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DNA damage response mechanisms and structures fundamental to cancer research progress

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Due to constant damage from endogenous and exogenous sources, all life depends upon DNA repair as well as replication. In every human cell the essential ability to both accurately repair and replicate genomic information depends upon the coordinated actions of complex functional protein-nucleic acid assemblies. Defects in repair and cell cycle regulation are in turn responsible for the cancer hallmarks of genome instability and replication stress (Hanahan and Weinberg, 2000). Furthermore, it has become evident that the complexes responsible for DNA replication and repair stress as well as inflammation and immune responses are keys to cancer susceptibilities as well as markers and targets for cancer intervention. Due to their complexity and multiscale connections to cancer cell biology, the development of unified knowledge and integrated efforts on complexes controlling genome stability from molecules to humans benefits from conferences to stimulate intellectual and experimental collaborations. In this context, DNA replication and repair researchers convened to discuss how these processes contribute to biomolecular function as well as the onset, progression, and prognosis of cancer. The presenters discussed emerging data and advanced methods for examining relevant responses in atomic-level structural detail and in cells. The resulting integrated ideas and efforts support new approaches and rationales for how these complexes and their networks act in regulating biological outcome in organisms from microbes to humans. To make this information broadly accessible, eight exemplary biophysics papers that came into being from work emerging from the presentations and discussions at this symposium are represented here for this Progress in Biophysics and Molecular Biology themed volume.

A review (Cunningham et al., 2019) and a research paper (Bacolla et al., 2019) examine the genome or chromosomal instability (CIN) that is a cancer hallmark (Hanahan and Weinberg, 2000). CIN creates tumor heterogeneity by increasing the rate of genetic alterations, which is both a major challenge and a possible target for the development of advanced therapeutic strategies. The authors note how such DNA instability arises from replication stress, defective DNA damage repair, and ineffective telomere maintenance in cancer cells. In fact, many cancer drugs target replication stress and the DNA repair inhibitors for PARP are already successful in the clinic (Lord and Ashworth, 2017). Cunningham and colleagues provide a thought-provoking and comprehensive consideration on types and mechanisms of genomic instability. Indeed, the authors note that by looking at the interaction networks

among CIN pathways in cancer, loss-of-function and gain-of-function alterations can be targeted. Furthermore, the environment determines if high CIN cells are selected for or against providing a means to control the development of a heterogenous tumor. The ideas and information provided, such as the table of inhibitors targeting CIN in clinical trials (from clinicaltrials.gov), should prove valuable to students and experts alike.

The complementary research paper on genome instability employs a computational approach to uncover mechanisms underlying mutational burden in cancer (Bacolla et al., 2019). The authors show that G4 DNA-forming sequences are enriched twofold at translocation breakpoints. This finding extends other data showing the role of non-B DNA forming sequences in genome instability (Bacolla et al., 2016). The authors furthermore find that breakpoints are overrepresented at G4 DNA-forming sequences and generally occur in tumors with mutations in tumor suppressor genes, such as TP53. Unexpectedly, correlation analyses between mRNA levels and exome mutational loads revealed the strongest positive correlations originated from genes that are not currently listed as cancer genes. The new findings implicate mitochondrial dysfunction plus cell cycle and DNA repair as two main avenues through which gene expression is linked to somatic mutation in cancer. These correlation analyses thus elucidate three underappreciated overall processes driving the accumulation of somatic mutations in cancer cells: G4 DNA, activation of cell cycle and DNA repair pathways, and mitochondrial dysfunction.

Kinases provide the signals for responses to DNA damage and replication stress. A thought-provoking review links two serine/threonine kinases, ATR and mTOR, that respond to stress and belong to the phosphatidylinositol 3-kinase (PI3K)-related kinase (PIKK) family (Lamm et al., 2019). This PIKK family includes the other major kinase regulators of the DNA damage response (ATM and DNA-PK). ATR (ataxia telangiectasia and rad3 related) kinase is the main regulator of the replication stress response from yeast to humans. On the other hand, the mechanistic target of rapamycin, mTOR kinase, regulates multiple cellular responses to environmental stress (e.g. deficiencies in oxygen, nutrients, and growth factors). The authors therefore consider emerging evidence that uncover the role for mTOR in DNA damage and replication stress responses that drive oncogenic transformation. Unbiased transcriptome and transcriptome analysis induced by mTORC1/2 in breast cancer cells indicates DNA replication proteins are the most differentially expressed.

TORC1 regulates FANCD2 (important for repair of both dsDNA breaks and stalled replication forks) and stimulates dNTP biosynthesis important to overcome replication stress by stimulating ribonucleotide reductase activity. The insights presented show that mTORC1 helps balance anaerobic processes (dNTP production, rRNA synthesis + ribosomal proteins + global protein production) with catabolic processes (cell-cycle arrest, autophagy, cell death).

Recombination activities are critical for effecting replication fork rescue and double-strand break repair. A scholarly review cleverly considers a model recombination system and its relationship to more complex eukaryotic systems (Caldwell and Bell, 2019), specifically the Red recombination system of bacteriophage lambda, comprising a resecting exonuclease (homologous to human DNA2) and a single-strand annealing protein (homologous to human

RAD52), for binding and annealing the overhang to a complementary strand. This streamlined phage system unveils fundamental features of recombination: DNA end-resection and linked annealing by homologous pairing. As highlighted in the nuclease structures discussed, DNA steering to nuclease active sites seems a general regulatory feature, as seen for example in other work presented at the conference on the human replication and repair nuclease FEN1, that re-enforces the review theme on the conservation of core DNA repair activities across the domains of life (Tsutakawa et al., 2017). Thus, the comparative analysis discussed enhances our knowledge base and provides implications for genome engineering and eukaryotic mechanisms.

The exciting impacts of cryo-EM on DNA repair structural biology are brought out in a comprehensive review of the structures of the PIKK family kinases ATM, ATR and DNA-PKcs and their complexes (Baretic et al., 2019). The authors build upon and extend the observations and insights from their own cryo-EM studies. Insights presented include novel conformations of ATR in complex with its partner ATRIP and novel dimeric arrangements of DNA-PKcs. Combined X-ray scattering and EM data support the idea that conformational changes in the helical solenoid (HEAT repeats) influence enzymatic activation. As this paper usefully examines ATM, ATR and DNA-PKcs cryo-EM studies in the light of X-ray results and along with the details of the sample preparation and EM setup, it will prove to be a resource for some time. The PIKK regulatory domain (PRD) conformation is emerging to be a function of regulatory inputs switching the activity on and off, but the comparative structural biochemistry suggests variation in this control. Currently, the shift of the PRD between closed and the open dimers suggests mechanisms for ATM and ATR regulation that may involve activation activities from the MRN ATPases nuclease complex (Syed and Tainer, 2018).

Despite the importance of ATM in homologous recombination and ATR in replication fork rescue, in human cells most dsDNA breaks are repaired by the non-homologous end-joining (NHEJ) pathway driven by the PIKK kinase DNA-PK. This makes DNA-PK and the NHEJ critical for both genome integrity and responses to breaks from ionizing radiation used in cancer therapy. Three papers provide in depth and state-of-the-art knowledge on this repair network and furthermore methods for the understanding of such multiprotein machines (Frit et al., 2019; Wu et al., 2019)(PBMB_2019_20). For DNA-PK the precise sequence threading to the structure is important to understand how phosphorylation of specific residues controls activity. A research paper formulates an integrative approach for this problem of sequence assignment to a partial backbone model (SSEThread: Integrative threading of the DNA-PKcs sequence based on data from chemical cross-linking and hydrogen deuterium exchange PBMB_2019_20). This method localizes previously disordered regions using chemical crosslinks and hydrogen deuterium exchange along with sequence connectivity. IMP:SSEThread thus provides a method to represent, sample, and assess alternative sequence threading into a given backbone model, based on multiple input data. Applying this method to the DNA-PKcs crystal structure produced ensemble models providing insight into phosphor-regulation with implications for the allosteric mechanism regulating NHEJ, including a predicted inter-molecular auto-phosphorylation mechanism.

The review focusing on X-ray and cryo-EM studies of DNA-PK shows how the powerful combination of these methods is ushering in the new era of structural biology for understanding the large multi-component assembly of NHEJ at the DNA damage sites and its structural relationship with upstream DNA damage response proteins and chromatin environment (Wu et al., 2019). The functionality of flexible regions is shown in structural differences of the DNA-PK N-terminal region that unveil the impact of interactions between the DNA end binding protein Ku and DNA-PKcs. Importantly, cryo-EM structures of DNA-free and DNA-bound DNA-PK uncover inactive and active functional states that show the conformational changes relevant to kinase activation. Activated DNA-PKcs becomes more compact and the N-terminal region including the helical HEAT1-HEAT8 repeats is uplifted. Indeed, we are finding that EM and X-ray structures of molecular machines can be combined with calculations to better define functionally important movements and how mutations causing mobility defects can promote cancer and other diseases, as seen in the dynamic structure of the multiprotein complex for initiating transcription and repair (Yan et al., 2019).

The eighth paper focuses on the human Ku-DNA hub (Frit et al., 2019) by considering structural and biological knowledge on its interactants and the different classes of Ku-binding motifs (KBMs) identified in several Ku protein partners. Notably, the first binder to dsDNA breaks is Ku, which also binds to single-ended dsDNA breaks during replication that are repaired by homologous recombination and so are removed to avoid NHEJ by the dual action of MRE11 nuclease (Shibata et al., 2014). DNA-PKcs acquires kinase activity after binding Ku making this heterodimer a dominant regulator of the NHEJ kinase. Ku binding motifs (KBM) are thus important for regulation as well as processing and are considered for APLF (A-KBM), XLF (X-KBM), PAXX (P-KBM) and X-family of DNA polymerases (B-KBM). Furthermore, the collective data for NHEJ supports the concomitant binding of most NHEJ components as a supra-molecular machine on the Ku anchor, as recently characterized by single-molecule force experiments as also presented at the conference (Wang et al., 2018). The dynamic flexibility discussed here and in the DNA-PK review allow proximity for activities without obstructing needed functional interactions, as also recently seen for the complex regulating the important cancer protein p53 (Zhou et al., 2019). By combining the available data, the authors develop an interesting integrated structural model of the core NHEJ network at the synapsis step. Importantly this model suggests possible added factors, provides useful insights, and will no doubt stimulate many further experiments.

Collectively, the eight papers and the systems described are largely informative of existing foundational knowledge on DNA repair and replication stress responses emerging from advanced mechanistic and biophysical investigations. There are a few missing emerging areas of damage responses of increasing interest for their cancer connections, notably RNA damage and responses (Thapar et al., 2019) and phase separations associated with unstructured regions of DNA repair proteins, such as 53BP1 (Piccinno et al., 2019). However together the papers in this themed volume examine the critical questions and describe advanced experimental approaches and results likely to prove useful for some time. Overall, they form a compass directing the way forward for defining and analyzing mechanisms and structures of DNA repair and replication stress responses as critical foundational knowledge to advance cancer biology and inform patient therapies.

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