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An abstract modelling framework for adverse outcome pathways

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Problem Overview

To develop a top-down modelling framework for Adverse Outcome Pathways (AOPs), that will be complementary to current bottom-up mechanistic models, with a view to using a combination of these approaches for risk assessment of chemical exposure to humans.

Background

Within toxicology there is a significant shift from qualitative descriptors of adverse endpoints in surrogate species, to quantitative models based on human biology. This shift was precipitated by, in large part, the National Research Council report 'Toxicity Testing in the 21st Century' (TT21C) [1]. One strategy, proposed in the TT21C report, is to recognise that toxic responses are generated through a limited number of so-called AOPs. These are normal gene regulatory and signalling networks that, when perturbed by a chemical (for example, by the chemical binding to a particular receptor or protein), lead to adverse health effects.

While the details of each AOP will differ, it is largely thought that for low levels of chemical exposure, AOPs will elicit an adaptive response, ensuring that normal cell function is maintained. However, at higher exposure levels, adaptation will fail and cells will switch to an adverse response (such as programmed cell death). Thus, from a safety-assessment perspective a key goal is to be able to identify the exposure of a chemical at which the adaptive-adverse switch occurs in humans. Of course, the dynamics of AOPs can only be studied to a limited extent in humans. Instead, TT21C strategy requires that the AOPs are experimentally characterised *in vitro*, and then these observations are used to determine safe levels of chemical exposure *in vivo*.

Several AOPs have been identified in the literature, including ones for oxidative stress, DNA damage and heat shock response [1]. Mathematical models of these pathways are typically based on known or hypothesised interactions within the networks (and are hence mechanistic in nature and are developed bottom-up). While these models have been successfully used to elucidate specific experimental observations on AOPs [2-4], such an approach typically requires that the relevant networks are experimentally well-characterised. In reality, this requirement will only be met by a few AOPs, and even for these, many network components or interactions will be missing. While simple mechanistic models can be used in concert with experiments to first hypothesise and then validate specific network components and interactions, this can be a slow and resource-intensive process. Moreover, since the networks will be incomplete, such an approach may not lead to good predictions on what exposure scenarios might cause cells to switch from an adaptive response to an adverse one, and therefore not be 'useful' from the perspective of safety assessment. Nevertheless, these approaches do allow for experimental data to be related back to model outputs for validation purposes, and the additional insight they yield (providing they are simple enough) can help increase confidence in a particular safety assessment.

Motivation: could abstract models be used to compliment mechanistic ones?

We wish to explore strategies that could help complement the 'bottom-up' approach offered by purely mechanistic models. In other fields of biology, various complex phenomena have been studied very effectively using so-called 'abstract' models. Here, rather than have the model

composed of variables representing specific molecular players and interactions, state variables will represent abstract concepts and in this sense provide a 'high-level' description of the system. For example, in environmental toxicology, Dynamic Energy Budget (DEB) models are used to study how chemicals effect the growth, maturation and reproduction of organisms at the population or eco-system scale [5]. Importantly, the state variables in DEB models are abstract concepts such as (energy) reserve and (organismal) structure, rather than individual molecular players, and are used to assess the impact of chemical exposure on the environment [5]. In the case of developmental biology, abstract models have been used to study phyllotactic patterns in plants (how leaves are organised along a stem). These patterns are thought to be driven by complex interactions between plant hormones and downstream regulatory networks, which are only partially understood. However, Douady and Couder [6] demonstrated that these patterns could be understood by assuming a few simple rules: new leaf primordia emerge at the stem apex, primordia emit an inhibitory field on other nearby primordia, and that as the stem grows the leaf primordia move out radially. This simple model is able to capture the complex phyllotactic patterns observed in nature in a quantitatively accurate manner. Vernoux and co-workers have since extended this approach so that the state variables and parameters of the abstract model can be related back to specific molecular players [7]. By doing so, the abstract model was then used to elucidate complex mutant phenotypes and identify new molecular mechanisms important to the patterning process [8]. Other examples include the work Riedel-Kruse et al. [9], wherein the segmentation of vertebrate embryos was studied using an abstract system of coupled oscillators, leading to new insights on how specific mutations cause complex developmental phenotypes to form. Thus, combining top-down abstract models with bottom-up mechanistic ones can provide a powerful tool for studying complex biological processes.

Problem specifics: oxidative stress and DNA damage

We are interested to explore whether an abstract modelling approach could be applied to studying AOPs. For simplicity, we wish to focus on two well-characterised pathways: oxidative stress and DNA damage. For example, with the oxidative stress pathway, alterations in the levels of reactive oxygen species (ROS) are detected by KEAP1-NRF2 complexes as follows. In normal cells, KEAP1 binds to NRF2 to form a stable complex, preventing NRF2 from entering the nucleus. However, for excess levels of ROS, KEAP1 is oxidised, causing NRF2 to unbind and translocate to the nucleus, allowing it to activate downstream genes and thereby promote a complex repertoire of antioxidant responses. Mechanistic models of this homeostatic aspect of the oxidative stress pathway have appeared in the literature (see for example [10]). However, excess ROS can also lead to increased lipid peroxidation, abnormal protein aggregation, and other adverse effects such as activation of inflammation pathways (via NF-κB signalling), or potentially programmed cell death (via P62 signalling). Thus, as with other AOPs, the oxidative stress pathway is potentially very large and complex, and any model developed bottom-up will likely be incomplete. The DNA damage AOP involves a similarly complex set of feedback loops involving p53, ATM and γH2AX (among others; see for example [2,3,11]).

Although the composition of both AOPs are quite distinct, the networks are similar from an abstract perspective in that cells: 1) sense the damage caused by a chemical insult; 2) induce mechanisms to repair the damage and possibly remove the chemical (adaptive response); 3) in the event that damage incurred is too great, an adverse response is initiated. A key challenge is to identify whether these or other abstract concepts can be used to form the basis of a model for studying the adaptive-adverse switch in these AOPs. A long term goal would then be to explore whether these ideas can be generalised so that they may be applied to other networks that are less well-characterised. It is desirable that, as with the abstract modelling examples from developmental biology and

environmental toxicology given above: 1) we can use our existing, incomplete, knowledge of the underlying biology to relate the abstract state variables back to known molecular players and processes; 2) the models enable one not only to predict certain responses, but also to understand how they are generated from conceptual perspective, which can then help better inform our mechanistic modelling efforts or direct future experiments.

Available data and resources

- Single cell measurements (flow cytometry and fluorescence -based imaging) on the key molecular players associated with DNA-damage and oxidative stress, taken for multiple chemicals at different concentrations and timepoints.
- End-point assays on responses to chemical stimuli (apoptosis, micronuclei etc), which provide information on level of chemical exposure at which the adverse-adaptive switches occurs.
- Data on transcriptomic responses (RT-PCR and microarray).
- Existing mechanistic models of DNA-damage [11] and oxidative stress pathways [10], which capture certain aspects of how the pathways respond to chemical insult.

Question to be addressed

1. From a high-level perspective, what are the commonalities and what are the differences between the oxidative-stress and the DNA-damage AOPs?
2. Can abstract models be used in combination with existing mechanistic ones to better understand what molecular mechanisms drive the adaptive-adverse switch?
3. Is it possible to use an abstract model to accurately predict AOP responses to chemical stimuli? What are the limitations of such an approach?
4. How can different chemical molecular initiating events (MIEs; i.e. how different chemicals might interact with the AOPs) be incorporated in the models? Can abstract models be used to systematize how MIEs are explored experimentally?

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