The extracellular matrix modulates asynchronous concurrent lactation in tammar wallaby (*Macropus eugenii*)

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Wallaby reproductive strategy

- short gestation
- relatively long lactation
- greater investment in development during lactation compared to eutherians
- milk composition and milk production altered significantly during lactation
Changes in milk composition during lactation
Changes in milk protein gene expression during lactation

Phase 1
Pregnant

Phase 2A
Permanent attachment to teat

Phase 2B
On and off teat
Always in pouch

Phase 3
In and out of Pouch, eating grass

ELP
β-cas  α-cas  α-lac  β-LG  B-1,3 galactosyltransferase

WAP

LLP-B
Domain-specific bioactives delivered at specific times

e.g. Cathelicidins
Found gastro-intestinal tract, respiratory system and the skin

1. antimicrobial
2. wound healing

Role in lactation not clear
MaeuCath1 differentially expressed during lactation

Wanyonyi. S. et al Comparative Biochemistry and Physiology, 2011

Spliced into two variants to

a. Cath 1a may provide antimicrobial defence to the young and mother
b. Cath1b may play a role in mammary growth during peak lactation
Asynchronous concurrent lactation (ACL)

The wallaby can produce both P2A and P3 milk concurrently.
Possible mechanisms for ACL?

1. Hormones
2. Sucking of the young
3. The milk? The ECM?
Possible mechanisms for ACL?

1
Hormones

Changing hormone concentrations does not significantly affect milk protein gene expression
Mechanism 2: Sucking by the young

Highly sensitive to oxytocin
Gentle but continuous sucking by the newborn causes sustained release of small amounts of milk

Low sensitivity to oxytocin
Only aggressive sucking by older sibling stimulates milk secretion

Mechanism 3: The Extracellular matrix (ECM)

Rationale

Stromal/epithelial interactions regulate mammary differentiation
ECM-Cell interaction

Cell comes in contact with ECM

Mechanical and biochemical signals transduced to the nucleus

Change in gene expression profile

New proteins secreted into ECM

Our hypothesis

P2A cells → P2B ECM = P2B phenotype

P2B cells → P3 ECM = P3 phenotype

P2A phenotype = P2A ECM ← P2B cells
Approach

Purify mammary extracellular matrix from various phases of lactation (P1, P2A, P2B, P3 and INV)

Culture wallaby mammary epithelial cells from P2A and P2B on ECM and stimulate with hormones

Assay expression of milk protein genes by qPCR
ECM controls mammosphere morphology

<table>
<thead>
<tr>
<th>Bright field</th>
<th>Confocal</th>
<th>Lactation Phase of ECM</th>
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<tr>
<td>![Bright field image]</td>
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<td>P1, P2A, P2B, P3</td>
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<tr>
<td>![Bright field image]</td>
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<td>INV</td>
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**β-casein is differentially expressed on ECMs**

**P2A cells**

**P2B cells**
ECM changes lactation phase phenotype of mammary cells

P2A cells

P2B cells
ECM changes lactation phase phenotype of mammary cells

P2A cells

P2B cells
ELP regulated independently of ECM and hormones

P2A cells

P2B cells
ECM may regulate the switch from one phase of tammar lactation to another and therefore direct ACL.

The ECM can only change the phase phenotype in a stepwise manner.

It may not be possible to reverse the phenotype to an earlier phase.

The ECM could programme ACL by enhancing the response of the mammary gland to hormones.
1. Can we deliberately change the phenotype of early lactation cells in a stepwise manner into a late lactation phenotype?

2. What are the molecular factors in the ECM that drive ACL?
Thank you
Tammar milk cathelicidins regulated by the ECM

P2B cells

Gene expression

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<tr>
<th></th>
<th>P1 ECM</th>
<th>P2A ECM</th>
<th>P2B ECM</th>
<th>P3</th>
<th>INV</th>
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<tr>
<td>Gene expression</td>
<td>5</td>
<td>6</td>
<td>3</td>
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<td>7</td>
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</table>
Heparan sulfate proteoglycan core protein and glycogen IV
Laminin 250kD tenascin 240kD
Fibronectin 230kD
Vimentin 57kD
Alpha-actinin-2
Annexin A2 39 kD
Serpin H1
β-casein 24.1 kD
Nidogen-1 precursor
Biglycan/Laminin 1
Decorin-like iso-form 1
Unidentified

ECM composition changes during lactation
MMP activity is increased during P3 and involution.