

The Importance of the Signature of Breast Cancer Prevention

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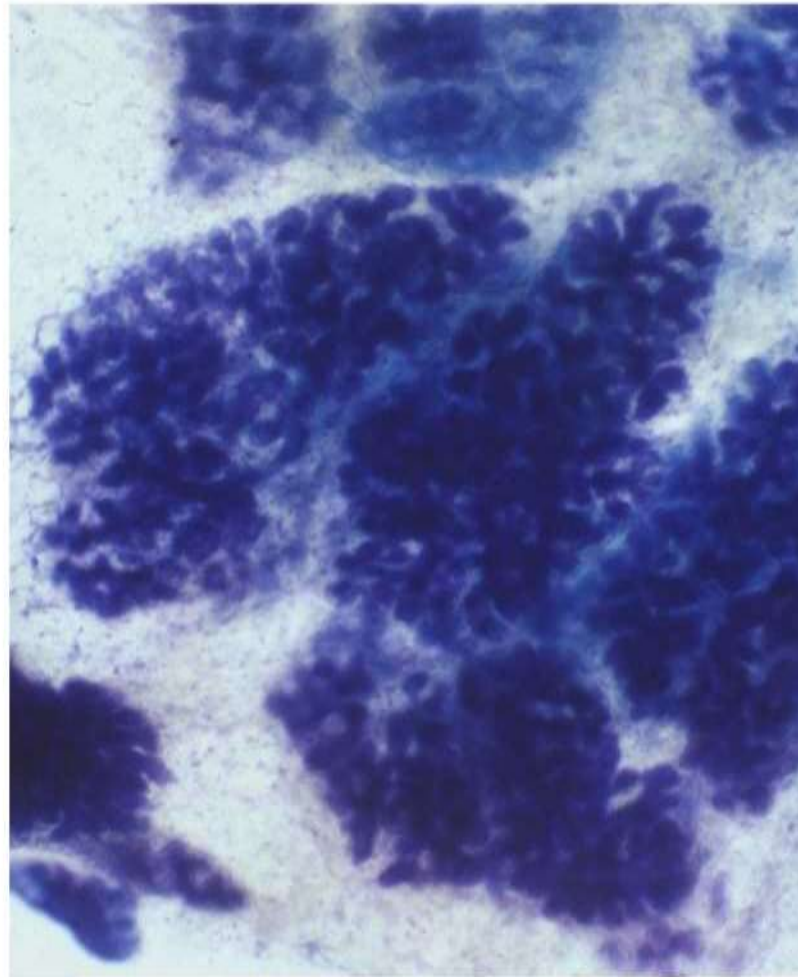
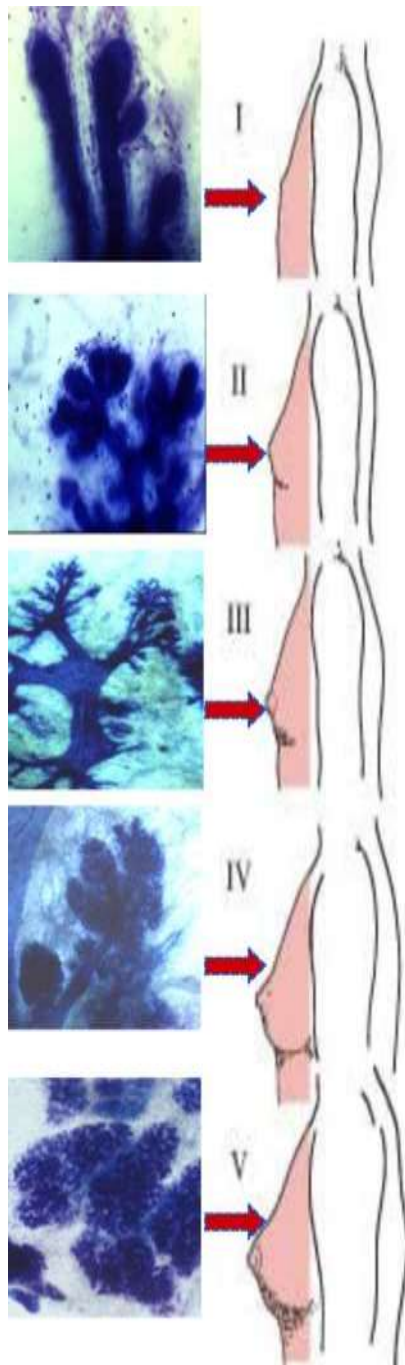
Protective effect of first full term pregnancy from developing breast cancer after menopause when:

- Early parity- First child born from early teens
to middle twenties
- Breastfeeding
- Multiparity in early parous women
- Each additional birth confers greater protection

Question 1

**Why Pregnancy
Produces Protection
Against Breast
Cancer?**

Life-time cycle of breast development



Terminal duct

4

Alveolar buds

4

Lobule type 1 (site of origin ductal carcinoma)

Lobule type 2 (site of origin lobular carcinoma)

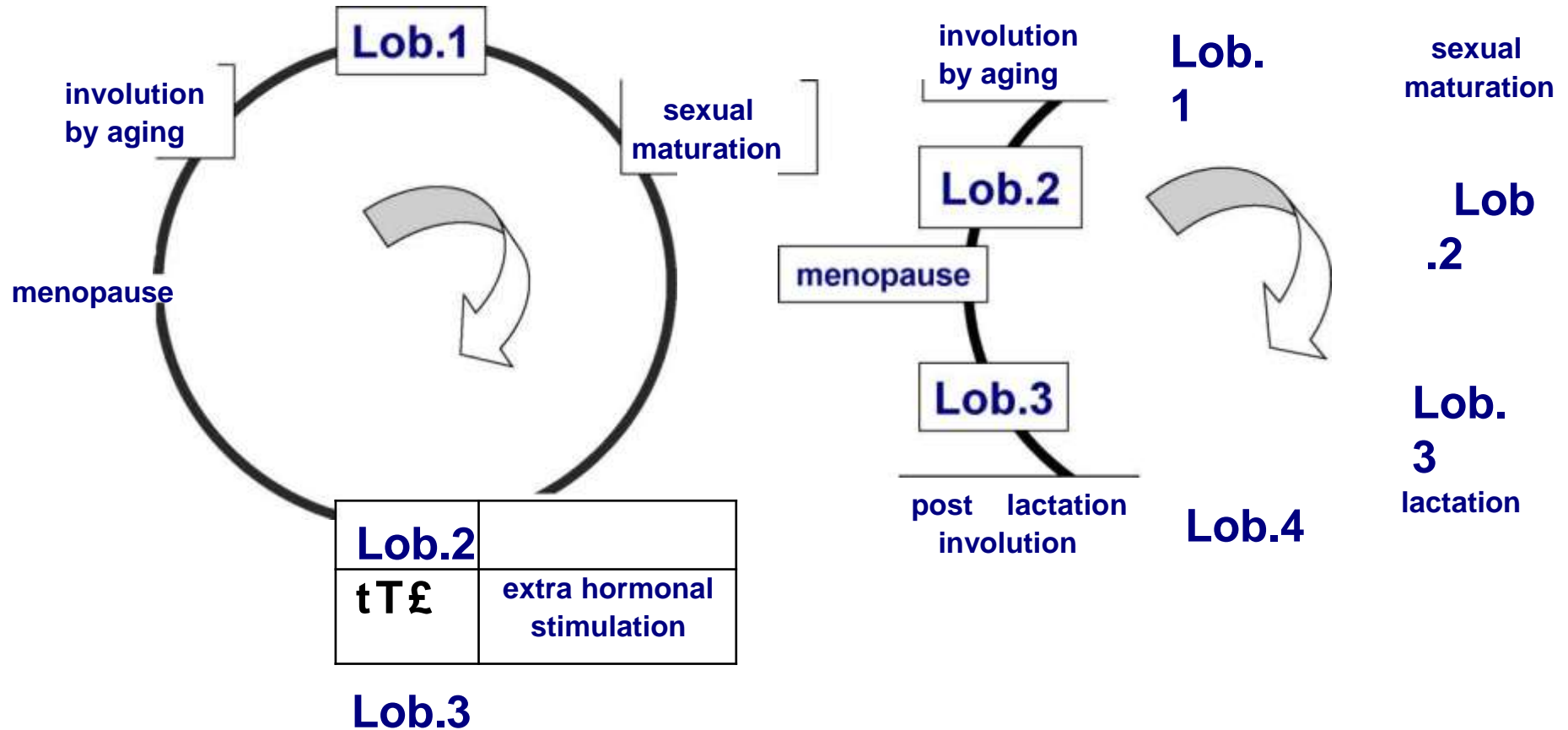
Lobule type 3

Russo, J. et al, Breast Cancer Res. and Treat. 23:211-218, 1992.

Russo, J. et al. JNCI. 2000 ,and Microscopy Res. & Tech., 2001

Life-time cycle of breast development

Nulliparous Parous



Working hypothesis

- J The breast of parous postmenopausal women exhibits a specific genomic signature that has been induced by a full term pregnancy during the hormonal protection window (HPW), i.e., 18-24 years of age.**
- This genomic signature controls cell differentiation, leading to breast cancer prevention.**

Study subjects

S Volunteers: Residents of Lulea, Sweden. SPost-menopausal: age 50-69
^Parous (P)= had a full term pregnancy
SNulliparous (NP)= never pregnant or uncompleted pregnancy

Interviewed: **389**



Included: **251** 111 (44%) nulliparous + 140 (56%) parous

Samples suitable for microarray: **128** (44 nulliparous + 84 parous)

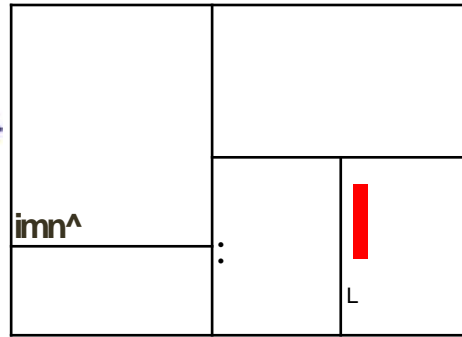
Eligible for the study: **113** (42 nulliparous + 71 parous)



Methodology



Breast Core Biopsy

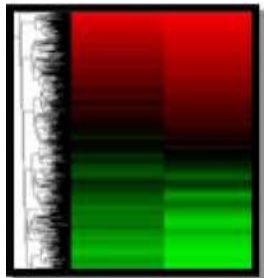


RNA isolation and QC assessment

Two-cycle target labeling for expression arrays



Human Genome U133 plus 2 Microarrays (Affymetrix)



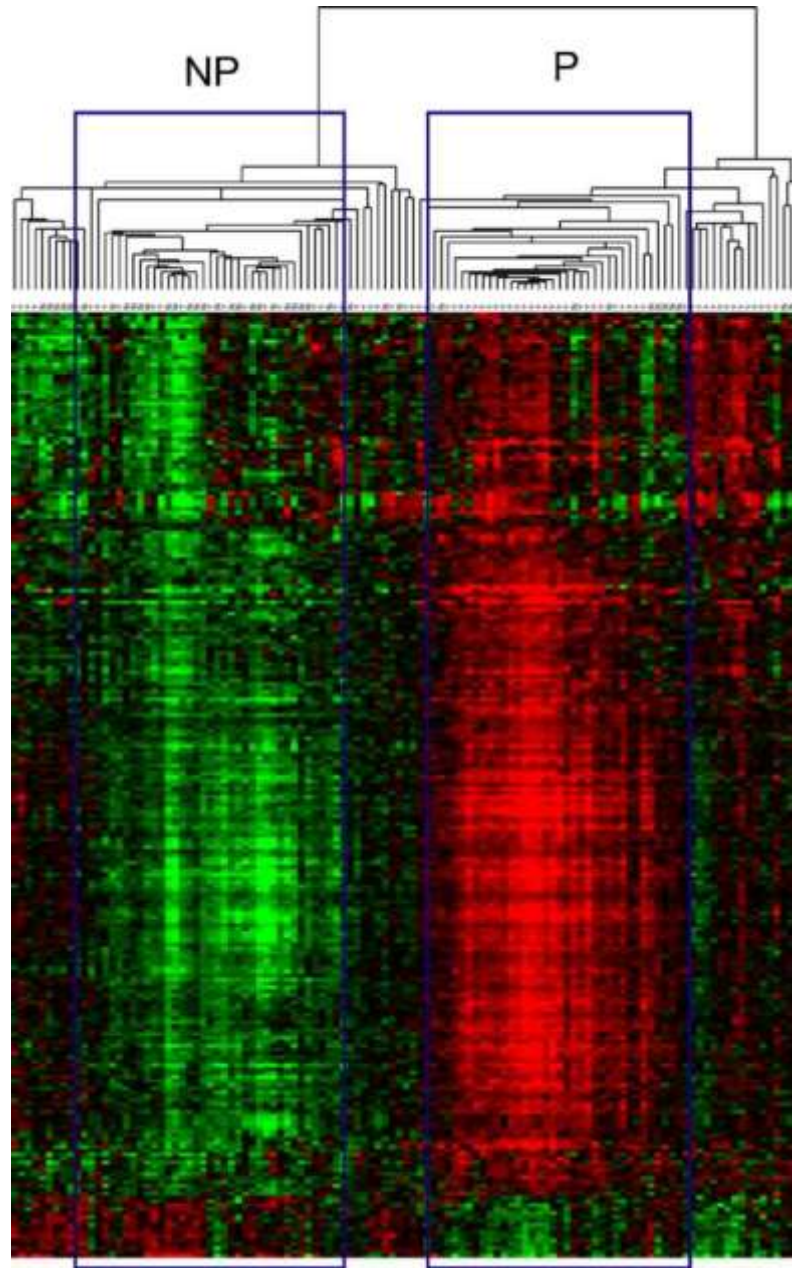
Microarray Analysis



Microarray Hybridization, washing and scanning



Genomic Signature of Pregnancy (GSP)



Up regulated genes



Down regulated genes

Question 2

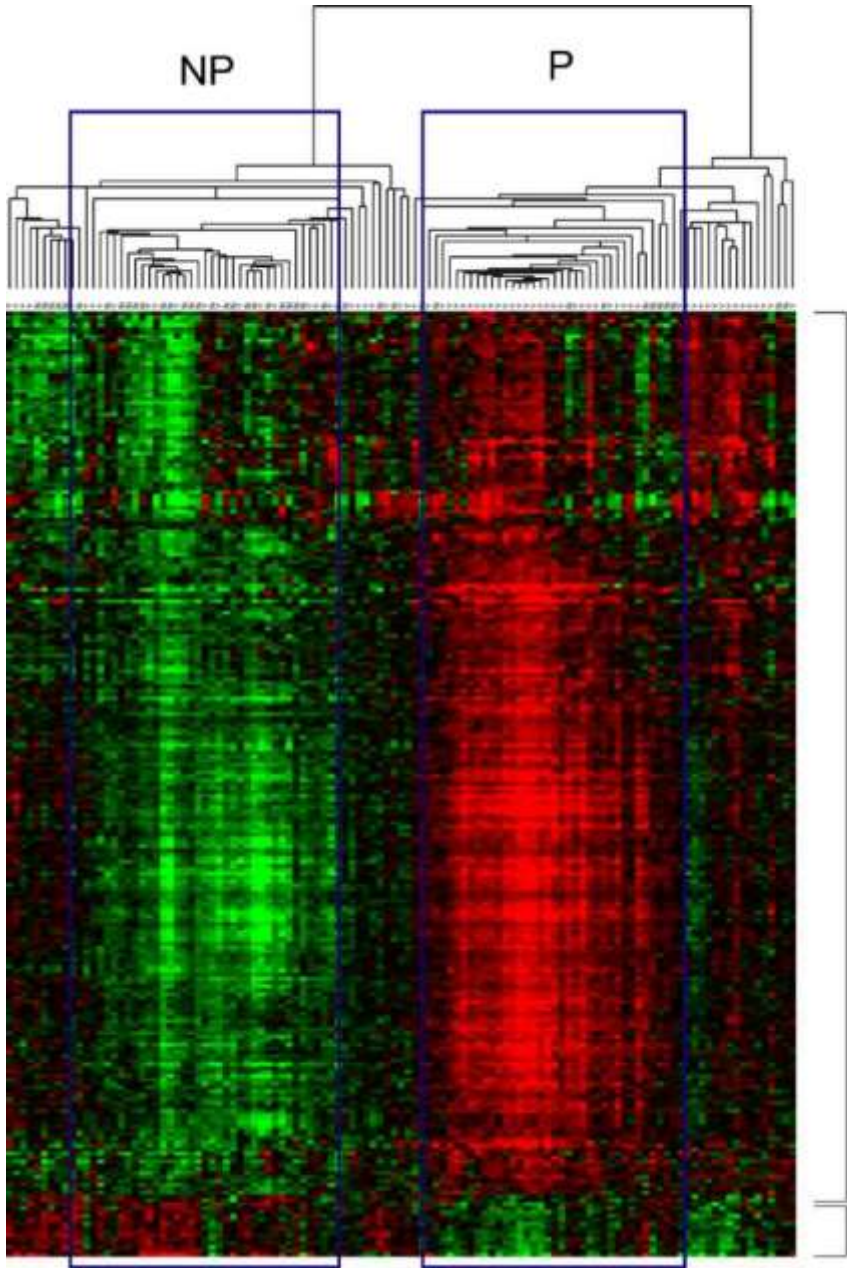
What is the meaning of the genomic signature of pregnancy?

How the genomic signature of pregnancy works?

Main Pathways of the GSP

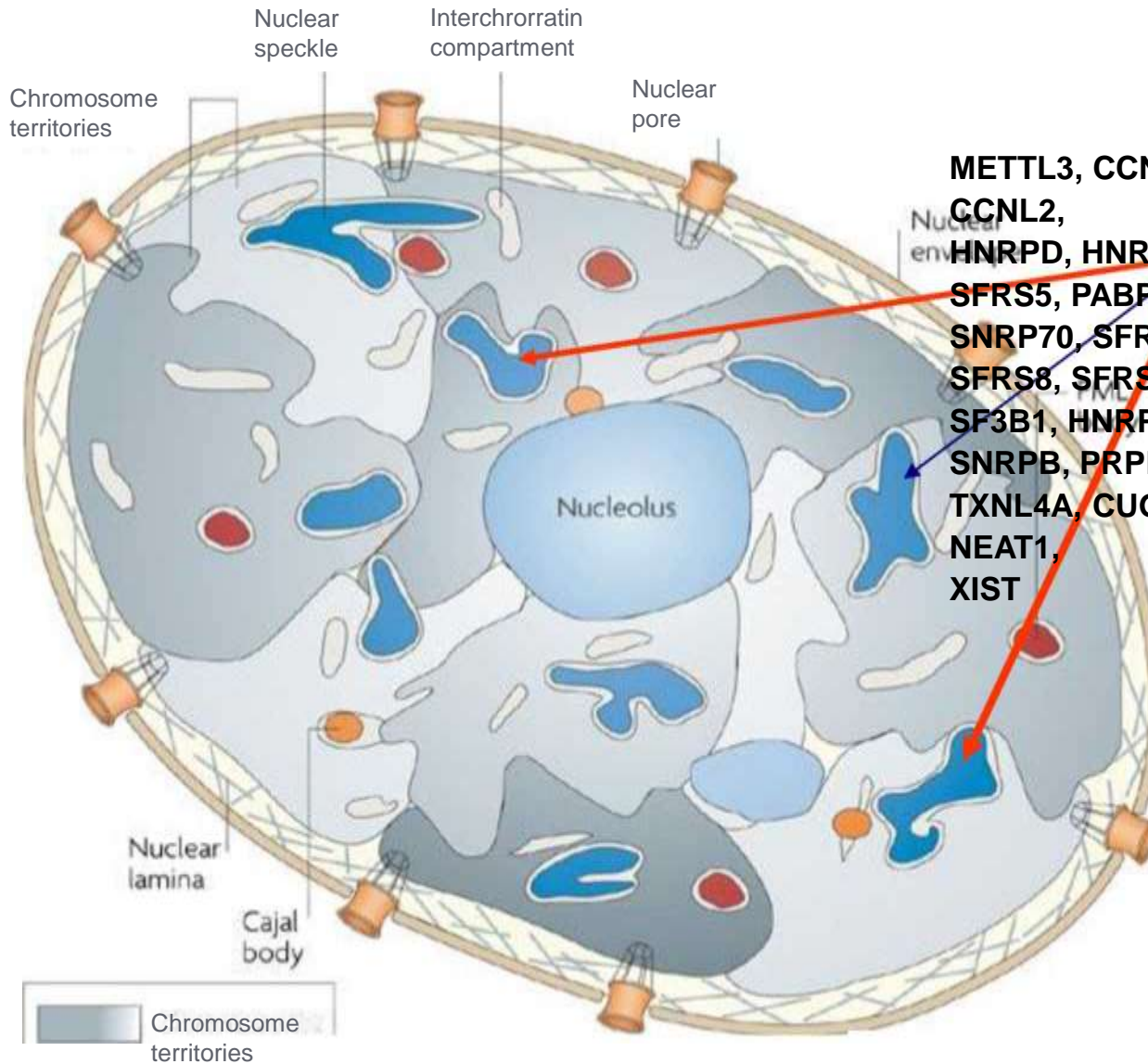
J Cell differentiation J Splicing
mechanism J Shifting of Stem or
Progenitor cells. J Chromatin
remodeling:

- Polycomb
- Methylation of Histones
- GpC methylation
- LncRNA



Up-regulated processes	Up-regulated Genes
cell-substrate junction assembly	KRT5, LAMA3, LAMC2
ectoderm development	COL7A1, KRT5, KRT15, LAMA3, LAMC2, NTF4, KLK7
epidermis development	COL7A1, KRT5, KRT15, LAMA3, LAMC2, NTF4, KLK7
hemidesmosome assembly	KRT5, LAMA3, LAMC2
RNA metabolic process	DDX17, CHD2, CBX3 , CIRBP, ZNF785, EZH2 , L3MBTL, GATA3, RBMX, ZNF789, HNRNPA1, HNRNPA2B1, HNRNPD, LUC7L3, PNN, PRPF39, ZNF83, METTL3, CREBZF, RBM25, RBBP8, RPS24, CENPK, SFPQ, SFRS1, SFRS5, SFRS7, ZNF814, ZNF207, PABPN1, RUNX3, FUBP1, PRPF4B, HNRPDL
RNA splicing	RBMX, HNRNPA1, HNRNPA2B1, HNRNPD, LUC7L3, PNN, PRPF39, RBM25, SFPQ, SFRS1, SFRS5, SFRS7, PABPN1, PRPF4B

Nuclear speckles



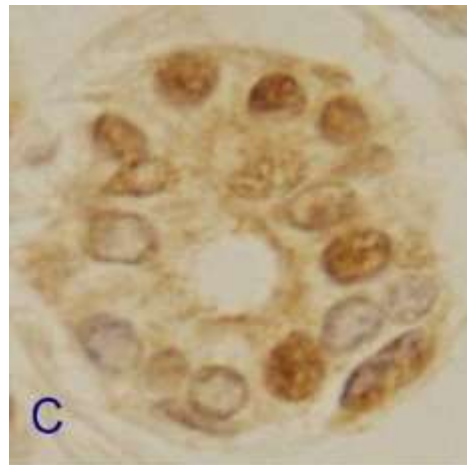
**METTL3, CCNL1,
CCNL2,
HNRPD, HNRPA2B1, PRPF4B, SFRS7, CLK4,
SFRS5, PABPN1, CSTF3, HNRPU, RBM5,
SNRP70, SFRS14, SNRPA1, CLK2, NXF1,
SFRS8, SFRS2, PTBP2, FUS, SFRS6, SFRS16,
SF3B1, HNRPA3,
SNRPB, PRPF3, SFRS12, U2AF1, PHF5A,
TXNL4A, CUGBP2, MALAT1,
NEAT1,
XIST**

Localization of CCNL2 in the nuclear speckles

CCNL2

cyclin L2

Transcriptional regulator which participates in regulating the pre-mRNA splicing process. Also modulates the expression of critical apoptotic factor, leading to A cell apoptosis



Nulliparous

B



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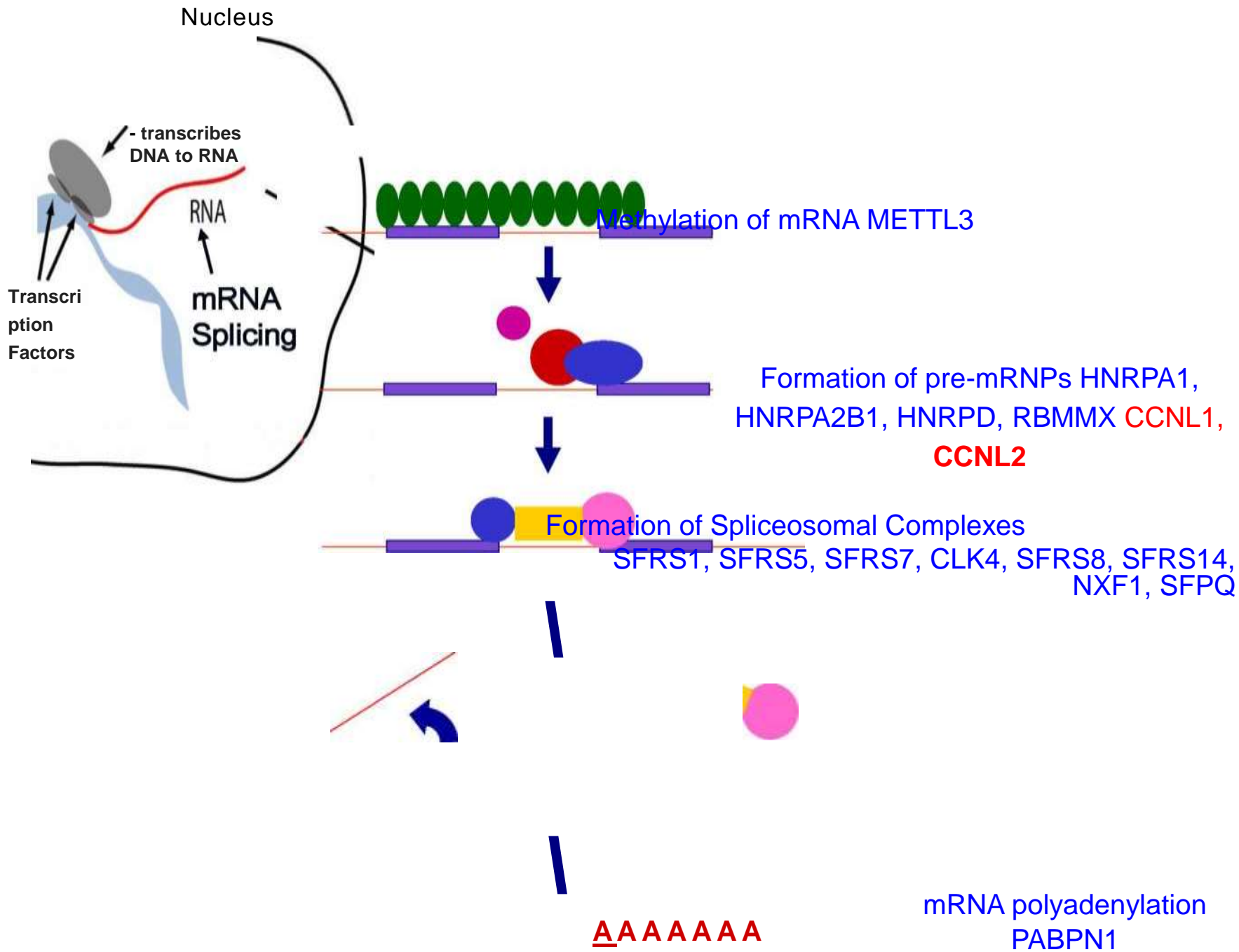


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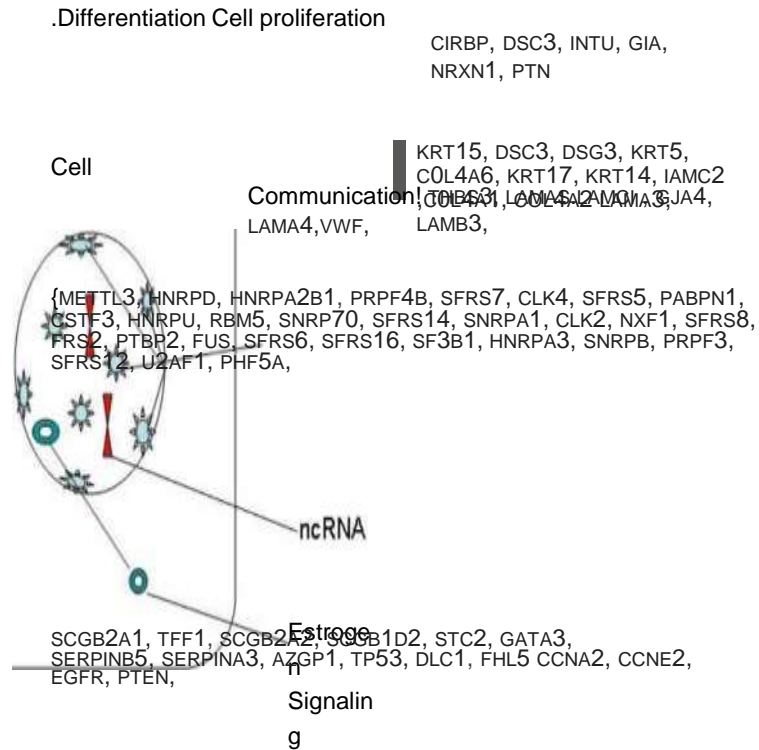


D

Parous



Pathways Affected by Full Term Pregnancy in the Breast Epithelial Cell



■ The differentiation of breast epithelial cells is centered in the mRNA processing reactome.

i The biological importance of the differential expression of genes that control the spliceosome could be an indication of a safeguard mechanism at post-transcriptional level that maintains the fidelity of the transcriptional process.

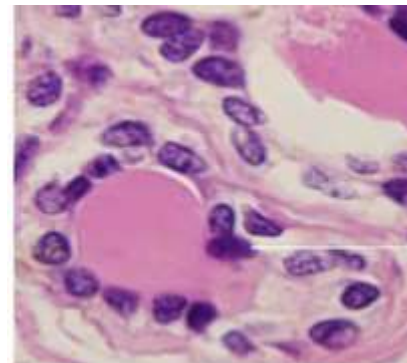
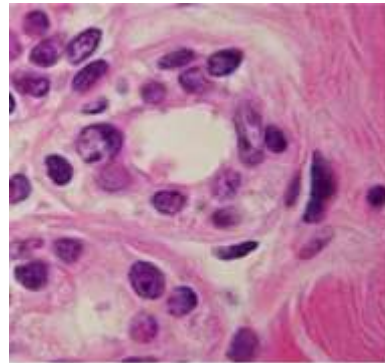
Nulliparous

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T-SSHHS

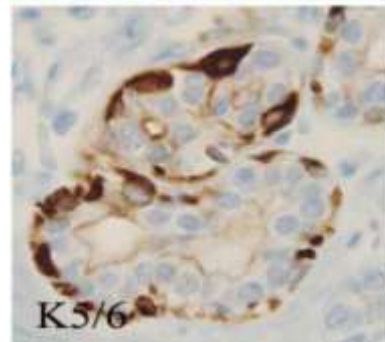
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Parous



HTN



Chromatin Condensation

- Chromatin condensation, limits the ability of RNA polymerase II transcription complex to access DNA, resulting in reduced mRNA and protein output.
- Chromatin condensation can be induced by epigenetic changes that can range from chemical modifications of histone proteins to modifications made to the DNA itself.

Histone 3 methylated at lysine

9

Nulliparous
Breast

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Parous
Breast

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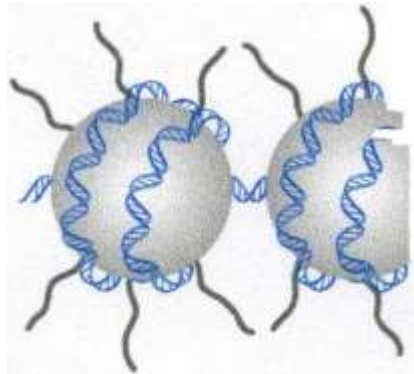
Histone 3 methylated at lysine

27

Nulliparous
Breast

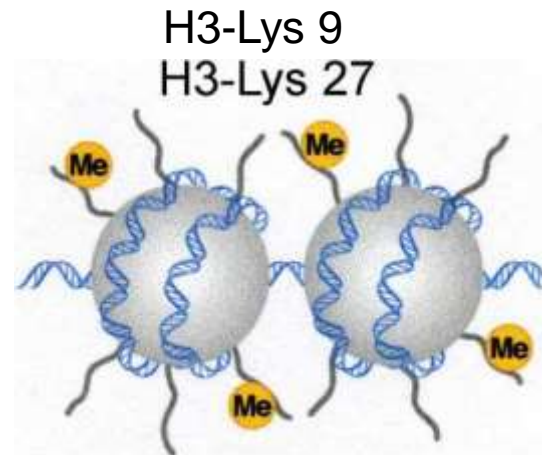
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<p>\ "</p> <p>« > i</p>	<p>% » * ■</p>	<p>sm » V'</p> <p>V • !</p> <p>"fi . 'A, ' *</p>

Parous
Breast



**Transcriptionally
Active Chromatin**

Parous Breast



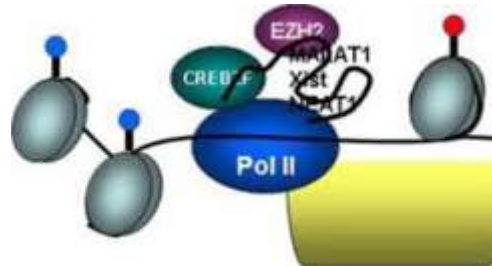
**Transcriptionally
Inactive
Chromatin**

Nulliparous Breast

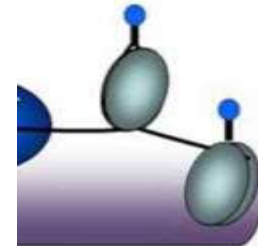
Table 1: Differentially expressed genes in the parous breast tissue			
Gene Symbol	Gene Title	Fold change	FDR q-val
SOX6	SRY (sex determining region Y)-box 6	-1.913	0.016
SOX17	SRY (sex determining region Y)-box 17	-1.956	0.011
CBX3	chromobox homolog 3 (HP1 gamma homolog, Drosophila)	2.110	0.001
EZH2	enhancer of zeste homolog 2 (Drosophila)	2.001	0.001
CHD2	chromodomain helicase DNA binding protein 2	1.956	0.005
GAT A3	GATA binding protein 3	2.217	0.002
CREBZF	CREB/ATF bZIP transcription factor	1.711	0.005
XIST	X (inactive)-specific transcript	2.230	0.001
NEAT1	Nuclear Paraspeckle assembly transcript I	1.980	0.015
MALAT1	Metastasis associated lung adenocarcinomas transcript 1	1.899	0.001

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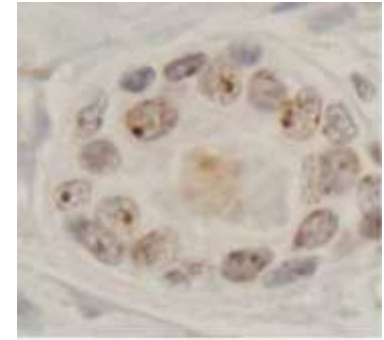
A



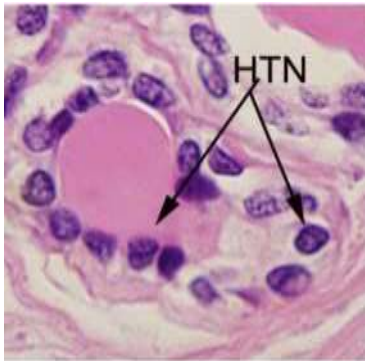
CpG island



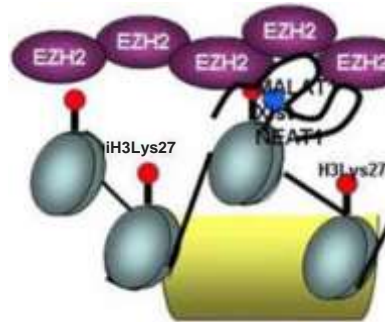
Gene



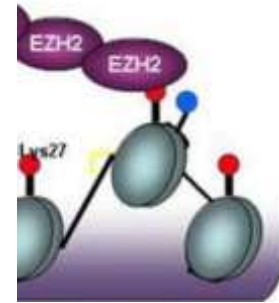
B



C



CpG island



Gene

« ,
 JL^

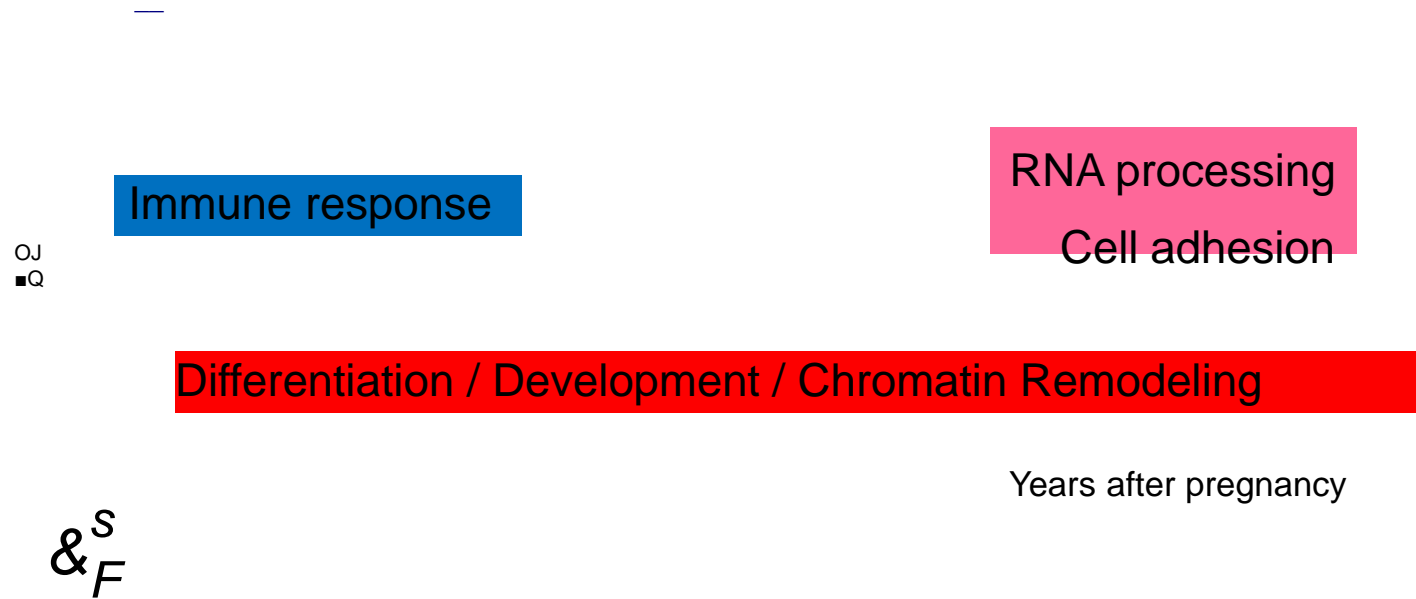


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Pre and post-menopausal women

Premenopausal period

Postmenopausal period



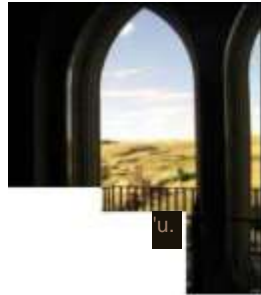
There are not many overlapping genes between both premenopausal and post-menopausal;

We observed that genes related to development, differentiation and chromatin remodeling are up-regulated after pregnancy and remain up-regulated in pre and post-menopausal women.

Impact of GSP

- J An established genomic signature of pregnancy would open myriad of research possibilities, including:
 - a surrogate end point to evaluate the degree of mammary gland differentiation.
 - its association with breast cancer risk.
 - for determining in which women parity has been protective.
 - development of interventions to induce protection against breast cancer.

Windows of Susceptibility



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%@+**

Period

Age



↑
Birth

↑
Weaning



Future Perspectives in Personalized Interventions in Breast Cancer Prevention

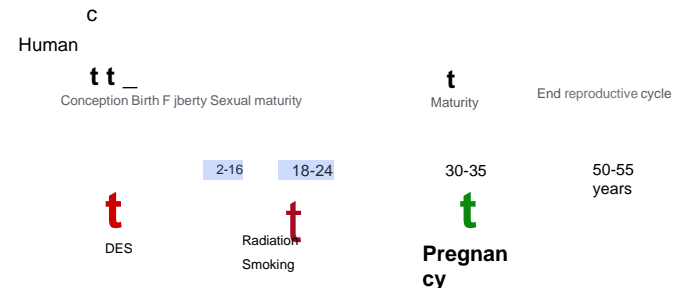
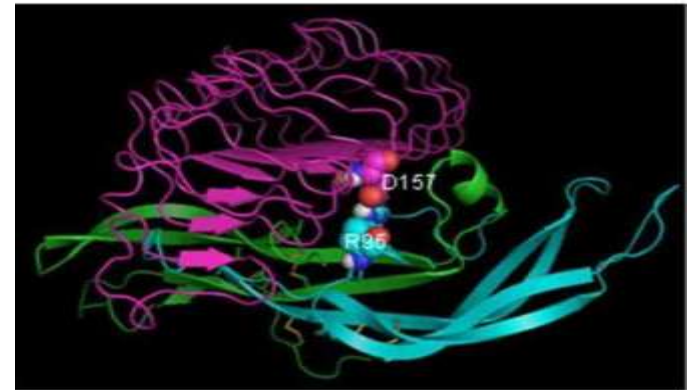
Transform the breast GSP to a blood Signature of Prevention.

To implement the hormonal treatment with hCG, that mimic pregnancy, to nulliparous women.

To target the preventive strategies in young women during the window of susceptibility.



Developing a Blood Test



Acknowledgments



The women of Norrbotten Count, Sweden for their contribution to the project.

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Slifker Luis

Sigal

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Maria Barton Dominic

Strohmeyer Derek

Jacob Colleen

O'Malley Sandy Weng

Cody Watson

A V O N
FOUNDATION



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v

Umea University