Defenders of development

Systems biologist Dr Thomas Knudsen outlines research that aims to create novel computational models to investigate the complex mechanisms underlying embryo development.

What are the main aims and objectives of the Virtual Embryo project? Is there evidence to suggest that chemicals in the environment can negatively affect foetal development?

As part of the Environmental Protection Agency (EPA)'s chemical safety research efforts, the Virtual Embryo project aims to build and deploy multiscale computer simulations to help visualise and predict how chemicals might impact pregnancy and development. Predictive modelling is a major focus, in which experimental methods and in silico approaches come together to address information in the public domain detailing thousands of genes and biological pathways that function in development. Emerging resources for in vitro profiling of thousands of chemicals in high-throughput screening assays of the ToxCast inventory, the EPA's toxicity forecaster of over 2,000 chemicals, are also a part of this effort.

Can you outline some of the key developmental models that have been created and describe how they are used to investigate the impact of environmental pollutants on the foetus?

The first simulation model demonstrating proof of concept was blood vessel development. This process is fundamental to growth and development, and the cardiovascular system is the first to function in a growing embryo. Its chemical disruption is a known mechanism of teratogenesis (the process by which chemicals cause birth defects) and causes adverse pregnancy outcomes in humans, including preterm labour, low birth weight, birth defects and miscarriage.

At the EPA's National Center for Computational Toxicology, we conceptualised an adverse outcome pathway (AOP) framework for embryonic vascular disruption. This included a comprehensive and searchable knowledgebase of relevant information for genes regulating blood vessel development and a predictive signature for AOP-based prioritisation of the ToxCast inventory (1,060 chemicals) based on assays for growth factors and cytokines, plasminogen activating systems and inflammatory chemokines.

Why were the four areas of focus – eye, vascular, limb and embryonic stem cell development – chosen?

You need to start somewhere! We selected systems known to be susceptible to environmental disruption, and focused on aspects simple enough that computer engineers and programmers could simulate them – but complex enough that a computer model is needed to understand their performance. A lot is known about genetic signals and responses that control the morphogenesis of these focal points as well as evidence for environmental susceptibility from human studies and animal teratogenesis. The list is growing to include other systems such as male reproductive development, which is sensitive to endocrine-disrupting chemicals. Our long-term goal is a toolkit of models that researchers can use to look across embryonic development, both spatially and temporally, and to make scientifically-based predictions on how development might be affected by different chemicals. Having more models is important because chemicals can affect biological systems in various ways.

How does combining a computational systems biology approach with a fundamentally multidisciplinary team of researchers contribute to the project’s success and novelty?

In developmental toxicity, if we posit that an adverse outcome emerges from a convergence of bad circumstances invoked by one or more molecular impairments, the critical steps may be hard to unravel using traditional in vitro methods that have been reduced to smaller subsystems for analysis. Such reductionist approaches have potentially lost the ability to self-organise a system’s properties of emergence (novel features growing out of simple interactions), criticality (threshold effects/phase changes), robustness (insensitivity to perturbation) and self-maintenance (capacity for repair/correction). The interdisciplinary team, working to build computer-based virtual models of embryo development, includes developmental toxicologists, computer engineers, programmers, bioinformaticians, molecular biologists and mathematicians.

Do you have any preliminary results from your research that you would like to highlight?

In addition to angiodysplasias (small vascular malformation of the gut), cell-based simulation models have been developed in which the in silico platform predicted the adverse phenotype of paw defects induced by genetic perturbation in the limb and palate, as well as teratogenic disruption by 5-fluorouracil (ectrodactyly) and dioxin (cleft palate). Simulation models are being developed for predictive modelling of hypospadias (a male urethral birth defect) and congenital heart malformations, but these are at a very preliminary stage.

What are your future hopes for this project?

Ultimately, our vision for 2020 (VISION 20/20) is a platform of experimental and computational models that capture system dynamics for predictive modelling of developmental processes and toxicities. In 2012, the team’s peers in the Office of Research and Development voted for the project as one of 12 Top Innovators in an internal competition called PeerOvation.
Growing pains

Scientists working for the US Environmental Protection Agency’s Office of Research and Development are developing advanced techniques to predict the consequences of exposure to chemicals

CONTERGAN AND TALIDEX could be the names of any number of unassuming chemical compounds sold over the counter in pharmacies across the world. In the form of nonessential medicines, cleaning products and various environmental sources, we come into regular contact with many chemicals throughout our everyday lives. For consumers, these substances – particularly when they come under unidentifiable trade names – are hard to distinguish, and their health implications difficult to determine. This is particularly important for expectant mothers, for whom seemingly innocuous chemicals can occasionally be detrimental to the sensitive development of their unborn children – exemplified by Contergan and Talidex – unfamiliar trade names for thalidomide.

The medical outbreak that was thalidomide is an extreme example of how pharmaceutical chemicals can affect the development of an unborn child. Despite the rarity of situations such as this, development can also be affected by a wide range of much more commonplace chemicals such as methylmercury and alcohol. In fact, the top cause of preventable mental retardation in infants worldwide is excessive alcohol consumption during pregnancy. Birth defects can be identified in around 3 per cent of babies born in the US. The causes of most birth defects remain undetermined; however, the majority are attributed to a complex interaction between the patient’s genetic makeup, intrauterine environment, maternal lifestyle and perhaps paternal factors as well.

COUNTLESS CHEMICALS

A major priority for the US Environmental Protection Agency (EPA) is ensuring the safety of chemicals but researching the impact of these on human health and the environment is a complex undertaking. More than 83,000 chemicals currently exist in the US market, and hundreds of new ones are introduced every year. Traditional testing strategies make use of studies on pregnant animal models to identify chemical toxicity to the developing foetus, but this process cannot effectively cover the possible exposure scenarios and hazard properties of every single chemical being used alone or in combination. What is more, it is a costly and time-consuming method of conducting research that has now led to considerable data gaps; alternatives to animal testing need to be evaluated, especially for prenatal hazards.

That is why EPA researchers launched the Virtual Embryo project; implemented under the Chemical Safety for Sustainability (CSS) Research Program with researchers at the National Center for Computational Toxicology (NCCT), National Health and Environmental Effects Research Laboratory (NHEERL), the National Exposure Research Laboratory (NERL) and the Texas-Indiana Virtual STAR Center. The project aims to develop new methods for predicting the effects of chemicals on development, making use of large biological databases, advanced computer modelling systems and high-throughput screening (HTS), with the ultimate goal of addressing the complex mechanisms underlying birth defects and developmental disorders. Using a number of chemical compounds, some known to affect development in animal models, the EPA scientists hope to determine whether it is possible to use a ‘virtual embryo’ to accurately predict developmental toxicity. “If the project is successful in broadly combining in vitro HTS for biological activity with in silico models of developmental processes, it could make a big difference in how the agency evaluates chemical safety,” postulates Dr Russell S Thomas, Director of the EPA’s NCCT.

MAKING MODELS

Project leader Dr Thomas Knudsen believes that working towards a functional suite of virtual screening models to reproduce the manifold interactions between a chemical and the biology of embryonic development begins with complex data inputs. To help realise this, Dr Sid Hunter from the NHEERL leads the EPA’s stem cell research that contributes data by exposing embryonic stem cells to chemicals and closely monitoring the results. Furthermore, an alternative experimental model, zebrafish embryos, is used to generate data because the developmental progression of toxicity can be monitored for pathways and processes of known importance to mammalian toxicity. This research is led by the NHEERL’s Dr Stephanie Padilla. Multicellular organotypic culture models, human induced pluripotent stem cells and microphysiological systems also provide a more novel context on important physical and spatial determinants of cellular responses to chemical exposure.

The Virtual Embryo team is therefore in the process of experimentally probing these in vitro models and computationally simulating their performance, in order to serially reconstruct the complexities of an embryonic system during chemical exposure. It is a laborious and time-consuming process, and one of the central questions being addressed is whether it is even possible to achieve an accurate predictive
methods for extracting physical parameters describing normal intersomitic vessel sprout growth, as well as models to simulate their growth in silico and determine how the spatiotemporal distribution of angiogenic signals changes during normal and perturbed vascular sprouting. These collaborations have helped advance the science of predictive modelling toward the goal of chemical safety in children’s environmental health protection,” enthuses Dr Elaine Hubal, Deputy Director of EPA’s CSS Research Program.

ACHIEVING CHEMICAL SAFETY

The EPA hopes the computerised platform that will be the ultimate product of this research will be useful to all researchers interested in developmental toxicology, allowing them to decrease their reliance on animal models and gain access to rapid decision-support tools for risk assessment. This is an ambitious goal, and the sheer scale of the project necessitates the combination of in vivo, in vitro and in silico data and techniques. The EPA scientists are essentially combining all the data that they have access to, and only time will tell how effectively their work can simulate a fully-formed virtual embryo.

THE STARS OF STAR

EPA has engaged external partners on the project, including DOW Chemical, the Finnish Center for Alternative Methods, the National Toxicology Program and contractors from Lockheed-Martin. It has also included academics from the Texas-Indiana Virtual STAR Center, which recently benefited from a US $3.2 million EPA grant. The primary objective of this multidisciplinary centre is to contribute to a more reliable chemical risk assessment through HTS of in vitro data and in silico simulation models for developmental toxicity, and its research at the University of Houston led to the development of transgenic zebrafish models expressing fluorescent proteins in specific tissues such as vascular endothelial cells and neurons, with cell imaging to address chemical perturbations of angiogenesis and neurogenesis. At Indiana University, researchers developed methods for extracting physical parameters