

Maintaining integrity

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Research on genome stability and integrity now extends far beyond the biochemistry of DNA repair to encompass signal transduction pathways that span numerous aspects of cellular life. Derailed genomic integrity pathways can result in debilitating genetic disorders, premature ageing, predisposition to cancer and degenerative conditions. Current progress in this rapidly expanding field was the subject of an EMBO workshop, *Maintenance of Genomic Integrity*, that took place in June 2004 in Galway, Ireland.

The importance of genomic stability has been recognized since the early days of molecular biology. The plasticity of the genome is manifest in normal DNA transactions that involve breakage and reunion of the DNA molecule; for example, meiotic recombination and the maturation of the immune system genes. Cellular DNA is continuously bombarded by reactive metabolic by-products and exogenous physical and chemical agents that readily find reactive targets in the DNA molecule.

Recent studies show that maintenance of genomic stability in the face of these ongoing assaults involves extensively branched signalling networks that activate DNA repair, alter chromatin organization, switch on cell-cycle checkpoints and modulate numerous cellular processes. Maintenance of genomic stability and integrity is thus a major component of an organism's homeostasis and of its ability to cope with environmental stresses. No wonder that defects in various junctions in this network result in a variety of diseases ranging from severe genetic disorders (collectively dubbed 'genomic instability syndromes') to chronic diseases, cancer predisposition and accelerated ageing.

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The field of DNA repair deals in parallel with structural modifications of DNA and lesions that lead to single- and double-strand breaks. Studies of single-strand breaks have provided ample information on repair enzymology and its relationship with the transcription apparatus, and have recently revealed an important component of the replication machinery that performs translesion synthesis. In parallel, studies of the fate of DNA double-strand breaks (DSBs) — an extremely cytotoxic lesion induced by ionizing radiation and radiomimetic chemicals — have been rewarding in elucidating the intricate pan-cellular damage-signalling mechanism, termed the 'DNA damage response'^{1–4}.

The unfolding response to DNA double-strand breaks

DSBs are repaired by an error-prone non-homologous end-joining process, or by a faithful mechanism based on homologous recombination between sister chromatids that operates in late the S or G2 phase of the cell cycle. Both mechanisms maintain intimate relationships with a fine-tuned system that signals DNA damage. The traditional view of this system depicts rapid sensing of the break by sensor proteins that convey the damage signal to transducers that in turn transmit it to numerous downstream effectors, each of which modulates a specific pathway. The prototype transducers are a group of conserved nuclear protein kinases, the 'phosphatidylinositol-3-OH kinase (PI(3)K)-related protein kinases' (PIKKs)⁵. Among the mammalian PIKKs are the DNA-dependent protein kinase (DNA-PK), ataxia-telangiectasia-mutated (ATM), the ATM and Rad3-related (ATR)

protein and hSMG-1. These protein kinases respond to DSBs (DNA-PK, ATM and ATR), UV damage (ATR and hSMG-1) and stalled replication forks (ATR). Of these proteins, ATM — the primary mobilizer of the DSB response — boasts the largest list of documented substrates, most of which are involved in activating the cell-cycle checkpoints. Many of these substrates may be shared with ATR. Loss or inactivation of ATM leads to a prototype genomic instability syndrome, ataxia-telangiectasia (A-T), which is characterized by cerebellar degeneration, immunodeficiency, chromosomal breakage and cancer predisposition.

New studies based on genetic, biochemical and molecular imaging approaches provide insights into the identity of the sensors, their functional relationships with the transducers and the rapid chain of events at DSB sites immediately after their formation, long before they are actually repaired (R. Rothstein, New York, NY; J. Bartek, Copenhagen, Denmark). The new data have turned the simple, linear hierarchy of the damage response process into a cyclical, self-reinforcing one in which the same proteins may have different roles at different stages of the process (Fig. 1; and see below).

An important factor at the sensor level is the trimolecular Mre11–Nbs1–Rad50 (MRN) complex. Endowed with a nuclease activity that resides in the Mre11 protein, the MRN complex is thought to perform initial processing of DSB ends. Hypomorphic mutations in the *NBS1* and *MRE11* genes cause two genomic instability syndromes, Nijmegen breakage syndrome (NBS) and A-T-like disease (ATLD). Whereas the NBS phenotype

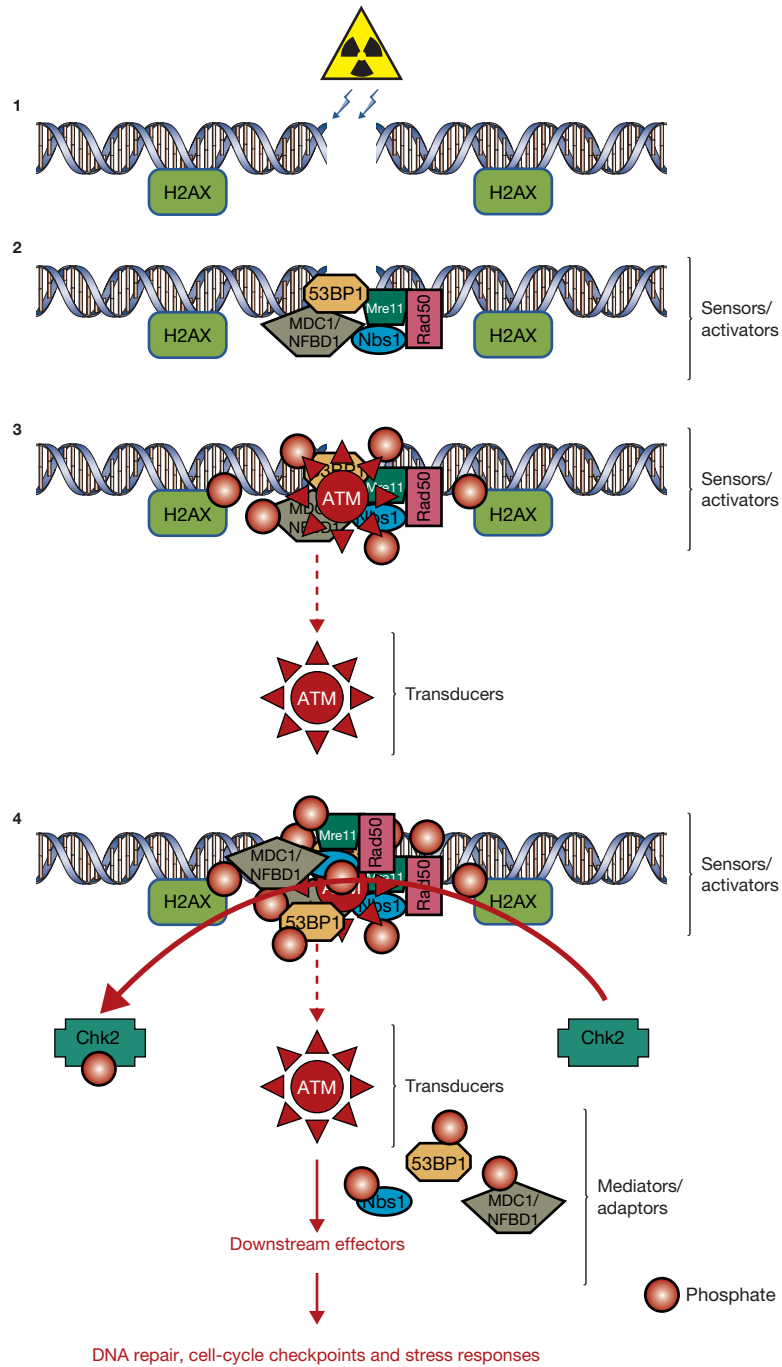


Figure 1 Early events in the cellular response to double-strand breaks (DSBs) in the DNA. For clarity, only ATM is shown as transducer of the damage signal, but ATR clearly joins in at later stages. Formation of a DSB (1), is followed within seconds by the assembly of several sensor-activators at the site of the damage, which is ATM-independent and unstable (2). Only several representatives of the expanding group of sensor-activator proteins are shown; Brca1, for example, may also function in a similar manner. The MRN complex precedes the other proteins of this group and is among the first to rush to the damaged site. The activators are involved in the activation of ATM and probably the recruitment and binding of a portion of it to the damaged sites, while another portion of activated ATM remains and phosphorylates various substrates in the nucleoplasm (3). ATM phosphorylates the sensors/activators at the damaged site, thereby

stabilizing the growing conglomerate of proteins that is now visible as a nuclear focus. Some of these proteins, now phosphorylated, may function as mediators/adaptors in the phosphorylation of other ATM substrates and function further downstream in specific signalling pathways (4). Certain ATM substrates, such as Chk2, which normally reside in the nucleoplasm, are temporarily recruited to the damaged sites in the chromatin, get phosphorylated there by the bound fraction of ATM, and leave the site as activated molecules to affect their respective substrates elsewhere in the nucleus. Further processes at the DSB site are repair through the non-homologous end joining or homologous recombination pathways. Extensive resection of DNA ends leads to the formation of single-stranded ends that are rapidly coated by replication protein A (RPA), a process required for the recruitment of the ATR-ATRIP complex to the damaged sites (not shown).

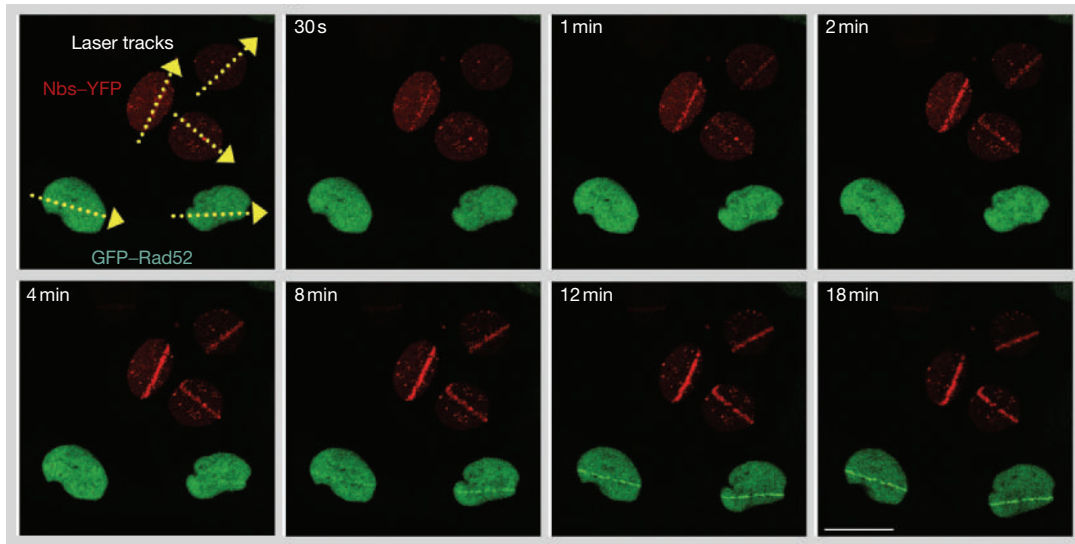


Figure 2 Real-time imaging of the recruitment of signalling and repair proteins to microlaser-generated sites of DNA damage. Human osteosarcoma cells (U2OS) stably expressing physiological levels of yellow fluorescent protein (YFP)-tagged Nbs1 and green fluorescent protein (GFP)-tagged Rad52 (a component of the homologous recombination pathway of DSB repair) were co-cultured, and the YFP and GFP signals were unmixed using spectral analysis to discriminate cells expressing Nbs1 and Rad52. The cells were microirradiated with a laser beam (337 nm) along 0.5–1 μm -wide tracks (the laser movement during microirradiation is indicated in the first frame). In cells pre-sensitized with halogenated thymidine analogues, such a treatment generates DNA

strand breaks spatially restricted to the laser-exposed sub-nuclear compartments. Immediately after microirradiation, redistribution of the respective fluorophore-tagged proteins was recorded by repeated scanning of the same fields. The selected images show that both proteins undergo a productive recruitment to the DSB areas, albeit with different kinetics. Whereas Nbs1 redistributes rapidly from the nucleoplasm to the damaged nuclear compartments (within less than 1 min), recruitment of Rad52 occurs later, and it becomes discernible around DSBs only after Nbs1 interaction with these regions reaches an equilibrium. (Contributed by J. Lukas and C. Lukas, Danish Cancer Society, Copenhagen, Denmark). Scale bar, 10 μM ; time, time after microirradiation.

partly overlaps with that of A-T, ATLD mimics the A-T phenotype almost in full. Evidence of the essential role of MRN in the sensor level of the DSB response comes from two directions: the first is the finding that full ATM activation, especially at low damage levels, requires MRN (Y. Shiloh, Tel Aviv, Israel; M. Weitzman, La Jolla, CA), which explains the phenotypic similarities among these syndromes; the second is the recruitment of MRN, within seconds, to DSB sites or collapsed replication forks, shown by real-time imaging in live mammalian and yeast cells (J. Bartek; R. Rothstein) (Fig. 2). Of note, the orthologue complex in *Saccharomyces cerevisiae*, MRX, is required for the recruitment of the ATM orthologue Tel1p to the damage sites (R. Rothstein).

Importantly, the MRN complex is also required for efficient interaction between ATM and some of its substrates, and their subsequent phosphorylation by ATM (T. Paull, Austin, TX). Thus, MRN also functions as an adaptor/mediator in that stage of the DNA damage response. On the other hand, previous work has shown that Nbs1 is an ATM substrate and that this is important for the activation of the S-phase cell-cycle checkpoint. M. Lavin (Brisbane, Australia) presented evidence that Mre11 is equally

phosphorylated by ATM, and that this phosphorylation seems, in turn, to be required for the G2–M checkpoint. Thus, the MRN complex or its components can function in different capacities at different stages of the DSB response: as sensor-activator, as adaptor-mediator or further downstream.

With its numerous roles in the DNA damage response, meiotic recombination, class switch recombination and telomere maintenance, the MRN complex is expected to be regulated by many post-translational modifications. A novel, unexpected one was presented by F.-M. Boisvert (Montreal, Canada), who found that the Mre11 protein was constitutively methylated by arginine methyltransferase-1 (PRMT1) on arginine residues within an arginine–glycine cluster located at its carboxy-terminus. This modification is essential for the recruitment of Mre11 to DSB sites and for its exonuclease activity. Mre11 methylation affects the activity and sub-cellular localization of several proteins, and this is its debut in the DNA damage response arena.

The sensor-activator layer in the DNA damage response is expanding to include additional proteins that may be important for ATM activation and recruitment to the damaged sites. Among them are several proteins

that contain a BRCT (BRCA1 C-terminal) domain, notably the *S. cerevisiae* Rad9 protein (N. Lowndes, Galway, Ireland). The group of BRCT damage-response proteins now includes 53BP1 (A. Doherty, Brighton, UK), MDC1/NFBD1 (S. Jackson, Cambridge, UK), and probably BRCA1 itself. Although these proteins may function ‘upstream’ of the transducers like MRN, they are also targets of ATM kinase activity and have roles in specific branches of the damage response. Thus, MDC1/NFBD1 functions in the intra-S checkpoint (M. Goldberg, Jerusalem; S. Jackson), 53BP1 is involved in the non-homologous end-joining pathway of DSB repair (A. Doherty) and BRCA1 is involved in the homologous recombination repair and cell-cycle checkpoint pathways (K. Hiom, Cambridge, UK; and previous reports). The initial aggregation of these proteins at the damaged sites is ATM-independent and unstable. However, subsequent cycles of recruitment and phosphorylation by ATM stabilize the growing conglomerates of proteins that finally emerge as nuclear foci marking these sites (Fig. 1). J. Haber (Waltham, MA) reported that the cohesin complex is also recruited to DSB sites in an MRN-dependent, ATM-independent manner.

Animal models mimicking many of the human genomic instability syndromes have been established. However, in certain cases complete knockout of the corresponding genes is embryonic lethal, attesting to the importance of these proteins in cellular proliferation; such is the case for all three MRN components (the corresponding human syndromes are caused by hypomorphic mutations). A. Nussenzweig (Bethesda, MD) used Cre-LoxP mediated recombination to circumvent this limitation, restricting *Nbs1* inactivation to B-lymphocytes. These cells showed impaired proliferation and extreme chromosomal instability, including changes in DNA ploidy that were not observed in *Nbs1* hypomorphic mutations. A marked defect in class switch recombination of the immunoglobulin genes was observed, pointing once again to the intimate link between the DNA damage surveillance system and the maturation of the immune system genes.

Another novel participant in the early stage of the DNA damage response, before the activation of the transducers, was described by A. Pelliccioli (Milan, Italy) and J. Haber. These investigators showed that in *S. cerevisiae*, Cdc28 (the *S. cerevisiae* cyclin-dependent kinase 1 (Cdk1) homologue) and cyclin B2 are required for checkpoint activation and maintenance after DSB formation. Furthermore, Cdc28/Cdk1 inactivation led to impairment of DSB repair by gene conversion, while enhancing non-homologous end-joining.

The mechanism by which ATM is activated continues to be a focal point of interest. The known autophosphorylation of ATM on Ser 1,981 functions as a hallmark of activated ATM, but M. Lavin reported additional phosphorylation sites that probably contribute to ATM activation. Although ATM is activated primarily by DSBs, the orthologue of ATM in *S. cerevisiae*, Tel1p, can be activated after formation of single-stranded DNA (ssDNA). In yeast cells lacking Mec1p, a close collaborator of Tel1p and an orthologue of the ATR kinase, attempts to replicate the DNA after heavy ultraviolet (UV) damage, which leads to extensive formation of ssDNA, causes Tel1p activation and subsequent phosphorylation of known Tel1p targets (M.P. Longhese, Milan, Italy). Furthermore, a Tel1p-MRX-dependent mitotic checkpoint, preventing the metaphase-to-anaphase transition and linked to the spindle assembly checkpoint operating in undamaged cells, was activated. Not much is known about DNA-damage-induced mitotic checkpoints, so this observation is of particular interest.

Z. Ronai (New York, NY) added a new target to the plethora of known ATM substrates:

activating transcription factor 2 (ATF2), a member of the bZIP family of transcription factors. Interestingly, this transcription factor, in response to DSBs, functions as a typical damage response protein: ATM dependent phosphorylation results in migration to damage-associated nuclear foci. Loss of ATF2 attenuated the recruitment of Mre11 to these foci and the subsequent ATM-mediated damage response independently of the ATF2 transcription factor function. Thus, certain proteins may function in the DNA damage response in novel capacities that do not necessarily overlap with their functions in undamaged cells.

The ATR protein, a close ally of ATM in the DNA damage response, has a critical, ongoing surveillance function in DNA replication. ATR (and its partner ATRIP) functions as the main transducer of the replication stress alarm and is quickly activated by collapsed replication forks. What are the proteins that link the replication apparatus to the stress-signalling system? R. Abraham presented evidence that a factor new to this field, the MCM7 component of the replication licensing complex, is required for ATR recruitment and subsequent phosphorylation of downstream targets, such as hRad17 and Chk1, in response to UV damage and replication stress. Thus, MCM7 may function as a link between the core replication and the genome surveillance machineries.

A unique protein capable of functioning both as sensor and adaptor in the replication stress response is Claspin (Chk1 large associated protein). W. Dunphy (Pasadena, CA) initially discovered Claspin in *Xenopus* extracts as a partner of the checkpoint kinase Chk1. Claspin is necessary for efficient activation of Chk1 by ATR-mediated phosphorylation after replication stress. To bind efficiently to Chk1, Claspin itself needs to be phosphorylated on two sites. Moreover, Claspin becomes phosphorylated on two additional sites: a potential ATR target that also functions as a docking site for the Polo-like kinase (Plk1) as well as a phosphorylation site for Plk1. Claspin phosphorylation by Plk1 creates an elegant feedback loop that eventually shuts off checkpoint activation by means of adaptation after replication stress. Claspin may also function with ATR and Rad17 as a sensor that binds to chromatin during DNA synthesis to monitor DNA replication. These regulatory loops are reminiscent of those that emerge in the DNA damage response. One of the targets of activated Chk1 is the Cdc25A phosphatase, whose activity is required for the mammalian S-phase checkpoint.

A long-standing question in the field has been whether a DNA damage checkpoint

exists during mitosis⁶. C. Rieder (Albany, NY) concluded that the effect of DNA damage on mitotic progression is very much dependent on the timing and extent of DNA damage. Needless to say, the best option for damaged cells at G2 is to activate the G2-M checkpoint so as never to enter mitosis. But what happens if cells do enter mitosis with damaged DNA, or if chromosomes are hit during mitosis? Working with mammalian cells in which mitosis can be conveniently dissected using real-time imaging, Rieder concluded that vertebrate somatic cells lack a conventional DNA damage checkpoint during mitosis. However, damaged DNA can prolong mitosis and delay the transition from metaphase to anaphase through the regular spindle assembly checkpoint, which is probably activated by perturbations of kinetochore structure. Importantly, this process is ATM-independent. The MAP kinase p38, known to function as a transducer of stress responses, turned out to be a major player in the late G2 checkpoint induced by drugs like topoisomerase II or histone deacetylase inhibitors that perturb chromatin structure.

Accurate DSB repair is based on homologous recombination between sister chromatids. This well understood multi-step process involves the Rad51 protein and other important recombination proteins, such as Rad52 and Rad54. The basic steps are: end resection; strand invasion; formation and movement of the Holliday junction while continuous strand exchange and DNA synthesis take place; and finally, resolution of the junction. An open question in this field is the identity of the elusive resolvase responsible for this last step. Elegant biochemical analysis provided evidence that a heterodimer of Rad51C and XRCC3 is essential (S. West, South Mimms, UK); however, the involvement of additional proteins cannot be ruled out.

One of the most studied processes that help maintain genomic stability is the extensive poly(ADP-ribosyl)ation of histones and nuclear proteins after DNA damage. Z.-Q. Wang (Lyon, France) described mice deficient for the prototype poly(ADP-ribose) polymerase, PARP-1, which exhibit genomic instability, radiation sensitivity and tumour predisposition (enhanced if p53 deficiency or Ku80 haploinsufficiency). Although PARP-1 was not involved in homologous recombination repair after DSB induction, it was found to modulate the action of the homologous recombination machinery at stalled replication forks by inhibiting the binding of the Rad51 protein to these sites.

Excision repair

Much is known about the mechanisms of base-excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR), but new implications of these pathways for human health are being revealed. TREX1/DNase III is implicated in the editing of mismatches generated during replication or repair. TREX1-deficient mice are viable with normal spontaneous mutation frequency, and no sensitivity to DNA damaging agents (T. Lindahl, South Mimms, UK). Nevertheless, the mice had a very short lifespan and died with a hugely enlarged heart caused by inflammatory myocarditis.

Uracil glycosylase (UNG) removes uracil from DNA near the replication fork. Ung^{-/-} mice die somewhat prematurely with a high incidence of lymphomas. Furthermore, during development of the immune response, the UNG deficiency results in a marked change in the somatic hypermutation spectrum and a decreased level of immunoglobulin class switching (T. Lindahl).

There is a growing body of evidence for the involvement of NER proteins in the ageing process. The XPD protein is a component of transcription factor TFIIH, which has dual roles in NER and transcription. Mutations in XPD result in either xeroderma pigmentosum or trichothiodystrophy⁷, a multisystem disorder without higher cancer incidence. A mouse with a TTD-specific mutation in the XPD gene had the features of TTD, and at age 1 year displayed many characteristics of premature ageing (J. Hoeijmakers, Rotterdam, Netherlands); endogenous oxidative damage is the probable cause of these ageing features. The prediction that different types of DNA damage accumulate in different organs, resulting in organ-specific manifestations of repair defects, is supported by studies of mice deficient in the ERCC1–XPF heterodimeric damage-specific nuclease. The liver and kidney are most severely affected in deficient mice, with gene expression patterns similar to those seen in 2-year-old ageing normal mice. A balance between ageing and cancer may account for the many different repair-deficient phenotypes. Survival with an elevated mutation rate results in cancer, whereas increased cell death leads to ageing.

Transcription-coupled repair (TCR), whereby damage in the transcribed strands of active genes is rapidly and preferentially repaired following UV irradiation, is specifically deficient in patients with Cockayne Syndrome, who carry mutations in either the CSA or CSB gene. To gain further insight into the mechanism and proteins involved in TCR, chromatin immunoprecipitation was

employed using antibodies to the elongating form of RNA polymerase II (PolIIo) (L. Mullenders, Leiden, Netherlands). CSB, CSA, TFIIH, XPF, XPA (but not XPC) and the p300 histone acetylase were found in extracts of irradiated (but not unirradiated) cells, by examining immunoprecipitates. The presence of CSA and TFIIH (and presumably the other proteins also) was dependent on the CSB protein, as these proteins were not found in extracts of CSB-deficient cells. The picture that emerges is that the blocked PolIIo recruits CSB, which in turn loads CSA and the rest of the NER machinery.

Interaction of DNA damage with DNA replication

In recent years, several specialized DNA polymerases have been discovered that synthesize DNA opposite damaged bases (translesion synthesis, TLS)^{9,10}. The best characterized is DNA polymerase η (pol η), deficient in the variant form of xeroderma pigmentosum. Both *in vitro* and *in vivo*, pol η is able to bypass UV-induced cyclobutane pyrimidine dimers by inserting usually the correct nucleotides. But, how does the cell bring about the switch from replicative to TLS polymerase at the stalled fork? When the replication machinery is stalled in human cells, the polymerase accessory protein PCNA becomes mono-ubiquitinated, and this increases its affinity for pol η , which binds only to the ubiquitinated form (A. Lehmann, Brighton, UK). The increased affinity provides a mechanism for bringing about the switch from replicative to TLS polymerase.

DNA polymerase ι (pol ι) is a paralogue of pol η , but its function in TLS has not yet been defined. Like pol η , it interacts with PCNA, and in the case of pol ι , this interaction increases its processivity *in vitro*. Pol ι has three putative PCNA-binding motifs, only one of which is required (R. Woodgate, Bethesda, MD). Mutagenesis of this motif abolishes not only PCNA binding and consequently the increase in processivity *in vitro*, but also the accumulation of pol ι in replication foci *in vivo*.

Pol η and pol ι belong to the recently defined Y-family of DNA polymerases, which seem to be involved specifically in TLS. DNA polymerase Q (PolQ or Pol θ) is a very recently discovered polymerase belonging to a family of DNA polymerases called the A family. PolQ has DNA polymerase activity and is able to perform TLS past AP sites *in vitro* much more efficiently than pol η , and past thymine glycols with a similar efficiency to pol η (R. Wood, Pittsburgh, PA). Unlike pol η , it cannot perform TLS past UV photoproducts.

Rev3 is the catalytic subunit of DNA polymerase ζ (Pol ζ) and has been known for many years to be required for UV mutagenesis in yeast cells. Chicken DT40 cells deleted for REV3 are extremely sensitive to crosslinking agents, such as cisplatin, and are sensitive to ionizing radiation (cells deleted in pol η are not sensitive to either of these agents). Furthermore, REV3-deleted cells are sensitive to ionizing radiation even when the cells are irradiated in G2, and this is associated with a marked increase in the number of chromosome breaks generated by ionizing radiation in G2 (S. Takeda, Kyoto, Japan). These and other results suggest a previously uncharacterized role for Rev3 in repair by homologous recombination.

Further links between stalled replication forks and recombination were revealed using an elegant system in *Schizosaccharomyces pombe*, (A. Carr, Brighton, UK). The RTS sequence is found at the mating-type locus in *S. pombe* and causes directional stalling of the replication fork. RTS sequences were inserted on either side of the *ura4* gene, so that replication fork progression was blocked at the *ura4* locus. Wild-type and most types of repair-deficient cells were able to tolerate this block. The exceptions were cells deleted for either the *rad51* or *rad50* genes, suggesting that the cells used homologous recombination to overcome the blocks.

Chromosome integrity, DNA replication and the DNA damage response

Genomic instability, the hallmark of malignant cells, has historically been detected as numerical and structural chromosomal aberrations. Numerical chromosomal aberrations result from errors in chromosome segregation and often from malfunction of the centrosome — the principal microtubule-organizing centre of somatic cells responsible for the formation of the mitotic spindle. E. Nigg (Martinsried, Germany) presented evidence that the centrosome amplification typical of tumour cells can also result from errors during mitosis or cytokinesis, rather than defects in the fine-tuned centrosome cycle.

The centrosome was the focus of two other presentations that provided some of the missing links between the chromosome segregation apparatus and the DNA damage response machinery. C. Morrison (Galway, Ireland) showed that damage-induced G2 arrest in chicken DT40 cells with impaired homologous recombination leads to centrosome amplification. Overriding the G2–M checkpoint using PIKK inhibitors strongly reduced this phenomenon, as did loss of ATM, albeit only partially. This work suggests that damage-induced

centrosome amplification is a mechanism for ensuring the death of cells that evade the DNA damage or spindle assembly checkpoints. J. Bartek showed that in interphase a portion of the checkpoint kinase Chk1 associates with the centrosome. Before mitosis, the centrosome houses the Cdk1–Cyclin-B1 complex, the chief propeller of the G2–M transition, which dissociates from the centrosome after entry into mitosis. Chemical inhibition of Chk1 resulted in premature centrosome separation and activation of centrosome-associated Cdk1, as did forced immobilization of kinase-dead Chk1 to centrosomes; wild-type Chk1, on the other hand, impaired activation of centrosome-associated Cdk1, resulting in DNA endoreplication and centrosome amplification. Collectively, the data suggest that centrosome-associated Chk1 shields centrosomal Cdk1 from unscheduled activation by cytoplasmic Cdc25B, thereby contributing to proper timing of the initial steps of cell division, including mitotic spindle formation.

Genome integrity is maintained by ensuring not only that the sequence of the DNA is kept intact in the face of damage and during DNA replication, but also that the sister chromatids separate correctly during mitosis. The principal function of cohesins is to keep sister chromatids together during and after DNA replication. Current models suggest that cohesins form a ring around the paired sister chromatids. In *S. cerevisiae*, one of the components of the ring is cleaved at the metaphase-anaphase transition to release the sister chromatids. Many cohesin molecules are bound on each chromosome, but is there any specificity to the chromatin-binding

sites? Chromatin immunoprecipitation across a whole chromosome revealed that cohesin molecules are bound at sites where two adjacent genes transcribed on opposite strands converge, and this binding is dependent on these genes being transcriptionally active (E. Uhlmann, London, UK).

An unexpected link between replication and cohesion was revealed by work with origin replication complex (ORC) proteins. ORC binds to origins of replication and remains bound during DNA replication. In yeast *orc2-1* mutants, the ORC2 protein is labile. *orc2-1* was put under the control of the GAL promoter. Cells with downregulated Orc2-1 nevertheless progressed through S phase and were delayed only at mitosis (S. Gasser, Geneva, Switzerland). This delay was mediated by both the DNA-damage and the spindle checkpoints, but cohesion was deficient, resulting in failure of sister chromatids to pull apart at anaphase. It was suggested that ORC functions in the correct distribution of cohesin along the chromosomes.

Evidence is rapidly accumulating that epigenetic mechanisms contribute to guarding genome stability, particularly those that shape and maintain chromatin structure. S. Polo (Paris, France) discussed the role of two histone chaperones, CAF-1 and HIRA, in nucleosome assembly. Each one is responsible for depositing a specific histone variant in nucleosomes: CAF-1 is active primarily at the time of DNA synthesis (replicative or repair), whereas HIRA functions independently of DNA synthesis, replacing histone molecules in existing chromatin fibres. Interestingly, CAF-1 was found to function in heterochromatin

duplication and to be overexpressed in cancer cells exhibiting genomic instability.

The meeting demonstrated the growing number of technologies and the different areas of cell biology that the genome stability field now involves. Particularly impressive is the power of real-time imaging in live cells, which allows the dissection of the fast and complex DNA damage response. It is no surprise — considering that a single, unrepaired DNA lesion, such as a DSB, can put the life of the cell at risk — that the cellular response to DNA damage is global, vigorous and uncompromising. Genome stability, crucial to our health and vitality, is expected to remain at the forefront of biomedical research for the foreseeable future. *A note from the authors: we apologize to the many participants in this wide-ranging meeting whose excellent contributions could not be mentioned here owing to space limitations. We also acknowledge the support of the Science Foundation of Ireland and the University of Galway.*

1. Bassing, C. H. & Alt, F. W. *DNA Repair (Amst.)* **3**, 781–796 (2004).
2. Aylon, Y. & Kupiec, M. *DNA Repair (Amst.)* **3**, 797–815 (2004).
3. Lieber, M. R., Ma, Y., Pannicke, U. & Schwarz, K. *DNA Repair (Amst.)* **3**, 817–826 (2004).
4. Wyman, C., Ristic, D. & Kanaar, R. *DNA Repair (Amst.)* **3**, 827–833 (2004).
5. Abraham, R. T. *DNA Repair (Amst.)* **3**, 883–887 (2004).
6. Morrison, C. & Rieder, C. L. *DNA Repair (Amst.)* **3**, 1133–1139 (2004).
7. Lehmann, A. R. *Genes Dev.* **15**, 15–23 (2001).
8. Karran, P. & Bignami, M. *Nucleic Acids Res.* **20**, 2933–2940 (1992).
9. Lehmann, A. R. *Mutat. Res.* **509**, 23–34 (2002).
10. Prakash, S. & Prakash, L. *Genes Dev.* **16**, 1872–1883 (2002).
11. Gruber, S., Haering, C. H. & Nasmyth, K. *Cell* **112**, 765–777 (2003).