and density of the adhesive ligand, surface topography, texture, rigidity and more. I will also describe specific siRNA screens, which were conducted to functionally map genes that are playing key roles in the formation of focal adhesion and driving cell migration. Specific attention will be focused on the molecular diversity of cancer cells, and difference in the tumorigenic behavior of cancer cell sub-populations differing in their adhesive behavior.

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SYMPOSIUM

DNA repair and genomic instability

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DNA damage and repair: from premature aging and cancer to longevity

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Genome stability mechanisms protect our DNA from damage by exogenous agents (e.g. UV, X-rays, chemicals) and from endogenous metabolism (ROS, lipid peroxidation). One of the most versatile DNA repair systems is nucleotide excision repair (NER), which removes a wide class of helix-distorting lesions. Two sub-pathways exist. Global genome NER operates genome-wide and prevents mainly mutations. Transcription-coupled repair removes damage that obstructs transcription, counteracting cytotoxic effects of DNA injury. Photosensitive inherited NER syndromes include xeroderma pigmentosum (XP, pigmentation abnormalities and high skin cancer predisposition) and the severe neuro-developmental conditions Cockayne syndrome (CS) and trichothiodystrophy (TTD).

Mutations in NER helicases XPB and XPD are associated with all three disorders. XPDTTD mice demonstrated that TTD is in fact a premature ageing syndrome. XPDXP/CS mutant mice are highly predisposed to cancer, but also display premature ageing, demonstrating that both phenotypes can co-exist. Different single and double repair mutants exhibit premature aging features limiting life span ranging from 15 months to 4 weeks depending on the severity of the repair defect. The correlation between severity of compromised repair and rate of onset and severity of the clinical ageing manifestations provides strong arguments for the DNA damage theory of ageing. Conditional mutants in which dramatic aging occurs only in e.g. the brain display many signs of neurodegeneration and only mild aging features in the remainder of the body. We propose that endogenous oxidative lesions hamper transcription/replication and trigger apoptosis-senescence and ageing. Microarray, functional and physiological studies have revealed that persisting DNA damage triggers a systemic downregulation of the IGF1 somatotrophic axis, causing a shift towards energy storage rather than energy production explaining the severe growth defect of the repair mutants. This 'survival' response also maximizes anti-oxidant defence. Interestingly, long-lived dwarf mice and caloric restriction exhibit a similar response. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and we provide evidence that it also implicates regulation by microRNA. These data link accumulation of DNA damage and the IGF1 control of life span.

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Chromatin signaling in DNA damage checkpoint response

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DNA damage repair and checkpoint proteins, chromatin modifying enzymes and other factors are rapidly recruited to DNA double strand breaks, forming a specialized chromatin domain. In metazoan cells, DNA damage foci likely persist until repair is complete and blocking foci formation sensitizes cells to irradiation. While the assembly, components, and functional significance of DNA damage foci remain to be fully defined, an attractive model is that foci facilitate DNA repair but also amplify DNA damage signaling. Via propagation of chromatin modifications and protein assembly over megabases of chromosomal DNA, even individual DNA strand breaks can induce apoptosis or delay cell cycle progression to allow repair and prevent aneuploidy.

Our work has been directed at examining the kinetics and molecular determinants of protein localization to double strand breaks and toward elucidating the functional consequences of disrupting protein recruitment. We are combining imaging and proteomic analysis of DNA damage foci in cancer cells using fluorescent protein fusions to the checkpoint signaling protein 53BP1. The rapid relocalization of GFP-53BP1 has facilitated kinetic analysis of DNA damage foci in living cells and identification of new protein components. We are also developing this approach to track DNA damage and repair in tumor xenograft models treated with radiation.

Taking advantage of the facile molecular genetics in yeast, we have identified determinants of activation of the 53BP1 ortholog Rad9 in G1, S phase and G2/M. In G1, phosphorylation of nucleosomal histone H2A adjacent to break sites by the ATM homolog Tel1 promotes Rad9 recruitment and checkpoint activation. Our data support combinatorial binding to modified chromatin, where tudor domains tether Rad9 to Dot1-methylated histone H3 while BRCT domains recognize phosphorylated H2A. After ionizing radiation in G1 or S phase or uncapping of telomeres in G2/M, mutations blocking signaling via H3 and H2A modifications impair phosphorylation of Rad9, prevent activation of the signal transducing kinase Rad53 and diminish checkpoint response. By contrast, defective Rfa1 single strand binding protein has little or no effect. These data suggest chromatin signaling may be necessary and sufficient for checkpoint initiation, independent of formation of single strand DNA. However, checkpoint persistence requires other factors. After irradiation in G1, Pho85 CDK activity is limiting for checkpoint recovery and S phase onset, while prolonged mitotic arrest after irradiation of nocodazole-arrested cells is independent of H3 and H2A modifications, exposing roles for the Cdc28 CDK, single strand DNA, spindle checkpoint proteins, and/or other factors.

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Mechanisms controlling the integrity of replicating chromosomes

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The checkpoint response and the SUMO-pathway counteract abnormal transitions at replication forks preventing the accumulation of reversed forks and cruciform recombination derivatives resembling hemicatenanes. Although the final targets of these regulatory processes are still unknown, the Sgs1 RecQ helicase and the Top3 topoisomerase have been implicated in the SUMO sub-pathway protecting damaged replication forks. We have investigated whether and how Top2 topoisomerase protect the integrity of replication forks. Our results indicate Top2 counteract torsional stress and sister chromatid entanglement at the forks, thus preventing the diffusion of topological changes along large chromosomal regions, abnormal chromosome transitions, DNA damage checkpoint activation and chromosome breakage during segregation. Altogether our results suggest that Top2 coordinates replication termination and S phase transcription.

We have also analyzed the dynamics of replication forks encountering a double strand break and we have unmasked a role for the Tel1-mediated checkpoint in preventing fork collapse at DNA breaks.

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An oncogene-induced DNA replication stress model for human cancer development

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Background: DNA damage checkpoint genes, such as p53, are frequently mutated in human cancer, but the selective pressure for their inactivation remains elusive. Further, most human cancers have chromosomal instability, but the genes whose mutation leads to this type of genomic instability have also remained elusive. We hypothesized that the presence of DNA double-strand breaks (DSBs) in cancer cells could explain both the presence of p53 mutations and the genomic instability.

Materials and Methods: We analysed a panel of human precancerous and cancerous lesions for the presence of DNA damage response markers using immunohistochemistry and for the presence of genomic instability by loss-of-heterozygosity analysis.

Results: In a panel of lung hyperplasias, all of which retained wild-type p53 genes, we found signs of a DNA damage response, including histone H2AX and Chk2 phosphorylation, p53 accumulation, focal staining of 53BP1 and apoptosis or senescence. Progression to carcinoma was associated with p53, 53BP1 or Chk2 inactivation and suppression of