

Signalling network modelling of skin appendages during development

K.Poterlowicz¹, Y. Peng¹, V.A. Botchkarev^{2,3}, T.Y. Sharova³, A.N. Mardaryev³, M.Y. Fessing³,
A.A. Sharov³ and E. Wang⁴

¹ School of Computing, Informatics and Media, University of Bradford, UK ; ² Centre for Skin Sciences, University of Bradford, UK;
³ Department of Dermatology, Boston University, MA, USA; ⁴ Biotechnology Research Institute, National Research Council, Montreal, Canada

Background

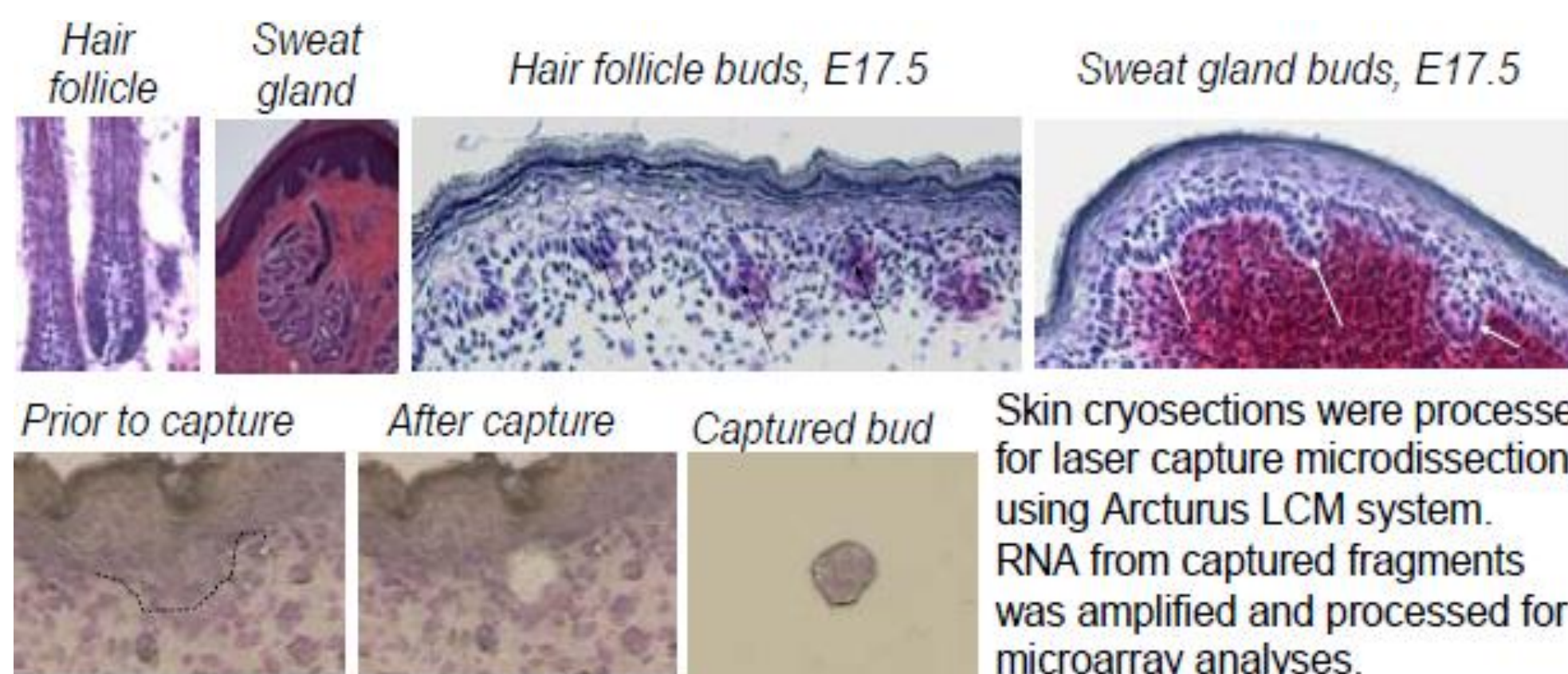
- Mammalian skin develops as an organ in which epithelium and mesenchyme interact with each other to give rise the formation of epidermis and a variety of appendages (hairs, scales, nails, glands).
- Morphogenesis of the hair follicle (HF) and sweat gland (SWG) begins from the focal thickening of the epidermis (epithelial placodes) and remodelling of the underlying dermis that later develop into the epithelial and mesenchymal compartments of these organs.
- Epithelial placodes further develop into epithelial buds invading into the dermis. However, molecular profiles of gene expression and signaling/transcription factor networks in the epithelial buds of sweat glands and hair follicles remain to be defined.

Specific Aims

- To characterize and compare the molecular signature of the epithelial buds of the hair follicle and sweat gland using laser capture microdissection, microarray and real-time PCR analyses
- To construct signalling networks for both hair follicle and sweat gland placodes by integrating literature-mining information with experimental microarray datasets and develop methods of computer simulation of the signalling relations in the networks to determine the key nodes and output changes.

Experimental approach

- Skin cryosections of C57BL/5 mouse embryos at E17.5 were used for laser capture microdissection using Arcturus system (Sharov et al., 2006)



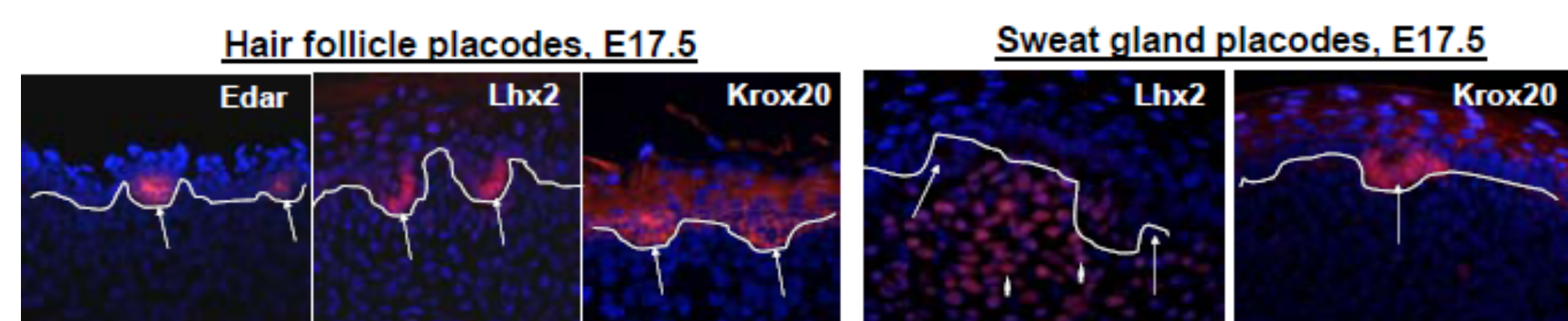
- RNA samples obtained after laser capture microdissection were processed for microarray analyses using Agilent mouse whole genome chip

Hair follicle bud Vs Inter-bud epidermis

Over-represented genes (848)		Under-represented genes (225)	
Signaling	Transcription	Signaling	Transcription
Shh 7.3↑	Lhx2 10.1↑	Ttrap 7.9↑	Notch3 8.1↑
Tgfb2 6.4↑	Sp5 9.7↑	Tm9sf2 3.1↑	Wnt10a 8.0↑
Calml3 4.1↑	Sox13 6.9↑	Rab5a 2.9↑	Rab15 5.5↑
Hdc 3.4↑	Sox21 6.0↑	Sdfr1 2.6↑	Map3k6 4.8↑
Sostdc1 3.3↑	Dach1 4.9↑	Tm4sf1 2.4↑	Dkk1 4.1↑
Fabp4 3.0↑	Egr3 3.8↑	Il10rb 2.4↑	Pdgfa 4.0↑
Edar 2.9↑	Foxc1 2.8↑	Mapk1 2.4↑	Cxcl14 3.9↑
Il17r 2.8↑	Krox20 2.4↑	Tank 2.3↑	Igf2 3.3↑
Dkk4 2.8↑	Foxo6 2.3↑	Ocmr 2.2↑	Wnt6 3.3↑
Tgfb3 2.7↑	Smad7 2.1↑	Hspa4 2.2↑	Catbip1 3.2↑
Pdgfa 2.6↑	Msx2 2.0↑	Rgs10 2.2↑	Tgfr2 3.0↑
Kcnmb4 2.5↑	Gtf2a2 2.8↑	Pgmrc1 2.1↑	Map3k10 2.8↑
Calcr1 2.3↑	Zfhx1b 2.4↑		Traip 2.7↑
Gmeb1 2.1↑	Twist 2.3↑		Gmeb1 3.2↑
	Helt 2.2↑		Ece1 2.6↑
	Pitx2 2.1↑		Bmp7 2.4↑
			Pparbp 2.3↑
			Sico2a1 2.1↑
			Ddr1 2.0↑
			Mekk1 2.0↑
			Map3k10 2.8↑
			Traip 2.7↑
			Gmeb1 3.2↑
			Ece1 2.6↑
			Bmp7 2.4↑
			Pparbp 2.3↑
			Sico2a1 2.1↑
			Ddr1 2.0↑
			Mekk1 2.0↑

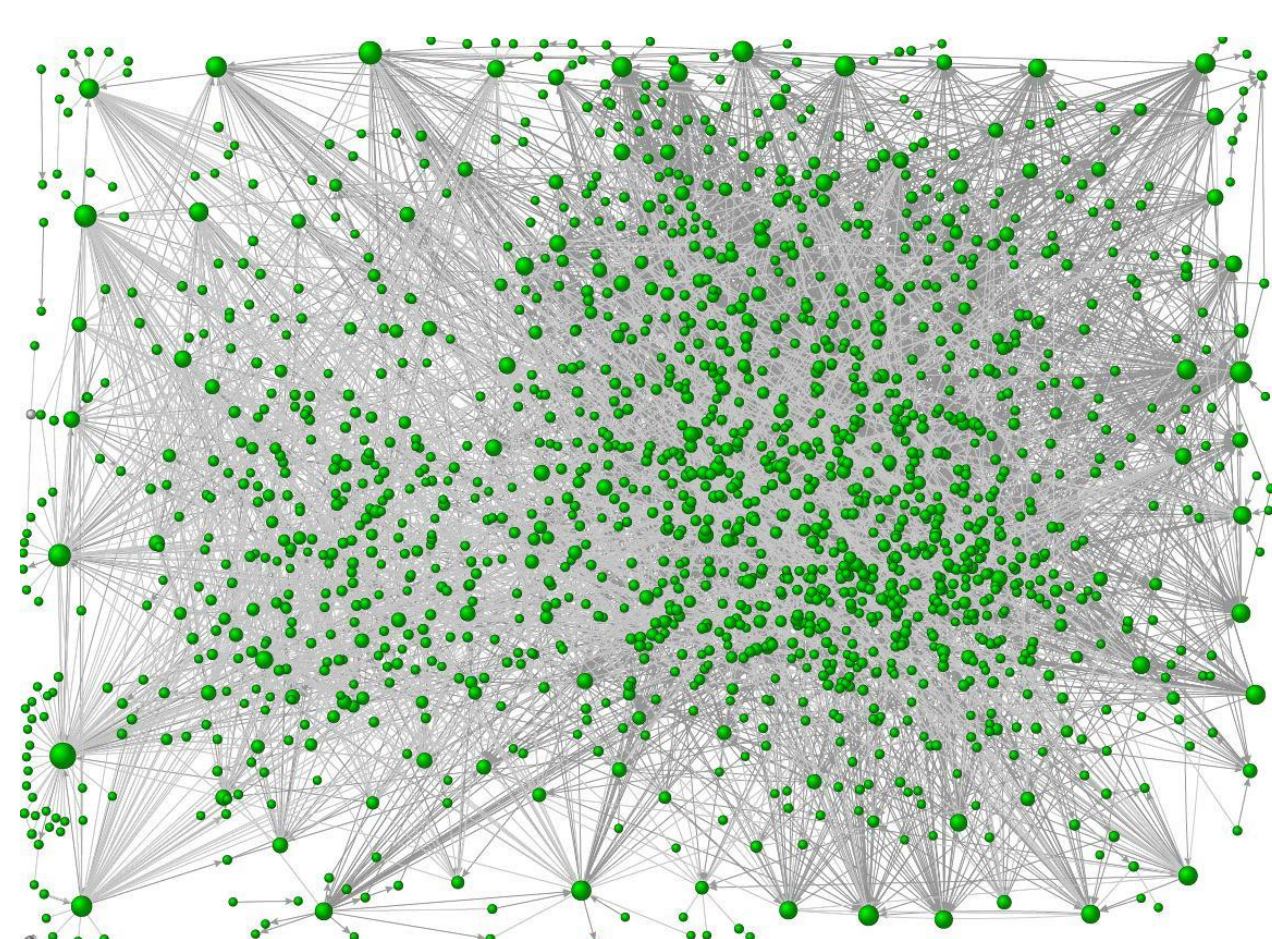
Sweat gland bud Vs Inter-bud epidermis

Over-represented genes (3592)		Under-represented genes (5477)	
Signaling	Transcription	Signaling	Transcription
Ttrap 7.9↑	Notch3 8.1↑	Zfp53 23.5↑	
Tm9sf2 3.1↑	Wnt10a 8.0↑	Scratch 18.1↑	
Rab5a 2.9↑	Rab15 5.5↑	Sox10 12.8↑	
Sdfr1 2.6↑	Map3k6 4.8↑	Yy1 4.1↑	
Tm4sf1 2.4↑	Dkk1 4.1↑	Krox20 4.0↑	
Il10rb 2.4↑	Pdgfa 4.0↑	Nfe2l2 2.2↑	
Mapk1 2.4↑	Cxcl14 3.9↑	Tfam 2.2↑	
Tank 2.3↑	Igf2 3.3↑	Htr1f 2.2↑	
Ocmr 2.2↑	Wnt6 3.3↑	Foxc1 2.1↑	
Hspa4 2.2↑	Catbip1 3.2↑	Foxd1 2.1↑	
Rgs10 2.2↑	Tgfr2 3.0↑	Foxf2 2.1↑	
Pgmrc1 2.1↑	Map3k10 2.8↑	Trp63 14.1↑	
	Traip 2.7↑	Klf3 9.7↑	
	Gmeb1 3.2↑	Klf5 7.6↑	
	Ece1 2.6↑	Klf4 5.9↑	
	Bmp7 2.4↑	Irx1 4.5↑	
	Pparbp 2.3↑	Tcf3 3.0↑	
	Sico2a1 2.1↑	Tbx1 2.9↑	
	Ddr1 2.0↑	Twist2 3.6↑	
	Mekk1 2.0↑	E2f2 3.3↑	
		E4f1 2.0↑	

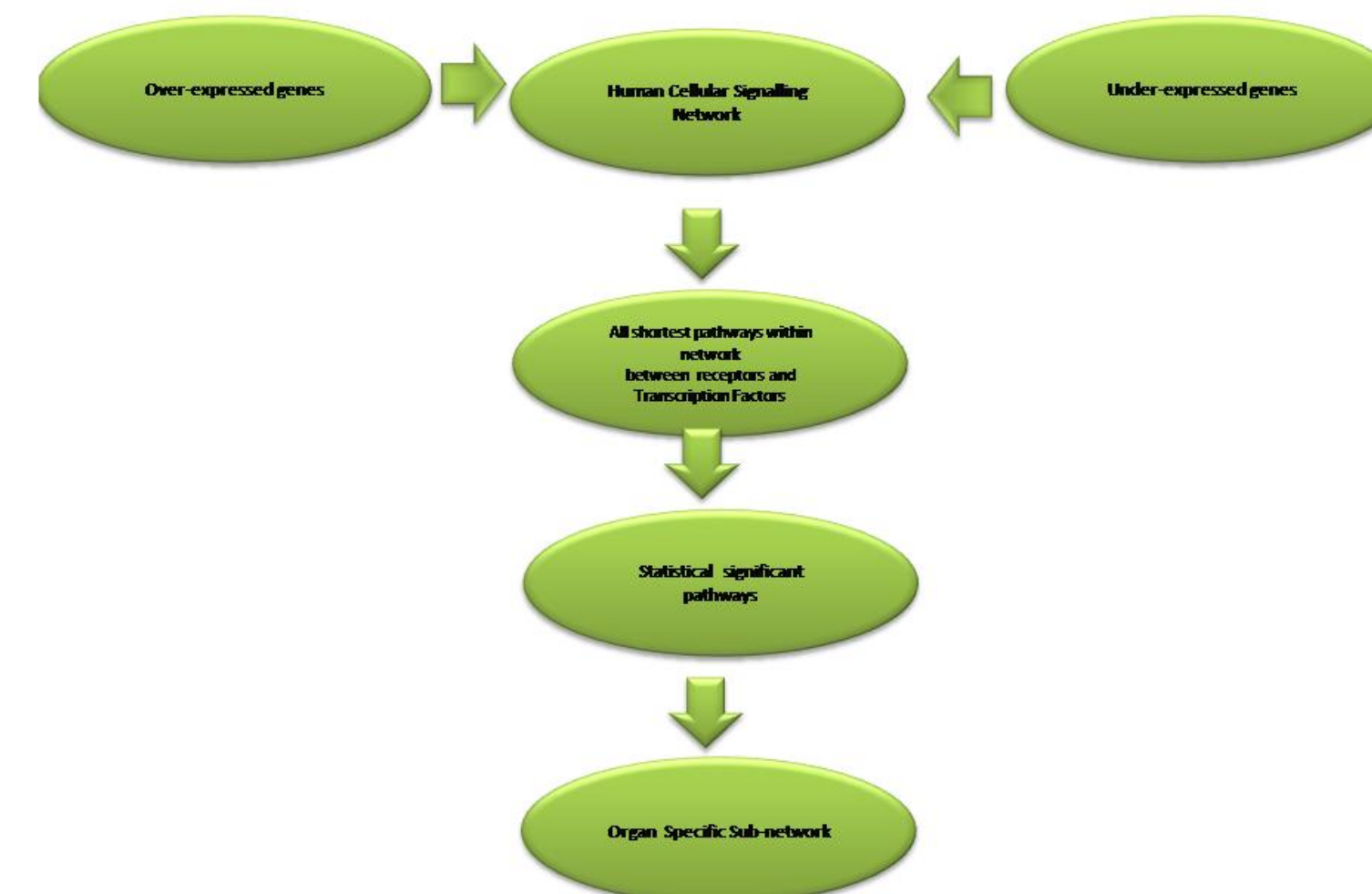


Human Signalling Network

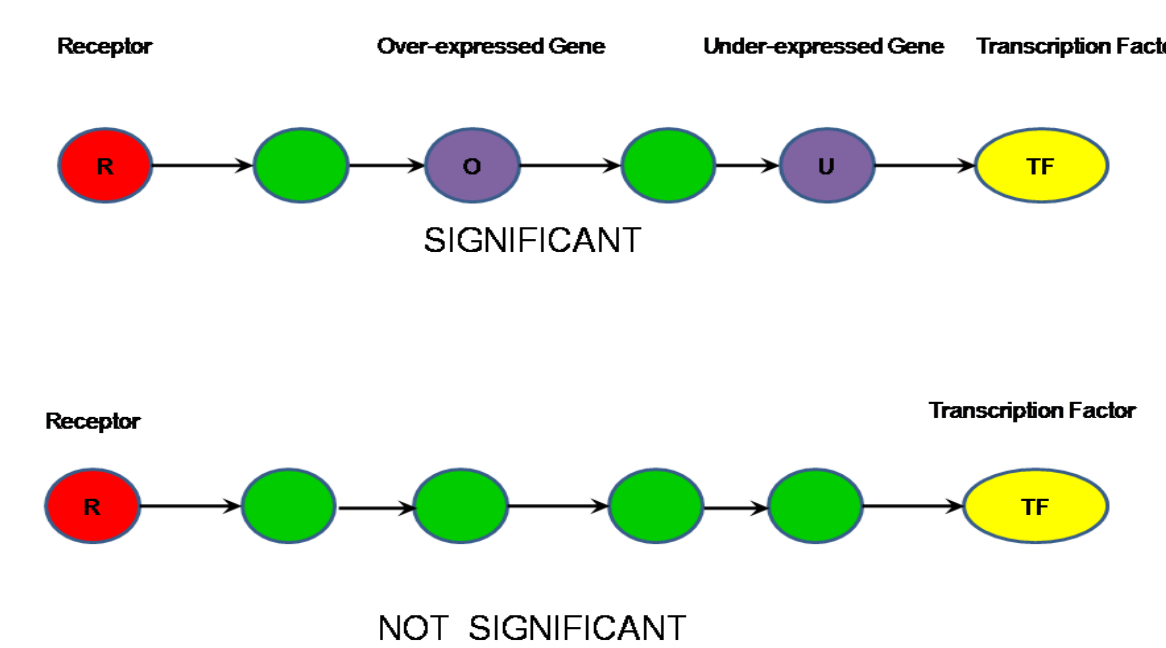
- Manually curated human cellular network (>1,600 proteins and >5,000 signaling relations), Cui et al., Mol Syst Biol, 3:152, 2007.



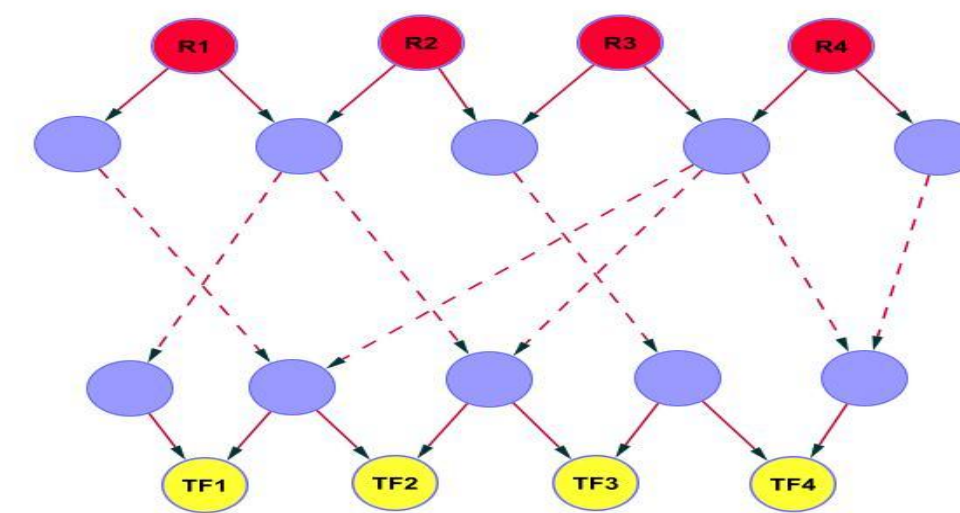
Network Construction



- Over and under-expressed genes has been mapped to Human Cellular Signalling Network.
- Shortest pathways between receptors and transcription factors has been extracted.
- Statistical permutation test has been used to select significant shortest pathways.



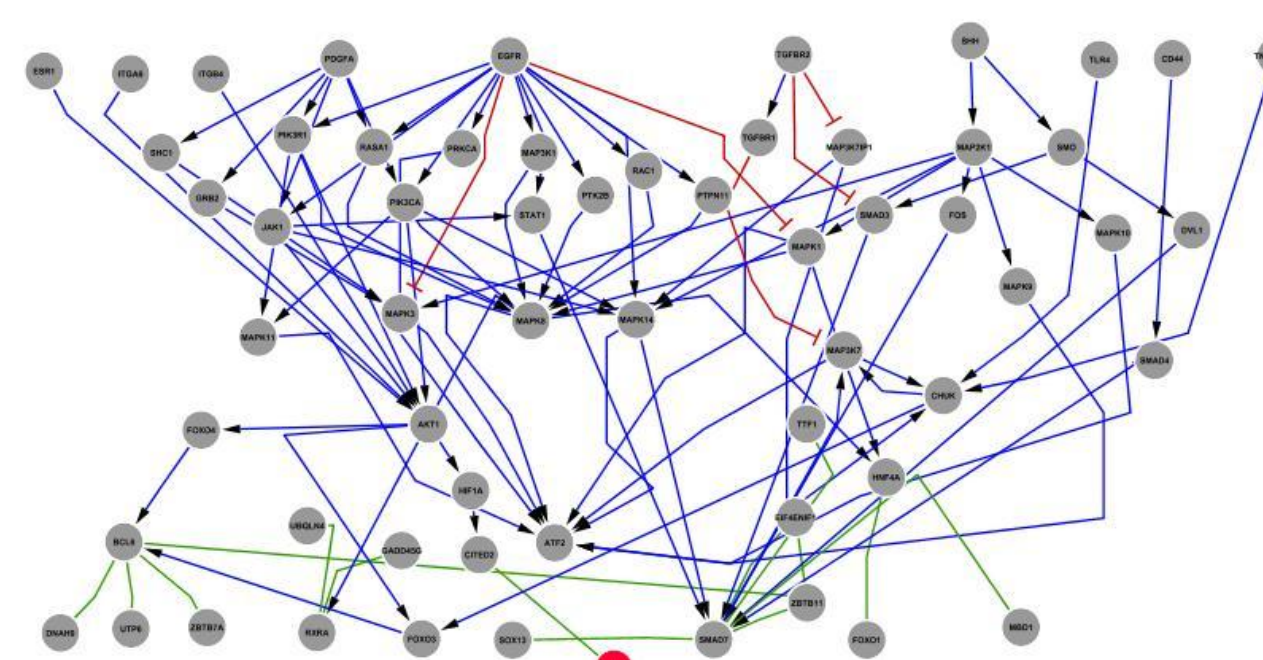
- Significant shortest pathways has been merged into organ specific sub-network.



Organ specific sub-networks

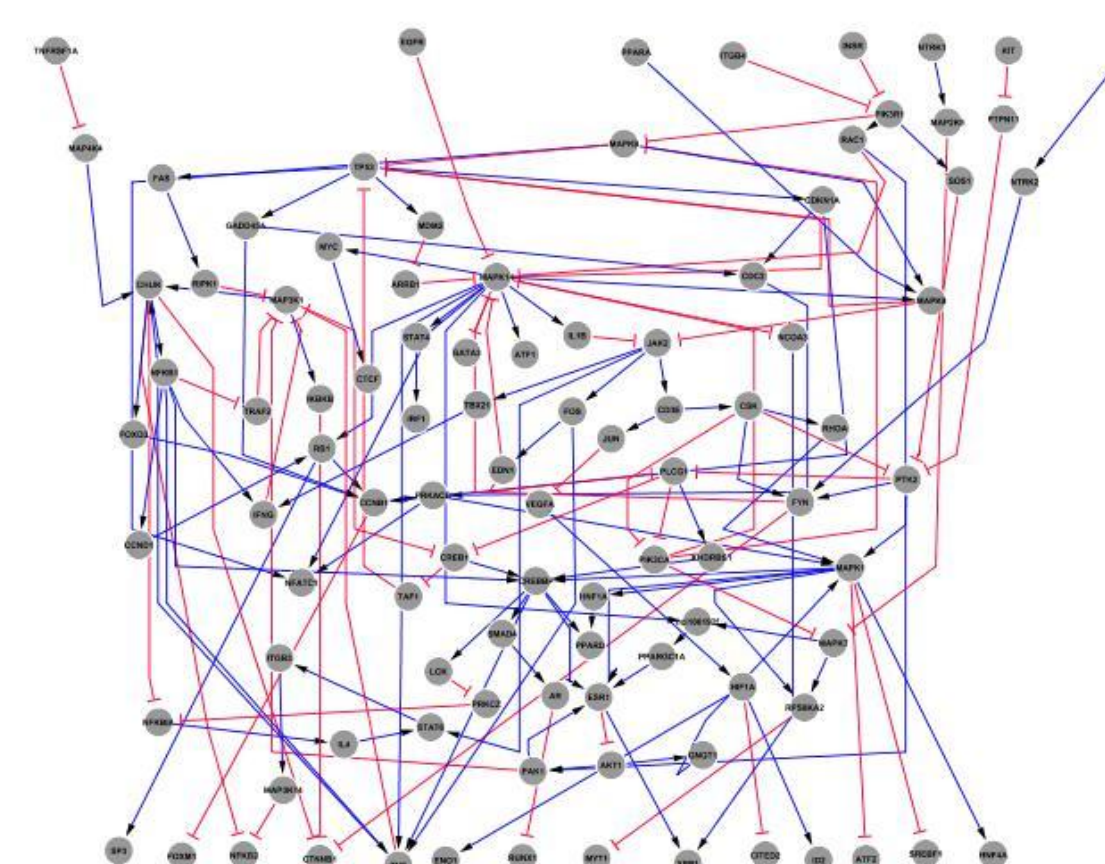
Hair Follicle Sub-network:

- 61 nodes, 100 connections.
- LHX2 has been identified as a key transcription factor.



Sweat Gland Sub-network:

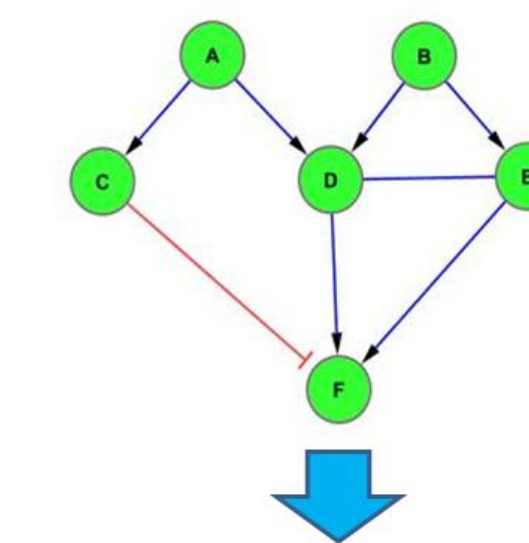
- 98 nodes, 143 connections.
- LHX2 has been not included in this sub-network.



Simulation

- Boolean networks have been used to simulate dynamical behaviour of the system
- Initial Conditions (Over-expressed genes ON, under-expressed genes OFF, rest of the genes set to RANDOM)

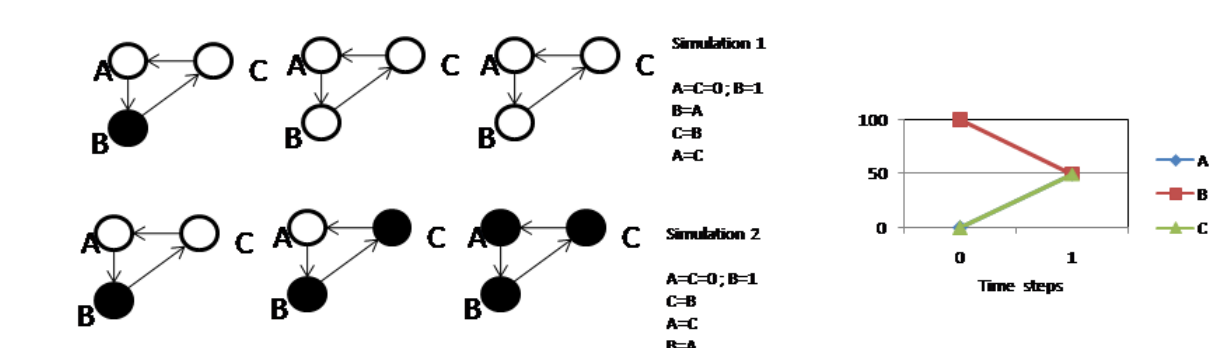
Boolean networks rules



C=A
D=A or B
E=B
F=(D and E) and not C
Closure =F

- Asynchronous update
- 1000 repetitive simulations
- 20 times points (all nodes reached steady state)

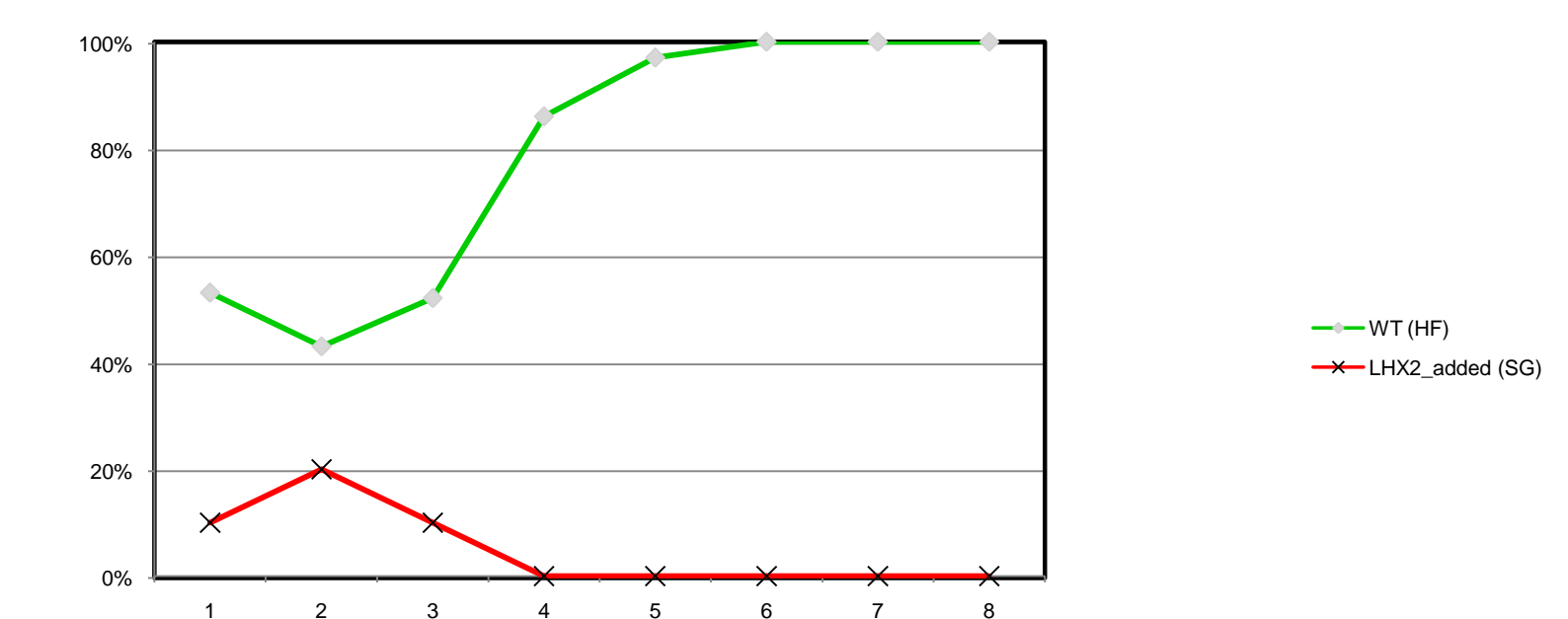
Asynchronous update



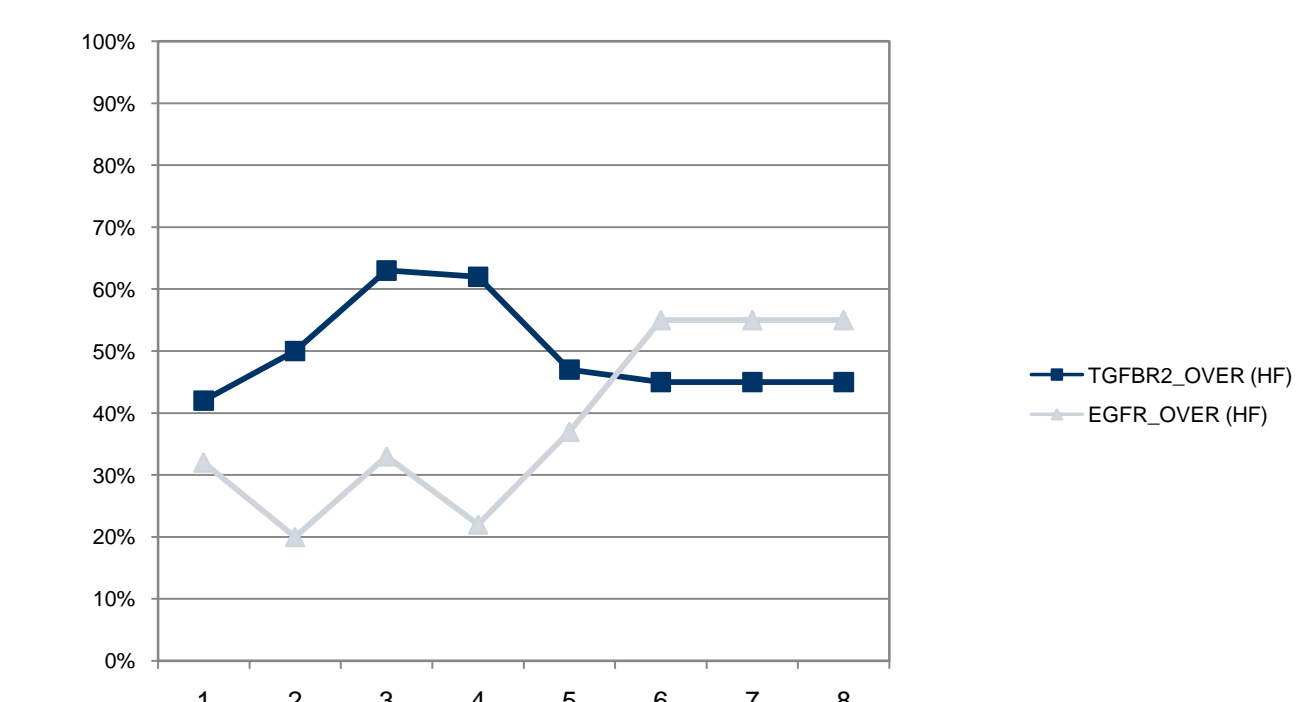
Asynchronous update
Updates are performed in random order and the states immediately reflect the changes
•Allow to sample timescale (qualitative information).
•Large number of repetitive simulations is needed

Results

- LHX2 has been confirmed as the key transcription factor in Hair Follicle development.
- Artificially inserted LHX2 not expressed in Sweat Gland.



- Up regulation of TGFBR2 and EGFR repress expression of LHX2.



References

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- Sharov AA, Sharova TY, Mardaryev AN, Brissette JL, Dotto GP, Botchkarev VA. Bone morphogenetic protein signaling regulates the size of hair follicles and modulates the expression of cell cycle-associated genes. *Proc Natl Acad Sci USA*, 2006, 103, 18166-77.