EPA'S VIRTUAL EMBRYO: MODELING DEVELOPMENTAL TOXICITY

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research&development

Science Question

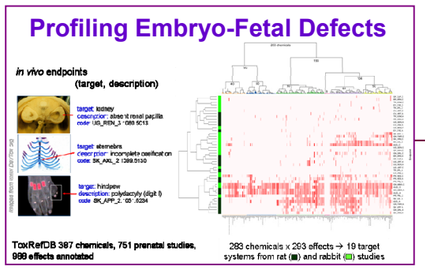
The present research addresses computational (*in silico*) systems that can execute a morphogenetic series of events and introduce chemical disruptions identified in ToxCast™ assays. A goal of the Virtual Embryo project (v-Embryo™) is to build such a system and demonstrate its usefulness in predictive modeling and mechanistic understanding of developmental toxicity. Effective Virtual Embryo modules that can support risk assessment should:

- implement knowledge of local cell signaling pathways and gene regulatory networks;
- enable relevant phenotypes to emerge from these lower-order interactions;
- propagate simple and complex alterations (genetic, chemical) across development; and
- extrapolate results from *in vitro* assay to *in vivo* architecture across dose and species.

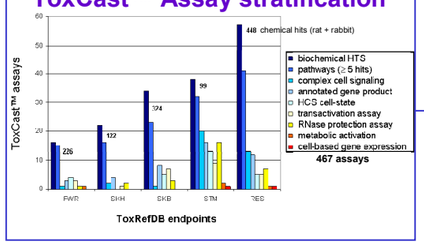
Research Goals

1. Create a virtual tissue (VT) knowledgebase, simulation engine & extensible interface.
2. Build small working prototypes of developmental systems and computable morphologies.
3. Analyze network-level function and state relationships for developmental trajectories.
4. Perform sensitivity analysis for *in vitro* assays in ToxCast™ predictive signatures.

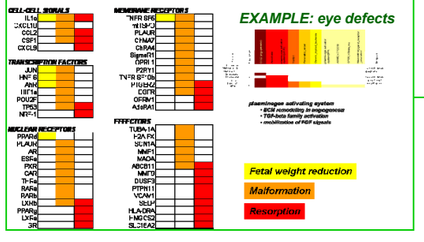
Profiling Embryo-Fetal Defects



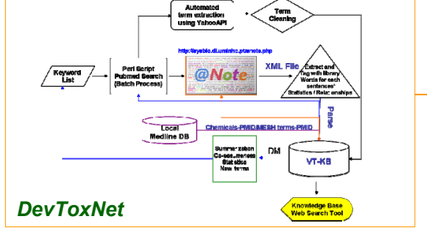
ToxCast™ Assay stratification



Predicted Molecular Targets

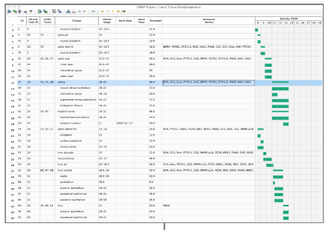


Workflow supporting VT-KB

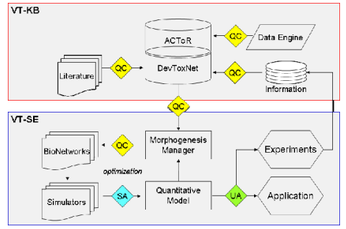


Methods/Approach

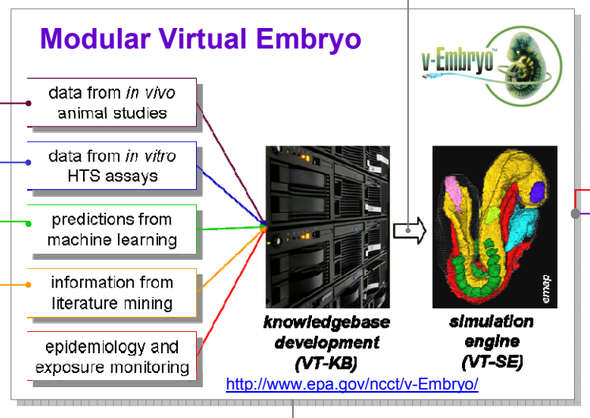
Morphogenesis Manager



v-Embryo™ workflow



Modular Virtual Embryo

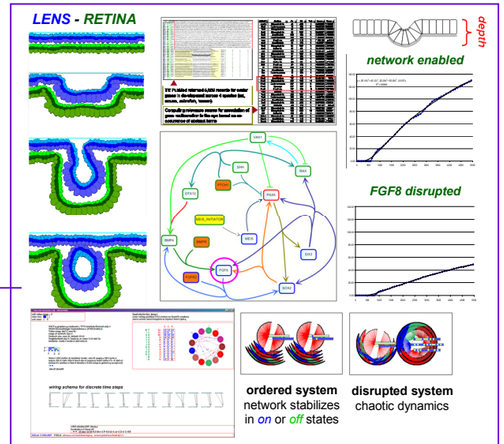


CRITICAL SOFWARE: open-source tools: http://sysbio.di.uminho.pt/anote.php, custom Perl-scripts and MySQL for literature-mining; CompuCell3D http://www.compucell3d.org and Python v2.5 for cell-based simulations; http://pureviolet.net/ganttpv/ for the Morphogenesis Manager interface; and SBW (www.sys-bio.org/), BioTapestry (www.biotapestry.org), and DDlab (www.ddlab.com/) for network structure and function.

DATA SOURCES: *in vivo* embryo-fetal endpoints culled as Lowest Effect Levels (LELs) from ToxRefDB [1] focusing on 283 chemicals, mostly pesticides with prenatal developmental toxicity studies in both rat and rabbit species; HTS data culled from 467 ToxCast™ *in vitro* assays as half-maximal inhibitory activity (AC50) [2]. Developmental gene expression from Mouse Edinburgh Atlas Project (EMAP) database (www.emap.org/).

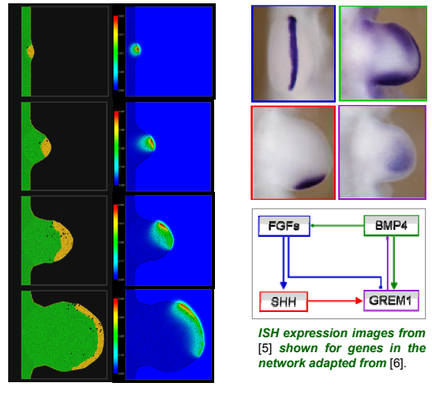
DATA MINING: significant assay-endpoint contingencies identified by Fisher's exact test ($P \leq 0.05$); positives and negatives (true, false) sorted by Relative Risk (RR); multivariate signatures predicting significant assay-endpoint associations constructed with ToxMiner™ machine-learning tools using relationships from the univariate contingencies.

EYE: lens invagination



CompuCell3D implementation of lens-retina induction. Cell shape-driven model for lens invagination, and prototype gene network built from VT-KB. Perturbation of FGF8 predicted to disrupt network state dynamics and slow lens invagination.

LIMB: polarized outgrowth



ISH expression images from [5] *shown for genes in the network adapted from* [6].

CompuCell3D implementation of signaling network for interactions between AER-mesenchyme, and programmed cell death. FGF8 signaling gradient is shown, adapted from chick limb model [3]. Predictive signatures for limb defects in ToxRefDB correlated with broad hits to kinase and phosphatase assays [4].

Results/Conclusions

Self-regulatory gene networks built with VT-KB and modeled in (topology) and DDLab (state relations).

Small working prototypes built in CompuCell3D showed computable state trajectories and the impact of network dysfunction.

Impact and Outcomes

High-fidelity computer models that link cell behavior with network-level function can resolve into normal and phenotypes.

Virtual models of modular embryonic systems can help unravel complexity in mechanisms of developmental toxicity.

The capacity to implement molecular lesions provides a powerful approach to assess combinatorial disruptions from high-throughput screening (HTS) assays.

Virtual Embryo systems-modeling makes HTS data potentially useful in the quantitative risk assessment of prenatal developmental toxicity.

Future Directions

- Expand agent-based models to more broadly array morphogenesis (eg, vasculogenesis, chondrogenesis, matrix remodeling, programmed cell death).
- Concatenate small working prototypes as building blocks for a more complete Virtual Embryo.
- Library of working models available from the Virtual Embryo website.

References

1. Profiling the Activity of Environmental Chemicals in Prenatal Developmental Toxicity Studies using the U.S. EPA's ToxRefDB. Reprod Tox 28: 209-219
2. Judson RS, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Mortensen HM, Reif DM, Richard AM, Rotroff DM, Shah I and Dix DJ (2009) Predictive in vitro screening of environmental chemicals – the ToxCast project. (submitted)
3. Poplawski NJ, Swat M, Gens S and Glazier JA (2006) Adhesion between cells, diffusion of growth factors, and elasticity of the AER produce the paddle shape of the chick limb. Phys A 373: 521-532
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5. Benazet J-D, Bischofberger M, Tiecke E, Goncalves A, Martin JF, Zuniga A, Naef F and Zeller R (2009) A self-regulatory system of interlinked signaling feedback loops controls mouse limb patterning. Science 323: 1050-1053
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