PL-1

Tracking the antibiotic resistome in the environment

Yong-Guan Zhu*

Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, China

Antibiotics have been widely used not only in humans but also in animals for growth promotion and infectious disease control. Antibiotic resistance is defined as the microbial ability to sustain and multiply in the presence of antibiotics. Antibiotic resistance is ancient and ubiquitous in environmental microbes, particularly soils where many antibiotics have been discovered so far, and this original resistance is viewed as intrinsic resistance. Nevertheless, the intensive use of antibiotics in humans and animals have undoubtedly increased the emergence and abundance of antibiotic resistance in the environment, and therefore threatening global human health. Numerous studies now have demonstrated that the amounts of antibiotics use and residual discharge into the environment is well correlated with the abundance of antibiotic resistance, and antibiotic resistance can spread via not only vertical gene transfer but also horizontal gene transfer (HGT), and eventually to human pathogens, and even the emergence of superbugs. In 2006 Pruden *et al*. explicitly proposed antibiotic resistance as emerging contaminants, and suggested that conventional environmental treatment systems were not designed to remove these emerging contaminants. This talk will first discuss the major sources of environmental antibiotic resistome-urban waste water system *vs.* intensive animal farming. Subsequently the talk will discuss the major pathways of the emission of antibiotic resistance genes to the environment (soil and water). Thirdly the talk will take China as an example to investigate the pollution status of antibiotic resistance genes in soils, rivers and estuaries. Finally, potential mitigation measures will be discussed. Throughout the talk, divers molecular tools and "omics" approaches characterizing the environmental antibiotic resistome will be highlighted.

PL-2

Food and physiological factors impacting bioavailability of dietary (poly)phenols

Mario G. Ferruzzi*

Plants for Human Health Institute, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081 USA

Epidemiological evidence across various populations supports the notion that diets high in fruits and vegetables are associated with a decreased risk of chronic disease and overall mortality[1]. Additionally, clinical studies have shown that diets high in polyphenols can improve risk factors for cardiovascular disease and neurodegenerative processes. Mechanisms of action have been postulated including the ability of select polyphenols to modify oxidative and inflammatory stress, impact gut microbial communities, and positively alter functional endpoints including blood flow and cognitive performance. With such promise, interest in the physiological delivery (bioavailability) of polyphenols and their metabolites to target tissues from foods has grown. Polyphenol bioavailability has been the subject of intense investigation. Prevailing opinion is that absorption of polyphenols is generally poor and modified by several factors including (1) chemical form of the polyphenol and composition of the food matrix; (2) potential sensitivity to intestinal conditions; (3) poor intestinal transport; (4) metabolism by mammalian and bacterial systems and (5) rapid excretion from the body[2]. As evidence mounts for a health-protective role for dietary polyphenols, the importance of understanding factors that can improve bioavailability of these compounds has increased. This lecture will present data from our past and ongoing efforts focused on understanding food matrix and select physiological factors that impact absorption, metabolism and tissue distribution of flavan-3-ols, a main dietary polyphenol class.

Through a combination of preclinical (in vitro digestion/Caco-2 intestinal cell and animal models) as well as clinical studies we have investigated the role of food form and formulation on digestive stability, bioaccessibility and bioavailability of flavan-3-ols from tea, cocoa and grape. Macro and micronutrient interactions within the food matrix were found to impact digestive stability and intestinal transport of flavan-3-ol from tea, cocoa and grape products. Co-formulation of tea and cocoa with ascorbic acid and carbohydrate were found to positively influence flavan-3-ol bioavailability while protein incorporation only had a modest impact to overall absorption in both animal models[3,4] and humans[5] despite the ability to enhance stability of flavan-3-ols. These results suggest that product formulation strategies can be leveraged to enhance acute absorption and potentially metabolism of flavan-3-ols from foods. In consideration of the complexity of long-term dietary patterns, we also investigated the impact of background diet (high versus low fat), repeated exposure (10 days or greater) and presence of risk factors (obesity and diabetes) on grape derived flavan-3-ol bioavailability and metabolism. While background diet did not influence polyphenol absorption[6], clear differences were observed in lean versus obese animal models and volunteers[7,8]. Furthermore, evidence of an adaptation in absorption and metabolism of polyphenols during periods of repeated exposure was observed with

an increase in flavan-3-ol bioavailability for rodents[9] and humans[8] over a 10-day repeated exposure to grape polyphenols. Metabolism of flavan-3-ols was also impacted with an increase in glucuronidation but not methylation of monomeric forms observed[10]. Mechanisms were explored using the Caco-2 human intestinal cell model and suggest an enhancement of trans-epithelial transport over time as a result of adaptation in select xenobiotic and metabolizing genes (COMT, ABCC2 and ABCB1) from exposure to flavan-3-ols form green tea and grape seed extracts[11]. Overall, these results suggest that both food and physiological factors can alter bioavailability and metabolism of polyphenols from diets and, that adaptation to polyphenol exposure must be considered when studying metabolite profiles and their association to disease risk and outcomes. Finally, understanding how food matrix and physiological factors can impact polyphenol bioavailability and metabolism will allow for the design of foods and diets consistent with delivery of bioactive polyphenols and their desired health benefits in target populations.

Acknowledgments

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SL-1

How to Write a Great Research Paper, and get Published in a Top Journal

Deirdre Dunne*

Environmental Sciences Department, Elsevier, Amsterdam, The Netherlands

Background: Knowing the best way of structuring your paper when writing it, and the most appropriate journal to send it to, really helps in getting your paper accepted. Also understanding how editors and publishers think and what they expect, and knowing how the peer review process works, is invaluable insight into the publishing process.

Results: After attending this workshop, one in the Elsevier Publishing Connect Workshop series, participants will have a clear idea of the steps needed to be taken before starting to write a paper. They will also be able to plan writing manuscripts using the logical step sequence – not the sequence in which the paper will be read. Authors are also made aware of what aspects of their papers Editors and Publishers look at critically, and to ensure that in taking care of these areas, their papers are much more likely to be accepted. Dealing with referees' comments and the art of polite rebuttal are also described such that these can be used to improve the submitted paper suitably. Sensitive areas such as publishing ethics, plagiarism, duplicate publishing, etc are also clearly explained such that participants have a clear understanding of what is allowed, and what is not permitted.

Conclusions: These insights into the publishing process will enable the participants to be more confident as an author in the world of science publishing, and will help them get their papers published more easily.

SL-2

The Korean Society for Applied Biological Chemistry (ABCH) Seminar on Research Ethics

Hoi-Seon Lee*

Department of Bioenvironmental Chemistry, College of Agriculture & Life Sciences, Chonbuk National University, Jeonju 54896, Republic of Korea

In present-day Korea, studies that explore the fundamentals of science are being actively pursued. Despite all the outstanding achievements of its researchers, however, the prevention and verification of research misconduct has become an important issue. Just as other countries have made systematic efforts to eradicate research misconduct for a long time, there is an urgent need in Korea for taking various measures and holding discussions to formulate a set of research ethics that keep pace with current demands. As a part of this effort, the Korea Ministry of Education (MOE) has authorized a revised version of "Guidelines for the Securing of Research Ethics" (an MOE directive; hereafter referred to as "Research Ethics Guideline," or REG) on November 3, 2015, to reinforce accountability and prevent research misconduct among researchers. The main points in the revised REG include clear guidelines and determining criteria for identifying the type of research misconduct, such as "plagiarism" and "improper authorship." In particular, "redundant publication" was added to the types of research misconduct, while detailed descriptions were provided for "plagiarism" and "improper authorship" (Article 12 of the REG). "Plagiarism" has been defined as the act of using another person's original idea or creation that is not common knowledge without proper citation, thereby causing a third party to perceive the work to be one's own creation. Further, "improper authorship" has been defined as omitting, without justification, to attribute authorship to someone who has contributed to the research content or results, or attributing authorship to a person who did not contribute to the research as a way of expressing gratitude or conferring an honor on him or her. In response to the present demand for the eradication of research misconduct and the establishment of research ethics, the editorial board of the journal of the Korean Society for Applied Biological Chemistry (ABCH) is striving to elevate the qualitative standards of the journal. It aims to do so by rejecting the review of any submitted manuscript with a plagiarism rate of 30% or higher (by using CrossCheck/iThenticate, a plagiarism checker program from Springer-Nature, and excluding references), as well as establishing disciplinary procedures for manuscripts with a plagiarism rate of 60% or higher.

IS1-1

Oxidative DNA lesions, a new insight into alpha-synuclein gene regulation and aggregation toward the pathogenesis of Parkinson's disease

Yoon-Seong Kim*

Burnett School of Biomedical Sciences, University of Central Florida, College of Medicine, Health Sciences Campus at Lake Nona, 6900 Lake Nona Blvd. Orlando, FL 32827, USA

Mutations in alpha-synuclein (α-SYN) encoding gene, *SNCA* were the first reported genetic cause for familiar forms of Parkinson's disease (PD) and it is the major component in Lewy bodies (LBs) and Lewy neurites (LNs), the accumulation of intraneuronal protein aggregates. However, the molecular mechanisms underlying the regulation of expression of α-SYN and pathological processes leading to the formation of LBs/ LNs are unclear. Increasing evidence suggest that oxidative stress is a major culprit in α-SYN pathology. The most frequent DNA lesion caused by oxidative stress is 8-oxo-7,8-dihydroguanine (8-oxodG) and it is often associated with neurodegenerative diseases including PD and aging processes. My lab has recently discovered how this oxidative DNA lesion contributes to α-SYN gene regulation and aggregation process.

First, the role of 8-oxodG in epigenetic regulation of SNCA will be discussed. We found that 8-oxodG accumulations in the substantia nigra (SN) of PD patients are significantly higher than age-matched control subjects. Under oxidative stress conditions, 8-oxodG incorporation in the intron1, a well-studied transcriptional regulatory region of *SNCA,* is elevated. Interestingly, this area is highly occupied by 8-oxoguanine-DNA glycosylase (OGG1), the 8-oxodG-specific repair enzyme. Inhibition of this enzyme reduced α-SYN expression, implying that OGG1-mediated 8-oxodG repair process can positively regulate α-SYN expression. We also investigated histone methylation pattern in the intron 1, identifying that the *SNCA* has both H3K4me3 (active) and H3K27me3 (repressive) present. α-SYN expression can be modulated by the genomic locus-specific modification of histone mark by the innovative CRISPR/Cas9-Suntag system.

Second, 8-oxodG-mediated transcriptional mutagenesis (TM) and its contribution to pathological aggregation of α-SYN will be discussed. In terminally differentiated cells like neurons, 8-oxodG DNA lesions in the transcribed strand could be bypassed by RNA polymerase II, and generate erroneous proteins through a process called transcriptional mutagenesis. We have recently identified a various TM-generated mRNA mutant species including S42Y and A53E from the brain tissues of PD patients. We have also found S42Y-positive LBs and LNs from postmortem brain samples of PD and dementia with Lewy bodies (DLB) using highly specific anti-S42Y antibody. This result strongly suggests that small amounts of various α-SYN mutants generated from 8-oxodG-mediated TM process potentially contribute to α-SYN aggregation and pathogenesis of PD.

Together our research introduces for the first time an intriguing novel mechanism underpinning how oxidative stress is critically involved in α-SYN expression and aggregation.

IS1-2

Novel epitope tags (2B8/3H7) and antibodies for protein detection, purification and other applications (The better alternatives of commercial Myc/Flag/His tags)

Seong Hee Bhoo*

Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Korea

Bacteriophytochromes are phytochrome-like light-sensing photoreceptors that use biliverdin as a chromophore. To study the biochemical properties of the Deinococcus radiodurans bacteriophytochrome (DrBphP) protein, two anti-DrBphP mouse monoclonal antibodies (2B8 and 3H7) were generated. Their specific epitopes were identified in our previous report. We present here fine epitope mapping of these two antibodies by using truncation and substitution of original epitope sequences in order to identify minimized epitope peptides. The previously reported original epitope sequences for 2B8 and 3H7 were truncated from both sides. Our analysis showed that the minimal peptide sequence lengths for 2B8 and 3H7 antibodies were nine amino acids (RDPLPFFPP) and six amino acids (PGEIEE), respectively. We further characterized these peptides in order to investigate their reactivity after single deletion and single substitution of the original peptides. We found that single-substituted 2B8 epitope (RDPLPAFPP) and dual-substituted 3H7 epitope (PGEIAD) showed significantly increased reactivity. These two antibodies with high reactivity for the short modified peptide sequences are valueble for developing new peptide tags for protein research.

IS1-3

Regulatory mechanism and functional characterization of ginseng saponin biosynthesis-related genes in a plant system

Ngoc Quy Nguyen, Jinhun Jang, and Ok Ran Lee*

Department of Plant Biotechnology, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea

Ginsenosides are glycosylated triterpenes, considered important pharmaceutically active compounds, from an adaptogenic herb *Panax ginseng* Meyer. Ginsenoside backbones, categorized as protopanaxadiol, protopanaxatriol, and oleanane saponin, are synthesized via the isoprenoid pathway by cyclization of 2,3-oxidosqualene mediated with dammarenediol synthase or beta-amyrin synthase, starting from the first committed step catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR). However, the regulatory mechanism underlying the triterpene saponin biosynthesis via the mevalonate pathway in ginseng remains unclear. Therefore, in this study, we first characterized two HMGR homologs (*PgHMGRs*) from *P. ginseng*. Heterologous overexpression of *PgHMGR* in *Arabidopsis* showed subcellular localization in the endoplasmic reticulum and a spherical vesicular structure similar to the previously reported endogenous HMGR. *PgHMGR1* is a functional ortholog of Arabidopsis *HMGR1*, and its expression is regulated at the transcriptional level. Treatment of ginseng with mevinolin, a competitive inhibitor of HMGR, led to a significant reduction in the total ginsenoside content, which suggests an important role of the mevalonate pathway in ginseng saponin biosynthesis. Continuous exposure to dark resulted in reduced total ginsenoside content in 3-year-old ginseng, which suggests a dark-dependent regulation of secondary metabolite biosynthesis in the ginseng plant. Other ginsenoside biosynthesis-related genes will also be addressed.

IS1-4

Structural analysis of PPARγ - lobeglitazone complex reveals key determinants for the recognition of antidiabetic TZD drugs

Young Jun Im*

College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea

Peroxisome proliferator-activator receptor (PPAR) γ is a member of the nuclear hormone receptors that plays a crucial role in the regulation of glucose and lipid metabolism. As PPARγ agonists, thiazolidindiones (TZDs) have been widely used for the treatment of type 2 diabetes. Recently, lobeglitazone (Duvie^{R}) was developed as a potent PPARγ agonist with reduced side effects by Chong Kun Dang pharmacetical co. To discover the structural determinants for the high efficacy of lobeglitazone compared to other TDZs, we determined the crystal structures of PPARγ in complex with lobeglitazone and pioglitazone at 1.5 and 1.8 Å resolution, respectively. Structural comparison of PPARγ bound to lobeglitazone and pioglitazone reveals that the binding mode of TDZs are well conserved. However, additional *p*-methoxyphenoxy group of lobeglitazone makes complementary hydrophobic contacts with the ligand binding pocket. Computational docking analysis using TDZ-bound structures suggests that lobeglitazone displays at least 10 times higher affinity to PPARg compared to rosiglitazone and pioglitazone. This structural difference correlates with the enhanced affinity and the lower effective dose of lobeglitazone compared to rosiglitazone and pioglitazone.

IS1-5

Plant Proteomics and International Plant Proteomics Organization (INPPO): A Road Map towards Future

Ganesh Kumar Agrawal[1,2*], Abhijit Sarkar[3], Sun Tae Kim[4], Antonio Masi[5], Randeep Rakwal[1,2,6]

[1]Research Laboratory for Biotechnology and Biochemistry (RLABB), GPO 13265, Kathmandu, Nepal, [2]GRADE Academy Pvt. Ltd, Adarsh Nagar - 13, Birgunj, Nepal, [3]Laboratory of Applied Stress Biology, Department of Botany, University of Gour Banga, Malda – 732 103, West Bengal, India, [4]Department of Plant Bioscience, Life and Industry Convergence Research Institute, Pusan National University, Miryang, 627-706, South Korea, [5]DAFNAE, University of Padova, Legnaro, Italy, [6]Faculty of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8574, Japan

Plant proteomics has progressed tremendously since the year 2000 when few proteomics studies were performed and published; i.e., plant proteomics was in its infancy. Progress involved the refinement of the existing techniques, starting with two-dimensional gel electrophoresis, the search for and development of new techniques, and the utilization of these techniques towards better understanding plant biology. This also opened the way to properly start addressing the food security, safety, and health issues at the global level. These progresses are now beginning to transform the plant proteomics field, i.e., the extended roles of proteomics in accelerating the process of understanding plant biology, expansion of its disciplines such as nanoproteomics, applications in addressing global ground-level issues such as food and health under the umbrella of term translational plant proteomics, and community-based organization. During my presentation, I will provide few examples to explain how plant proteomics is being modified or adapted. Establishment of International Plant Proteomics Organization (INPPO; www.inppo.com) – a non-governmental, non-profit, global interactive platform, consisting of mainly plant biologists (those who are working and / or interested in plant proteomics – the "plant proteomers") – will be also presented with its roles in creating a physical as well as virtual '*knowledge network*' for development, improvement and dissemination of plant proteomic knowledge worldwide. Last but not the least, INPPO involvement in drafting a road map for the future of plant proteomics will be highlighted.

IS1-6

Violacein: It's Production and Activity against Multidrug-Resistant *Staphylococcus aureus*

Robert J. Mitchell*

School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST)

According to a recent announcement from the World Health Organization (WHO) (http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed), new antibiotics are desperately needed to combat multidrug resistant pathogens and their infections. One potential candidate that has gathered attention lately is the bisindole violacein. This compound is produced by several different Gram-negative bacterial strains through a condensation reaction involving two tryptophans and is known to be particularly effective against Gram-positive bacterial strains. Consequently, we are characterizing the activity of violacein against MRSA within our lab as well as considering other applications for this antibiotic.

In the first portion of this talk, I will describe the isolation and characterization of a novel violacein producing strains, *Duganella violaceinigra* str. NI28, as well as our effort to produce this compound in recombinant bacterial strains. We found the activity of the purified violacein against *S. aureus* ATCC 25923 and *S. aureus* CCARM 3840 was identical. The ATCC strain is not resistant to any antibiotics while the latter (CCARM 3840) is resistant to six antibiotics, *i.e.*, ciprofloxacin, clindamycin, oxacillin, erythromycin, gentamycin and tobramycin. For both strains, the minimum inhibitory concentration (MIC) for violacein was 15 μM. Similar results were also obtained with a clinical isolate that was resistant to the same six antibiotics plus rifampin. These findings demonstrate that, although these strains are resistant to numerous antibiotics, this resistance does not diminish the efficacy of violacein. We also show that violacein acts as a bacteriostatic agent at 15 μM as it prevented growth of the MRSA populations, while higher concentrations were bactericidal towards this pathogen. In tests with 60 μM violacein, the viable *S. aureus* ATCC 25923 and clinical isolate populations both decreased by 2-log after 24 hours. Once more, these results show the multidrug resistant nature of the clinical isolate offered no benefit.

In the latter half of my talk, I will describe efforts on our part to develop anti-MRSA fabrics in collaboration with a clothing company. In addition to being an antibiotic, violacein is also a noteworthy bacterial dye. Combining these two characteristics, we developed fabrics that are effective against MRSA for use in medical settings. The dyed cottons were found to be very effective, preventing *S. aureus* growth by more than 2-log. Moreover, even after the dyed fabrics were washed ten times in a washing machine, they still retained their activity against *S. aureus*, illustrating the potential reuse of these anti-MRSA fabrics.

S1-1

Lab-on-a-Chip Technologies for Molecule Studies

Chiwan Koo*

Department of Electronics & Control Engineering, Hanbat National University, Daejeon 34158, Korea

Lab-on-a-chip or microfluidics is the micro-technique that allows small samples of fluids to be prepared and analyzed within the confines of a microchip, using unique arrangement of microchannels that serve as pathways for the movement of fluid samples on a glass or polymer substrate. The advantages of microfluidic devices are scale-down, integration and rapid process and these properties have made microfluidics attractive candidates to replace traditional experimental approaches. Several lab-on-a-chip devices for molecules studies such as a stand-alone portable real-time PCR system and microfluidic electro-sonoporation device will be introduced.

S1-2

Strategies for Enhanced Production of Stilbene Compounds in Plant and Cell Culture Systems

Yu Jeong Jeong, Chul Han An, Ji Hye Park, Jae Cheol Jeong, and Cha Young Kim*

Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 181 Ipsin-gil, Jeongeup-si, Jeonbuk 56212, Korea

The biosynthesis of flavonoids such as anthocyanin and stilbenes has attracted increasing attention because of their potential health benefits. Anthocyanins and stilbenes share common phenylpropanoid precursor pathways. We previously reported that overexpression of sweetpotato R2R3-type *MYB* gene *IbMYB1a* (*IbMYB1a*-OX) induced anthocyanin pigmentation in transgenic *Arabidopsis* and tobacco plants. Here, we also generated transgenic plants overexpressing a stilbene synthase gene (*STS*) to obtain a high-level production of stilbene compounds in transgenic plants. However, low levels of resveratrol compounds were produced in transgenic tobacco plants (*STS*-OX) plants. Therefore, to improve the production of stilbene compounds in plants, we cross-pollinated flowers of *STS*-OX or *ROST*-OX and *IbMYB1a*-OX transgenic lines (SM and RSM). Phenotypic changes in vegetative and reproductive development of SM and RSM plants were observed. Furthermore, by HPLC and LC-MS analyses, we found enhanced production of stilbene compounds such as piceid, piceid methyl ether, resveratrol methyl ether *O*-hexoside, and 5-methyl resveratrol-3,4′-*O*-β-D-diglucopyranoside in SM and RSM cross-pollinated lines. Thus, we suggest that coexpression of *RpSTS* and *IbMYB1a* via cross-pollination can induce enhanced production of resveratrol compounds in plants by increasing metabolic flux into stilbenoid biosynthesis. In addition, as an alternative approach, we employed the cell culture system of grape (*Vitis vinifera*) for production of resveratrol derivatives. Here, we established the elicitation and secretion conditions for high-level production of resveratrol and its oligomers such as viniferin, a resveratrol dehydrodimer in grape cell suspension cultures. In particular, we developed the conditional production of resveratrol and viniferin in the culture media using different solubilizers. In this presentation, our current studies will be addressed.

S1-3

Transcriptional Elongation Requires DNA Break and Damage Response Signaling

Heeyoun Bunch[1*], Brian P. Lawney[2], Xiaofeng Zheng[3], Adam Burkholder[4], Shmulik Motola[5], Christopher C. Ebmeier[6], Gabriel Birrane[1], Stuart S. Levine[5], David Fargo[4], Yaoyu E. Wang[2], Benjamin P.C. Chen[7], Guang Hu[3], Dylan J. Taatjes[6], Stuart K. Calderwood[1]

[1]Harvard University, Boston, Massachusetts 02115, USA, [2]Center for Cancer Computational Biology, Dana Farber Cancer Institute, Boston, Massachusetts 02130, USA, [3]Laboratory of Molecular Carcinogenesis, National Institutes of Health, RTP, North Carolina 27705, USA, [4]Integrative Bioinformatics, National Institutes of Health, RTP, North Carolina 27705, USA, [5]BioMicro Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA, [6]Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80303, USA, [7]University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA

RNA polymerase II (Pol II) promoter-proximal pausing is a major transcriptional regulatory step in a number of protein coding and non-protein coding genes. Pol II pausing occurs during the transition between the early and processive transcriptional elongation. Initially, we identified Tripartite motif-containing 28 (TRIM28), a protein known as a transcriptional repressor and DNA repair factor, to bind to the non-template DNA including the Pol II pausing site of human *hsp70* through an unbiased method. Biochemical, cell biology, and genomic analyses have shown a biphasic characteristic of TRIM28: TRIM28 stabilizes Pol II pausing during the un-induced state of transcription, and is also phosphorylated at serine 824 by Ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK), key kinases of DNA damage response signaling, during Pol II pause release. Consecutively, we have found that topoisomerase II and DNA repair proteins such as γH2AX, DNA-PK, and TRIM28 are recruited and activated on many stimulus-inducible protein-coding genes during Pol II pause release. Inhibiting topoisomerase II or DNA-PK, thus blocking DNA double strand break or DNA damage response signaling, respectively, interferes with Pol II pause release and productive elongation. Together, our data suggest the coupling of transcriptional elongation with DNA damage response signaling. We propose that DNA break and damage response signaling is required for Pol II pause release and processive elongation in humans.