indicated. Lane 1 shows the input level of E2F8 (10%). (**F**) Each of the subunits E2F7 and E2F8, or the indicated DBD or DD mutant derivatives (1 μg), were co-transfected into U2OS cells together with E2F1-luciferase (1 μg) and CMV-βgal (as the internal control). Cells were collected as described previously. (**G**) Levels of ectopic proteins expressed in transfected cells. E2F8 levels were detected by anti-Myc, and E2F7 by anti-HA; E2F7 DBD was detected by anti-Flag. GST, glutathione-*S*-transferase; HA, haemagglutinin; WT, wild type.

promoter, this is likely to be mediated through the ability of E2F7 and E2F8 to repress E2F1 transcription. The E2F1 protein is an interesting member of the E2F family. E2F1 has been implicated not only in the control of cell-cycle progression, but also in regulating apoptosis (Johnson *et al*, 1993; Qin *et al*, 1994; DeGregori *et al*, 1997; Hallstrom & Nevins, 2003; Denchi & Helin, 2005). A model has been suggested to account for the context-dependent physiological roles ascribed to E2F1, in

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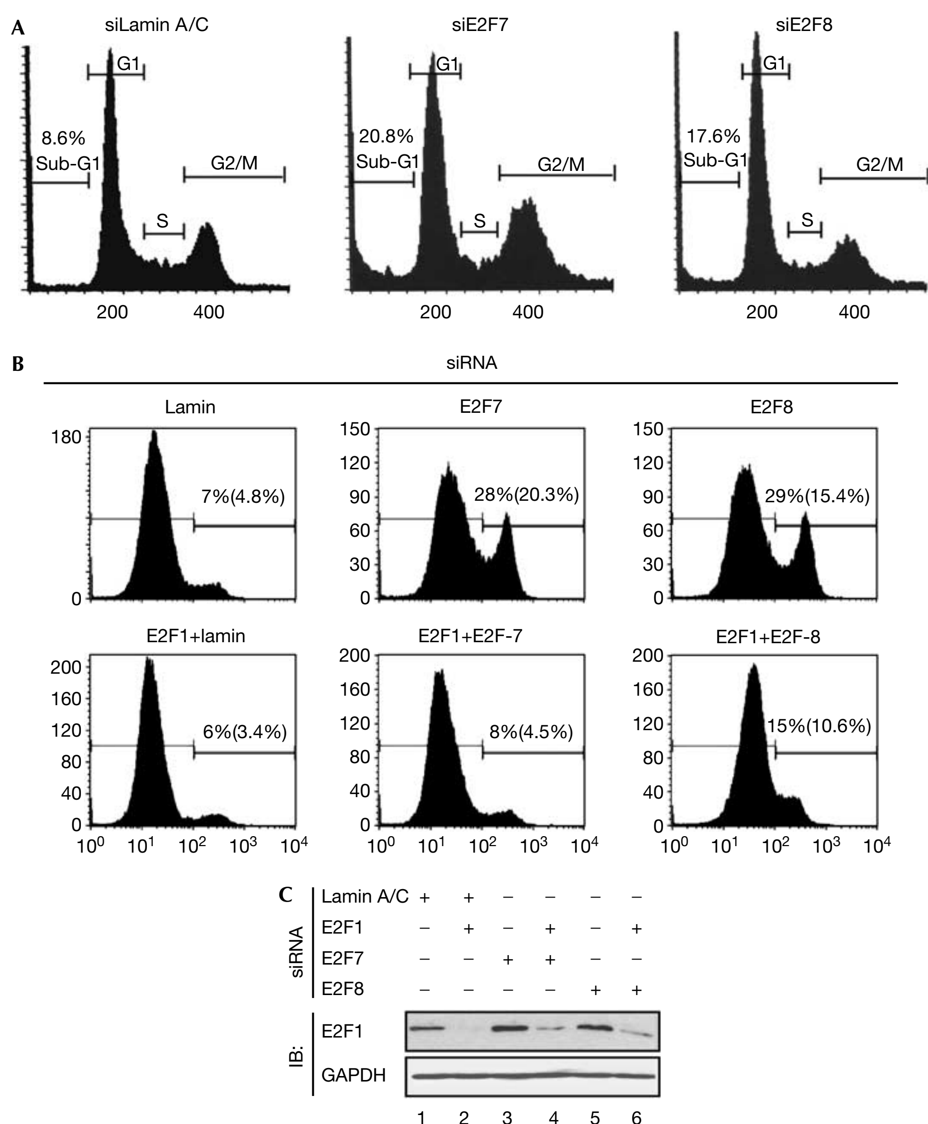

Fig 4 | E2F7 and E2F8 suppress E2F1-dependent apoptosis. (**A**) U2OS cells were treated as previously described with E2F7, E2F8 or control lamin A/C siRNA (50 nM) for 72 h. The cell-cycle profiles are shown and were determined by FACS. The level of sub-G1 cells is indicated as a percentage of the whole population for one representative experiment. Similar enrichments in the sub-G1 population were observed in two other independent experiments. (**B**) U2OS cells were treated as in (**A**) with E2F7, E2F8 or control lamin A/C siRNA together with E2F1 siRNA. Annexin 5 expression was measured as described previously, and the percentage of annexin 5 positively stained cells are indicated. The percentage of annexin 5-positive cells that were negative for popidium iodide staining is indicated in parenthesis. (**C**) Level of E2F1 protein in U2OS cells, treated as described in (**B**). FACS, fluorescence-activated cell sorting; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IB, immunoblotting; siRNA, short interfering RNA.

particular, that the level of E2F1 protein influences the cellular consequence of E2F1 activity (Trimarchi & Lees, 2002; La Thangue, 2003). For example, levels of E2F1 above a certain threshold, such as that occurring during the DNA-damage response (Blattner *et al*, 1999; Lin *et al*, 2001; Stevens *et al*, 2003), might lead to apoptosis. Conversely, lower levels of E2F1, for example, during cell-cycle progression, might fail to activate apoptosis but instead affect cell-cycle progression. Therefore, the level of E2F1 is likely to be finely balanced and critically important in regulating cell viability.

An overriding conclusion from the studies on *E2F1*$^{-/-}$ mice is that cells and tissues differ in their dependency and sensitivity to E2F1 activity; *E2F1*$^{-/-}$ mice suffer from tissue atrophy, tumour predisposition and defective apoptosis during T-cell ontogeny (Field *et al*, 1996; Yamasaki *et al*, 1996). As *E2F1*$^{-/-}$ mice are viable, many tissues are unaffected by the loss of E2F1. By contrast, in tissues where the absence of E2F1 coincides with tumour predisposition, the function of E2F1 is likely to take on a tumour suppressor-like role, perhaps through its ability to influence apoptosis through a checkpoint response. Therefore, it

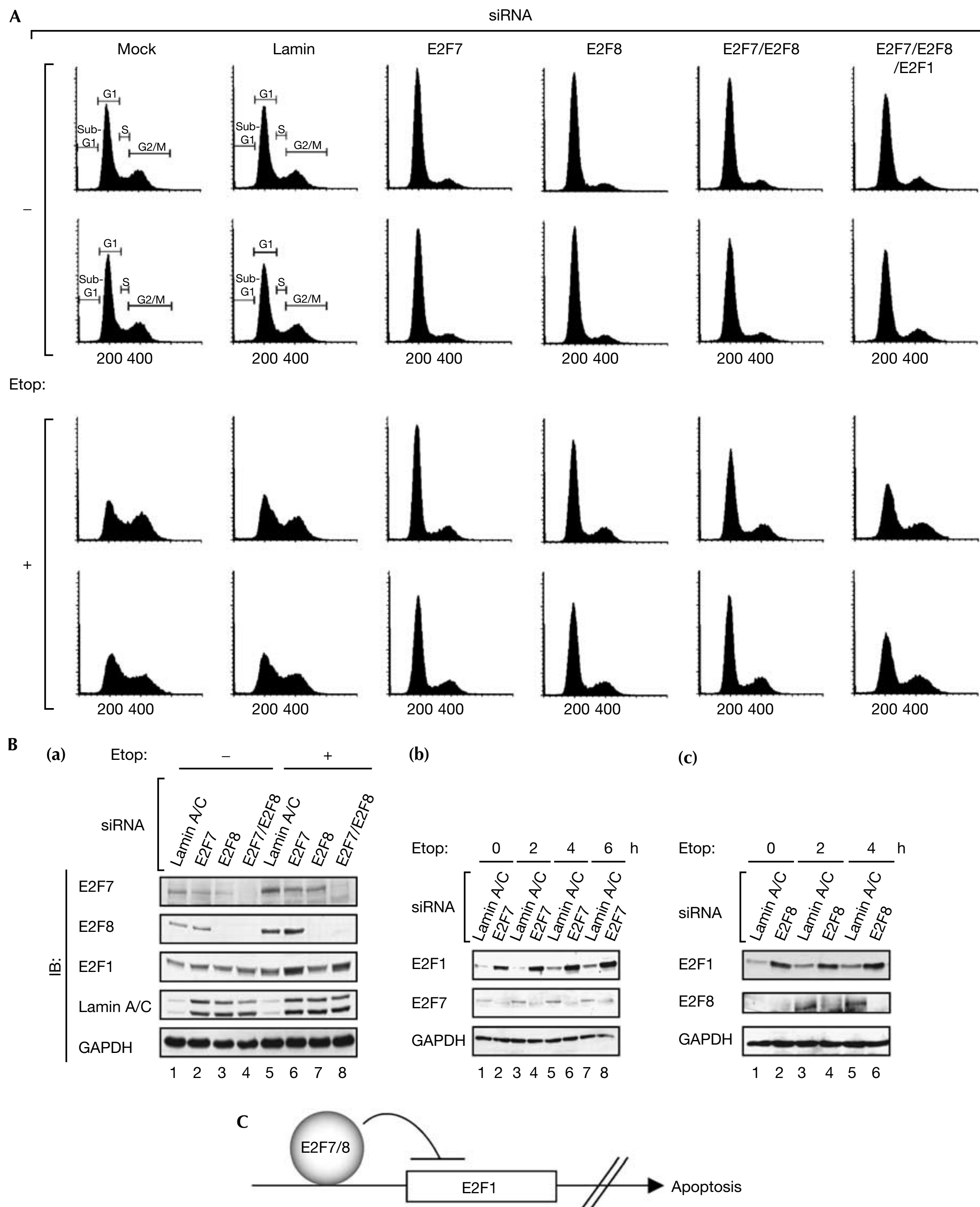

Fig 5 | The effect of E2F7 and E2F8 short interfering RNA in DNA-damaged cells. (**A**) U2OS cells were treated, as indicated, with E2F7, E2F8, E2F1 or lamin A/C siRNA (60 nM) and after 56 h treated with etoposide (Etop: +, 10 μM) for a further 16 h. At 56 h, the cells were washed and replaced with fresh medium. The cell-cycle profile was assessed by using flow cytometry; note that apoptotic cells were excluded from this analysis. (**B**) U2OS cells were treated, as indicated, with E2F7 (**a**, **b**), E2F8 (**a**, **c**) or control lamin A/C (60 nM) siRNA as indicated and after 66 h treated with etoposide (10 μM), then collected at the indicated time points, and immunoblotted (IB) with anti-E2F7, E2F8, E2F1, lamin A/C or GAPDH, as indicated. (**C**) Summary of the control of the *E2F1* gene by E2F7 and E2F8. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; siRNA, short interfering RNA.

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is tempting to speculate that the ability of E2F7 and E2F8 to control the expression of the *E2F1* gene is crucial in dictating the effect of E2F1 in cells (Fig 5B). On the basis of the studies described here, we would predict that cells that express high levels of E2F7 and E2F8 show an intrinsically lower sensitivity to E2F1-dependent apoptosis.

METHODS

Cell culture and transfection. Cells were transfected, according to the manufacturer's instructions, by using Lipofectamin 2000 (Invitrogen, Paisley, UK) or GeneJuice (Merck, Nottingham, UK), and, in the case of RNA interference (siRNA), by Oligofectamine (Invitrogen). The siRNAs used for targeting E2F1 were as described previously (Youn *et al*, 2005), and siRNA for lamin A/C was purchased from Dharmacon, Cramlington, UK. The siRNA for E2F7, E2F8 and E2F1 were: E2F7 siRNA1: AAAGGTACGACG CCTCTATGA; E2F7 siRNA2: AACAGAAGAGCGAGGTCGTAA; E2F8 siRNA1: AATGTTGAACGTCGACGCATT; E2F8 siRNA2: AA ACAGCCGCAAAGACAAGTC; E2F1 siRNA1: ACTGACCATCAG TACCTGGUU; E2F1 siRNA2: GAAGTCCAAGAACCACATCUU.

Immunoblotting, immunoprecipitation and protein extracts. Immunoblotting was carried out according to standard procedures (Logan *et al*, 2004) using the following antibodies: Myc antibody 9E10, Flag, E2F7, E2F1, PCNA, GAPDH, CDC6, caspase 3, p73, p53, lamin A/C, APAF1 (Santa Cruz, Heidelberg, Germany), HA antibody HA11 (Babco, Richmond, CA, USA) and E2F8 antibody M01 (Abnova, Taipai City, Taiwan).

Real-time and semiquantitative RT–PCR. RNA was extracted from treated U2OS cells using Trizol reagent (Invitrogen). A total of 400 ng RNA was reverse transcribed and subsequently quantified by semiquantitative PCR or with real-time PCR using the Brilliant II SYBR Green 1-step QRT–PCR master mix (Stratagene), according to the manufacturer's instructions on a MX3005P QPCR system (Stratagene). 18S ribosomal RNA was used as the internal control. PCR primers used were as follows: E2F7 forward: 5′-GGAAAGGCAACAGCAAACTCT-3′, E2F7 reverse: 5′-TGGGAGAGCACCAAGAGTAGAAGA-3′; E2F8 forward: 5′-GCAGCCAATGATACCTCAAAGG-3′, E2F8 reverse: 5′-ATGAG CACTGCGTGAGAGGGATTA-3′; E2F1 forward: 5′-ATGAGACC TCACTGAATCTGACCACC-3′, E2F1 reverse: 5′-AGTCACAGTCG AAGAGGTCTCTG-3′; GAPDH forward: 5′-CCATCAATGACCCC TTCATTGACC-3′, GAPDH reverse: 5′-GAAGGCCATGCCAGTG AGCTTCC-3′ and 18S forward: 5′-GATACCGAACGAGACTCT GGC-3′, 18S reverse: 5′-CCATCCAATCGGTAGTAGCG-3′.

Chromatin immunoprecipitation. The primers used were as follows: 5′-AGGAACCGCCGCCGTTGTTCCCGT-3′ (E2F1 forward); 5′-GCTGCCTGCAAAGTCCCGGCCACT-3′ (E2F1 reverse); 5′-TGGG GTTGACAGAAGAGAAAAGC-3′ (Albumin forward); 5′-TACATTGA CAAGGTCTTGTGGAG-3′ (Albumin reverse).

Supplementary information is available at EMBO reports online (http://www.emboreports.org).

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