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ARF stimulates XPC to trigger nucleotide excision repair by regulating the repressor complex of E2F4

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The tumour suppressor ARF (alternative reading frame), which is mutated or silenced in various tumours, has a crucial role in tumour surveillance to suppress unwarranted cell growth and proliferation. ARF has also been linked to the DNA-damage-induced response of p53 because of its ability to inhibit murine double minute 2 (MDM2). Here, however, we provide genetic evidence for a role of ARF in nucleotide excision repair (NER) that is independent of p53. Cells lacking ARF are deficient in NER. Expression of ARF restores the repair activity, which coincides with increased expression of the damaged-DNA recognition protein xeroderma pigmentosum, complementation group C (XPC). We provide evidence that, by disrupting the interaction between E2F transcription factor 4 (E2F4) and DRTF polypeptide 1 (DP1), ARF reduces the interaction of the E2F4–p130 repressor complex with the promoter of XPC to ensure high-level expression of XPC. Together, our results point to an important 'care-taker'-type tumour-suppression function for ARF in NER through the increased expression of XPC.
Keywords: ARF; NER; XPC; E2F4; p130

INTRODUCTION

The Ink4A/ARF locus (cyclin-dependent kinase inhibitor 2A (CDKN2A) in humans) encodes two tumour-suppressor proteins, p16[INK4a] and p19[ARF] (p14ARF in humans; Quelle *et al*, 1995; reviewed in Sherr, 2006). ARF, derived from an alternative reading frame of the INK4A locus, is mutated, deleted or silenced in a significant number of human cancers, is coupled to the p53 pathway. In response to sustained hyperproliferative signalling,

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ARF is activated and, in turn, stabilizes and activates p53 by antagonizing its negative regulator, murine double minute 2 (mdm2; Kubbutat *et al*, 1997; Kamijo *et al*, 1998; Pomerantz *et al*, 1998; Zhang *et al*, 1998; Weber *et al*, 2000a,b; Llanos *et al*, 2001). Activation of the ARF–p53–MDM2 pathway results in cell-cycle arrest or apoptosis and is considered to be a checkpoint that protects cells from tumorigenesis.

Interestingly, there is a growing body of evidence to suggest that ARF has additional tumour-suppressive activities that are independent of p53. The p53-independent function first became apparent when it was observed that ARF retained its ability to cause cell-cycle arrest in cells that were p53^{−/−} or p53^{−/−}MDM2^{−/−} (Carnero *et al*, 2000; Weber *et al*, 2000a,b; Eymin *et al*, 2001). Reintroduction of ARF into p53^{−/−}MDM2^{−/−}ARF^{−/−} mouse embryonic fibroblasts (MEFs) caused a delayed G1-phase growth arrest (Weber *et al*, 2000a,b). These findings imply that ARF could not be solely linked to the p53 pathway and has led to the discovery of many new targets by which it regulates the cell cycle.

ARF has been shown to inhibit ribosomal-RNA processing (Sugimoto *et al*, 2003), to interact with topoisomerase I (Ayrault *et al*, 2004) and to interact physically and re-localize several transcription factors, including E2F transcription factor 1 (E2F1; Martelli *et al*, 2001), forkhead box M1B transcription factor (Kalinichenko *et al*, 2004) and c-Myc (Datta *et al*, 2004; Qi *et al*, 2004). Consequently, it has been reported that ARF affects gene expression (Eymin *et al*, 2001; Martelli *et al*, 2001; Datta *et al*, 2002, 2004; Kuo *et al*, 2003; Rocha *et al*, 2003; Aslanian *et al*, 2004; Qi *et al*, 2004). We reported previously that ARF could specifically regulate E2F target gene expression by binding to DRTF polypeptide 1 (DP1), the functional partner of E2F family of factors, and inhibiting the interaction between DP1 and E2F1. Upon ARF expression there was a loss of DP1 binding to the promoter of an E2F target gene (Datta *et al*, 2005).

The role of ARF in DNA repair has not been investigated in depth. One study implicated ARF in processing DNA photo-products that are generated following exposure to ultraviolet radiation (Sarkar-Agrawal *et al*, 2004). DNA damages incurred by ultraviolet radiation are repaired by nucleotide excision repair (NER). NER involves three basic biochemical steps consisting of

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damage recognition, dual incision and gap-filling DNA synthesis (Li *et al*, 1998). It is currently acknowledged that repair proteins act in a highly coordinated manner and assemble sequentially on the site of DNA damage to return it to its native state (Volker *et al*, 2001). Seven of these repair proteins belong to the xeroderma pigmentosum (XP) group (XPA-G) (de Laat *et al*, 1999; Masutani *et al*, 1999; Mullenders & Berneburg, 2001).

Through its crucial role in DNA-damage recognition and initiation of NER, the xeroderma pigmentosum, complementation group C (XPC) protein epitomizes a first line of defence against carcinogenesis (Sugasawa *et al*, 1998; Volker *et al*, 2001). Cells deficient in XPC are impaired in the removal of cyclobutane–pyrimidine dimers (CPDs) and 6-4 pyrimidine pyrimidones (6-4PPs) from the global genome, emphasizing the important function that XPC has in these processes (Venema *et al*, 1991; Emmert *et al*, 2000). In global genomic repair, XPC recognizes CPDs and 6-4PPs and recruits the transcription/repair factor TFIIH, which is crucial in the assembly of the NER complex that leads to excision of the damaged strand. XPC, however, is dispensable for the removal of DNA lesions from the transcribed strand of active genes (Venema *et al*, 1990; Emmert *et al*, 2000). Although the biochemical properties of XPC in repair have been characterized in some detail (Venema *et al*, 1991; Sugasawa *et al*, 1998, 2001; Emmert *et al*, 2000; Volker *et al*, 2001), much remains to be elucidated with regards to its regulation and interaction with other tumour-suppression pathways. Here, we provide evidence for a link between the tumour suppressor ARF and the repair factor XPC.

RESULTS AND DISCUSSION
ARF is required for efficient NER
To investigate whether ARF has a role in NER, we compared the extent of DNA repair in wild-type MEFs and in ARF-null MEFs treated with ultraviolet irradiation. The repair activity was measured by unscheduled DNA synthesis (UDS), an assay that measures repair re-replication following excision of the damaged DNA strand. In UDS, the level of repair is correlated with the amount of tritium-labelled thymidine (3H-TdR) incorporated into the DNA of mammalian cells, which are not in the S phase of the cell cycle. UDS was performed using strategies described by Smith *et al* (2000). Briefly, the cells were pre-labelled using 3H-TdR to identify the S-phase cells. The cells were then subjected to ultraviolet irradiation (10 J/m^2) and maintained in a medium containing 3H-TdR, in the absence of serum for 3 h. The cells were then incubated in a medium containing unlabelled TdR for 1 h, fixed and coated with emulsion (EM-1), and developed for UDS measurement. We found that repair synthesis—grains corresponding to 3H-TdR incorporation—in the ARF-null MEFs was significantly reduced compared with the wild-type MEFs, as can be seen in representative nuclei (Fig 1). The quantification shows that most nuclei counted for wild-type MEFs showed 200–300 grains per nucleus, whereas most nuclei in ARF-null MEFs showed 100–200 grains per nucleus (Fig 1).

To determine whether the function of ARF in NER was dependent on p53, we analyzed p53$^{-/-}$Mdm2$^{-/-}$ (double knock-out (DKO)) MEFs and p53$^{-/-}$Mdm2$^{-/-}$ARF$^{-/-}$ (triple knockout (TKO)) MEFs. By comparing the four genotypes—wild-type, ARF$^{-/-}$, p53$^{-/-}$Mdm2$^{-/-}$ (DKO) and p53$^{-/-}$Mdm2$^{-/-}$ARF$^{-/-}$ (TKO) MEFs—we observed that the extent of repair was comparable
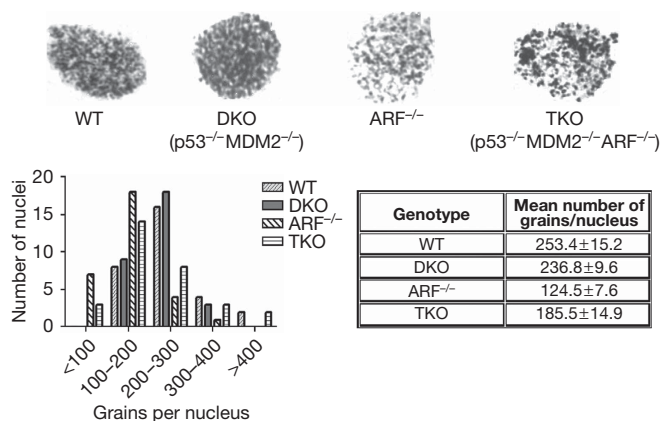

Fig 1 | ARF has a role in nucleotide-excision repair that is independent of p53. The nucleotide-excision-repair activity following ultraviolet irradiation was measured in WT, ARF$^{-/-}$, p53$^{-/-}$MDM2$^{-/-}$ (DKO) and p53$^{-/-}$MDM2$^{-/-}$ARF$^{-/-}$ (TKO) MEFs by UDS (Smith *et al*, 2000). UDS was quantified by counting the number of 3H-thymidine grains per nucleus in 30 nuclei per cell type. Autoradiographs of the grains in nuclei are shown in the upper panel, and grains per nucleus and means ± s.e.m. are shown in the lower panel. TKO compared with DKO $P = 0.0058$ and WT compared with ARF$^{-/-}$ $P < 0.0001$. ARF, alternative reading frame; DKO, double knockout; MDM2, murine double minute 2; MEF, mouse embryonic fibroblast; TKO, triple knockout; UDS, unscheduled DNA synthesis; WT, wild type.

between the wild-type and DKO MEFs, and that it was significantly more than the repair activity observed in the ARF$^{-/-}$ and TKO MEFs (Fig 1). Together, these results suggest that ARF has a function in NER independently of p53.

We hypothesized that if ARF has a function in NER then by expressing it in ARF-null MEFs we should see a reversal of the repair-deficient phenotype. We used an adenovirus expressing ARF to infect the ARF$^{-/-}$ MEFs and, for comparison, we infected the ARF$^{-/-}$ MEFs with a control adenovirus. As expected, re-introduction of ARF restored the degree of repair to a level similar to that seen in the wild-type MEFs (supplementary Fig S1 online).

Involvement of ARF in the removal of 6-4PPs and CPDs
As UDS measures DNA synthesis after the excision of DNA fragments containing 6-4PPs and CPDs, we compared the rates of removal of CPD and 6-4PP in MEFs lacking ARF (TKO and ARF$^{-/-}$) with wild-type MEFs and with DKO MEFs using a previously described procedure (Eveno *et al*, 1995; Li *et al*, 2006). Genomic DNA was isolated from the MEFs at various time points after ultraviolet irradiation. The DNA was denatured and subjected to dot-blot assays. The membrane was assayed using antibodies against 6-4PPs (clone 64M2) and CPDs (clone TDM2) in each cell type. Quantification was obtained by using the Image J program and calculated by comparing the intensities of the signals produced at the indicated times to that of the corresponding signal at time zero. We found that the TKO MEFs and the ARF$^{-/-}$ MEFs were considerably impaired compared with the wild-type or DKO MEFs in removing 6-4PPs (Fig 2). CPD removal, however, was much less efficient in the MEFs, especially at an early time point (4 h).
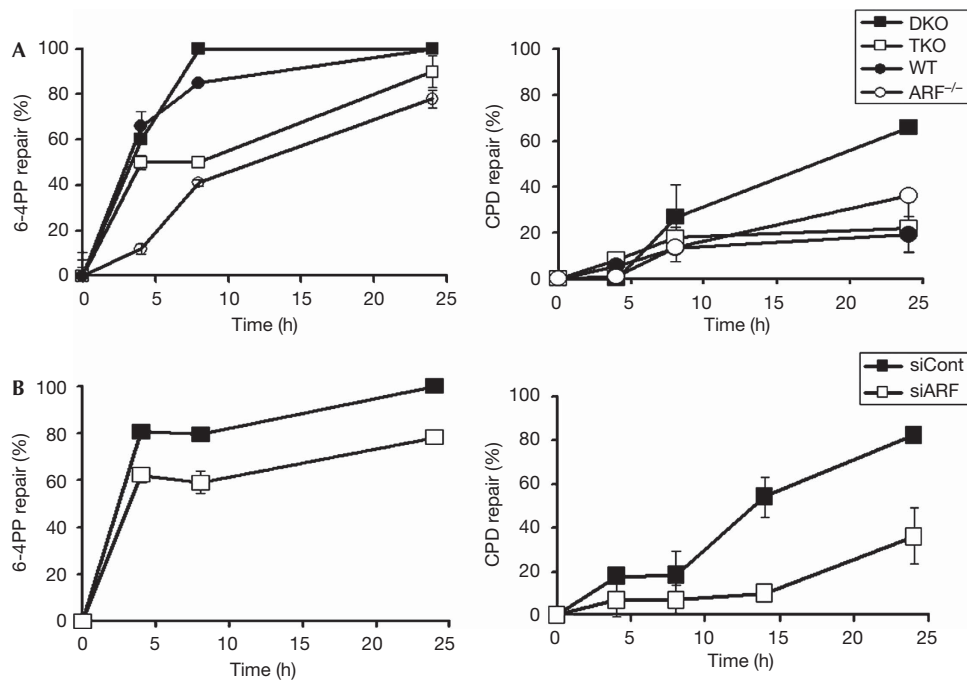
Fig 2 | ARF increases 6-4PP and CPD removal. (**A**) 6-4PP and CPD removal rates were compared between DKO, TKO, WT and ARF$^{-/-}$ MEFs, and (**B**) in DKO cells in which ARF was knocked down by the expression of an ARF shRNA (see supplementary information online for methods). Percentages of 6-4PP and CPD removed in three experiments were quantified and plotted. 6-4PP, 6-4 pyrimidine pyrimidone; ARF, alternative reading frame; Cont, control; CPD, cyclobutane pyrimidine dimer; DKO, double knockout; MEF, mouse embryonic fibroblast; shRNA, short-hairpin RNA; TKO, triple knockout; WT, wild type.

Surprisingly, we did not see any significant difference in CPD removal between wild-type MEFs and ARF-null MEFs (Fig 2). However, the DKO MEFs showed high levels of CPD repair. The extent of CPD removal in DKO MEFs was much higher than that reported for rodent cells (Tang *et al*, 2000). For example, at 24 h post-ultraviolet irradiation, close to 70% of the CPDs were removed in DKO MEFs, whereas in the other MEFs only about 20% were removed (Fig 2). We hypothesize that the efficient removal of CPD in the DKO MEFs results from the high expression of ARF in those cells (Datta *et al*, 2005; supplementary Fig S2 online), as similar efficient removal was detected by overexpressing ARF in ARF$^{-/-}$ cells (data not shown).

To confirm further the role of ARF in the repair of 6-4PPs and CPDs, we used DKO cells in which the level of ARF had been knocked down by the expression of an ARF short-hairpin RNA. Knockdown of ARF was confirmed by using Western blot (supplementary Fig S2 online). The DKO cells, with or without ARF knockdown, were compared for the removal of CPDs and 6-4PPs. Clearly, depletion of ARF caused a significant impairment in the removal levels of CPD and 6-4PP (Fig 2, lower panels), confirming the idea that ARF has a significant function in the repair of ultraviolet-damaged DNA.
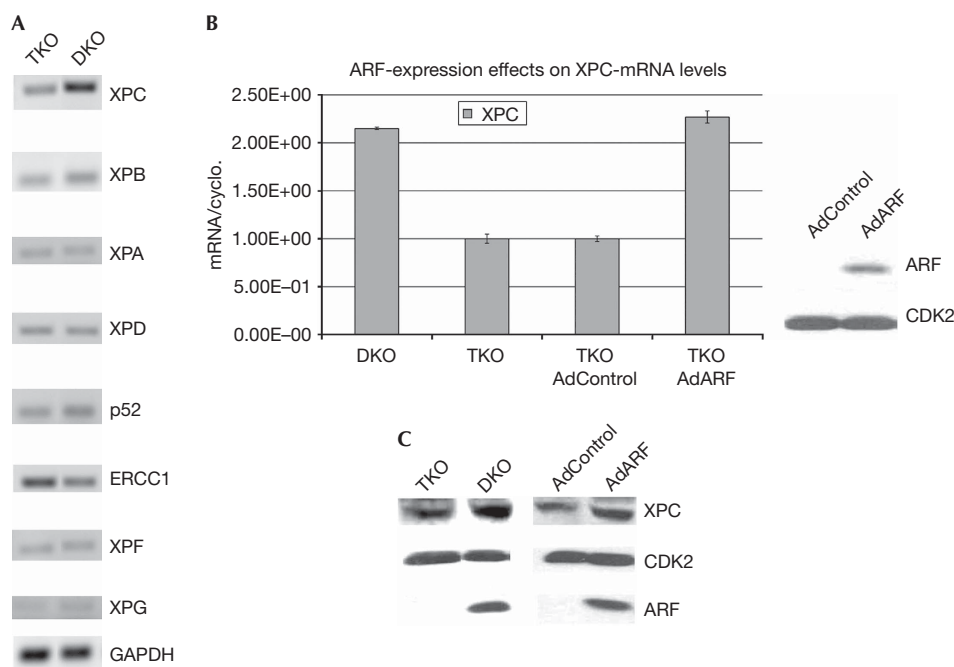
ARF stimulates the expression of XPC protein

To determine the mechanism by which ARF participates in NER, we investigated whether the genes that are essential for NER are regulated by ARF. We compared the RNA isolated from ARF + cells (DKO MEFs) with those from ARF− cells (TKO MEFs) using semi-quantitative reverse transcription PCR (RT–PCR). The messenger-RNA (mRNA) levels of XPC, XPB, XPA, XPD, p52 (one of the subunits of TFIIH), excision repair cross-complementing rodent repair deficiency, complementation group 1 (ERCC1), XPF and XPG were analysed (Fig 3A). We observed that the transcript level of XPC in ARF + cells was substantially higher than in ARF− cells. This observation was also seen by using quantitative RT–PCR (Fig 3B). Furthermore, we found that infecting ARF− (TKO MEFs) cells with ARF-expressing adenovirus brought the transcript levels of XPC to that observed in the ARF + cells (DKO MEFs; Fig 3B). Moreover, expression of ARF increased the protein level of XPC (Fig 3C). The observation that ARF stimulates the expression of XPC is significant because XPC has an invaluable role in DNA-damage recognition and the initiation step of NER (Sugasawa *et al*, 1998; Volker *et al*, 2001). We did not see any significant difference in the level of the XPE gene product damage-specific DNA binding protein 2 (DDB2) in the ARF-null MEFs in response to ARF expression (data not shown).

ARF disrupts E2F4 repressor complexes to stimulate XPC

As there was a substantial increase in XPC transcript level on ARF expression, we directed our attention to the promoter of XPC. A previous report (Cam *et al*, 2004) indicated that the XPC gene is repressed by the E2F4–p130 repressor complex. Previous studies have shown that the tumour suppressor ARF binds to DP1 to inhibit the interaction between DP1 and E2F1 (Datta *et al*, 2005). We considered the possibility that ARF would disrupt the interaction between the E2F4–p130 repressor complex and the XPC promoter by dissociating the interaction between E2F4 and DP1, which would result in the loss of DNA binding. To

Fig 3 | ARF stimulates the expression of XPC. (**A**) Semi-quantitative RT–PCR and (**B**) quantitative RT–PCR assays were used to measure the expression of genes that are essential for nucleotide-excision repair in TKO and DKO MEFs. (**C**) Protein levels of XPC in the extracts (0.15 mg) of DKO and TKO MEFs were compared using Western-blot assays. A comparison of XPC level in TKO cells infected with control or ARF-expressing adenovirus is shown on the right lanes. Ad, adenovirus; ARF, alternative reading frame; CDK2, cyclin-dependent kinase 2; DKO, double knockout; ERCC1, excision repair cross-complementing group 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MEF, mouse embryonic fibroblast; mRNA, messenger RNA; RT–PCR, reverse transcription PCR; TKO, triple knockout; XPC, xeroderma pigmentosum group C.
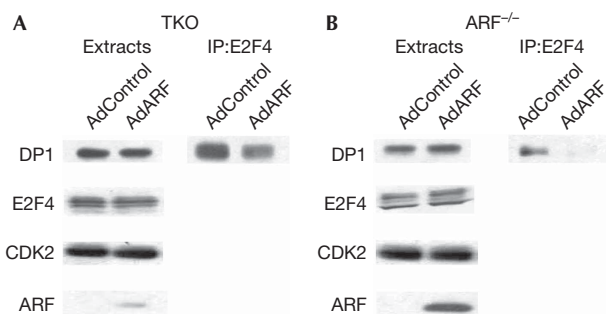

Fig 4 | ARF dissociates the E2F4–DP1 interaction. (**A**) TKO MEFs or (**B**) ARF-null MEFs were infected with control or ARF-expressing adenovirus for 16 h. Cell extracts (1 mg) were immunoprecipitated (IP) using the E2F4 antibody, and the immunoprecipitates were subjected to Western blot analysis using the DP1 antibody. The levels of E2F4, DP1 and ARF in the extracts (0.1 mg) of the infected cells are also shown. Ad, adenovirus; ARF, alternative reading frame; CDK2, cyclin-dependent kinase 2; DKO, double knockout; DP1, DRTF polypeptide 1; E2F4, E2F transcription factor 4; MEF, mouse embryonic fibroblast; TKO, triple knockout.

investigate whether ARF reduces the interaction between E2F4 and DP1, we infected TKO MEFs and ARF-null MEFs with either control adenovirus or ARF-expressing adenovirus. Extracts from the infected cells were immunoprecipitated with the E2F4 antibody and the immunoprecipitates were assayed for the presence of DP1 by Western blot using an DP1 antibody. Consistent with our prediction, there was indeed a reduced association between E2F4 and DP1 in the presence of ARF expression in both cell types (Figs 4A,B).

To determine whether the expression of ARF has a regulatory effect on the E2F4–p130 repressor complex and its interaction with the promoter of XPC, we carried out chromatin-immuno-precipitation (ChIP) experiments. The mouse XPC promoter contains a canonical E2F binding site between −3 and +5 positions relative to the transcription start site. We designed primers to detect E2F4 and p130 binding to this region of the XPC promoter. TKO MEFs were either infected with control adenovirus or ARF-expressing adenovirus. At 16 h post infection, the cells were cross-linked and the chromatin was sonicated and processed for ChIP using E2F4 and p130 antibodies. The amounts of XPC promoter DNA associated with the immunoprecipitated chroma-tin were quantified by using real-time PCR. As shown in Fig 5A,B, the expression of ARF resulted in a considerable decrease of E2F4 and p130 bound to the XPC promoter. To confirm that the XPC promoter is indeed a target of ARF-mediated activation, we generated a luciferase reporter construct with a −1.5 kb fragment of the XPC promoter. ARF-stimulated expression from the native promoter is shown in Fig 5C, upper panel. To assess further the role of the E2F site in the XPC promoter, sequences between −51 and +7 positions of the mouse XPC promoter, with intact or
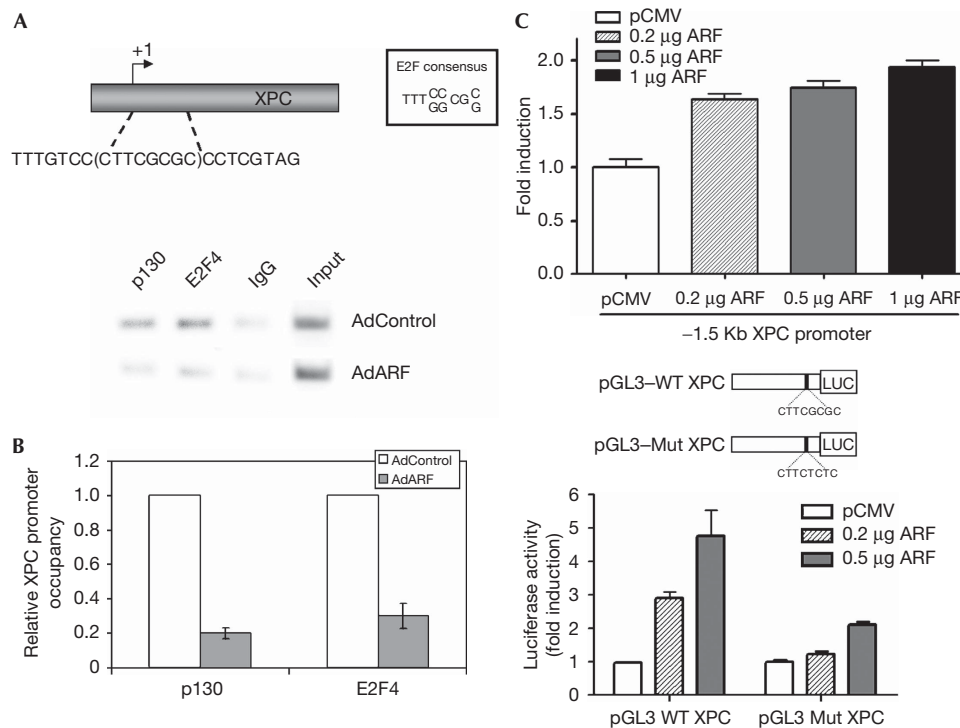
Fig 5 | ARF stimulates the expression of XPC by disrupting the repressor complex of E2F4. TKO MEFs were infected with either control or ARF-expressing adenovirus and subjected to ChIP assay. XPC promoter DNA associated with immunoprecipitated chromatin was quantified by using real-time PCR. An aliquot of the RT–PCR sample was analysed by agarose gel electrophoresis (**A**), and the quantitative RT–PCR results are shown in panel (**B**). In panel (**C**), NIH-3T3 cells were serum starved for 12 h followed by transfection with a 1.5 kB fragment of the XPC and a smaller fragment of the promoter (−51 to +7) containing the E2F site or a mutant promoter construct along with the indicated amounts of ARF. Luciferase activity was measured. Results from three experiments were plotted. ARF, alternative reading frame; ChIP, chromatin immunoprecipitation; E2F4, E2F transcription factor 4; MEF, mouse embryonic fibroblast; Mut, mutant; RT–PCR, reverse transcription PCR; TKO, triple knockout; WT, wild type; XPC, xeroderma pigmentosum group C.

mutated E2F sites, were used to generate luciferase reporter constructs. Expression of the reporter gene was measured in serum-starved cells, a condition in which the repressor complex of E2F4 is abundant. The expression of ARF clearly stimulated transcription from the promoter with an intact E2F site, whereas little stimulation was observed from the promoter construct with a mutated E2F site (Fig 5C, lower panel). Among the NER genes, the ERCC1 and XPC promoters contain E2F binding sites; however, we detected a decrease in ERCC1 expression in DKO MEFs that overexpress ARF. It is possible that ERCC1 is a target of the activator E2Fs, which are inhibited by ARF (Martelli *et al*, 2001). Taken together, these observations show that the expression of ARF stimulates XPC transcription by downregulating the repressor complex of E2F4.

To determine whether the repair defects in the ARF-deficient cells resulted from reduced expression of XPC, we added back XPC in ARF-deficient TKO cells by infecting them with XPC-expressing lentivirus. Cells infected with either control virus (pLVx) or XPC-expressing virus (pLVx-XPC) were selected for three days with puromycin. XPC expression was confirmed using Western blot (supplementary Fig S2 online). These cells, along with DKO cells, were subjected to the UDS assay. Expression of XPC in ARF-deficient TKO cells caused a significant stimulation of UDS (supplementary Fig S3 online). The expression of XPC

stimulated UDS in TKO cells to a level comparable with the DKO cells. These results further confirm the idea that NER deficiency in cells lacking ARF results from the reduced expression of XPC.

Here, we have established that ARF functions as a 'care-taker' type of tumour suppressor and has a important function in the maintenance of genomic integrity. We have shown that ARF is required for high-level expression of XPC, a factor involved in damage recognition and a crucial component in the first step of NER. Previous studies (Adimoolam & Ford, 2002) have indicated a role for p53 in stimulating the expression of XPC after DNA damage. Consistent with that, we observed an increase in XPC mRNA in ultraviolet-irradiated wild-type MEFs (1.6-fold increase after correcting for loading; supplementary Fig S1 online). However, the DKO MEFs, which lack both p53 and MDM2, express XPC at a much higher level (supplementary Fig S1 online). The DKO cells, unlike other MEFs, also overexpress ARF (supplementary Fig S1 online). Our observations made in DKO cells suggest that ARF is able to stimulate XPC expression independently of p53. ARF activates XPC expression by de-repressing the promoter through disruption of the E2F4 repressor complex. De-regulation of the E2F4 repressor complex is a new mechanism, and it is likely that ARF uses this mechanism to stimulate other genes that are repressed by the E2F4–p130 repressor complex.

Speculation

The E2F4–p130 repressor complex regulates the expression of several DNA-repair genes, including *RAD54, BARD1, MLH1* and *MSH2* (Ren *et al*, 2002); E2F4 also regulates the expression of the checkpoint genes *CHK1* and *MAD2* (Ren *et al*, 2002). Therefore, the observation that ARF disrupts the interaction of the E2F4–p130 complex with a target DNA-repair gene provides support to the idea that ARF would stimulate pathways that are significant in maintaining genomic integrity. For example, RAD54 participates in homologous-recombination-mediated double-strand break repair, whereas MLH1 and MSH2 are crucial for mismatch repair. It will be interesting to determine whether ARF stimulates these pathways. It is noteworthy that ARF$^{-/-}$ cells show defective DNA-damage response after ionizing-radiation treatment, which is partly due to impaired activation of p53 in these cells (Khan *et al*, 2004). There is some evidence that nucleolar ARF re-localizes to the nucleoplasm after DNA damage (Lee *et al*, 2005), which might be important in stimulating the DNA-damage-response genes regulated by the E2F4–p130 complex. Furthermore, ARF expression is stimulated by supra-physiological mitogenic signals. We speculate that the 'care-taker' function of ARF would be beneficial for a cell responding to strong mitogenic signals.

METHODS

Cells. Wild-type, ARF$^{-/-}$ (Kamijo *et al*, 1999), p53$^{-/-}$Mdm2$^{-/-}$ARF$^{-/-}$ (TKO; Weber *et al*, 2000a,b), p53$^{-/-}$Mdm2$^{-/-}$ (DKO) and NIH-3T3 MEFs were maintained in Dulbecco modified Eagle medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Hyclone, Logan, UT, USA), supplemented with 1% L-glutamine (Invitrogen) and 1% non-essential amino acids (Invitrogen).

UDS assay. NER was measured using UDS as described previously (Smith *et al*, 2000). Cells were plated overnight on coverslips. The next morning, cells were pre-labelled in serum-free media for 1 h with 3H-TdR1 > (10 µCi/ml) to identify the S-phase cells. Cells were then irradiated using ultraviolet subtype C radiation at a total dose of 10 J/m^2 in the absence of culture medium. After this irradiation, the cells were again placed in 3H-TdR in serum-free media for 3 h. The cells were then incubated with non-radioactive thymidine for an additional hour, fixed, treated with EM-1 (Amersham, Pisctaway, NJ, USA) and developed for UDS measurement.

CPD- and 6-4PP-removal assay. Removal rates of ultraviolet-induced photoproducts were measured as reported previously (Eveno *et al*, 1995; Li *et al*, 2006). After ultraviolet irradiation, cells were allowed to repair for various times in fresh culture medium. Cells were collected and genomic DNA was extracted using Easy-DNA (Invitrogen). For CPD removal, 1 µg of genomic DNA was denatured by adding NaOH at a final concentration of 0.4 M. EDTA was added at a final concentration of 10 mM. Samples were boiled for 10 min at 100 °C. DNA was then neutralized by adding 2 M ammonium acetate (pH 7). For the removal of 6-4 PP, 1 µg of DNA was boiled at 100 °C for 5 min followed by cooling for 5 min on ice. The DNA was bound to membrane using the Bio-Dot Microfilitration Apparatus (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol for DNA blotting. The membrane was then probed with an antibody specific for CPD or 6-4PP, the chief photoproducts produced by ultraviolet subtype C treatment (anti-thymidine dimer, clone TDM2; or anti-6-4PP antibody clone 64M2) and used at a dilution of 1:3,000 and 1:100, respectively. The

quantification was obtained by using Image J and was calculated by comparing the intensities of the dots produced at the indicated times to that of the corresponding dot at time zero when there was no opportunity of repair and all 100% CPDs were present.

ChIP assay. TKO (p53$^{-/-}$Mdm2$^{-/-}$ARF$^{-/-}$) cells were either infected with control adenovirus or ARF-expressing adenovirus and processed 16 h post infection for ChIP assays. Cells were first cross-linked by the addition of formaldehyde to a final concentration of 1%, the chromatin was sonicated and immunoprecipitation was carried out using 2 µg of E2F4 antibody (C20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and 2 µg of p130 antibody (clone C20; Santa Cruz Biotechnology). ChIP was carried out using a ChIP assay kit (Upstate, Lake Placid, NY, USA) according to the manufacturer's protocol. DNA released from the precipitated complexes was amplified by PCR alongside 0.1% of the input chromatin used to carry out the immunoprecipitation. Mouse XPC promoter (-68 to $+63$ bp)-specific primers (XPC forward 5′-CGGGAACAGGAACTCAGAAA-3′ and XPC reverse 5′-CAGCCCAGGGTAGGACCAC-3′) were used to carry out PCR. The PCR products were separated on agarose gels and visualized by ethidium-bromide staining.

The details for immunoprecipitation, Western blot and luciferase assays can be found in the supplementary information online. The primers used for the RNA assays are also described in the supplementary information online.

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

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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