DNA Damage Response and Repair Mechanisms
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4 FP6 Integrated Project: DNA Repair

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The Project
The realisation of the importance of preservation of genetic integrity is one of the major developments within the field of biomedical genetics over the last decade. Alteration of genetic information has major impacts not only on carcinogenesis, but also on ageing and other aspects of human health (see Figure 1). Understanding the detailed mechanisms through which genome integrity is maintained, including DNA damage repair mechanisms, is thus of pivotal importance for improvement of numerous quality of life issues. This project intends to unravel the mechanisms that safeguard the integrity of the genome, using an integral approach ranging from molecules to human disease. The project brings together leading groups with multi-disciplinary and complementary expertise to cover all pathways impinging upon genome stability, ranging from molecules to mouse models and human disease. The results should provide a more solid basis for rational design of preventive, diagnostic and therapeutic options for DNA damage-related diseases notably cancer and ageing-associated disorders, which dominate health care in the developed world.

DNA damage: Induction, consequences and repair
Right part, top: common DNA damaging agents; middle: examples of lesions that can be introduced in the DNA double helix by the above agents; bottom: the most relevant DNA repair mechanism responsible for the removal of the indicated lesions. Left part, top and middle: acute effects of DNA damage on cell cycle progression (leading to transient arrest in the G1, S, G2 and M cell cycle phases) and on various aspects of DNA metabolism (notably interruption of transcription, replication and chromosome segregation), which can induce apoptosis or necrosis; bottom: long term consequences of DNA injury causing permanent changes in the DNA sequence (point mutations affecting single genes or chromosome aberrations which may involve multiple genes) and their biological effects.

Abbreviations: MMC: mitomycin C and Cis-Pt: Cis-Platin (both DNA cross-linking agents); (6-4)PP: 6-4 photoproduct and CPD: cyclobutane pyrimidine dimer (both induced by UV light); BER and NER: base and nucleotide excision repair respectively.
The objectives

1. To gain a detailed understanding of the biochemical mechanism of DNA repair and checkpoint pathways.
2. To obtain detailed insight into the cellular functioning and consequences of defects in one of the genome surveillance pathways.
3. To collect information on the coordination and interplay between the different genome surveillance processes within the cellular framework.
4. To monitor the reprogramming of intracellular processes in response to DNA damage.
5. To identify new components of DNA damage response pathways.
6. To extend knowledge from model organisms to humans.
7. To provide training in the field of DNA damage response to diverse target audience.

The workpackages

To achieve our goals the project is broken down into six work packages:

WP1  Biochemistry and structural biology of DNA damage response and repair mechanisms
WP2  Cell Biology of DNA damage response and repair mechanisms
WP3  Generation and analysis of (Mouse) models of DNA damage response and repair mechanisms
WP4  Systems biology of DNA damage response and repair mechanisms, genomics, proteomics and bioinformatics
WP5  From models of DNA damage response and repair mechanisms to humans
WP6  Training in DNA damage response and repair mechanisms
WP7  Management
DNA Damage and Repair

What is DNA repair?
Every day our cells battle against genetic damage that could lead to cancer. DNA repair is an innate insurance policy against the normal daily toil on our genes, damage to which results from genotoxic stress, like smoking and UV radiation. Even the essential elements in water and air can lead to DNA damage. Central to our daily metabolism, oxidation and hydrolysis reactions occur in their billions within our bodies producing reactive oxygen species that wreak havoc on the helical backbone housing our genes. The damage response comprises a number of important pathways, which cope with different kinds of environmental insult:

**BER**: base excision repair—replaces damaged bases in the DNA code

**NER**: nucleotide excision repair—replaces a string of bases if one or more is damaged

**NHEJ**: non-homologous end joining—fixes double-strand breaks in the DNA double-helix

**HR**: homologous repair—fixes double-strand breaks in and interstrand cross-links in DNA

**MMR**: mismatch repair—corrects mismatches in the sequence of bases in DNA

Why is DNA repair so important?
In short: to combat the ‘time-dependent erosion of the genome’. The slightly longer answer is that thousands of problems with DNA arise every day in every body cell, each of which has to be successfully detected and, if necessary, amended. The DNA repair system detects and co-ordinates a response to such onslaught, effecting measures to prevent cell death, or remove cancerous cells from the system. Embodied in a series of proteins that police the cell nucleus, genome caretakers maintain the integrity of DNA, protecting us from cancer and ageing-related diseases, keeping our immune system healthy and more importantly preserving our genes for our children.

Introduction

Genotoxins
Polonium is five million times more toxic than hydrogen cyanide and a single gram can self-heat to 500 °C. Unsurprising then that Alexander Litvinenko died very shortly after ingesting an unknown quantity of this radioactive element. Half a century ago Marie and Pierre Curie’s daughter was accidentally exposed when a sealed capsule of polonium exploded on her lab bench. A decade later she died of leukaemia. On a broader scale this genotoxin has been ingested by those smoking tobacco grown using phosphate fertilisers. However, the particles emitted during alpha-decay of polonium only wreak havoc in body tissues if ingested. In contrast, beta and gamma radiation are more severe and act across much greater distances, as has been apparent in the aftermath of nuclear tragedies such as Hiroshima and Chernobyl. Ionizing radiation snaps the DNA backbone as easily as we might break a hair. One break is sufficient to kill a cell. Genotoxins are all around us and they don’t just come from dangerous radioactive chemicals. Cosmic rays from outer space and UV from sunlight are absorbed by cells, causing breaks and cross-links in DNA strands, which hamper replication. The oxygen that fills our lungs every few seconds helps us to metabolise food, but produces chemicals like hydrogen peroxide that can cause DNA damage. Car
exhaust fumes spew forth a complex cocktail of hydrocarbons that fuel a huge diversity of biochemical alterations to our chromosomes.

**Natural defence**

Given the 3 billion base pairs present in every copy of your genome, it is a small wonder that the battery of daily assaults on a single cell, let alone the billions of cells in our body, don’t cause more damage. For this, we are indebted to the evolutionary process that has graced us with a sophisticated DNA repair system. Even bacteria have a natural armoury to protect against insults to their genes.

So how does a cell know it has been assaulted? Are there sentinels on duty day and night to keep attackers at bay? The cell employs both signalling networks and DNA repair pathways, each comprised of around ten to twenty proteins. These pathways do not exist in isolation, but overlap on many levels with a host of shared players. Depending on the extent of the damage, the cell has to decide whether to repair the fault, ignore it and risk mutation, or bin itself to forego any ill-effects on the whole organism.

The components involved in such decisions will vary according to the way in which the genome has been damaged, who it belongs to and its overall stability. Take for example the marvellous *Deinococcus radiodurans*—literally the ‘strange berry that withstands radiation’—discovered by accident around 50 years ago, during an attempt to sterilise tins of meat with gamma-radiation. This hardy bacterium evaded nuclear blasting, living to tell the tale by spoiling the meat.

‘Conan the bacterium’ has extremely fast acting DNA repair machinery and multiple copies of the genome in its single-celled body. The latter strategy affords it a useful way in which to re-synthesise DNA in the event of backbone breakage. Should part of the genome be lost in this way, the duplicate copies act as templates for its reconstruction. Versions of the players in the bacterial repair response have been found in yeast, mice and humans. For example, bacterial RecA-a repair protein - is very similar in sequence and function to yeast and human RAD51.

Breaks in the DNA backbone are picked up by ‘checkpoint’ proteins, which sit at the top of complex signalling cascades that hail repair troops to the damage site. ATM is one such ‘caretaker of the genome’ in humans. When it spots a double-strand snip in DNA, it acts as a loudspeaker activating other proteins to initiate one of two possible repair responses: homologous recombination (HR) or non-homologous end-joining (NHEJ).

HR is jolted into action when a single-strand of the DNA double-helix is severed, but if both strands snap, NHEJ kicks in too. Steve Jackson (Cancer Research UK Laboratories, Cambridge UK) works on the latter pathway. When double-strand breaks are generated, “Ku proteins jump onto broken ends” he explains. “These ring-shaped molecular policemen clamp the ends,” allowing other proteins called ligases to ‘glue’ them together again.

If both strands of the double-helix break, HR is triggered, HR is performed by a host of proteins, including the RAD proteins. When a cell is exposed to ionising radiation, RAD proteins rally together, visibly clumping at damage sites in the cell nucleus. RAD51 picks up the broken end and searches for a
complementary base-sequence. Once a suitable template is located DNA polymerases can do the job of resynthesising damaged strands. The EU is supporting research into this area through the DNA Repair Integrated Programme which is already making strides along new therapeutic avenues in collaborations with companies such as Kudos pharmaceuticals and DNage BV). We are also likely to benefit through caretaker strategies such as are offered by the fast-growing nutraceuticals industry. Antioxidants, although convincing data are still forthcoming, look like pretty good candidates for mopping up DNA-damaging chemicals we produce as by-products of normal metabolism. Radioactive genotoxins might be rare, but internal genotoxic stress is a daily reality. A healthy DNA repair system protects us from all sorts of ills, whether caused by deliberate or accidental damage, including cancers and leukaemia (see DNA repair and cancer), premature ageing (see DNA repair and ageing) immune deficiencies, neurodegenerative diseases like Alzheimer’s, certain cardiovascular disorders, diabetes and many other ageing-related disorders. The importance of DNA repair is highlighted by the existence of a number of rare inherited DNA repair defects that cause early death (see DNA repair and human disorders). Understanding more about our DNA repair systems and how to support these will therefore have great implications for health and disease.

**DNA repair and ageing:**

**Life-span, diet and DNA**

Why do tortoises live so long? It is not uncommon for a giant tortoise to reach 150 years in age. Some have even suggested there is a Galapagos tortoise old enough to have met Charles Darwin. Darwin himself only lived for half as long – still rather longer than the average human of his day. Since the 1800s, improvements in lifestyle and medicine now mean that humans in developed nations live on average 20 years longer. Not quite tortoise potential.

Scientists have come up with some interesting ideas, which might cast light on why different species have different life-spans. One such theory relates to metabolism. Humans and other mammals have higher metabolic rates than their reptilian counterparts. We make all our own heat rather than absorbing it from the sun. As we breathe in the air around us, oxygen diffuses into our cells, fuelling the combustive process of respiration, the driving force behind our metabolism, growth and development. While we make energy from food in this way, hazardous by-products are created that can damage our DNA, so-called reactive-oxygen species (ROS). The higher the metabolic rate, the greater the damage potential and the more likely our cells are to mutate and malfunction. Reptiles, like tortoises might be less susceptible to DNA damage caused by ROS, because they produce lower levels of these reactive chemicals.

We don’t know how much DNA damage speeds up ageing or indeed how much it is relevant to the natural ageing process, but recent research suggests that knowing more about our genetic maintenance might improve our quality of life. There’s no point in living as long as a tortoise if you’re not fit enough to enjoy it.

**Life-span, diet and DNA**

Dietary research on mice, monkeys, rats, spiders, fruit-flies and worms further emphasizes the link between metabolism and life-span. Severely restricting calorie intake (60-70% of our daily intake) can prolong life-span, given sufficient vitamins, minerals and other nutrients. The thinking is that fewer calories will result in a lower metabolic rate, less ROS and therefore less damage to DNA.

“That is the secret behind calorie restriction prolonging life-span in a natural manner,” says Jan Hoeijmakers.
(Department of Cell Biology and Genetics, Erasmus MC, Rotterdam), whose team is researching the role of DNA damage in ageing. He and others support the view that calorie restriction reduces metabolism, lowering ROS and the resultant stress on the DNA repair system thereby keeping cells healthier for longer.

Reactive oxygen species are charged molecules that can disrupt or alter energy bonds between other molecules. Chemicals like superoxide and hydrogen peroxide result from respiration in the powerhouses (mitochondria) of our cells. Neither chemical alone can harm DNA, but in the presence of iron or copper ions they form hydroxyl radicals that can damage organic bases (A, T, C or G) in DNA, which can translate through to protein function. Removal of damaged bases is estimated to occur 20 000 times a day in each body cell. Needless to say adequate measures must be taken to prevent chaos in the cell. Luckily we have a network of sophisticated DNA repair systems policing our genes and keeping genetic order. Scientists have identified well over a hundred genes involved in the various DNA repair pathways that both signal damage and effect a repair response. Ongoing research efforts continue daily to find pieces of this complex molecular jigsaw puzzle.

While DNA damage hasn’t been shown to cause ageing directly, a number of rare human disorders, caused by mutations in DNA repair genes, include symptoms of premature ageing. Only a few years ago, Jan Hoeijmaker’s team at the Erasmus MC described a new ageing syndrome in a teenage boy who encountered the fate of an old man before he even reached puberty (Niedernhofer et al, Nature 2006), The patient had mutation in a gene (called XPF) involved together with its partner ERCC1 in DNA repair. The two-protein complex (called XPF/ERCC1) protects against the kind of DNA damage caused by UV sunlight, which can mess up the DNA sequence (see DNA in human disorders). Mutations in the XPF gene are known to cause a rare condition known as Xeroderma pigmentosum (XP). Patients with XP are so sensitive to sunlight, they must completely cover themselves when they go outside and when indoors, live with curtains and shutters drawn. Failure to do so results in skin-cancer.

Patient ‘XFE’ was sensitive to sunlight, but more dramatic in his case, was the wizened, wasted appearance he developed by the age of 15, not characteristic of XP patients, who usually die from cancer later in life. He was blind and deaf and many of his body organs had wasted away. Jan explains that mutations in the XPF gene can be mild to extreme, mild mutations associating with cancers, in particular skin cancer, and severe mutations with premature ageing, as in the case of patient ‘XFE’.

The Dutch team has created mouse models defective in the XPF/ERCC1 protein complex that map closely to the clinical conditions of patient ‘XFE’. Mice with a defect in the
ERCC1 protein also age prematurely and die after a few weeks. When Jan’s group analysed genes in the liver of defective mice, well-over 1500 genes showed altered activity when compared to age-matched normal mice. The team confirmed that the same alterations to liver, a key player in metabolism, occur in naturally aged mice. Among such changes is a low level of insulin-like growth factor-1 (IGF-1). This protein-hormone, made and released into the bloodstream by the liver, normally boosts growth. Jan argues that the low levels of IGF-1 in aged and DNA-repair defective mice embody a stress-response that shifts priority from growth and development to maintenance and repair in the face of increasing DNA damage. “Using the rapidly aging mouse mutants, our intention is to efficiently identify compounds in food or drugs that improve the health status and life span of the mice. So I started up a company called DNage, whose mission is to provide solutions for medical/health care problems associated with ageing.”

The links between the growth hormone axis, the DNA repair system and the ‘ageing process’ warrant further research, of which the above mentioned studies are an important step in the right direction. Jan is hopeful that with a better understanding of DNA damage, diet and ageing, we can significantly improve the quality of life for those living longer.

DNA Repair in Human Disorders

Living in the dark

Despite all the hype about global warming, sunlight still cheers most of us up. But for a few individuals, who suffer from a rare genetic disorder, sunshine is a serious health hazard. So-called ‘children of the night’ share no such sun-filled pleasures with the great majority. One in 250,000 people worldwide—who suffer from Xeroderma pigmentosum (XP)—are two thousand times more likely to get skin cancer than the rest of us. Also known as ‘moon children’, because complete protection from sunlight would only be afforded by an astronaut’s suit, when exposed for even a few moments to normal ambient light intensities their skin can severely blister causing skin tumours. Trips outside require serious gear, including powerful UV-masks, caps and gloves.

Waves of ultraviolet radiation streaming forth from the sun range from 100-400 billionths of a metre (nm) in length, on the whole posing little risk for humans. But at a wavelength of around 300nm, they can zap through skin into cells causing chemical mayhem. DNA strands stick together where they shouldn’t, hampering replication and running the risk of mutation. In healthy individuals the chaos is quelled by a process called nucleotide excision repair (NER).

Lesions from light

“Individuals with XP cannot repair UV-damage to DNA owing to defects in NER,” explains Alan Lehmann (University of Sussex, Brighton, UK), expert in DNA repair disorders. The consequences of the disorder range from a generous freckling in some sufferers to extremely severe skin lesions leading to cancer in others. “With no protection,” he warns, “XP sufferers will get thousands of skin cancers.” Although there is no cure, protection is an effective prevention. However, “patients protected from skin cancers could still die from neurological abnormalities.”

“I’ve known patients, wheelchair bound and unable to speak by the age of 16, that have died before reaching...
30. Quite a lot of sufferers in this country have neurological abnormalities depending on which gene is mutated,” Alan confirms. To inherit XP, mutant genes must be inherited from both parents. Mutations can arise in one of about eight genes that make proteins involved in NER, compromising the cell’s ability to cope with daily UV-induced wear and tear and in some cases seriously messing up normal development.

The NER pathway replaces damaged chunks of DNA in a cut and paste manner. Damage is recognized by one or more proteins (including XPC and XPA), which assemble at the damage site. DNA is unwound by a massive protein-enzyme called TFIIH (made in part from XPB and XPD proteins). This produces a ‘bubble’ from which the damaged area is cut out by the XPF and XPG proteins. Fresh DNA is then synthesized by special polymerase enzymes (delta and epsilon), before another enzyme (a DNA ligase) glues the repaired patch into the DNA backbone.

Weak links at different nodes in the repair system are reflected by varied sets of symptoms between XP patients. Mutations in XPA, which directs repair troops to DNA damage, seem to cause the most severe symptoms, neurological problems arising in early childhood and supersensitive skin leaving patients completely open to skin cancer. Knocking out XPA obliterates the repair process, whereas some other defects in the pathway don’t seem to be quite as debilitating.

The whole NER system relies on the integrity of over 30 proteins, defects to any of which compromise repair in different ways. Faulty NER systems have been found in a number of other rare syndromes including TTD (trichothiodystrophy). The features of this syndrome, sulphur-deficient brittle hair and scaly skin, have little in common with XP. Patients’ cells are sometimes sun-sensitive, but there is no increased risk of skin cancer, although premature ageing is pronounced. Getting a feel for how NER-malfunction can cause such different syndromes has been a significant challenge. Mouse models have been very revealing in this respect.

Jan Hoeijmakers (Erasmus MC, Rotterdam, Holland) and his team have created mice with specific mutations in DNA repair genes. One such model mimics a specific mutation observed in human TTD patients, a mutation in the XPD gene that affects a single amino-acid in the protein. Mice bearing the same defect have very similar features: brittle hair that grows and breaks, falling out and leaving bald patches. They die young, like their human counterparts, and have a curved spine, wizened appearance, grey hair and wasted limbs. Defects in XPD are frequently found in XP patients. How do mutations in the same gene result in premature ageing in TTD patients, but susceptibility to skin cancer in XP patients? Jan’s feeling is that the answer lies in the triple function of this protein. While XPD is a cog in two distinct DNA repair pathways, as part of the TFIIH enzyme, it also participates in transcription. His hunch led him to question whether brittle hair might result from defects in the transcriptional role of XPD, with skin cancer and premature ageing the result of compromised repair in the other two pathways.

Sure enough, some mutations in XPD have been found to compromise repair, leading to cancer and XP, while closer inspection of mutant cells from humans with TTD reveals subtle defects in transcription. So
there is a clear link between genetics and physical characteristics, although not all cases of human disease can be easily related to underlying genetics. Many challenges remain for scientists who are trying to translate molecular truth into clinical practice. There are no treatments for XP or TTD. Such genetic faults run throughout the entire organism, pervading every cell. However, understanding these diseases stands to benefit us all.

“Everyone wants to know when we will cure cancer,” says Alan, “but studying these rare genetic diseases is fundamental to understanding how DNA repair works, which will help us to fight cancer.”

DNA Repair and Cancer

Cancer: cause and cure

Around one in three people in the EU will be diagnosed with cancer during their lifetime, most commonly with bowel cancer. Breast cancer follows closely and lung cancer causes a fifth of total deaths in the EU. Yet, despite what we know about the evils of tobacco, almost a third of the EU population smoke. Perhaps they already know that only one in ten smokers will develop lung cancer. So if smoking isn’t the major cause, then what is?

“This is difficult, because the damage comes mainly from inside of us,” explains Jiri Bartek (Institute of Cancer Biology, Denmark). Every day there are tens of thousands of DNA injuries inside every normal body cell. Most of these result from chemicals produced during metabolism. Luckily, most of us come fitted with an insurance policy against such damage in the form of our DNA repair machinery. Jiri affirms that “we need to understand the pathways underlying the DNA damage response” in order to understand cancer.

His team of researchers is investigating how DNA lesions are detected and how cells then get sent to the recycle bin. “When you understand the pathway you can find a way to interrupt it.” But why would we want to interrupt it? “Radiotherapy and chemotherapy work by causing damage,” killing cancer cells, but a lot of our normal body cells too. If we could make cancer cells more sensitive to chemotherapeutics then we could lower the dosage, preserving healthy body cells and reducing the traumatic side-effects of chemotherapy.

The thinking behind the therapy

“We are trying to sensitize cancer cells towards irradiation,” explains Jiri. “In most cancer cells, the G1 checkpoint is missing.” The G1 stage in the life-cycle of a cell runs a safety check. With the help of checkpoint proteins like p53, the cell decides what to do next: divide or rest. The ‘master watchman’, p53, protects the cell from cancer by picking up on genetic damage, activating DNA repair pathways and stopping cell division if necessary.

“So, cancer cells differ from normal cells in that they mostly rely on the G2 checkpoint phase”, Jiri continues, which follows G1 and precedes cell division. “If you could somehow silence the G2 checkpoint, you would push them into cell division without repair to the damage,” they would divide with a mess of broken chromosomes and die. Knocking out G2 in normal cells wouldn’t be a problem, because they are protected by the G1 checkpoint. So inhibitors of this protein would specifically target cancer cells.

What kind of small molecules are we talking about? “CHK1 inhibitors, for example,” says Jiri. CHK1 is an essential checkpoint protein for G2. Inhibitors of this protein are currently under trial, heralding a new wave
in anti-cancer drugs. New strategies like G2-blocking represent a broader move towards improving treatment for cancer patients. “But we have to watch out for toxic side-effects,” he warns, “and look for alternatives, like combination treatments that also target DNA repair mechanisms. Cancer cells are often defective in both signalling and repair pathways. “If we block faulty repair pathways in tumour cells, we increase damage in the tumour, but not normal cells.” “This all has implications for individualised cancer therapy and for family counselling,” Jiri explains. Cancers result from defects in different DNA repair pathways. If you are born with a defect in these pathways you are more likely to develop cancer. “We could use markers to assess the DNA damage response status in every tumour,” paving the way for personalised medicine. “With the current technology and the human genome as a yardstick, screening individuals for specific defects is a realistic goal. Coupled with an increased understanding of the DNA repair machinery and the consequences of specific repair defects, doctors will be in a better position to advise families on prognosis, but also to tailor treatments to their particular shortcomings.

Josef Jiricny (Institute of Molecular Cancer Research, University of Zurich, Switzerland) agrees. “We can prevent colon cancer if we know who’s at risk.” Josef’s team has studied families with mutations in the mismatch-repair (MMR) pathway. “We’ve identified 300 families in Switzerland and found 108 mutations that segregate from family to family.” Colon cancer can be prevented by colonoscopy, regular internal screens for polyps that might turn cancerous. “Now that we can identify the mutation carriers, we can screen them,” says Josef. “And there’s a double positive for the family,” he adds. “At the moment a whole family is screened, yet only 50% of them inherit the mutation.” Those with MMR mutations live with the risk of colon cancer, but the other half without mutations can be relieved of their fear; “they can just live a normal life. Those with mutations are assured that if they go through the screening programme, their chances of getting cancer are limited. So far, we have been able to prevent cancer in 90% of the males.” “In females, it’s a bit more complicated, because they also have the tendency to get endometrial and ovarian cancer,” Josef warns, “and this is not quite so easy to detect early with limited invasiveness. An endometrial endoscopy is a bit more unpleasant, and you can’t look at ovaries in this way, so you have to use ultrasound, which is not as precise as colonoscopy. But even then, the prevention rate is 70% plus.” So, for those who need anti-cancer treatment, how can this be improved? “We are trying to find a way of specifically targeting the MMR-deficient cells, to wipe them out with fewer side-effects, by a kind of gene-therapy approach,” Josef explains.

Traditional methods are only 1.5-fold more effective at wiping out cancer cells than normal cells. Josef’s method is 25-fold more effective at killing cancer cells than normal cells, at least in a model system. “Of course, we’re trying to find the basic causes of colon cancer. When people inherit these mutations, are there environmental factors, you know: diet, nutrition and genetic factors that trigger the process of transformation in the epithelial cells in the colon and the endometrium?” Looking at the bigger picture, Josef reveals that “13-15% of all colon cancers worldwide are MMR-deficient.” Around 4% are inherited from our ancestors, but the majority, the remaining 9% are sporadic, caused by mutations we acquire as our cells divide. In some cases the MMR repair gene is completely switched off by a process of epigenetic silencing. Unlike
mutation, this fault is reversible. Having the tools to decipher what has gone wrong in individual patients will pave the way for individualised treatment.

How can the DNA repair Integrated Project help? “The most important goal is trying to get the clinical link,” Josef confirms. “The whole programme relies heavily on animal models; these are very useful but not always faithful to human pathology. We need to forge a link between the model system and the patient. To this end, we’re using biochemistry to study the individual proteins involved in repair, how they work in human tissue and how relevant that is to human disease.” The EU has awarded several million euros in support of DNA repair research. “Hopefully the programme in the long-term will benefit the patients.”

DNA Structure

Through the eyes of an enzyme

When Galileo noted—through his telescope—that the earth was round rather than flat, the universe started to make more sense. Understanding a whole system like the universe is impossible without architectural insight. This is very much the case when it comes to studying the inner life of a cell. Figuring out how cells work relies on a clear picture of what they look like. Molecular investigators work on the biological blueprints that cell biologists and geneticists can use to model the mechanics.

When Karl-Peter Hopfner (University of Munich, Germany) puts on his molecular glasses, he is trying to see the cell nucleus through the eyes of an enzyme. “Enzymes see a vast amount of DNA in the nucleus,” which means they need to be able to distinguish between healthy and damaged DNA. Why, for example, is smoking toxic and how does it promote cancer? Smoke forms DNA adducts, tiny chemical bolt-ons to the double-helix, which can damage the way our cells operate if not removed. “If you are smoking actively or passively,” explains Karl-Peter, “the DNA repair machinery can’t work properly.

A DNA adduct itself isn’t often a problem. The problem occurs when enzyme function is disturbed, adducts aren’t removed, or there are subsequent changes in the DNA sequence. One very important enzyme is the kind involved in making new DNA before cells divide. “DNA polymerases can make mistakes causing mutations or breaks in the DNA backbone. This genome instability can lead to all sorts of mayhem in the cell; different genes get activated and cell physiology changes dramatically. “In order to understand the process, we need to understand the chemistry underlying how enzymes recognise lesions.”

Using the knowledge

Thwarting the DNA repair machinery can be a useful anti-cancer strategy. The drug, cisplatin, used in cancer chemotherapy, interferes with normal cellular processes by causing DNA lesions. These lesions form cross-links between DNA bases that can interfere with several cellular processes. The DNA repair or surveillance system kicks in and cells are killed off. While the drug helps get rid of cancer cells, normal cells suffer too, which is why patients receiving chemotherapy lose all their hair. Big problems arise when cancer cells find a way around the drug. “For instance, cancer cells try to make polymerases that can replicate over cisplatin,” Karl-Peter explains, so they can survive drug treatment, because the DNA repair checks are evaded, and cells aren’t sent to the recycle bin. Structural biologists want to understand how such enzymes can recognise the cisplatin lesion. What is the active site chemistry of the interaction? “Understanding this”, explains Karl-Peter, “you could design other chemotherapeutics that
Deciphering the world within

So how does Karl-Peter’s work provide a basis for new treatments? “We provide the toolbox for modulating enzyme function,” he confirms. “Take this polymerase, for example. If you have the crystal structure you can start to think about how to design a drug that can interact with it.” To get the crystal structure, you need to purify the protein. In fact, around 90% of his time is spent trying to purify proteins to ‘grow’ crystals for analysis.

“We try to derive a three-dimensional picture of all the atoms in a molecule like an enzyme. The most powerful and best way is to use X-ray crystallography. We use the interaction of X-ray photons with electrons in a molecule.” X-rays (photons) are literally bombarded against the crystal and are diffracted off the electron ‘clouds’ surrounding atoms.

As Karl-Peter explains, collecting these diffraction patterns is a task in itself. “In order to resolve the distances between atoms, you need a means to measure the waves between atoms, which are separated by distances of around 1 angstrom”, a hundred-billionth of a meter. There is currently no such thing as an X-ray microscope because of the difficulty in making a lens that can capture the tiny X-ray waves.

“So why do we need a crystal? We use diffraction patterns from millions of molecules because we can’t reconstruct the patterns through a lens. We need to grow crystals, because the atoms are all orientated in the same way.” Millions of molecules are needed to get enough ‘signal’ for the structural information. Owing to the lens problem, “half of the information (phases of light) is lost, so we need to run computer programmes to get the lost phases back.” Another difficult job. In fact, admits Karl-Peter, “this is often a PhD thesis!”

Once diffracted waves are run through a computer programme, “We get the electron density distribution and model the protein structure, something we do nowadays at synchrotrons.” A synchrotron is an enormous electron accelerator, which is used to produce high-intensity X-ray beams. There is one in Grenoble, an impressive 1 km across, which cost over a billion euros to build. Synchrotrons “accelerate electrons to almost the speed of light.” Although there are several in Europe, you need to apply for time to use them. This is often funded by a grant for one day’s worth of measurement.

Karl-Peter and his team make regular trips to Grenoble carrying their little 0.1mm wide crystals in liquid nitrogen. The one disadvantage of the X-ray method of deciphering protein structure is that, “you need lots and lots of protein.” Although 10mg doesn’t sound like much, “it takes a lot of time to purify this much protein.” Also, you often want to verify X-ray crystallographic models by complementary analysis. For instance, electron-microscopy is useful, because you need 100-fold less protein.

Through deciphering the molecular architecture of the cell, “We can really envision the chemistry of DNA repair.” With a clear picture of the tools used by cells we can improve therapeutic strategies. As Karl-Peter notes, ‘slash-hammer’ methods of killing cancer cells are not specific and kill every cell that divides. Harnessing the ingenuity of viruses, for example, which naturally re-programme cells, holds promise, but without having a clear model of the molecular infrastructure, strategies will remain unrefined.

These texts are also available from the IP’s website: www.dna-repair.nl
Jan Hoeijmakers has supervised, for more than 20 years, the research in the laboratory on mechanisms of genome (in)stability and their biological impact. Research is focused on all major DNA repair mechanisms known in mammals including NER, DSBR (homology dependent and NHEJ), BER, repair of interstrand DNA cross links, TLS, and the response to DNA injury of cell cycle control systems. The research in these areas covers the molecular and cellular level extending to mouse mutants and human syndromes, enabling genotype-phenotype relationships to be explored. His group has identified many of the known NER complementation groups, was the first to clone a human DNA repair gene followed by many others, discovered the strong evolutionary conservation of some repair pathways, unravelled the function of a number of the components, elucidated the basis of several human DNA repair syndromes and in close collaboration with participant 14 identified TFIIH as a dual functional transcription-repair factor. His team has generated a large variety of mouse mutants in genome care-taking systems including several models for human DNA repair disorders (CS, TTD). Recently, his laboratory disclosed a relationship between compromised genome functioning and ageing and discovered a connection between DNA damage and a ‘survival’ response, that boosts defences at the expense of growth and promotes longevity under protective conditions. He has longstanding productive interactions with almost all of the other participants.

Roland Kanaar studied chemistry at Leiden University and obtained his PhD degree in 1988 for research on the action of an enhancer in site-specific DNA recombination and the elucidation of how nucleoprotein complexes assembled at distant sites along a DNA chain communicate with each other to provide selectivity during recombination. His post-doctoral work with at the University of California, Berkeley, aimed at understanding mechanisms of homologous recombination (with Nick Cozzarelli) and at understanding how proteins and RNA interact to achieve accurate but flexible recognition of splice sites (with Don Rio). His current research addresses the mechanisms and biological relevance of genome surveillance processes with particular emphasis on homologous DNA recombination and DNA double-strand break repair. Genome surveillance is essential to prevent chromosomal abnormalities, which in their turn may lead to hereditary diseases, cancer or cell decay. In 2000 he was appointed Professor of Molecular Radiation Genetics. He is a scientific co-founder of the biotechnology company DNage, which is focused on the development of products for medical and health problems associated with ageing. He serves on the scientific advisory board of the FIRC Institute of Molecular Oncology Foundation in Milan, Italy, and on the editorial boards of a number of scientific journals including EMBO Journal and Molecular Cell. In 2002 he was elected as a member of the European Molecular Biology Organization.

The group of Wim Vermeulen has extensive expertise on different aspects (genetic, biochemical and cellular) of mammalian nucleotide excision repair (NER). He has established a micro-needle injection infrastructure, allowing studying DNA repair at the level of (intact) single cells. In addition, he played a major role in dissecting the genetic complexity of rare NER-deficient disorders. In a combined effort with participant 14, these studies formed the basis to the discovery that transcription factor TFIIH has a pivotal role both in repair and transcription, culminating into the concept of a new genetic en-
tity: ‘repair/transcription syndromes’. He developed a new ‘comparative immuno-fluorescence assay’ to directly monitor and compare cellular protein expression level between different cells. He initiated (in close collaboration with Dr. A.B. Houtsmuller) a new line of research, i.e. the study of DNA repair in living cells using GFP-tagging, photobleaching technology and computer simulation. This new area of biological research resulted in novel insights into the highly dynamic organisation of DNA repair in living nuclei. Recently, he extended his research interest towards the interplay between DNA damage and chromatin modifications.

The group of Dik van Gent has extensive expertise on different aspects (genetic, biochemical and cellular) of mammalian non-homologous end-joining (NHEJ). He developed several procedures to study NHEJ in vivo and in vitro. He provided the first evidence that DNA-PK auto-phosphorylation is important for the transition of a closed to an open configuration, which is necessary for subsequent DNA end processing and joining. Furthermore, he has characterized dynamics of NHEJ proteins in living cells using GFP-tagging, combined with laser induced local damage induction and bleaching techniques. Finally, he has also contributed to our understanding of molecular defects in severe combined immunodeficiency (SCID) patients, identifying a unique ligase IV mutation and the first DNA-PKcs patient mutation.
The laboratory of Jiri Bartek & Jiri Lukas studies the molecular mechanisms underlying the major transitions during the mammalian cell division cycle and the way these mechanisms respond to various types of genotoxic stress. The group has contributed to this field by elucidating the molecular pathways coupling mitogenic signalling with cell cycle progression, and by identifying the mechanisms that lead to an instant cell cycle arrest, cessation of DNA replication and chromatin rearrangement in cells exposed to DNA damage. Together with participant 4, the group has been involved in characterization of Mdc1, a founding member of a new class of the checkpoint proteins (‘mediators’) that play important roles in the assembly of diverse signalling molecules on the sites of DNA lesions. Recently, the group has initiated a systematic effort to monitor the dynamics of checkpoint reactions in live cells. These studies revealed the unique ability of the Chk2 kinase to couple the focal DNA damage sites with the pan-nuclear cell cycle effectors. In the latter area, the group has greatly benefited from the ongoing interaction with participant 1.
From left to right: Jiri Bartek and Jiri Lukas
Leon Mullenders studied biophysical chemistry at the University of Nijmegen, The Netherlands. His PhD study on the regulation of DNA replication in mammalian cells had its focus on the role of chromatin structure in the organization of the replication machinery. His postdoctoral work at the Department of Radiation Genetics and Chemical Mutagenesis was focussed on the regulation of nucleotide excision repair particularly the role of chromatin remodellers and chromatin structure. A main achievement was the discovery of a defect in transcription-coupled repair in cells of patients suffering from the rare disorder Cockayne syndrome. His current research focuses on the identification of factors involved in the performance and regulation of early and late steps of DNA damage excision repair particularly the role of chromatin remodellers and chromatin structure. We employ single cell approaches as well as chromatin immuno-precipitation techniques to isolate repair complexes from living cells, biochemistry and mass spectrometry to identify post translational modifications and genome wide technologies to map repair events along the genome. Another part of his research deals with investigations towards the relationship between early (repair, cell cycle arrest, apoptosis) and late events (mutagenesis, cancer) employing animal systems with defined mutations in DNA damage response genes complemented with repair and mutation analysis in cultured cells. He is head of the Department of Toxicogenetics at LUMC since 2001.
Stephen Jackson has been a senior group leader at the Wellcome Trust/Cancer research UK Institute for over ten years. The main focus of research in his lab is on the processes by which cells detect and signal DNA double strand breaks. The aim of his work is to understand the mechanisms by which cells detect DNA damage and then signal these changes to the DNA repair, transcription, cell cycle and apoptotic machineries. Understanding how these pathways operate to maintain viability of cancer cells and how underlying defects in these pathways may lead to cancer, neurodegenerative disease and/or premature ageing may open the way for the development of better therapies for these conditions. Professor Jackson was instrumental in elucidating the non-homologous end-joining (NHEJ) pathway of DNA double-strand break repair. He has also played key roles in establishing mechanisms of DNA damage and signalling, showing how DNA damage repair and signalling proteins function in telomere maintenance. Professor Jackson also founded the company KuDOS Pharmaceuticals Limited, which is exploiting our increasing knowledge of DNA-damage responses to developing novel ways for treating cancer.
Alan Lehmann is Chairman of the Genome Damage and Stability Centre at the University of Sussex. Prior to that he was Section Head of Molecular Biology at the MRC Cell Mutation for many years. He has worked for 35 years on DNA repair with particular emphasis on genetic disorders associated with defects in DNA repair. He was responsible for the discovery of the defect in post replication repair in XP variants, the defect in RNA synthesis recovery in CS cells, the characterisation of defects in TTD cells and the establishment of a large collection of repair-deficient cell strains from patients. He has carried out a molecular analysis of mutations in CS-B, XP-D and XP-V cells and discovered that the site of the mutations in the XPD gene correlates with the clinical phenotype. Most recently he has been investigating the ways in which cells replicate damaged DNA by translesion synthesis with the Y-family DNA polymerases. These, include DNA polymerase eta, defective in XP variant cells. He has contact with numerous clinicians worldwide, who send him samples of patient material for diagnosis and research.

Penelope Jeggo is a senior scientist at the Genome Damage and Stability Centre. She is an international expert on cellular responses to ionising radiation, and their links to human disorders. She identified rodent mutants defective in DNA non homologous end joining, which have substantially contributed to our understanding of the significance and mechanism of this process. Using these rodent mutations, Dr Jeggo identified the dual role of non-homologous end joining in the repair of radiation induced double strand breaks and V(D) J recombination and, together with participant 4, identified Ku and DNA-PKcs as genes functioning in DNA non-homologous end joining. She identified LIG4 syndrome as a disorder conferred by mutations in DNA ligase IV and has recently described a mouse model for this disorder. She has also identified two causative defects for Seckel Syndrome; one being a mutation in the ATR gene, a key gene involved in the checkpoint response to DNA damage, and the second in pericentrin, a key component of the centrosome. Her studies on human damage response disorders have highlighted links between deficiencies in DNA damage responses, immunodeficiency and neurodegeneration. More recently, Dr. Jeggo has described a role for the damage response signalling kinase, ATM, in DNA double strand break repair and shown that ATM is required for the repair of a subset of double strand breaks that localise to heterochromatin. These studies provide insight into the impact of higher order structure on double strand break repair and the role ATM plays in this.

Anthony Carr is Director of the MRC/University of Sussex Genome Damage and Stability Centre (GDSC), a multi-disciplinary and cross organism research Centre. Researchers at the GDSC have been involved in defining and identifying DNA repair and checkpoint genes in both human and model organisms.
and have been at the forefront of understanding the cellular consequences of DNA damage for many years. Carr has identified and characterised most of the key checkpoint proteins in fission yeast. He has played a major role in defining the organisation of the checkpoint pathways, which underlie the DNA damage response (DDR). More recently, the Carr laboratory has led in the biochemical analysis of the checkpoint proteins in fission yeast and his work has been central to establishing this organism as an excellent model to study the structure and function of the DDR pathways. Carr is the world expert in checkpoint analysis in fission yeast. Several of the key human checkpoint proteins (ATR and Chk1) were identified and characterised by Carr and his collaborators. His team’s recent work has combined genetics, bioinformatics and biochemical analysis to make several major contributions to the checkpoint field, including the identification of a role for cyclin-dependent kinase in DSB repair, the identification of a novel mechanism regulating ribonucleotide reductase and insights into the underlying organisation of the checkpoint proteins and how they interact to generate the signal.
Stephen West has been a leader in the recombination/repair field for many years, and is head of the Genetic Recombination Laboratory at the CR-UK Clare Hall laboratories, one of the leading European research institutes with interests in DNA repair. Dr West’s research focuses on the mechanisms of recombination in relation to DNA repair, and he has increasing interests in the mechanisms of genome stability and its relationship to inheritable human disease. His group is known for the groundbreaking recombination studies carried out using E. coli as a model system and was the first to purify and characterise the RecA, RuvA, RuvB and RuvC recombination proteins. Much of our understanding of the mechanisms by which recombination intermediates (Holliday junctions) are formed and processed comes from studies carried out in his lab. More recently, his group moved towards developing an understanding of recombination processes in mammalian systems where he immediately made impact by purifying the human RAD51, RAD52, and DMC1 proteins, and by the development of elegant recombination assays. In his recent work, after an 18 year search, he identified Holliday junction resolvases from yeast and human cells, and has shown that they resolve junctions by a mechanism similar to that exhibited by RuvC. He has also defined the role that the breast cancer tumour suppressor BRCA2 plays in the regulation of RAD51 function, and how defects in interactions between RAD51 and BRCA2 result in a loss of ability to promote recombinational repair, leading to genomic instability.

Work in the laboratory of Jesper Svejstrup has been focused on understanding the cellular responses to DNA damage occurring in the coding region of active genes. Such lesions lead to stalling of the advancing polymerase and are subject to transcription-coupled DNA repair. However, recent work in the Svejstrup lab has uncovered that cells also use an alternative pathway to deal with the problem a damage-stalled RNA polymerase poses, namely RNAPII ubiquitylation and degradation. In general, there is a substantial, but so far only poorly investigated, connection between damage-induction and proteolysis. For example, the majority of yeast genes that are up regulated in response to a number of different DNA damaging agent turn out to be involved in ubiquitylation or proteolysis pathways. The work in the Svejstrup laboratory takes advantage of the powers of yeast genetics combined with biochemical reconstitution of important cellular reactions. This is extended with biochemical studies on the homologous human proteins. Work on human CSB is in collaboration with participant 1, and contacts with participant 14 on different aspects of research on TFIIH and CSB have been close over several years.
Stephen West (left) and Jesper Svejstrup (right)
Josef Jiricny is Director of the Institute of Molecular Cancer Research of the University of Zurich and a Full Professor at the medical faculty of the University of Zurich. In 2001 he was elected joint member of the faculty of sciences and on 1st Oct 2003 he was elected to the independent chair of Functional Genomics at the Swiss Federal Institute of Technology (ETH).

For the past fifteen years, Dr. Jiricny has been studying the molecular mechanisms of mismatch repair. Together with his collaborators he identified several enzymes capable of correcting G/T and G/U mismatches in the DNA of different organisms. Dr. Jiricny was also the first to identify the human mismatch binding factor hMutSα. Dr. Jiricny and his collaborators are currently focusing on the identification of new components of the mismatch repair pathway and on the reconstitution of the entire process from purified recombinant proteins. They are also studying the role(s) of the mismatch repair process in other pathways of DNA metabolism and in DNA damage signalling.
Marco Foiani is professor at the University of Milan and scientific director of the FIRC Institute of Molecular Oncology (IFOM) in Milan. His group has been working in various aspects of chromosome metabolism, including DNA replication, recombination, topology and repair. In recent years he has been studying the regulatory mechanisms that control the integrity of replicating chromosomes. His main focus is on the ATM and ATR-mediated checkpoint responses. He was able to show that the ATR pathway is controlling the stability of stalled replication forks while ATM is more specialized in controlling the integrity of terminal forks. He has been also involved in characterizing the DNA damage response pathway that is activated in response to double strand break (DSB) formation and in particular the role of the cyclin dependent kinase CDK1 in modulating DSB processing and checkpoint activation.

The group is mainly using as a model system the yeast Saccharomyces cerevisiae.
Paolo Plevani has directed for more than 25 years the lab of DNA metabolism at the University of Milano. Research in the group was initially focused on the enzymology of DNA replication and its regulation during the cell cycle. In the last 10 years, with Marco Muzi Falconi acting as co-PI, the major interest shifted toward the identification of genes involved in the DNA damage checkpoint and in the study of the functional and physical interplay between the DNA damage checkpoint and other DNA transactions, such as repair, replication and recombination. This work has been carried out mainly in the yeast Saccharomyces cerevisiae, although more recently the group extended its interest to human cells. The group has a long-standing expertise in the purification and characterization of proteins, and the major finding in this field was the first identification and characterization of the yeast DNA polymerase-primase complex. By developing novel genetic screens, new genes required for proper cellular response to DNA damage were identified and characterized in yeast and showed to be conserved from yeast to man. In general, the group has a strong expertise in yeast molecular genetics and in the study of protein-DNA and protein-protein interactions.
From left to right: Marco Muzi Falconi and Paolo Plevani
Karl-Peter Hopfner is a full professor at the Gene Centre of the University of Munich and director of the Department of Chemistry and Biochemistry of the Ludwig-Maximilians University Munich. Hopfner has 14 years expertise in protein X-ray crystallography and 10 years expertise in the structural biology of enzymes involved genome maintenance and replication. His group studies mechanisms of DNA repair and associated processes in genome stability by X-ray crystallography and biochemistry. Central aspects are the determination of high-resolution crystal structures of genome maintenance enzymes to determine the underlying molecular mechanisms of DNA damage detection and signalling, hybrid structural biology methods and recognition of pathogenic nucleic acids (damaged DNA and viral RNA) in the cell. Key results of K.-P. Hopfner in the last years revealed the first structural mechanism of a Swi2/Snf2 remodeling enzyme and its DNA complex, structural studies on the Rad50 and Mre11 proteins including identification of the zinc-hook tethering motif, the structural biochemistry of DNA repair helicases, and the role of superfamily 2 helicases in innate immunity. His group recently discovered a new type of small molecule second messenger associated with DNA damage signalling in prokaryotes and could show how the translesion synthesis DNA polymerase eta can replicate cisplatin lesions, a DNA adduct used in chemotherapy.
Hans Krokan has headed the DNA repair research group in Trondheim for 19 years, as professor and head of UNIGEN Centre for Molecular Biology, as head of Institute of Cancer Research and Molecular Biology (1998-2002) and now as head of Department of Cancer research and Molecular Medicine (2006-present). The department has now fused with the clinical cancer department and molecular physiology and has been renamed. His research is concentrated on BER, using a broad approach that comprises genomics, proteomics, structural biology, cell biology, animal models and clinical disease. The most important contributions are within repair of uracil-containing DNA. In this area his group has carried the research all the way from protein purification to human disease: purification of the protein and cloning of the UNG-gene encoding nuclear and mitochondrial forms, structure of the UNG catalytic domain, phenotypic changes in gene disrupted mice, and disrupted B-cell function and hyperIgM syndrome (HIGM) in humans carrying UNG-gene mutations. The laboratory has also contributed significantly in other areas, recently most notably by the discovery of the AlkB homologous hABH2 and hABH3, their function in repair of DNA-alkylations (m1A and m3C), and the surprising repair of the same alkylations in RNA by hABH3. Lately he has also headed the Norwegian branch of a multi-centre study headed by IARC/WHO on single nucleotide polymorphisms (SNPs) predisposing to lung cancer.
Magnar Bjørås is the leader of this participant after the death of Erling Seeberg in December 2004. The groups of Arne Klungland and Torbjørn Rognes are also directly involved in the project of this participant. Major scientific contributions have been made in the field of BER of alkylation and oxidative damage in several organisms from bacteria to mammals. Studies on DNA repair are carried out in mammalian, yeast and microbial systems and aim to identify and characterize genes for DNA base repair, genome maintenance, chromatin modification and RNA modification by bioinformatics, biochemical and genetic approaches. The three group leaders of this participant are part of the Centre for Molecular Biology and Neuroscience (CMBN) (www.cmbn.no) that was established as a Norwegian Centre of Excellence in 2002 by the Research Council of Norway. The aim of the Centre is to elucidate DNA damage and repair in relation to neurological disorders and disease with emphasis on the ageing aspects and accumulation of endogenous DNA damage.
From left to right: Magnar Bjørås, Luisa Luna, Arne Klungland and Torbjørn Rognes
Noel Lowndes’ research career spans two decades of effort in leading research laboratories. During that time he has built an international reputation as an expert on the mechanisms of cell cycle control and DNA damage-dependent checkpoint regulation. A thorough understanding of these mechanisms will be important for future cancer treatments. In 2001, he was appointed to the Chair of Biochemistry at the National University of Ireland Galway and leads the Genome Stability Laboratory. Researchers in the Lowndes lab investigate the mechanisms of sensing and responding to DNA damage using yeast and, more recently, DT40 chicken cells as their principal model systems. More specifically, two areas of focus of the Genome stability laboratory are the function and regulation of the checkpoint mediators, Rad9 and 53BP1 as well as the function and regulation of the PIK kinases, Mec1 and ATR.

In addition to his scientific track record and since his recent move to Galway, Professor Lowndes has played a pivotal role in positioning NUI Galway at the forefront of cancer biology within Ireland. In particular, he has led the development of an internationally recognised and expanding cluster of excellence in cell cycle and genome stability studies as founding director of the Genome Stability Cluster, which recently became the Centre of Chromosome Biology (http://www.chromosome.ie/). The CCB currently consists of ten laboratory heads who direct over 60 full time researchers engaged in studies related to the function and regulation of checkpoints, centrosomes, chromatin, DSB repair, DNA synthesis, nucleoli, translesion DNA synthesis and telomeres.
Jean-Marc Egly, a member of the French Science Academy, is Directeur de Recherche at the INSERM. He has been involved on a research project on mechanisms of gene expression since more than 25 years. Research is focused on protein coding gene transcription and associated mechanisms such as transcription activation, DNA repair, splicing and apoptosis. His group has identified some of the factors involved in basal transcription such as TBP, TFIIB, and TFIIH as well as regulatory factors. In addition they were the first to define the role of all the ten subunits of TFIIH thus providing insights for the understanding not only of the transcription initiation reaction but also of the nucleotide excision repair mechanism. In a close and very productive connection with J.H.J. Hoeijmakers, his group elucidate the basis of several disorders such as xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome. His laboratory underlines a strong connection between the hormonal induction and TFIIH in both transcription and DNA repair depicting a crucial step of the hormonal response.
Mark O’Connor is Chief Scientist at KuDOS Pharmaceuticals, a drug discovery company focussed on the identification and development of inhibitors of DNA damage response pathways. He started at KuDOS as Head of New Target Research in 1999 and was one of the first people to use the differential proteomics technology of 2D-DiGE applying it to provide new insights into mammalian DNA damage response. As well as expanding the number of proteins known to be involved in DDR pathway networks, this work has also led to the identification of a new co-factor for p53 (HnRNPK) and more recently a novel DNA-PK-associated protein required for NHEJ. He has also spent the last few years as the translational science lead for olaparib, a novel PARP inhibitor currently in Phase II clinical trials. KuDOS currently has a number of DDR inhibitors in development including inhibitors of DNA-PK, ATM and ATR and has an interest in identifying new targets for DDR inhibitors that may represent future treatments for cancer. KuDOS is also experienced in the development of medium to high throughput assays at both the in vitro and cellular level.