

izations to conduct a project intended to demonstrate how a deep knowledge of cell signaling pathways could be directly used to conduct human health risk assessments. Using a case study approach focused on a few key prototype nuclear receptor and stress-response pathways, we are applying an integrated systems toxicology approach to map and model these pathways. The goals of this program are to: (1) develop *in vitro* assays for relevant compounds in appropriate cells/tissues; (2) use a suite of tools to create a dense data stream on dose response; (3) apply bioinformatics tools to infer pathway circuitry while generating a computational systems biology model of the circuitry, and 4) create dose-response curves for the various assayed endpoints. In a related effort, a consortium of researchers led by

Dr. Thomas Hartung at The Johns Hopkins University, have begun to map estrogenic pathways in human breast cancer cells using a combination of transcriptomics and metabolomics (<http://altweb.jhsph.edu/news/current/caatnihgrant.html>). In this talk, we describe these research consortia in more detail. We also describe the suite of technology platforms used in these studies and show how these tools are being and have been applied in our studies. We can now identify specific technologies and experiments that will accelerate completion of the first-phase of pathway mapping and modeling. We also discuss how these integrated data packages are shaping, informing and modifying our conventional views of toxicity pathways.

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## From integrated signaling networks to high content microscopy: Systems approaches for TT21C

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**Abstract:** Toxicogenomics has played a major role in the past decade to uncover cellular stress responses that underlie chemical-induced adverse reactions at the cellular as well as organ level and subsequently apply transcriptomics-based classifiers to predict adverse outcome. The question remains what functional role these stress response pathways as well as the individual genes that underlie these stress responses play in the onset of adversity. We have integrated transcriptomics, phosphoproteomics, and RNA interference (RNAi) approaches and used time-resolved live cell high content imaging of cellular stress responses to identify critical cell signaling components that determine the breaking point from adaptation to cell stress versus maladaptation and onset of cell death. In pluripotent stem cells, DNA damage triggers loss-of-pluripotency and apoptosis as a safeguard to exclude damaged DNA from the lineage. An intricate DNA damage response (DDR) signaling network ensures that the response is proportional to the severity of the damage. We combined an RNAi screen targeting all

kinases, phosphatases, and transcription factors with global transcriptomics and phosphoproteomics to map the DDR in mouse embryonic stem cells treated with the DNA crosslinker cisplatin. Integrated networks derived from canonical pathways shared in all three datasets, were implicated in DNA damage repair, cell cycle and survival, and differentiation. Experimental probing of these networks identified, amongst others a novel, p53-independent mode of DNA damage-induced Wnt signaling that limits apoptosis. Our findings reveal a balance between p53-mediated elimination of stem cells, through loss-of-pluripotency and apoptosis, and Wnt signaling that attenuates this response to tune the outcome of the DDR. We currently explore several other newly identified signaling networks that modulate the outcome of the DDR. To further reveal the complexity of dynamic toxicity-related signaling events we have developed systems microscopy technologies. Here, quantitative live cell confocal imaging of dynamic cell biology processes is followed by quantitative multiparameter image analysis to pro-

vide cell-to-cell dynamic data for systems biology modeling. A range of BAC-GFP reporters has been developed and expressed in HepG2 lines for this purpose. We have used this approach to study the complex signaling involved in drug-induced liver injury (DILI), an important clinical problem that involves crosstalk between drug toxicity and the immune system. Transcriptomics analysis established critical drug-induced toxicity pathways that act in synergy with the pro-inflammatory cytokine tumor necrosis factor  $\alpha$  (TNF $\alpha$ )

to cause cell death of liver HepG2 cells. Live cell imaging of oscillatory NF- $\kappa$ B cytoplasmic-nuclear translocations and activation of distinct gene reporters provided further insight into the joint regulation of these pathways by TNF and compounds associated with DILI in humans. Focused RNAi experiments and pharmacological inhibition is currently employed to probe these pathways for critical hubs in liver cells that are targeted by drugs and pro-inflammatory cytokines and control life/death decisions.

## Mitochondrial toxicity and new China goals for TT21C/Adverse Outcome Pathways

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**Abstract:** Mitochondria are major sources of cellular energy and reactive oxygen species (ROS), playing fundamental roles in regulation of cell survival and death under pathological conditions. Numerous chemicals have shown to significantly induce mitochondrial toxicity in targeted organ such as liver and heart. Early identification of mitochondrial toxicity for chemicals is important for avoiding hazard exposure in human or environment. However, due to the complexity of mitochondrial toxicity which may involve multiple mechanisms, the assessment of mitochondrial toxicity based on animal test is unsatisfactory. The release of *Toxicity Testing in the Twenty-First Century: Vision and A Strategy* by the US National Research Council brings new perspectives to the strategy and methods on the assessment of chemical-induced mitochondrial toxicity. The development of toxicity testing of mitochondrial toxicity is also transforming expensive and lengthy traditional *in vivo* animal tests to *in vitro* high-throughput alternative methods with quantitative parameters analysis and mechanistic exploration. Recently, many *in vitro* alternative methods have been established for accessing chemical-induced

mitochondrial toxicity, such as high content screening, mechanism-based toxicity testing (eg, mitochondrial membrane potential, ATP production, oxygen consumption rate, oxidative phosphorylation, and mitochondrial superoxide generation). In particular, adverse outcome pathway (AOP) for mitochondrial toxicity is highlighted to get a better mechanistic understanding and *in vitro* to *in vivo* extrapolation. Mitochondrial AOP integrates from chemical characterization to molecular initiating event (MIE) and population response. A significant effort has been made by Chinese scientists to establish and develop Chinese TT21C/AOP. Based on previous studies in toxicological alternatives, many new projects have been carried out using TT21C strategies to access toxicity of various chemicals.

**Key words:** mitochondrial toxicity; toxicity testing; risk assessment

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