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3rd US-EU Workshop Systems level understanding of DNA damage responses

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Abstract

The 3rd US-EU Workshop on Systems level understanding of DNA damage responses was held from March 30 - April 1, 2009 in Egmond aan Zee, The Netherlands. Objectives of the workshop were (1) to assess the current science of the DDR, in particular network level responses to chemotherapeutic and environmentally induced DNA damage; and (2) to establish the basis for a reciprocal scientific exchange program between the EU and US in the relevant areas of DDR research. Here we report the highlights of the meeting program and conclude that this third meeting in 2009 refined the role of DDR networks in human disease.

Functional impairment of the DNA damage response (DDR) has been implicated in a plethora of human diseases. While many of the relationships among DDR factors and pathways are known in broad outline, much is yet to be learned about functional relationships and the molecular mechanisms that coordinate DNA-damage/replication signaling with DNA repair and cell cycle control.

Three recent joint EU/US workshops were convened to discuss and promote systems biological approaches to understanding DDR. The first Workshop was held in Cortona, Italy in 2003, the second in Stowe, Vermont, USA in 2005, and the third Workshop¹, the subject of this report, was held March 30-April 1, 2009 at Egmond aan Zee, The Netherlands. The first two Workshops considered the systems level properties of core functions of eukaryotic DNA repair and replication networks, seeking conserved elements, common core functions and protein-level organization that could be applied to higher eukaryotes such as human cells.

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The system biology focus of the third Workshop was on the role of DDR networks in human disease and responses to environmental exposures that are expressed through changes in DDR. The Workshop sessions focused on recent developments in three broad areas: (1) the structure and cellular dynamics of molecular machines that mediate DDR; (2) insights from functional genomics that can be used to identify critical gene and protein interactions that constitute the human DDR; and (3) the use of this information to define biomarkers and therapeutic targets for disease identification and treatment.

The workshop included five plenary sessions, each with a keynote speaker. Session topics were: DNA damage sensing/triggering the signaling system; Cell cycle regulation and DNA repair; Structural biology processes and network dynamics; Proteome and chromatin dynamics; and Systems biology approaches to disease. This report summarizes each keynote lecture in detail, as well as key outcomes from the entire Workshop.

Background

Cells utilize highly complex mechanisms for mitigating the potentially deleterious effects of agents that damage DNA. These mechanisms are collectively known as the DNA damage response (DDR). Extensive studies demonstrate that DNA repair and cell cycle checkpoints are key features of the DDR, and that the DDR is mediated by enzymes and proteins that alter the cellular physiological state and modulate expression and activity of multiple downstream targets, typically via global transcriptional or proteome reprogramming. Post-translational modifications to DDR components, including phosphorylation cascades, also play an important role in mediating the DDR.

Three layers of the DDR have been characterized: sensors/activators, transducers and effectors. However, there is cyclical feedback within the three layers of the response. Key DDR proteins include ataxia telangiectasia mutated (ATM), ataxia telangiectasia-related (ATR), the Mre11/Rad50/Nbs1 (MRN) complex, p53, Chk1 and Chk2. Complex downstream networks are formed by protein targets of these signalling proteins (Figure 1).

Two non-redundant checkpoint pathways have been identified and characterized in mammalian cells: the ATR-ATRIP-Chk1 pathway, which is induced by stalled replication forks and activated by gapped duplex DNA; and the ATM-mediated Chk2 pathway, which is induced by DNA double strand breaks (DSBs). ATR is an essential protein in all proliferating cells, but ATM is not.

ATM was first identified as the protein product of the defective gene that causes a rare human autosomal recessive disease, ataxia telangiectasia (AT). Cells from AT patients are hypersensitive to DSB-inducing agents, identifying ATM as a central player in the response to DSBs. ATM belongs to a family of protein kinases, which includes ATM, ATR, SMG-1, DNA-PKcs, mTOR/FRAP and TRRAP. These proteins share several common domains, including a PI3-kinase-like domain and FAT and FATC domains. Activation of ATM, which correlates with autophosphorylation and probably other post-translational modifications of ATM, occurs at high stoichiometry within minutes after induction of DSBs. In unstressed cells, ATM exists as an inactive dimer; however, ATM dissociates into monomers once activated. The DSB response involves massive recruitment of members of the sensor/activator group to sites of DNA lesions, activation of ATM, and ultimately, formation of γ -H2AX DNA repair foci. In the ATR pathway, RPA is recruited to ssDNA, followed by ATR-ATRIP and the 9-1-1 complex in separate steps. TopBP1 binds the phosphorylated tail of the 9-1-1 complex, and activates ATR through its BRCT domain. These protein complexes stabilize stalled replication forks, prevent replication fork collapse, and promote repair of DNA lesions and DNA replication restart. The ATR/ATRIP and ATM

checkpoints can be activated in a single cell, and there is significant cross-talk between and cross-activation of the ATR/ATRIP and ATM pathways.

DDR activation leads to a cellular decision point between cell death and cell survival. Survival in cells with DNA damage depends on DNA repair (removal of the damage). The seven major DNA repair pathways, which have unique but overlapping specificities for specific DNA lesions, are base excision repair (BER), single strand break repair (SSBR), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), and DSB repair via non-homologous end joining (NHEJ). Translesion DNA synthesis (TLS) by specialized DNA polymerases also contributes to cell survival in cells with replication blocking DNA lesions. While DNA damage is being repaired, cell cycle progression is arrested by checkpoint mechanisms, which mitigate the potentially lethal effects of the DNA damage. Many studies of DDR are modelled on the cellular response to DNA double-strand breaks (DSBs), which cause rapid strong activation of the DDR in mammalian cells.

Workshop Overview

Systems Biology Delivers

Systems biology is no longer an “emerging” research field, but is beginning to deliver on its promise, in the form of insights into human biology and disease. These insights are being revealed by both top-down high throughput screening approaches and bottom-up analyses of the interaction matrices of all cellular components, including protein-protein, protein-DNA, protein-RNA and protein-metabolite interactions and transactions. Combined with powerful bioinformatics, the data yield gross and fine-structural information about the genetic and protein interaction networks that mediate normal cellular functions, as well as the dysfunctional processes that cause human disease. Systems-based studies have highlighted the redundancy of stress response and signaling pathways, and generated system “parts lists” and interactome maps for DDR pathways and subpathways. Thus, systems biological research is applying powerful technologies and effective bioinformatics tools to expand our understanding of and ability to manipulate biological pathways, including DDR.

As reported at the Egmond aan Zee Workshop, systems level discovery research is revealing new nodes and edges in the DDR network. Some of this research is being fueled by recent technological advances in proteomic technologies, such as LC-MS/MS or phosphoproteome analysis, while other studies are exploiting established methods such as TAP-MS or 2D-DIGE to analyze targeted protein interactions in a specific biological context. Jesper Olsen screened and studied dynamics of the human cell proteome and phosphoproteome at different stages of the cell cycle, providing one of the first efforts to quantify cell cycle dependent changes in the phosphoproteome at the systems level. The scope and scale of this effort is unprecedented, such that tens of thousands of phosphosites on thousands of proteins have been identified. This unbiased global screen of the phosphoproteome, as well as targeted screens focused on specific DDR kinases, have identified novel putative substrates of ATM and ATR, and their downstream effectors.

Anne Claude Gavin and collaborators conducted a large scale *in vitro* screen for novel biologically important protein-lipid interactions in yeast. The screen confirmed and validated many lipid-protein interactions that were predicted; the screen also revealed >300 novel interactions, many of which were not predicted from protein sequences, based on our current understanding of protein-lipid interaction motifs. Cryptic lipid binding domains were identified on yeast RasGAP and RhoGAP proteins, and many novel sphingolipid binding proteins were discovered.

Building on Bioinformatics

Bioinformatic tools are essential for analyzing and interpreting the large datasets that -omics technologies generate. The bioinformatics toolbox for this purpose is continuing to expand, and several novel and well-established algorithms were described at the Egmond aan Zee Workshop. These include: SPIKE, EXPANDER, MATISSE, AMADEUS and ALLEGRO – a set of algorithms developed by Ron Shamir for analyzing diverse types of genomics-based expression data as well as human clinical data; R2, a database with a suite of bioinformatics tools developed in Rogier Versteeg's laboratory for analyzing and integrating multiple types of -omics data in a clinical and cancer focused context; ng-LOC, an algorithm that predicts subcellular protein localization, and a predictive algorithm for protein domain-domain interactions (DDIs), both developed in C. Guda's laboratory; and Cytoscape, a data visualization tool developed by Trey Ideker and collaborators. Because data processing is the most time consuming, and perhaps the most challenging, aspect of systems biological research, continued development and improvement of bioinformatics tools and infrastructure, including shared databases, is critical to progress in the field. To promote faster research progress, systems biologists at this Workshop and elsewhere are distributing and sharing newly developed algorithms and bioinformatics tools now (*i.e.*, Cytoscape and ng-LOC) or are planning to do so in the near future.

Novel Transcriptional and Post-transcriptional Mechanisms Regulate DDR

One of the most interesting and unanticipated research areas emerging at the Egmond aan Zee Workshop involves novel transcriptional, post-transcriptional and translational controls that optimize the DDR response. Karen Adelman demonstrated that promoter-proximal stalling in *Drosophila* cells is a mechanism to rapidly induce transcription of a large number of genes involved in cellular stress response pathways, including innate immune response genes. At the post-transcriptional level, Joris Pothof identified three groups of miRNAs that are induced in different kinetic patterns in UV-irradiated yeast. Of particular interest, he reported that miR16 down-regulates expression of *CDC25a* in UV-irradiated yeast and that a significant fraction of DNA-damage-inducible miRNAs are dysregulated in human lung cancer cells. Pothof also showed that miRNA-mediated effects on gene expression in UV-irradiated yeast are slower than protein post-translational modifications, but faster than transcriptionally-mediated changes in expression of target mRNAs, possibly providing another layer of temporal control of cell cycle progression in cells with activated DDR pathways. The responsiveness of miRNAs to UV was also demonstrated in humans (Figure 2). In addition, a novel post-transcriptional mechanism to optimize expression of a subset of yeast DDR genes was described by Tom Begley. This mechanism involves optimized translation of AGA-rich transcripts, which occurs in the presence but not in the absence of the tRNA methyltransferase, Trm9. Yeast *Trm9* is a new node in the yeast DDR network, because it is required for resistance to MMS in yeast. These results show that, in addition to alterations in gene expression and protein stability and/or activity, novel mechanisms and additional layers of complexity are involved in activation and regulation of DDR pathways in stressed cells.

Insights into Structure of DDR Effectors and Kinetics of the DDR response

Structural biological studies are an essential complement to systems level studies, because they reveal the molecular/atomic interaction, which is implied by, but which can not be ascertained from, an edge in a network diagram. Tom Ellenberger, John Tainer, and Phoebe Stewart described recent studies of DNA ligase III, the MRN complex and its subcomplexes, and DNA-PKcs, respectively. Ellenberger and colleagues identified a domain-domain interaction surface in human DNA ligase III, disruption of which selectively inactivates blunt-end DNA joining, without inhibiting nick ligation. If small molecule inhibitors can be targeted to and block this intermolecular domain-domain interaction, it may be possible to

selectively inactivate this essential function of DNA ligase III, and explore its potential as a therapeutic target.

New insights have been gained regarding the spatial and temporal aspects of ATM function in DDR. Penny Jeggo, Chris Bakkenist and Junjie Chen described striking changes in DDR signalling and ATM function, depending on where and when data is collected from within the damage-induced cell. Thus, specific signalling function and activities vary significantly, depending on the kinetic phase of the DSB response and subcellular or subnuclear region of the cell. Chris Bakkenist demonstrated that selective inhibition of ATM with KU55933 from +15 to +75 minutes post-irradiation is sufficient to radiosensitize cells without inhibiting ATM autophosphorylation or ATM-induced cell cycle arrest.

Workshop Buzzwords: Synthetic Lethality and Personalized Medicine

Because constitutive activation of oncogenes in cancer cells often leads to constitutive loss of DDR subpathways, it has been suggested that agents that target functional DDR pathways in cancer cells with a partial DDR-defect will induce cancer cell-selective synthetic lethality. This concept has been validated by Mark O'Connor and his colleagues and several other research groups, who demonstrated that *BRCA1*- and *BRCA2*-deficient cells and tumors are highly sensitive to killing by olaparib, an inhibitor of poly-ADP-ribose polymerase (PARP). Olaparib is currently undergoing clinical development for the treatment of *BRCA1/2*-defective breast, ovarian, pancreatic and colorectal tumors and melanoma. Because olaparib may be widely useful as a therapeutic agent in HR-defective human cancer cells, ongoing studies are aimed at developing biomarkers for HR-deficiency, and correlating HR genotype with olaparib sensitivity. It is hoped that patient selection for HR-deficiency is a feasible approach to improve the therapeutic index for olaparib. Synthetic lethality was also investigated by Trey Ideker, in the context of a high throughput genetic screen covering hundreds of genes in budding yeast, an approach which could help identify many novel potential targets for cancer therapeutics.

The concept of personalized medicine is based on the idea that therapeutic response is highly context-dependent, and that therapeutic response could potentially be predicted from clinical or genetic information about individual patients. At the level of the cell, it is also known that cells respond to DNA damage in a context-dependent genotype-driven manner. Michael Yaffe described studies showing that knockdown or inhibition of ATM or *chk2* causes chemosensitization in p53-defective cells and chemoresistance in p53-proficient cells and that this has important implications for clinical use of small molecule inhibitors of checkpoint kinases in cancer therapy (Figure 3). Most importantly, checkpoint inhibitors are contra-indicated in patients with p53-proficient tumors.

Future Vision: Significant Translational Impact

The ultimate goal of systems level studies of DDR is to develop sufficient knowledge and understanding of the DDR network, and the extent to which DDR capacity is polymorphic in the human population, to generate diagnostic or prognostic tools for clinical use. Results and discussion at the Egmond aan Zee Workshop provide evidence that this goal is achievable and that systems biological studies have significant translational potential. Better understanding of DDR pathways, as developed by systems biological studies, will support development of effective individualized treatment for human diseases including cancer. Development of clinical decision trees based on molecular characterization of tumors in individual cancer patients may be the new holy grail of oncology and the beginning of the much anticipated era of personalized medicine.

TEXT BOX 1

Key Workshop Outcomes

- **Systems biology approaches can be used to infer and analyze global physical and functional interactions between diverse cellular components including genes, proteins, RNA and metabolites.** Such approaches reveal novel biologically important interactions, which can not be predicted from individual gene and protein sequences or other existing data; such studies can also confirm and validate known or previously documented interactions.
- **A large suite of sophisticated bioinformatics tools and customized databases continue to be developed to support systems biology research.** Examples include: SPIKE, EXPANDER, MATISSE, AMADEUS, ALLEGRO, R2, ng-LOC, and Cytoscape.
- **Global phosphoproteomics has identified novel phosphorylation/dephosphorylation events in cells exposed to DNA damage.** Putative ATM targets are enriched among phosphosites that undergo cell-cycle-dependent phosphorylation at high occupancy during S phase.
- **ATM/ATR DDR responses are mediated by downstream effectors Chk2 and Chk1, respectively. p38 activates a third DDR subpathway, whose primary effector is MAP-KAP kinase 2 (MK2). MK2 is effectively “Chk3.”**
- **Cells respond to DNA damage in a context-dependent genotype-driven manner, such that knockdown or inhibition of ATM or Chk2 causes chemosensitization in p53-defective cells and chemoresistance in p53-proficient cells.**
- **The Mre11/Nbs1/Rad50 (MRN) complex is one of the first responders at DNA DSBs. MRN and each of its components are sophisticated protein machines with multiple conformations and multiple functions.**
- **CryoEM structure of the DNA-dependent protein kinase catalytic subunit at subnanometer resolution reveals alpha-helical domains and potential ssDNA and dsDNA binding clefts.**
- **Human DNA Ligase III Δ ZnF (deleted for the zinc finger domain) and DNA Ligase III K323E and R327E mutants are completely unable to conduct blunt-end DNA ligation, but retain a near wild-type level of nick ligation activity.** Residues K323 and R237 lie in a putative interface between the zinc finger and DNA binding domains of human DNA ligase III.
- **Euchromatic DSBs in human cells are largely repaired in an ATM-independent manner, while DSBs localized to heterochromatin require ATM, KAP-1 and 53BP1 for repair.**
- **Inhibition of ATM during a one hour post-DNA damage kinetic window is sufficient to induce radiosensitization.**
- **The *in vivo* spatial and temporal distribution and dynamics of YFP-tagged XPB vary dramatically in different cell types.**
- **Olaparib-sensitivity of triple-negative human breast and human ovarian cancers correlates with expression of six core HR genes.**
- **Promoter-proximal stalling, DNA-damage inducible microRNAs and tRNA-methylation-dependent optimization of protein translation are novel**

transcriptional, post-transcriptional and translational controls that optimize the DDR response.

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Appendix

Session I Keynote Address Systems biology approach to the ATM-mediated damage response Yosef Shiloh (Tel Aviv University)

Complete understanding of the ATM signalling network requires systematic and comprehensive functional characterization of specific phosphorylation sites on validated substrates of ATM protein kinase. **Yosef Shiloh** and colleagues are applying both global and targeted approaches to achieve this goal, such as unbiased screens of phosphoprotein targets of ATM and ATM protein-protein interactions or direct analysis of the phosphorylation status of candidate ATM substrates. Size fractionation followed by co-immunoprecipitation has been used to identify binding partners of ATM, which exists in the cell in large complexes up to 2 mDa in size. Recent studies showed that ATM-associated proteins in these complexes include several novel substrates of ATM. One example is heterodimer of the RING finger proteins RNF20 and RNF40, which together constitute an E3 ubiquitin ligase that monoubiquitinates histone H2B. This monoubiquitination usually occurs at transcribed sites, but following DSB induction it is localized at the damaged sites.

Bioinformatic approaches have been used to compile, analyze and interpret large amounts of information about ATM-mediated signalling pathways. A novel bioinformatic tool for this purpose is an integrated knowledgebase called SPIKE (Signalling Pathway Integrated Knowledge Engine; <http://www.cs.tau.ac.il/~spike>). The power and value of SPIKE is enhanced by the fact that it is a resource both developed and used by entire scientific community studying the DDR and other cellular networks. Transcriptome dynamics have been analyzed on DNA damage-induced wild type cells and cells treated with RNAi targeted to components of ATM-mediated DDR. These studies reveal early (2-3 h) and late (4-6 h) kinetic phases of the ATM-mediated transcriptional response. Comparison of these transcriptomic data and clustering of co-regulated genes using an algorithm called CLICK revealed specific subnetworks of the ATM pathway. An algorithm called PRIMA (Promoter Integration in Microarray Analysis) has been used to identify putative transcription factor (TFs) targets of ATM, and the downstream subnetworks within the larger ATM-mediated DDR pathway. PRIMA scores promoter regions of network genes for enrichment in TF regulatory binding sites. Putative TFs in the ATM signalling pathway identified by PRIMA include p53, NF- κ B, FOXO4, AP-1, and Oct1.

Session II Keynote Address A systems biology approach identifies p53 as a binary switch that re-wires DNA damage signaling-response pathways Michael Yaffe (Massachusetts Institute of Technology)

It has been said that the holy grail of oncology is to develop monotherapeutic treatments that target only cancer cells. However, systems level understanding of cellular DDR pathways now suggests that this view might be too simplistic. To better understand intrinsic susceptibilities of cancer cells to stress perturbations and to design more effective cancer

therapies, **Michael Yaffe** and colleagues undertook systems level network analysis of perturbed cells. The perturbations examined in this study were exogenous TNF α with or without doxorubicin. After perturbation, cells were subject to dense sampling (13 time points over 24 h measuring cell cycle progression, caspase cleavage, expression and activation of multiple signalling kinases) and the data were processed to identify correlated cellular responses using principle component analysis and partial least square regression. A two component model was developed which accurately predicted cellular response (*i.e.*, survival or death) under the conditions tested. The model showed that one component, the death/survival component, was dominated by p38 and γ -H2AX, while the second component was dominated by p53 and Akt. While it is well recognized that ATM- and ATR-dependent stress responses are mediated by downstream effectors chk2 and chk1, respectively, a study by Manke, Yaffe and colleagues reported that p38 activates a third DDR subpathway and that MAP-KAP kinase 2 (MK2) is the downstream effector of this DDR subpathway (Figure 3A). Importantly, knockdown of MK2 reduces survival of p53-deficient but not p53-proficient MEFs exposed to doxorubicin *in vitro*, and knockdown of MK2 enhances chemosensitivity of p53-deficient xenograft tumors in nude mice. Furthermore, p53-deficient MK2-depleted cells lose both the G1/S and G2/M checkpoints, and undergo mitotic catastrophe-induced cell death after DNA damage.

MK2 and chk1 are activated by the same cellular stresses and phosphorylate the same peptide sequence in downstream protein targets. This raises the question of why chk1 and MK2 are not redundant in p53-deficient cells. The answer is that chk1 and MK2 are differentially localized to the nucleus and cytoplasm of stressed cells, respectively. Localization of MK2 to the cytoplasm requires p38 and a nuclear export signal on MK2, and cytoplasmic chk1 (carrying the MK2 nuclear export sequence) complements the phenotype of MK2-depletion.

The above results demonstrate that cells respond to DNA damage in a context-dependent genotype-driven manner. This could have important implications for clinical use of small molecule inhibitors of checkpoint kinases in cancer therapy. Yaffe and colleagues confirmed this idea using *in vitro* and *in vivo* model systems. The results show that knockdown of ATM or chk2 causes chemosensitization in p53-defective cells; in contrast, knockdown of ATM or chk2 causes chemoresistance in p53-proficient cells. The clinical implications of this result depends on the relative frequency of p53-proficient tumors. A survey of approximately 400 human NSCLC and SCLC tumor samples indicated that loss of ATM expression is rarely coincident with loss of p53 expression, and that a significant fraction of tumors are ATM-deficient and p53-proficient. In breast cancer patients who received chemotherapy, the worst prognosis was observed for patients whose tumors were ATM-deficient and p53-proficient. A survey of p53 genotype in cancer cell lines and tumor samples also indicated that p53 defects are significantly more common in cell lines (60-70%) than in human tumor samples (32%). Thus, the frequency of p53-proficient human cancers may be higher than previously thought. While this may present a clinical challenge, appropriately customized therapy, based on understanding of DDR signalling pathways in human cells, could potentially be used to sensitize and neutralize inherently chemoresistant p53-proficient ATM-deficient tumors. One feasible approach would be to limit use of checkpoint inhibitors to patients with p53-deficient ATM/chk2-proficient cancers. Patients with p53-proficient, ATM/chk2-proficient or p53-deficient, ATM/chk2-deficient cancers would be treated with a DNA damaging agent, while patients with refractory p53-proficient, ATM/chk2-deficient tumors would be treated with both a DNA damaging agent and an inhibitor of DNA-PKcs (Figure 3B). Details on this proposal can be found in article by Reinhardt et al, 2009.

The above results and discussion provide evidence for the translational potential of systems biological studies and their ability to support development of effective individualized cancer treatments, which will have fewer adverse effects on healthy tissues and cells. Development of clinical decision trees based on molecular characterization of tumors in individual cancer patients may be the new holy grail of oncology and the beginning of the much anticipated era of personalized medicine.

Session III Keynote Address Functional specialization of mammalian DNA ligases Tom Ellenberger (Washington University)

ATP- and NAD(+)-dependent DNA ligases catalyze nucleotidyl transfer to polynucleotide 5' ends via covalent enzyme-(lysyl-N)-NMP intermediates. Domain mapping and early structural studies of DNA ligases revealed that these enzymes have a shared catalytic core (CC), composed minimally of a nucleotidyltransferase domain and an OB-fold domain. Human ligases III and IV also have a DNA binding domain (DBD) proximal to the CC and a BRCT protein interaction domain distal to the CC. Human DNA ligase III is unique among members of the human DNA ligase family in carrying an N-terminal Zn finger domain (ZnF), which has been described as a link sensor. Biochemical studies of full length and truncated forms of human ligase III revealed that the ZnF domain and DBD domain bind nicked DNA with low affinity and specificity, but the combined ZnF/DBD region of DNA ligase III as well as the CC region of DNA ligase III bind nicked DNA with high efficiency and specificity. Deletion of the ZnF domain (LigIII Δ ZnF) moderately reduces the efficiency of nick-joining by full length DNA ligase III, but completely blocks intermolecular ligation of DNA substrates with blunt or short-overhang containing DNA ends. The weak nick-ligation activity of a truncation mutant carrying only the CC domain of DNA ligase III is stimulated *in trans* by the DBD but inhibited by the ZnF/DBD region.

The crystal structure of the human LigIII Δ ZnF DNA co-complex revealed striking structural homology to human DNA ligase I. However, attempts to crystallize DNA ligase III have been unsuccessful to date. Small angle X-ray scattering (SAXS) has been used to obtain additional structural information and to gain insight into the location of the DNA ligase III ZnF domain during DNA binding. SAXS analysis of DNA ligase III and LigIII Δ ZnF provided evidence that the ZnF domain adopts an extended conformation (larger D_{max} and R_g values) and that LigIII Δ ZnF adopts a more compact protein conformation than DNA ligase III in the presence and absence of a 20mer DNA substrate. Modeling studies identified a positively-charged groove, which could be a putative interface between the DBD and ZnF domains of DNA ligase III (Figure 4). This putative interface region includes residues K323 and R237. DNA Ligase III K323E and R327E mutants are completely unable to conduct blunt-end DNA ligation, but retain a wild type level of nick ligation activity. This result is consistent with functional requirement for the ZnF/DBD region in blunt-end DNA ligation by DNA ligase III. Because blunt-end ligation by human DNA ligase III could play critical biological roles in the mitochondrion or in NHEJ-mediated DNA end-joining in stressed cells, DNA ligase III could be a useful therapeutic target. Small molecule inhibitors that target the putative ZnF/DBD interface in human DNA ligase III are currently being developed and tested for possible therapeutic applications.

Session IV Keynote Address Biochemical approaches to biomolecular networks Anne-Claude Gavin (European Molecular Biology Institute)

Tandem affinity purification-mass spectrometry (TAP-MS) is a highly selective and specific MS method based on epitope-tagging and two step immunoaffinity purification. **Anne-Claude Gavin** and colleagues recently used TAP-MS to conduct a comprehensive analysis

of protein-protein interactions and protein socioaffinity in yeast. More than 5000 yeast strains were generated, each expressing a single TAP-tagged protein as a “bait” molecule, which allowed capture and identification of its co-purifying interacting partners. TAP-MS was used to purify approximately 2000 TAP-tagged protein complexes. This screen covered approximately 60% of the yeast proteome, all sub-cellular locations and assessed proteins expressed over a wide dynamic range. Reproducibility was approximately 69%, and 64% of the complexes were purified more than once, indicating that the screen approached saturation. Heterogeneity and dynamic changes in protein complexes were evaluated. The tendency for proteins to co-purify when they were “bait” or “non-bait” components of a complex or under different experimental conditions was evaluated. In several cases, alternative states of a single complex were characterized. A mathematical model was developed to quantify each protein's socioaffinity, a measure of the strength and reproducibility (confidence) of its protein-protein interactions. This approach is one of the first attempts to characterize the dynamic nature of the yeast protein interactome.

Gavin and collaborators recently interrogated protein-lipid interactions in yeast using a targeted screening approach. An array of lipids and lipid analogs were immobilized on a membrane surface using appropriate chemistry and optimized linkers. The lipid array was then co-incubated with extracts from yeast strains expressing a single TAP-tagged candidate lipid binding protein. The immobilized compounds included 50 physiological lipids or lipid metabolites and 6 non-physiological lipid analogs. Candidate lipid-binding proteins included 172 proteins and enzymes with a known or predicted lipid binding domain (91 candidates) and/or a role in lipid metabolism (32 candidates) as well as other signaling proteins or randomly selected controls (49 controls). Affinity bound proteins were analyzed by mass spectrometry and Western blot. A total of 530 lipid-protein interactions were identified. The data were collated into a series of lipid-binding footprints. Approximately 60% of known lipid-protein interactions were confirmed (based on literature survey), but 68% of the 530 interactions were novel (not previously predicted or reported). For example, novel lipid binding domains were identified in Ecm25, a yeast RhoGAP protein, and Ira2, a yeast RasGAP protein whose human homolog is NF1. The false-negative rate of the screen was estimated to be 40% and the accuracy of the screen was estimated to be >50%.

Sphingolipids are important cell signaling molecules with few known cellular effectors. Surprisingly, a large number of novel PH-domain containing sphingolipid-binding proteins were identified in the protein-lipid interaction screen described above. Because the screen only demonstrates *in vitro* lipid binding capacity, 48 of the putative sphingolipid-binding proteins and 32 control proteins were expressed as GFP fusions, and their sphingolipid binding capacity was confirmed *in vivo*. This experiment showed that membrane association of several of the putative sphingolipid binding proteins was inhibited by myriocin, an inhibitor of sphingolipid biosynthesis. For Slm1-PH, membrane association was unexpectedly shown to require both phosphatidylinositolphosphate lipids and sphingolipids. These studies show that systems biological approaches can be applied to protein-lipid interactions. The protein-lipid interaction screen described above was an effective tool for discovering novel biologically important interactions, which could not be predicted from protein sequence or other existing data, and for confirming and validating known or previously documented protein-lipid interactions. Additional studies will focus on protein-lipid and other protein-metabolite interactions in distinct cell types or in perturbed cells.

Session V Keynote Address Translational responses to exposures optimize the DNA damage response Tom Begley (University of Albany)

Tom Begley and colleagues used genome-wide screens in *E. coli* and yeast to identify genetic modulators of DDR. A significant number of DDR modulators identified in these

screens play functional roles in mRNA translation. These included proteins associated with mRNA binding, translation factors, and tRNA modification systems. *Trm9* is one such gene that is involved in translation and is required for MMS and bleomycin resistance in yeast. *Trm9* is a conserved tRNA methylase that methylates the uridine wobble base of tRNA-Arg and tRNA-Glu. tRNA methylases *Trm4* and *Trm7* were also identified in the screen for modulators of DDR in yeast. The human and mouse homologs of *Trm9* are fused to an *AlkB*-homologous domain. *AlkB* like protein have been shown to be involved in the direct repair of 1-methyladenine and 3-methylcytosine lesion, and the domain fusion of *AlkB*-*Trm9* suggests a possible linkage of translation to the repair of alkylation DNA damage in higher eukaryotic cells. Because *Trm9* alters the codon pairing specificity of tRNA-Arg, leading to enhanced pairing with AGA codons during translation, *Trm9* might promote translation of AGA-rich transcripts. Global computational analysis identified a set of AGA-rich transcripts in yeast, which includes yeast elongation factor 3 (*YEF3*) and ribonucleotide reductase subunits *RNR1* and *RNR3*. Yeast *Trm9* mutants express very low levels of Yef3 protein, but a normal level of *Yef3* mRNA. A similar result was observed for *RNR1* and *RNR3*. In contrast, translation of transcripts that are not AGA-rich was not altered in *Trm9* mutant cells. The DNA damage phenotype in *trm9* mutants was linked to decreased *Rnr1* and *Rnr3* protein levels, which corrupt the DNA damage response by leading to decreased ribonucleotide reductase activity. The decrease in Yef3, *Rnr1*, and *Rnr3* protein levels, independent of transcription, is consistent with a model in which *Trm9* is required for efficient translation of a subset of mRNAs, some of which play key roles in DDR. Coordinated effects of multiple proteins that modulate translational efficiency, including other tRNA methylases, could also help optimize expression of DDR proteins in stressed cells. Preliminary studies indicate that this model may also be valid in human cells and that *Trm9* plays a role in optimizing stress signalling in mammalian cells. Human *Trm9* is found on the short arm of chromosome 8 and published data has demonstrated that transcripts, which include human *TRM9*, are lost in many cancers of the colon and breast. Taken together with yeast functional data there is a distinct possibility that *Trm9* plays a tumor or growth suppressive role in cancer

Abbreviations

AT	Ataxia telangiectasia
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia related
CC	catalytic core
DBD	DNA binding domain
DDR	DNA damage response
DSB	Double strand break
HR	Homologous recombination
HRD	HR-defective
MEF	mouse embryo fibroblast
MMS	methyl methanesulfonate
MMR	mismatch repair
NER	nucleotide excision repair
NHEJ	non-homologous end joining

SSB	single strand DNA binding protein
PARP	poly-ADP-ribose polymerase
SAXS	small angle X-ray scattering
SCE	sister chromatid exchange
TAP-MS	tandem affinity purification/mass spectrometry

Figure 1. The ATM-regulated network

The ATM protein kinase is a master regulator of an intricate web of cellular responses induced by DNA double strand breaks. In the presence of these lesions, ATM sets off a wide array of signaling pathways by directly phosphorylating numerous substrates. The map was drawn by one of the algorithms contained in the SPIKE knowledge base (<http://www.cs.tau.ac.il/~spike>). The interactions shown in the map are selective: only extensively documented functional ATM interactions are shown. The emerging ATM-dependent network contains hundreds of proteins. Violet nodes represent single genes/proteins (denoted by their official symbols), yellow nodes represent protein families, and green nodes represent protein complexes. Blue edges denote regulation relations (→: activation; —|: inhibition; ---○: undetermined). Green arrows denote containment relations among nodes. Red and green dots within a node indicate that not all the regulations and containment relationships stored in SPIKE for that node are displayed.

Figure 2. Differential regulation of miRNA after DNA damage

Heatmap of UV responsive microRNAs. Human dermal fibroblasts (hTERT-immortalized) were exposed to UVC (8J/m²) or mock treated. At 4 and 24 hours total RNA was isolated from both the UVC-treated and mock-treated cells, and microRNA profiling was performed using the Exiqon platform as described (PMID: 19536137). Here UV-responsive microRNAs are shown in a heatmap (using fold changes) that have a false discovery rate (FDR) <5% and were at least 1.5 fold up or down regulated.

Figure 3. DDR Signaling Should influence Chemotherapy Regimens

(A) In response to DNA damage Eukaryotic cells activate a complex signaling network to arrest cell cycle, initiate repair, or induce apoptosis. In addition to ATM/Chk2 and ATR/Chk1 a third DNA damage signaling pathway mediated by p38 MAPK-activation of MAPKAP kinase 2 (MK2). Adapted and reprinted with permission from *Mol Cell*. 2005 Jan 7;17(1):37-48. (B) Detailed knowledge of DNA damage induced signaling pathways has great potential for personalized cancer treatments. Using Xengraft models Yaffe and colleagues have demonstrated that ATM inhibition can result in either pronounced chemoresistance or chemosensitization to DNA damaging agents. They have demonstrated how DNA damage signaling networks can be rewired and showed how pathway focused diagnostics can be used to successfully predict therapeutic outcomes in cancer treatment. Special thanks to H. Christian Reinhardt for creating the figure; adapted and reprinted with permission from *Cell Cycle*. 2009 Oct 1;8(19):3112-9.

Figure 4. Targeting Ligase III Function with Small Molecules

Structural studies on DNA ligase III have provided information on its ability to bind DNA and suggest a way to inhibit ligase activity. It has been proposed that the putative ZnF/DBD interface could be targeted by small molecules and used therapeutically to inhibit NHEJ and kill cancer cells.