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ECHO: the executable chondrocyte

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Introduction: We recently presented ANIMO (Analysis of Networks with Interactive Modeling), a software tool for modeling dynamic molecular networks for use by biologists [1, 2]. We used ANIMO to generate a computational model of articular cartilage.
Materials and methods: Based on a large-scale literature study [3] and our own experiments, we developed ECHO (Executable Chondrocyte), a computational model of the key processes that regulate expression and activity of SOX9 and RUNX2, two master transcriptional regulators of the chondrocyte phenotype. ECHO consists of 93 nodes with 274 interactions that describe the expression and activity of 52 genes and proteins. Simulations in ECHO were performed to investigate the robustness of the chondrocyte network. To validate ECHO predictions, we used FRAP to measure mobility of SOX9 and RUNX2, which we have shown to be a faithful readout of their activity.
Results: In its unperturbed form, ECHO displays two stable states in which activities of SOX9 and RUNX2 are mutually exclusive. SOX9 represents a stable articular cartilage phenotype, while RUNX2 represents transient hypertrophic cartilage. We tested *in silico* the hypothesis that addition of WNT (performed with a few clicks of the mouse) will change permanent cartilage into transient cartilage by inducing hypertrophy. Indeed, when we add WNT, a known regulator of bone formation, the permanent or $SOX9^+$ state changes to a transient or $RUNX2^+$ state in the model. However, it is known that healthy articular cartilage is resistant to hypertrophic differentiation. Our group has previously shown experimentally that this was probably due to the secretion of DKK1, FRZB and GREM1[4, 5]. We therefore added nodes to ECHO representing DKK1, FRZB and GREM1 (fig. 1). GREM1 and DKK1 are able to stabilize the permanent cartilage or $SOX9^+$ state even after addition of WNT in ECHO.

We observed that in our model activation of WNT leads to a switch from a SOX9 + state to a RUNX2 + state. To prove that WNT/β-catenin signaling can directly regulate SOX9 function, we investigated the response of SOX9 mobility to WNT3A in live primary chondrocytes. Addition of WNT3A to human chondrocytes transfected with SOX9-GFP resulted in a significant decrease of the immobile SOX9 fraction from 53% to 34% within 15 minutes after addition, indicating a loss of transcriptional activity of SOX9.
Discussion and conclusion: Using ECHO we predicted the stimuli that prevent hypertrophic differentiation of articular cartilage, and tested this experimentally with FRAP using SOX9 and RUNX2 mobility as a read-out.

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Disclosures: The authors have nothing to disclose.

References:

1. Scholma, J, et al., Gene, 2014. 533(1): p. 379.
2. Schivo, S, et al., Biomedical and Health Informatics, IEEE Journal of 2013. PP[99]: p. 1
3. Kerkhofs, J, et al., PLoS One, 2012. 7[4]: p. e34729.
4. Leijten, JC, et al., Arthritis Rheum, 2012. 64(10): p. 3302-12.
5. Leijten, JCH, et al., Arthritis Res Ther, 2013. 15(R126).

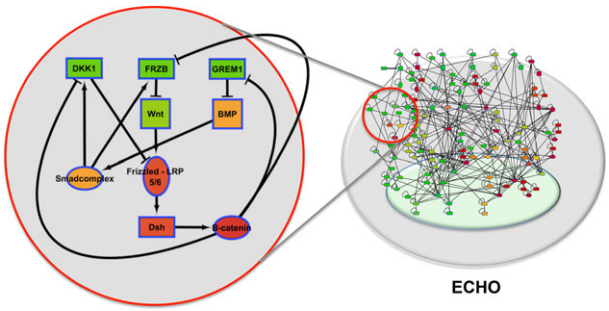

Figure 1 A complex network of many signal transduction pathways determines the development of either transient or permanent cartilage. We perform *in silico* experiments in the complete network, or in smaller areas.

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ANIMO: a tool for modeling biological pathway dynamics

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Introduction: Computational methods are applied with increasing success to the analysis of complex biological systems. However, their adoption is sometimes made difficult by requiring prior knowledge about the foundations of such methods, which often come from a different branch of science.

The software ANIMO (Analysis of Networks with Interactive MOdeling, [1]) allows the tissue engineer to add dynamic behavior to traditional static models of signaling events. We use ANIMO to optimize cartilage tissue engineering.
Materials and methods: Starting from a signaling network as traditionally represented in books, ANIMO allows biologists to take advantage of their expertise, enriching the symbolic description with quantitative parameters. The underlying computational model is based on the formalism of Timed Automata [2] and is automatically generated and analyzed by ANIMO. Implementation as a Cytoscape [3] plug-in makes the interface intuitively usable: for example, an existing network topology can be extended with few mouse clicks, adding new nodes and

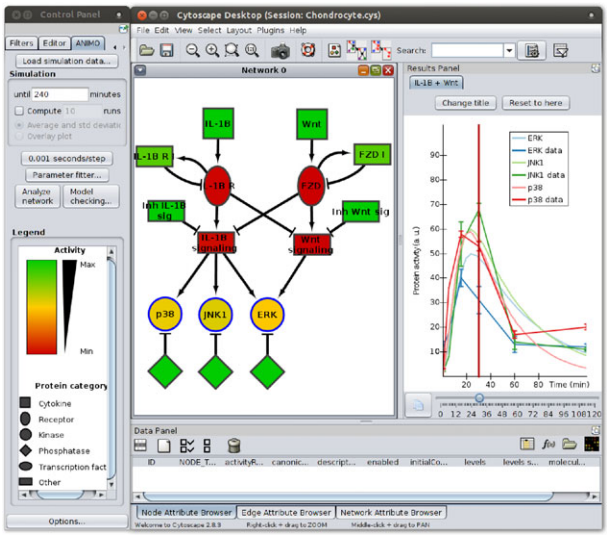

Figure 1 ANIMO user interface. The vertical red line in the graph on the right corresponds to the point in the simulation run on which coloring of nodes in the network is based.