Transcription – Guarding the genome by sensing DNA damage

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Competing interests statement

The authors declare no competing financial interests.

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OPINION

Transcription — guarding the genome by sensing DNA damage

Mats Ljungman and David P. Lane

Abstract | Cells induce the expression of DNA-repair enzymes, activate cell-cycle checkpoints and, under some circumstances, undergo apoptosis in response to DNA-damaging agents. The mechanisms by which these cellular responses are triggered are not well understood, but there is recent evidence that the transcription machinery might be used in DNA-damage surveillance and in triggering DNA-damage responses to suppress mutagenesis. Transcription might also act as a DNA-damage dosimeter where the severity of blockage determines whether or not to induce cell death. Could transcription therefore be a potential therapeutic target for anticancer strategies?

The integrity of cellular DNA is constantly challenged by both endogenous and exogenous sources. Oxygen metabolism generates oxygen radicals within cells, whereas ultraviolet (UV) light, ionizing radiation and other genotoxic agents add environmental exposure to our DNA. During the course of evolution, numerous genes coding for proteins involved in the surveillance and restoration of the integrity of DNA have evolved. Loss of function of any of these gene products could lead to genomic instability — the driving force of carcinogenesis.

Considering that the DNA in a diploid human cell is about 2 metres in length and that the DNA is packaged by histones into a compact chromatin structure, how does the cell scan its DNA for DNA lesions and how does it determine the severity of damage? Replication and transcription are two processes that 'undress' the DNA from its histone coat and 'scan' the DNA by separating the two strands of DNA. Whereas replication takes place only in proliferating cells and specifically during the S phase of the cell cycle, transcription of genes takes place whether the cells are proliferating or not and during all phases of the cell cycle, except during mitosis when the chromatin is condensed and transcription is suppressed. Therefore, the transcription machinery might be used by cells to monitor DNA integrity and to activate DNA-damage signalling. Furthermore, transcription might act as a damage dosimeter, where the severity of damage, the ability to remove the lesions and the kinetics of mRNA-synthesis recovery determine whether the cell lives or dies.

Transcription is performed by three different RNA polymerases in eukaryotic cells: RNA polymerase I transcribes ribosomal DNA into ribosomal RNA used in the biogenesis of ribosomes; RNA polymerase II transcribes genes into mRNA; and RNA polymerase III synthesizes transfer RNA and small nuclear RNA. Although RNA polymerase I and III transcribe DNA at a high rate, the genomic length of DNA transcribed

is rather small compared with the length of all mRNA genes transcribed by RNA polymerase II. So, RNA polymerase II would be expected to 'scan' a much larger portion of the genome than RNA polymerase I and III, and could therefore be considered to be a 'better' sensor than the two other RNA polymerases. Moreover, when RNA polymerase II complexes are blocked at sites of DNA lesions, they are able to recruit DNA-repair proteins to perform transcription-coupled repair (TCR)[1], a specialized nucleotide-excision repair (NER) pathway that removes transcription-blocking lesions and that does not seem to be associated with RNA polymerase I or III[2,3]. For these reasons, we argue in this article that RNA polymerase II is perfectly suited to act as a sensor of DNA perturbations in the template and to alert the cell by activating DNA-damage signalling pathways.

DNA lesions blocking the elongation of RNA polymerase II can have severe consequences for the cells, such as the induction of apoptosis. To counteract apoptosis, transcription-blocking lesions are removed by TCR. Blockage of transcription also triggers the activation of DNA-damage response pathways involving the tumour suppressor p53 (BOX 1). The surveillance and repair of DNA are of particular importance in proliferating cells before DNA replication in the S phase or chromosome segregation during mitosis. A specific set of DNA-damage signalling proteins have evolved to keep damaged cells arrested at specific points of the cell cycle to allow cells more time to repair their DNA before entering S phase or mitosis. There is a fine balance between the repair of transcription-blocking lesions by TCR to avoid apoptosis and the subsequent loss of DNA-damage signalling. If TCR is too efficient, cells will survive, but they might not have time to repair all the lesions before they are permanently fixed during replication, a condition promoting carcinogenesis. On the other hand, a less efficient TCR pathway would lead to excessive apoptosis, a condition promoting ageing.

A better understanding of the processes induced following blockage of RNA polymerase II as well as the fine balance between TCR and DNA-damage signalling could be very valuable for the development of novel anticancer therapies. Several anticancer chemotherapeutic agents that are used successfully in the clinic at present seem to preferentially kill tumour cells by interfering with transcription. So, how does damage sensing work in this situation?

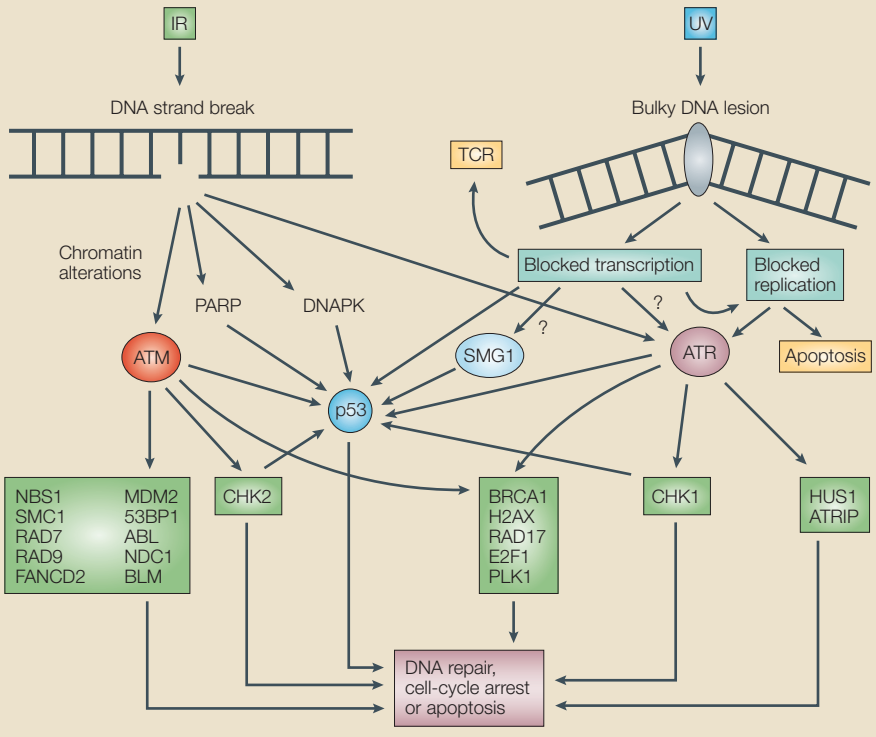
Cytotoxic versus mutagenic lesions

Damage to DNA can be classified as either cytotoxic or mutagenic[4]. Cytotoxic DNA damage, including bulky lesions, interstrand crosslinks and double-strand breaks, interfere with transcription, replication or chromosome segregation. Mutagenic lesions, on the other hand, are defined as lesions that do not necessarily interfere with DNA metabolic processes, but if not removed before replication could cause miscoding resulting in mutations and subsequent carcinogenesis. DNA-damage signalling mechanisms seem to have evolved primarily to respond to cytotoxic lesions, whereas DNA-repair mechanisms have evolved to deal with both classes of DNA lesions.

Bulky lesions such as UV-light-induced pyrimidine dimers and cisplatin adducts, which cause great distortion in the DNA helix, can act as both cytotoxic and mutagenic lesions depending on their localization in the genome. If they are located in the transcribed strand of active genes they are

Box 1 | **DNA-damage signalling pathways and the p53 response**

Following exposure to ionizing radiation (IR) or ultraviolet (UV) light, the induction of DNA-damage signalling pathways is triggered by different mechanisms (see figure). Among many types of base lesions, ionizing radiation induces DNA strand breaks that, through chromatin alterations, are thought to trigger the activation of the ataxia telangiectasia mutated (ATM) kinase[139], leading to the induction of the p53 response. By contrast, induction of the DNA-damage signalling pathway following UV-light irradiation seems to be triggered by blockage of either transcription or replication[20]. The ataxia telangiectasia and Rad3 related (ATR) kinase has been indicated to be a sensor of replication blockage[140] and might have a similar role in sensing blocked transcription complexes, although evidence for this is still lacking. SMG1 is a newly discovered member of the DNA-damage-inducible phosphatidylinositol-3-kinase-related kinase family, which also includes ATM, ATR and DNA-dependent protein kinase (DNAPK)[141]. The SMG1 kinase might respond to both DNA damage and truncated mRNA species that might arise as a result of blocked transcription. All of these protein kinases can directly phosphorylate p53 at the serine-15 site, a modification that is thought to stimulate further protein modifications, leading to the activation and stabilization of the p53 protein[20]. In addition to the phosphorylation of p53, ATM and ATR phosphorylate some common and some unique substrates, ultimately resulting in the activation of pathways dictating the fate of the cell, such as DNA repair, cell-cycle checkpoints or apoptosis. 53BP1, p53-binding protein 1; BLM, Bloom syndrome; NBS1, Nijmegen breakage syndrome 1; PARP, poly-(ADP-ribose) polymerase; PLK1, polo-like kinase 1; SMC1, structural maintenance of chromosomes 1.



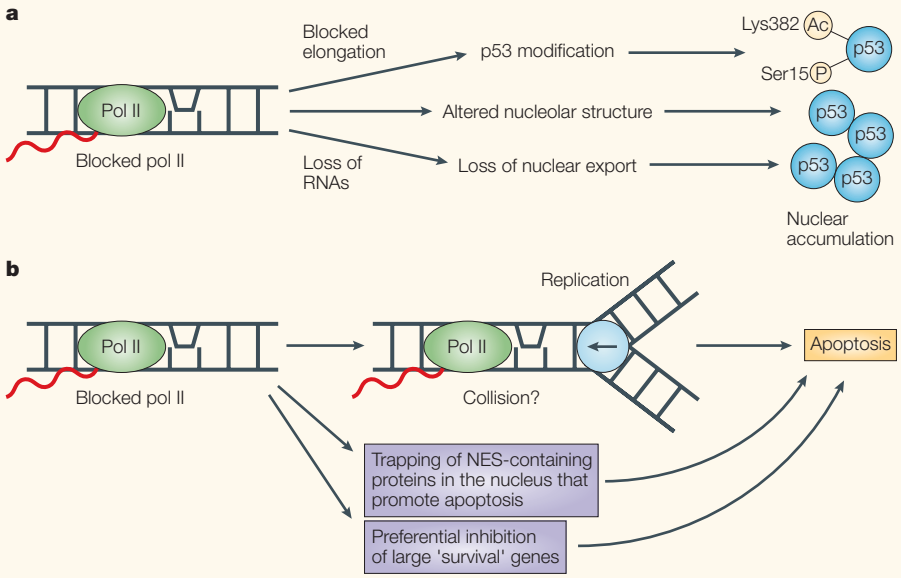


Figure 1 | **Cellular consequences of blocked transcription. a** | Blockage of the elongating form of RNA polymerase II (pol II) activates a stress-response pathway, resulting in specific modifications at the Ser15 and Lys382 sites of p53, which stabilizes the protein (REF. 30). Blockage of transcription might also lead to the accumulation of p53 in the nucleus because of attenuated nuclear export of proteins resulting from the loss of mRNAs on route to be exported out of the nucleus[51]. Finally, nuclear export of p53 might be attenuated if the structure of the nucleoli is perturbed because of inhibition of transcription. **b** | If the blockage of transcription is not resolved in a timely manner, cells might undergo apoptosis. The induction of apoptosis following ultraviolet (UV)-light irradiation is linked to persistent blockage of transcription[5] and occurs preferentially in the S phase of the cell cycle[61]. It is possible that oncoming replication forks might collide with blocked transcription complexes, resulting in apoptosis. Alternatively, apoptosis might be caused by the inhibition of nuclear-export signal (NES)-mediated nuclear export because of diminished availability of mRNAs that assist in nuclear export leading to the inappropriate nuclear accumulation of proteins. Finally, certain types of agents such as UV light might preferentially inhibit 'survival' genes because they are larger on average than pro-apoptotic genes[55], resulting in tipping the balance of gene expression in favour of cell death.

cytotoxic, as they can induce apoptosis by blocking transcription[5–12]. The cytotoxicities of these lesions are suppressed by their removal through TCR, whereas similar lesions elsewhere in the genome are removed by another NER system, global genomic repair (GGR). TCR is a specialized repair pathway using RNA polymerase II stalled at DNA lesions for recruitment of DNA-repair enzymes, whereas GGR requires specialized proteins for the detection of DNA lesions and recruitment of DNA-repair factors. So, the function of TCR is primarily to counteract DNA-damage signalling and suppress apoptosis, whereas GGR removes premutagenic lesions and thereby suppresses carcinogenesis. In fact, there is a strong correlation between the incident of lung[13–16] and breast cancer[17,18] and the capacity for NER of an individual.

Transcription blockage signalling

The tumour-suppressor protein p53 has an important role in determining the fate of cells following exposure to DNA damage and other types of cellular stresses[19,20]. Following these insults, p53 accumulates in the nucleus of cells, where it can act as a transcription factor. Depending on the cell type and the level of damage induced, p53 can increase the survival of exposed cells by transactivating genes encoding DNA-repair enzymes and cell-cycle inhibitors, or induce cells to undergo apoptosis (BOX 1). In either case, both mutagenesis and, subsequently, carcinogenesis are suppressed.

Using human cell lines with specific defects in NER, it has been shown that the triggering mechanism for p53 accumulation and apoptosis following UV-light irradiation is dependent on DNA damage[5,21–24]. Furthermore, the triggering of p53 and apoptosis is not dependent on DNA-repair-induced DNA strand breaks, but rather on persistent lesions specifically in the transcribed strand of active genes[5,21–24]. Studies using human fibroblasts treated with 2-acetylaminofluorene, the polycyclic aromatic hydrocarbon DMBA or cisplatin, which — like UV light — induce bulky DNA lesions, have shown that p53 accumulation and apoptosis are induced at much lower doses in cells that are deficient in the removal of transcription-blocking lesions compared with proficient cells[6,7,11,12,25].

Importantly, the finding that persistent lesions in the transcribed strand of active genes trigger p53 and apoptosis has been confirmed using mouse models with specific genetic defects in GGR or TCR. In animals with defects in TCR, such as *Csb*$^{-/-}$ and *Xpa*$^{-/-}$ mice, the development of sunburn as a result of apoptosis occurs at much lower doses of UV light than in corresponding wild-type littermates[9,26–29]. By contrast, *Xpc*$^{-/-}$ mice with proficient TCR but defective GGR show no hypersensitivity to UVB-light-induced sunburn[9,29]. A similar hypersensitivity for UVB-light-induced induction of p53 has been shown in the skin of *Xpa*$^{-/-}$ and *Csb*$^{-/-}$ mice, but not in the skin of *Xpc*$^{-/-}$ mice[9]. The hypersensitivity to UVB-light irradiation correlates closely with the reduced ability of these cells to recover RNA synthesis following irradiation[9,26,28,29].

These results show that the triggering signal for p53 accumulation and the induction of apoptosis following exposure to agents inducing bulky adducts originates specifically from persistent lesions in the transcribed strand of active genes, indicating that blockage of transcription might be involved in p53 signalling and in the induction of apoptosis[5–12,29].

How is p53 induced following blockage of RNA polymerase II transcription? Many RNA polymerase II complexes normally arrest shortly after transcription initiation. So, how do cells avoid inducing p53 and apoptosis in this situation? It seems that there is a difference in stress-response signalling induced following arrest at the early stages of the RNA polymerase II transcription cycle compared with inhibition of transcription elongation. Inhibition of transcription during the elongation stage results in the nuclear accumulation of Ser15- and Lys382-modified p53 proteins[30]. These modifications are normally associated with stress-induced stabilization of p53 (FIG. 1a). By contrast, inhibition of RNA polymerase II at the transition between initiation and elongation by inhibitors of the phosphorylation of the RNA polymerase II carboxy-terminal domain — such as 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) — results in the nuclear accumulation of p53 without concomitant phosphorylation of the Ser15 site or acetylation of the Lys382 site of p53 (REFS 30–32). Therefore, it seems that only after RNA polymerase II is fully phosphorylated at its carboxy-terminal domain and engaged in elongation does blockage trigger

the induction of a stress-response pathway, resulting in modifications of p53. In this way, cells avoid activating a stress-response inappropriately, allowing normal promoter-proximal stalling by RNA polymerase II complexes.

How can p53 accumulate in the nucleus following DRB treatment without Ser15 phosphorylation? The MDM2 protein is a key regulator of both p53 localization and stability by actively participating in the nuclear export of p53 (REFS 33–38). It has been noted that the cellular level of MDM2 rapidly diminishes following blockage of transcription. Therefore, it is possible that accumulation of p53 following transcription blockage is linked to the decreased level of MDM2 expression[9,39–42]. Surprisingly, we have found that overexpression of MDM2 in cells is not sufficient to reverse the nuclear accumulation of p53 induced by the transcription inhibitor DRB[43]. Moreover, the accumulated p53 proteins were found to interact with MDM2 and were highly ubiquitylated, indicating that the p53 proteins were not stopped from interacting with MDM2. Normally this would lead to nuclear export and degradation of p53, so these data indicate that the accumulation of p53 following inhibition of transcription is not merely due to decreased MDM2 protein levels.

Can p53 be induced by blockage of RNA polymerase I and/or III? Blockage of transcription that is mediated by RNA polymerase II seems to be sufficient for the induction of p53 and apoptosis in human cells. There are several findings that support this. First, TCR is a process that operates on RNA polymerase II genes but not on RNA polymerase I or III genes[1–3], and cells with defective TCR are hypersensitive to the induction of p53 and apoptosis following UV-light irradiation (see above). Second, inhibitors of RNA polymerase II, such as α-amanitin, DRB and H7, can induce p53 and apoptosis in human cells[6,7,21,44,45]. DRB and H7 also cause non-transcriptional effects in the cells, but α-amanitin is very specific for RNA polymerase II. Cells expressing α-amanitin-resistant RNA polymerase II are completely resistant to this drug[44]. Finally, microinjection of antibodies against RNA polymerase II into the nucleus of human cells induces the nuclear accumulation of p53, whereas microinjection of immunoglobulin G or anti-actin antibodies does not (H. O'Hagan, F. Derheimer and M. L., unpublished observations).

Although these findings indicate that inhibition of RNA polymerase II in the absence of DNA damage is sufficient for the induction of p53 and apoptosis, it is possible that the induction of p53 and/or apoptosis by DNA-damaging agents is also triggered by blockage of RNA polymerase I and/or III. In fact, microinjection of the RNA polymerase I transcription factor UBF has been shown to trigger the induction of p53 accumulation in human cells, indicating that inhibition of RNA polymerase I can trigger the accumulation of p53 (REF. 46). This study did not directly measure whether transcription mediated by RNA polymerase I was inhibited, but it was observed that the normal nucleolar structure was disrupted in these cells. It has been suggested that under normal conditions p53 might be escorted out of the nucleus by MDM2 through the nucleolus[47,48]. Therefore, disruption of the nucleolar structure might inhibit the nuclear export of p53, resulting in the nuclear accumulation of p53. As inhibition of either RNA polymerase I[46,49] or RNA polymerase II[31,46,50] results in the breakdown of the nucleolar structure, it is possible that the nuclear accumulation of p53 following blockage of both RNA polymerase I- and II-mediated transcription is because of inhibition of the nuclear export of p53 through the nucleolus[46] (FIG. 1a). Further studies are required to elucidate in more detail the potential role of blockage of RNA polymerase I and/or III in the regulation of p53.

Does loss of mRNA nuclear export contribute to nuclear accumulation of p53? An alternative mechanism by which p53 accumulates in the nucleus following inhibition of transcription is that its export might be linked to the nuclear export of mRNAs. It was recently shown that by specifically inhibiting two key components of the mRNA nuclear-export machinery — TAP and NUP160 — the general nuclear export of proteins containing nuclear-export signals (NES) was suppressed[51]. So, NES-mediated nuclear export of proteins seems to be linked to the nuclear export of mRNAs. As the p53 protein contains several NES sequences[52,53], it is likely that the export of p53 is also assisted by the nuclear export of mRNAs. In fact, it has been shown that p53 can interact with RNA and that this interaction suppresses p53 function[54]. The NES sequences of p53 can be activated by MDM2-mediated ubiquitylation[52] and become inactivated by Ser15 phosphorylation[53]. We have found that microinjection of anti-TAP or a dominant-negative NUP160 expression vector to specifically inhibit mRNA nuclear export results in significant nuclear accumulation of p53 (H. O'Hagan, F. Derheimer and M. L., unpublished observations). Therefore, as transcription is inhibited, less RNA will be available for p53 to associate with, resulting in a decreased nuclear export of p53 (FIG. 1a).

Taken together, blockage of transcription by DNA damage results in the induction of p53 accumulation in the nucleus by both p53 protein modification and decreased nuclear export of p53, perhaps because of loss of mRNAs available for nuclear export. This connection between the transcription machinery and the p53 response pathway ensures a rapid detection and response to DNA damage, ultimately leading to tumour suppression.

Transcription as a lesion dosimeter

The mechanism by which cells undergo apoptosis following inhibition of transcription is not clear. One mechanism might involve the loss of expression of 'survival' factors at a faster pace than the loss of expression of pro-apoptotic factors. This would lead to a shift in the balance between survival and death, so that at some point pro-apoptotic factors would tip the balance in favour of death. A recent study by McKay *et al.* indicated that transcription might act as a DNA-damage dosimeter[55]. As UV-light-induced DNA lesions efficiently block transcription and are randomly induced in the genome, the likelihood of a particular gene being inactivated by UV-light-induced DNA lesions is dependent on its chromosomal gene size. If pro-apoptotic genes are smaller than anti-apoptotic genes, UV light would preferentially inactivate larger anti-apoptotic genes and the balance would switch in favour of apoptosis. In fact, it was shown that many pro-apoptotic genes are compact and not as affected by UV-light irradiation as many anti-apoptotic genes. In this way, the degree of damage, the gene size of apoptosis-regulating genes and the ability of cells to recover RNA synthesis all contribute in a 'passive' way to suppress carcinogenesis by eliminating highly damaged cells.

In support of the hypothesis that transcription might act as a DNA-damage dosimeter that determines the fate of a cell is the evidence that there is a very close correlation between the inability of cells to recover RNA synthesis and the induction of apoptosis following exposure of cells to UV light or cisplatin[10,11,56–58]. Interestingly, the induction of apoptosis by UV light in human fibroblasts is, in contrast to rodent cells, not dependent on the induction of p53 (REF. 59). In fact, loss or mutation of p53 sensitizes some human cells to UV-light- or cisplatin-induced apoptosis[11,57,58,60]. The protective role of p53 against UV-light-induced apoptosis seems to be linked to faster recovery of RNA synthesis[57,58].

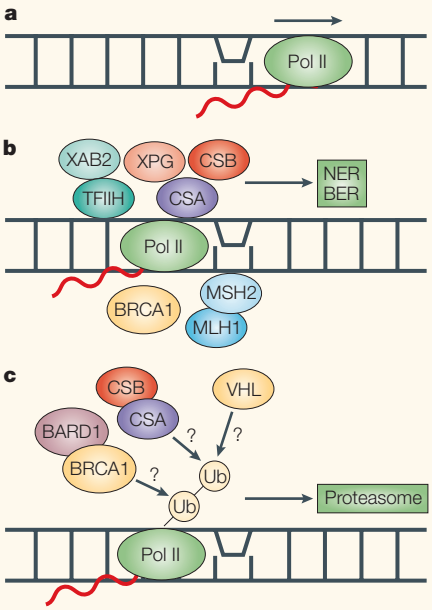


Figure 2 | **Recovery of RNA synthesis.** RNA polymerases stalled by bulky lesions or by an MLH1-dependent mechanism following exposure to alkylating agents or oxidative stress might attempt to bypass the blocking lesion (**a**), recruit either nucleotide-excision repair (NER) or base-excision repair (BER) enzymes for the removal of the blocking lesion by transcription-coupled repair (TCR; **b**), and/or undergo ubiquitylation and degradation to facilitate the recovery of RNA synthesis (**c**). BARD1, BRCA1-associated RING domain; CS, Cockayne's syndrome; pol II, RNA polymerase II; TFIIH, transcription factor IIH; VHL, von Hippel–Lindau; XAB2, XPA-binding protein 2; XPG, xeroderma pigmentosum complementation group G.

Although induction of p53 following UV-light irradiation is cell-cycle independent[21], the induction of apoptosis seems to be associated with S-phase progression[61]. How can it be reconciled that the induction of apoptosis following transcription blockage is linked to entry into S phase? It has been shown that transcription can take place during the S phase and that RNA and DNA polymerases might occasionally collide[62]. It is thought that these collisions can lead to the stalling and breakdown of replication forks. It is likely that DNA damage that blocks the elongation of RNA polymerases would lead to an increase in collisions between transcription and replication complexes (FIG. 1b). In support of this hypothesis are findings that blockage of S-phase entry by the drug mimosine reduces the amount of apoptosis induced by UV light[61] or DRB[63]. Similarly, it would be expected that the induction of a p53-mediated G1 arrest would reduce the potential collisions between stalled RNA polymerase complexes and the replication machinery. It is possible that such a mechanism, in addition to its role in activating the G1 checkpoint, transactivating DNA-repair genes and enhancing the recovery of RNA synthesis (mentioned above), contributes to the protective effect of p53 against DNA-damage-induced apoptosis in human cells.

Finally, as inhibition of transcription inhibits NES-dependent nuclear export[51], apoptosis induced following blockage of transcription might result from the trapping of NES-containing proteins in the nucleus. In fact, ectopic expression of a mutant form of the nucleocytoplasmic shuttling protein cyclin B1 that preferentially localizes to the nucleus is sufficient to trigger apoptosis[64]. Future studies should be aimed at elucidating the molecular mechanisms by which blockage of transcription leads to the induction of apoptosis. A better understanding of these mechanisms should be valuable for the development of novel anticancer therapeutics based on interference of transcription (see below).

Role of mismatch-repair proteins

Certain mutagenic base adducts, such as those caused by alkylating agents or oxidative stress — for example, thymine glycols, 8-hydroxyguanine and O^6-methylguanine — are bypassed by RNA polymerases *in vitro*[65–67]. However, recent evidence indicates that these lesions hamper RNA synthesis in cells[68,69]. So, there might be cellular factors that bind to these lesions and convert them from non-blocking to transcription-blocking lesions and therefore trigger a DNA-damage response through blockage of transcription.

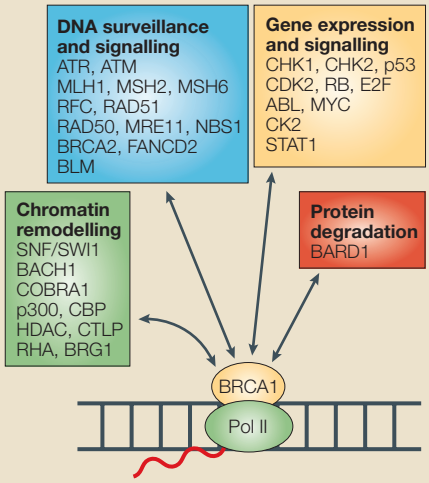
The mismatch repair (MMR) pathway detects and removes mismatched bases added by DNA polymerases during replication. MMR proteins also bind certain DNA lesions and direct TCR, DNA-damage signalling and apoptosis. MMR proteins, such as MSH2 and MLH1, have been shown to bind to certain DNA lesions, such as O^6-methylguanine and, possibly, thymine glycols and 8-oxyguanine adducts[70,71]. It has been suggested that the binding of MMR proteins to these adducts triggers the induction of p53 and apoptosis[71–73]. It has recently been reported that mRNA synthesis is significantly attenuated in a MLH1-dependent fashion in the colon cancer cell line HCT116 exposed to alkylating agents or oxidative stress[69]. This inhibition of transcription correlated to the MLH1-dependent induction of p53 and apoptosis. These results are consistent with the hypothesis that MMR proteins bind to certain DNA lesions and induce p53 and apoptosis by blocking transcription. It is possible that this transcription-blocking function contributes to the tumour-suppressing function of MMR proteins. In addition to MMR proteins, it is possible that there are other DNA-damage-binding factors that can 'flag' potential mutagenic DNA lesions and block transcription, so triggering DNA-damage response pathways and suppressing tumorigenesis.

Recovery of RNA synthesis

Transcriptional bypass of blocking lesions. As blockage of transcription can lead to cell death, it is urgent for the cells to rapidly resolve the blockage and recover RNA synthesis. The simplest mechanism to restore transcription would be for the blocked RNA polymerases to find ways to bypass the DNA lesions (FIG. 2a). Although UV-light-induced DNA lesions efficiently block transcription elongation both *in vitro*[74,75] and *in vivo*[76], some studies have indicated that mRNA synthesis can recover before any significant number of lesions are removed from the transcribed strand[77,78]. Therefore, blocked RNA polymerases might, with the aid of unknown factors, bypass certain transcription-blocking lesions. *In vivo* bypass of other lesions capable of directly or indirectly blocking transcription, such as thymine glycol, 8-oxyguanine and O^6-methylguanine, has also been reported[79]. As blockage of transcription might have a tumour-suppressing role by triggering stress responses in cells with damaged DNA, there must be a fine balance established between lesion bypass and loss of DNA-damage signalling. Furthermore, lesion bypass might lead to the production of mutant transcripts — 'transcriptional mutagenesis'[79] — and, therefore, should be carefully balanced to both promote survival and minimize transcriptional mutagenesis.

Transcription-coupled DNA repair. Depending on the nature of the lesions blocking transcription, either NER or base-excision repair (BER) factors are recruited to remove the blocking lesions through TCR and lead to recovery of RNA synthesis. The Cockayne's syndrome factors CSA and CSB[80,81], the MMR proteins MLH1 and MSH2 (REF. 82), and the xeroderma pigmentosum complementation group A binding protein XAB2 (REF. 83) couple stalled RNA polymerase II with the NER machinery (FIG. 2b). The coupling of blocked transcription to the BER pathway, which removes small base damages such as those induced by alkylating agents and oxidative stress, requires CSA, CSB [81] and the breast cancer susceptibility factor BRCA1 (REFS 84,85). The precise functions of these proteins in promoting TCR with NER or BER are still not fully understood.

Box 2 | **Potential role of BRCA1 in transcription**



BRCA1 is a tumour suppressor that has important roles in the protection against genomic instability. Cells lacking BRCA1 are hypersensitive to many DNA-damaging agents such as ultraviolet light and ionizing radiation[142], and show defects in both S- and G2/M-checkpoint activation following exposure to ionizing radiation[143]. BRCA1 is a target for both ataxia telangiectasia mutated (ATM)- and ataxia telangiectasia and Rad3 related (ATR)-mediated phosphorylation[144,145]. BRCA1 can interact with numerous proteins through its many protein–protein interaction domains[146,147](see figure). Interestingly, BRCA1 interacts under normal conditions with the elongating form of RNA polymerase II[103–105], but following genotoxic insult BRCA1 becomes phosphorylated and dissociates from the transcription complex[105]. So, BRCA1 and associated DNA-damage surveillance factors might be associated with the elongating form of RNA polymerase II and might monitor the elongation success of the RNA polymerase. When transcription halts at a lesion or is disrupted in some other way, BRCA1 and associated factors might relocate to recruit DNA-repair enzymes and to activate cell-cycle checkpoints. BARD1, BRCA1-associated RING domain; BLM, Bloom syndrome; CBP, CREB-binding protein; CDK2, cyclin-dependent kinase 2; COBRA1, cofactor of BRCA1; FANCD2, Fanconi anaemia complementation group D2; HDAC, histone deacetylase; NBS1, Nijmegen breakage syndrome 1; pol II, RNA polymerase II; RB, retinoblastoma; RFC, replication factor C; STAT1, signal transducer and activator of transcription 1.

Previous studies have shown that the TCR pathway has a crucial role in protecting cells against apoptosis induced by both UV light[5] and cisplatin[11,86]. However, whether TCR contributes to tumour suppression is not clear. In fact, TCR might promote cancer, as increasing the efficiency of TCR would lead to a faster recovery of transcription and a faster shutdown of DNA-damage signalling. This would lead to a decreased induction of p53-responsive genes such as *CDKN1A* (which is involved in cell-cycle arrest), *DDB2* (which generates XPE) and *XPC* (which is involved in GGR). Indeed, patients with type C xeroderma pigmentosum who have cells that are proficient in TCR but deficient in GGR are highly cancer prone. Conversely, patients with Cockayne's syndrome who have cells that are hypersensitive to the induction of UV-light-induced apoptosis because of the lack of TCR and have a proficient GGR pathway that is able to remove potentially mutagenic lesions in surviving cells are not prone to UV-light-induced carcinogenesis[4,5,87]. Therefore, although increasing the survival of cells, TCR might actually contribute to mutagenesis by allowing less induction of and less time for GGR.

Interestingly, inactivation of the *Csb* gene leading to loss of TCR in tumour-prone mice has been shown to significantly reduce the incidence of cancer in these animals, indicating that lack of TCR actually promotes the suppression of tumorigenesis[88]. Furthermore, the anticancer agent ET-743, which is now undergoing clinical trials, has antiproliferative activity that seems to be linked to its specific interaction with TCR[89]. Two other interesting drugs in clinical trials that, like ET-743, seem to specifically target TCR are the natural product illudin S and its synthetic analogue irofulven. It is thought that prolonged blockage of transcription complexes induced by these agents results in apoptosis, and cells lacking TCR are especially vulnerable to these drugs[90]. Taken together, these studies indicate that specific targeting of the TCR pathway in cancer cells might be a powerful anticancer strategy, either alone or in combination with agents that induce transcription-blocking lesions.

Transcription blockage and RNA polymerase II degradation. In cases when RNA polymerases do not find ways to bypass the DNA lesions, it seems that the RNA polymerases do not readily continue elongation following removal of the blocking lesions[91]. For cells to resume RNA synthesis and to avoid potential collisions between the blocked transcription complexes and the replication machinery, stalled RNA polymerase II complexes must be removed from the DNA template. It has been shown that the largest subunit of RNA polymerase II is subjected to ubiquitylation and degradation following exposure to agents that block RNA polymerase elongation[92–96]. Furthermore, it was recently shown that the proteasome — the complex that induces degradation of polyubuitylated proteins — associates with active transcription units especially following UV-light irradiation. This indicates that the blocked RNA polymerases might be degraded right at the site of the blocking lesion[97].

One candidate ubiquitin ligase that might target the blocked RNA polymerase II for ubiquitylation is the tumour suppressor von Hippel–Lindau (VHL) protein[98,99]. It was recently shown that the VHL protein binds to the hyperphosphorylated, elongating form of RNA polymerase II following UV-light irradiation and targets it for ubiquitylation[99]. Furthermore this VHL-mediated ubiquitylation seems to have a protective effect on cells, as it has been shown that the presence of functional VHL protects cells from UV-light-induced apoptosis[100]. In addition to VHL, the CSA and CSB proteins seem to contribute to the regulation of RNA polymerase II ubiquitylation and degradation[93,94,101]. The CSA protein can be found in a multiprotein complex that has ubiquitin-ligase activity, and the association of this complex with the phosphorylated RNA polymerase increases after UV-light irradiation[102]. A third ubiquitin-ligase complex that might be involved in targeting stalled RNA polymerases is the BRCA1–BARD1 (BRCA1-associated RING domain 1) complex. This complex associates with the elongating RNA polymerase II holoenzyme[103–105] and mutation of BRCA1 results in loss of transcription recovery of plasmids carrying 8-oxyguanine lesions[85]. As BRCA1 is associated with the elongating RNA polymerase II, it might also have important roles in TCR and in the activation of DNA-damage signalling following transcription blockage (BOX 2). Taken together, the VHL, CSA and BRCA1 proteins participate in three distinct complexes that might be involved in the recovery of mRNA synthesis by targeting stalled RNA polymerases for ubiquitylation and subsequent degradation (FIG. 2c).

DNA damage, ageing and cancer

It seems that there is a fine balance between the induction of apoptosis, ageing and cancer (FIG. 3). Although a responsive apoptotic pathway would efficiently remove damaged cells and therefore suppress carcinogenesis, such increased cell loss is thought to promote accelerated ageing[4,5,7,11,106,107]. On the other hand, if apoptosis was delayed by overexpression of anti-apoptotic factors such as BCL2 or BCL-X_L, elimination of damaged cells and subsequent suppression of mutagenesis and carcinogenesis would be compromised. A similar situation would arise if the TCR pathway was made more efficient so that the lesions that normally trigger DNA-damage signalling and apoptosis would be removed faster. This would result in reduced induction of expression of p53 and p53-responsive genes involved in cell-cycle arrest and DNA repair, and therefore would compromise the ability of the GGR pathway to remove mutagenic lesions with subsequent increased probability of carcinogenesis. So, TCR must be tightly regulated so that it does not negate the p53 signalling and apoptosis triggered by stalled transcription on damaged DNA. Conversely, if the operation of the TCR pathway is less than optimal, apoptosis will increase, leading to the reduction of carcinogenesis at the expense of ageing.

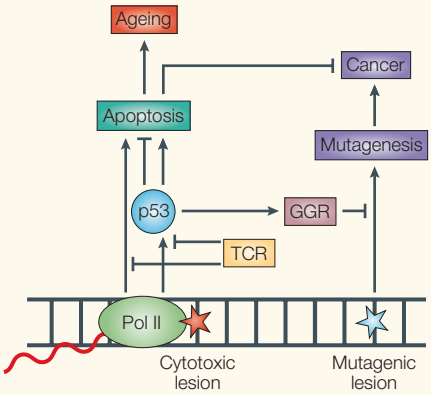


Figure 3 | **Balance between DNA-damage responses, cancer and ageing.** A lesion might be either cytotoxic or mutagenic, depending on where in the genome it is located. If it blocks transcription, it might lead to cell death and, therefore, it is cytotoxic. However, if the lesion is located in non-transcribed regions of the genome, the lesion might have no acute effects until the DNA is replicated and a resulting mutation might occur. Transcription-blocking lesions are primarily repaired by transcription-coupled repair (TCR), whereas mutagenic lesions are removed by global genomic repair (GGR). Patients with defective TCR are not predisposed to cancer, whereas individuals lacking GGR are highly cancer prone. Depending on the cell type and the level of DNA damage, the induction of p53 might either protect cells by stimulating the recovery of RNA synthesis or eliminate the cells by inducing apoptosis. The hyperinducible p53 and apoptotic pathways in TCR-deficient cells might be protective against cancer by compensating for the TCR defect. However, this type of cancer protection comes at the cost of premature neurological degeneration and ageing[5].

Cells from patients with the premature-ageing syndrome Cockayne's syndrome have been shown to have defects not only in the recovery of RNA synthesis following UV-light irradiation, but also in general transcription. It is possible that oxidative damage from endogenous sources causes transcription blocking and that the repair of these blocking lesions and recovery of RNA synthesis requires the Cockayne's syndrome factors. Similarly, premature-ageing phenotypes have been observed in mice that have a specific mutation in *Xpd*, giving rise to another premature-ageing sydrome, trichothiodystrophy (TTD)[26]. *Xpd* codes for a DNA helicase involved in both DNA repair and transcription[28]. These studies indicate that persistent DNA damage has an important role in promoting ageing. Specifically, lesions blocking transcription, which are linked to the induction of apoptosis[5], might be responsible for the accelerated-ageing phenotype in TTD[26]. This increased sensitivity of damaged TTD and Cockayne's syndrome cells to apoptosis coupled with proficient GGR might explain the low cancer incidence among individuals with these syndromes[5,26].

The connection between transcription deficiencies and ageing is not limited to Cockayne's syndrome and TTD (TABLE 1). In fact, many other premature-ageing syndromes and knockout mice with premature-ageing phenotypes show defects in general transcription. For example, cells from the premature-ageing syndromes Werner's, Bloom's and Rothmond–Thomson's that have specific defects in RecQ DNA helicase activity, seem to have reduced transcription efficiency compared with cells from normal individuals[108–110]. Another premature-ageing syndrome, Hutchinson–Gilford progeria syndrome, was recently shown to be caused by mutations in lamin A/C[111,112], which encodes proteins that form part of a filamentous network in the nuclear envelope. Interestingly, it has been shown that expression of dominant-negative forms of lamin A significantly reduces transcription by RNA polymerase II[113,114], indicating that a transcription deficiency might contribute to the premature-ageing phenotype in Hutchinson–Gilford progeria syndrome. Finally, it has been found that the *Ku86*-knockout mouse expresses a premature-ageing phenotype[115]. KU86 (KU80 in humans) is key in initiating a major pathway in repair of DNA double-strand breaks called non-homologous end-joining, but was recently also implicated in the transcription process mediated by RNA polymerase II[116–118].

The common feature of these premature-ageing syndromes is that their genetic deficiencies negatively affect RNA polymerase II and therefore promote apoptosis. So, reduced transcription might contribute to the premature-ageing phenotype in these disorders. Although some of these syndromes, such as Cockayne's syndrome and TTD, are associated with a low incidence of cancer, this is not true for all of the premature-ageing syndromes. As some of these syndromes have defects in genes involved in DNA repair and DNA-damage signalling, it is likely that the contributions of these defects to genomic instability outweighs the tumour suppression afforded by an increased apoptotic response.

Transcription as a therapeutic target

Inhibition of transcription has been shown to induce apoptosis in several cancer cell lines[45,119]. Moreover, the induction of apoptosis by transcriptional inhibitors seems to be more pronounced in transformed cells than in their non-transformed counterparts[44]. Perhaps transformed cells in general have a less stringent G1 checkpoint, allowing more cells to enter S phase even if cells have blocked RNA-polymerase complexes. This would then potentially lead to more frequent collisions between replication forks and blocked RNA polymerase II complexes, leading to higher rates of apoptosis. The increased apoptosis induced in transformed cells does not seem to be dependent on loss of a p53-mediated G1 arrest, as transcription inhibitors can induce a G1/early S-phase arrest[120] or apoptosis[42,44] in cells regardless of their p53 status. However, contradictory results have been reported[45]. As transcription inhibitors seem to preferentially kill transformed cells, and apoptosis is induced independently of p53 status, at least in some tumour types, further studies of the potential usefulness of transcription inhibitors as cancer therapeutic agents are warranted.

Many of the common chemotherapeutic agents used today act by damaging DNA and therefore might interfere with transcription. Indeed, the DNA-damaging agent cisplatin[7,86] and the topoisomerase I inhibitor camptothecin[91,121] have been shown to inhibit the elongation of RNA synthesis (TABLE 2). Moreover, cells with

Table 1 | **Transcription defects, DNA-damage signalling, cancer and premature ageing**

Syndrome or model	Defective function	Transcription	Apoptosis*	Predisposition to cancer?	Premature ageing?	References
Cockayne's	TCR, targeting blocked RNA polymerase II for degradation	Defective	++ (UV)	No (yes in mice)	Yes	4,5,26
Trichothiodystrophy	Nucleotide-excision repair	Defective	++ (UV)	No (yes in mice)	Yes	28,107
Werner's	DNA helicase	Defective	++ (4NQO, camptothecin, DNA crosslinks)	Yes	Yes	26,108
Bloom's	DNA helicase	Defective (RNA polymerase I in yeast)	++ (IR)	Yes	Yes	106,109
Huthinson–Gilford progeria	Lamin A, nuclear-envelope architecture	Dominant-negative lamin A inhibits RNA polymerase II	?	No	Yes	111–114
Ku80$^{-/-}$ mouse	Double-strand-break repair, telomere maintenance	Defective	++ (IR)	Yes	Yes	115–118

*Following exposure to agents in brackets. UV, ultraviolet light; IR, ionizing radiation; 4NQO, 4-nitroquinoline 1-oxide.

defects in TCR are hypersensitive to these agents[11,86,122], indicating that the combination of agents inhibiting transcription with drugs such as ET-743, which interacts with TCR[89], might be a useful anticancer strategy. The topoisomerase II inhibitor doxorubicin also inhibits transcription, but instead of affecting elongation, it causes structural alterations in promoter DNA[121]. Nucleotide analogues represent another class of agents for which cytotoxicity has been linked to their effects on RNA synthesis. For example, 5-fluorouracil[123,124] and the nucleotide analogue fludarabine[125], which is the most effective drug used to treat patients with chronic lymphocytic leukaemia, inhibit general transcription. It has also been shown that certain cyclin-dependent kinase (CDK)-inhibitory drugs used in clinical trials, such as roscovitine and flavopiridol[126,127], cause global inhibition of transcription[128–130]. Therefore, inhibition of transcription is a common mechanism of action for many chemotherapeutic agents.

Recent findings indicate that drugs that block transcription can act in synergy with other agents to induce apoptosis[131–133]. It has been reported that induction of p53-mediated transactivation by ionizing radiation is significantly increased if the irradiated cells are post-incubated with the transcription inhibitor DRB[32,132]. It was suggested that the synergy of the combination treatment was because of the ability of these agents to activate separate pathways leading to a stronger p53 response. Similar conclusions have been drawn from experiments using the transcription inhibitor actinomycin D and the nuclear-export inhibitor leptomycin B[131,134]. For tumours in which the activation of p53 is important for therapeutic efficacy[135], radiotherapy might become more effective when combined with agents such as DRB or leptomycin B that efficiently trap p53 in the nucleus[32,136–138]. Combination of the CDK and transcription inhibitor roscovitine with ionizing radiation resulted in synergistic induction of apoptosis regardless of the p53 status of the cancer cells used[133]. Therefore, the combination of transcription inhibitors with radiation or chemotherapy might be applicable to most tumours. Future investigations should be aimed at studying the radio- or chemotherapy-sensitizing potential of agents that inhibit transcription.

Table 2 | **Examples of chemotherapeutic agents that inhibit transcription**

Agent	Action	Inhibition phase	References
Cisplatin	DNA intrastrand crosslinks	Elongation	7,86
Camptothecin	DNA topoisomerase I inhibition	Elongation	121,122
Doxorubicin	DNA topoisomerase II inhibition	Initiation	121
5-Fluorouracil	Thymidylate-synthase inhibition, incorporation into DNA and RNA	Elongation?	123,124
Fludarabine	Ribonucleotide-reductase inhibition, incorporation into DNA and RNA	Elongation?	125
Roscovitine	Cyclin-dependent kinase inhibition	Transition into elongation	128
Flavopiridol	Cyclin-dependent kinase inhibition	Transition into elongation	129

Conclusions and future directions

In this article we have argued that the RNA polymerase II transcription machinery might act as a guardian of the genome by sensing DNA damage and activating DNA repair, p53 and apoptosis. Although induction of p53 by blockage of transcription seems to be cell-cycle independent, recent results indicate that the link between blocked transcription and apoptosis is manifested during the S phase. The underlying molecular mechanism by which inhibition of transcription is linked to these responses awaits further exploration. Furthermore, studies of the interplay between the induction of p53 and apoptosis following exposure to transcription-blocking lesions with ageing and cancer will be of great importance for a better understanding of these conditions. Finally, the concept of transcription as a therapeutic target for anticancer agents and in sensitizing cancer cells to anticancer therapy is exciting and should stimulate new avenues of investigation.

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TIMELINE

Radiation oncology: a century of achievements

Jacques Bernier, Eric J. Hall and Amato Giaccia

Abstract | Over the twentieth century the discipline of radiation oncology has developed from an experimental application of X-rays to a highly sophisticated treatment of cancer. Experts from many disciplines — chiefly clinicians, physicists and biologists — have contributed to these advances. Whereas the emphasis in the past was on refining techniques to ensure the accurate delivery of radiation, the future of radiation oncology lies in exploiting the genetics or the microenvironment of the tumour to turn cancer from an acute disease to a chronic disease that can be treated effectively with radiation.

In the early days of development of radiation as an anticancer therapy, physics had the biggest contribution — the focus was on increasing the quality and quantity of radiation that could be delivered to a tumour. Radiobiology was a field split between understanding fundamental changes in irradiated cells and understanding the responses of normal tissue versus tumours to radiation. The early studies in experimental radiation oncology evolved from using large single doses to using small doses of radiation to kill tumour cells and spare normal tissues.

Radiation oncology emerged as a discipline when health and science professionals from numerous disciplines (from nurses to radiographers, from radiologists to pathologists, from surgeons to medical oncologists, from biologists and physicists to radiation oncologists) began to interact in their common search for more effective anticancer treatments. Collaboration between scientists and clinicians in radiation oncology has always been two-way: discoveries in radiation physics, chemistry and biology have stimulated the interest of clinicians to start implementing a novel technique, agent or strategy as soon as possible ('bench to bedside'), and the results in the clinic have guided scientific research and the development of new technologies.

We are now at a turning point in radiation oncology — techniques have been refined to allow accurate delivery and we now need the insight of molecular biology and genetics to further refine targeting. We need to know what the most important targets are that will increase cytotoxicity towards tumour cells and spare normal tissue. The development of new approaches and their implementation in clinical practice will again require an integrated effort between clinicians, physicists and biologists.

Scientific breakthroughs are rare and great advances in health care are even rarer. In the twentieth century, important discoveries and advances were made both in Europe and the United States. Recognition of the importance of radiation oncology by governments and societies also played a big part in these advances in many countries. There were four main schools of radiation oncology in the twentieth century (BOX 1): the German school (1900 to ~1920), the French school (1920 to ~1940), the British school (1940 to ~1960) and the United States then European Union school (1970 to date).

The discovery of X-rays, in 1895, by Wilhelm Conrad Röntgen in Germany (FIG. 1) and of natural radioactivity a few months later, by the French physicist Henry Becquerel, were two such breakthroughs that paved the way for a new era in science. Although the mechanisms of X-ray action were far from understood, the speed with which the pioneers worked together to develop and implement the first successful X-ray therapies is amazing (TIMELINE 1). Less than 60 days after the discovery of X-rays, clinical radiotherapy was born — Emil Grubbé treated an advanced ulcerated breast cancer with X-rays[1] in January 1896 in Chicago. Over the next century, discoveries in radiation physics, chemistry and biology informed approaches in the clinic to develop an increasingly more accurate, more efficient and less harmful anticancer therapy.

Radiation physics 1896–1945

X-rays. Röntgen discovered X-rays while he was experimenting with a Hittorf–Crookes cathode-ray tube[2]. This consisted of a pear-shaped glass chamber from which almost all the air had been evacuated, and into which two electrodes were sealed at opposite ends of the tube — the cathode (negative) and the anode (positive). When these electrodes were connected to a high-voltage source, ions in the residual gas were accelerated to high speeds. The cathode repelled the negative electrons, which then struck the opposite end of the glass tube with considerable energy — the impact of these fast electrons on the glass produced photon energy called X-rays. With these tubes, both the quality and the quantity of the rays depended on the internal gas pressure, which changed as the air was ionized and used up. Such equipment was difficult to control.

In 1913, the American William Coolidge produced a 'hot-cathode tube'[3], in which the electron source was a tungsten filament heated by a low-voltage circuit; electrons were freely released from the hot metal (thermionic effect). It was now possible to control the quality and quantity (dose) of radiation independently — the development of these tubes revolutionized radiology.

Throughout the first four decades of the twentieth century, technical developments were essentially based on improving X-ray generator and tube output, and led to the routine clinical application of low-energy