

## Protection against Breast Cancer by Phosphorylated Prolactin in Milk

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## Pregnancy/lactation and breast cancer

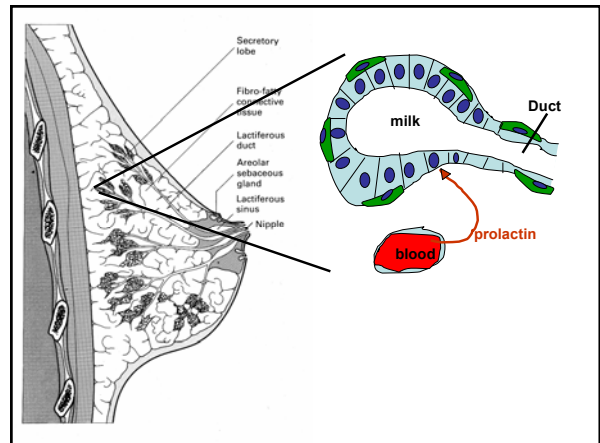
- An early pregnancy (<24 years) is protective against breast cancer
- This has been hypothesized to be due, at least in part, to a degree of differentiation of mammary stem cells

Reviewed in Russo *et al.* Breast Cancer Res 7: 131, 2005

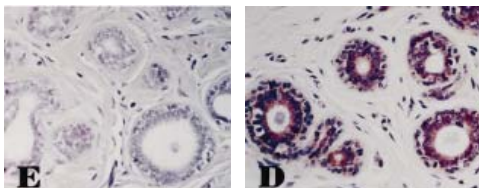
- Here we suggest that a phosphorylated form of the hormone prolactin plays an important role in this process, and that it occurs in an unexpected way

## Traditional View of Prolactin

- A hormone released in large quantities by the pituitary gland during pregnancy and in response to suckling
- Travels in the circulation to the mammary gland
- Major function to develop secretory portion of the mammary gland and promote the synthesis of milk proteins during lactation



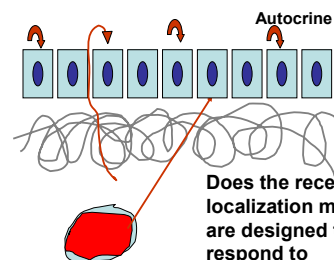
**BUT** in normal human non-pregnant and non-lactating breast, prolactin receptors are located throughout ducts and primarily on the opposite side of the duct than one would expect.



Work from Clevenger lab, first published in Am J Pathol 146:695, 1995

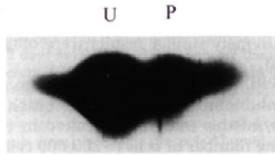
Also described by Vonderhaar lab (Gill *et al.* J Clin pathol 54:956, 2001)

In addition to pituitary prolactin, mammary cells make prolactin and proliferate in response to that prolactin



Does the receptor localization mean that cells are designed to primarily respond to autocrine/paracrine PRL?

Prolactin (PRL) comes in two varieties:  
unmodified (U) and phosphorylated (P)



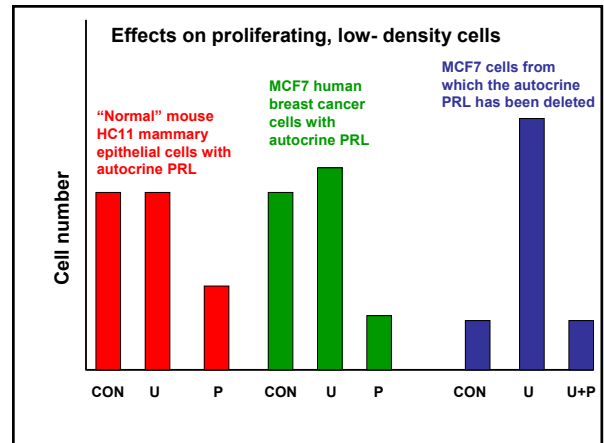
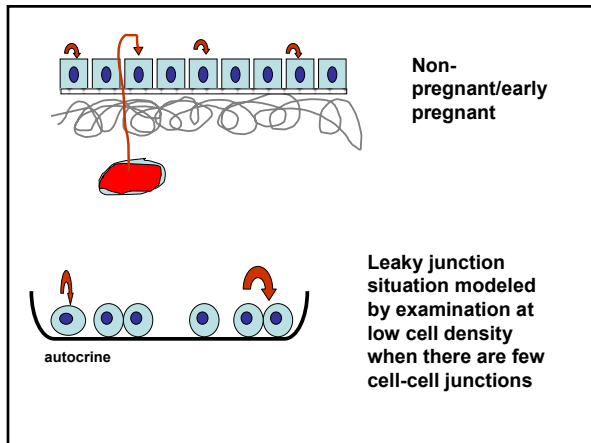
J Biol Chem 261:  
1649, 1986

Milk contains a lot of prolactin and 70-100% of rodent  
and human colostrum/milk prolactin is phosphorylated

[Ellis LA, Picciano MF.](#)  
Bioactive and immunoreactive prolactin  
variants in human milk.  
Endocrinology.136:2711, 1995.

[Grosvenor CE, Picciano MF, Baumrucker  
CR.](#)  
Hormones and growth factors in milk.  
Endocr Rev 14:710, 1993.

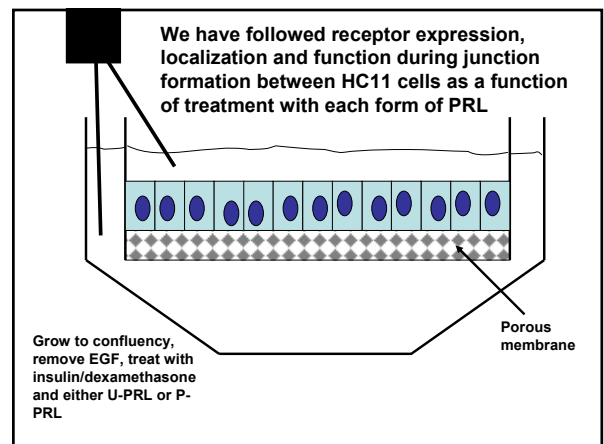
We have therefore examined the effect of both  
unmodified PRL and phosphorylated PRL on  
mammary epithelial cells



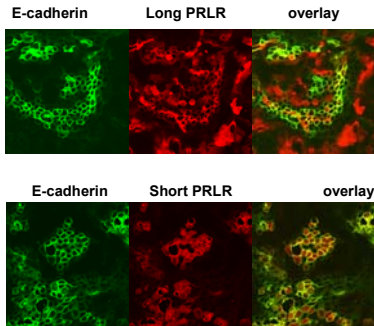
### In cells with few/loose junctions

- Most proliferation in response to PRL is dependent on autocrine PRL, which under these circumstances is unmodified PRL.
- This could be secreted on the apical face in the intact gland, accounting for placement of the receptors
- The effect of this autocrine unmodified PRL and exogenous U-PRL can be antagonized by P-PRL, which can come from the pituitary through the leaky junctions

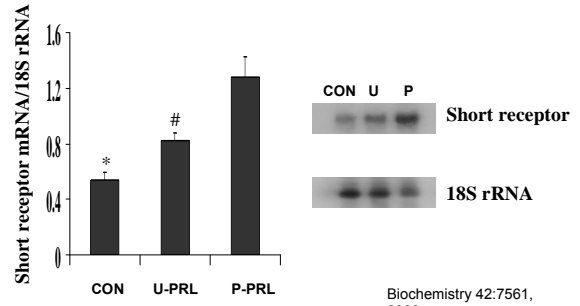
What happens as the junctions between cells  
become more extensive?



The Long prolactin receptor is expressed with and without cell-cell adhesion, whereas the Short prolactin receptor is only expressed after cell-cell adhesion

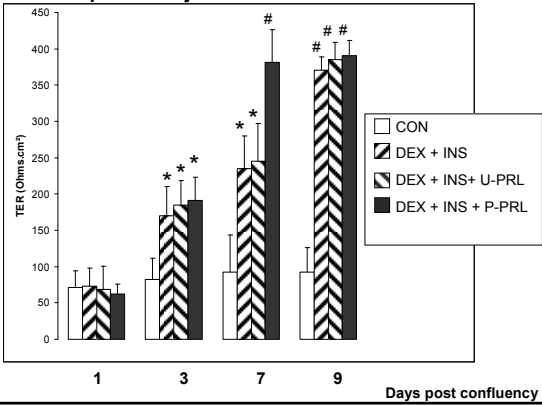


Expression of Short PRL receptors is increased by PRL treatment and P-PRL works best

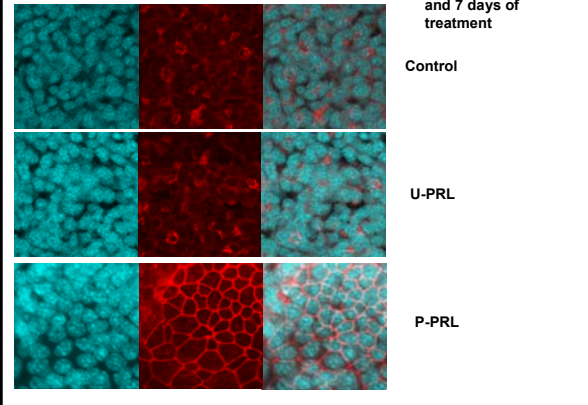


Biochemistry 42:7561, 2003.

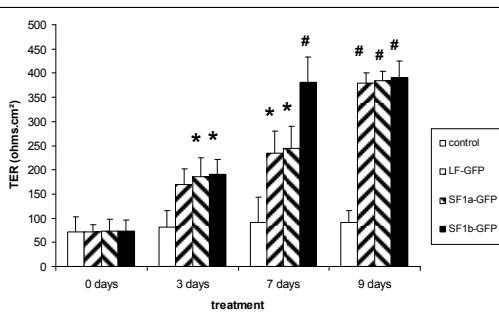
P-PRL promotes junction formation



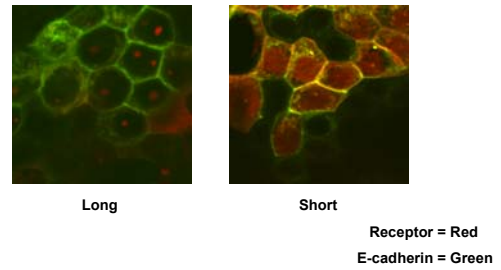
DAPI ZO-1 Overlay After Confluence and 7 days of treatment



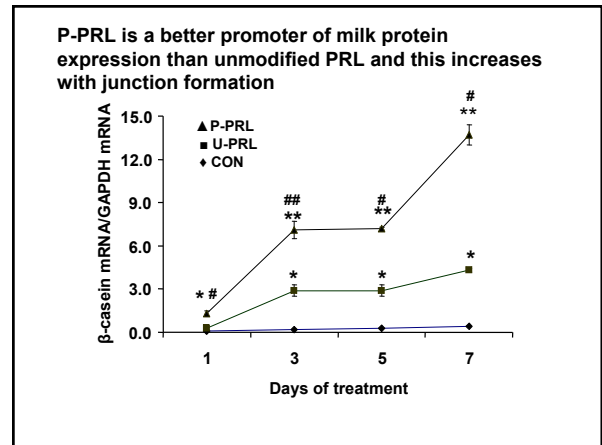
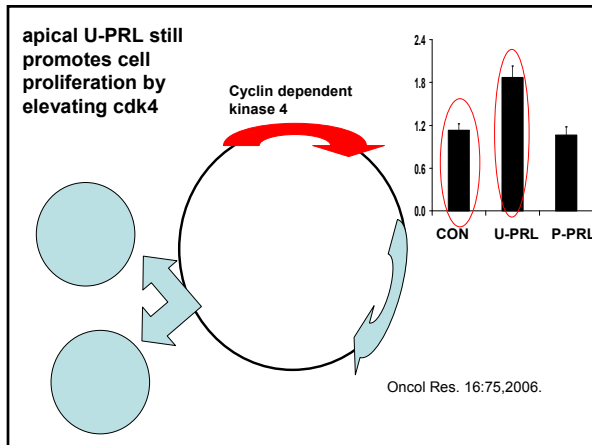
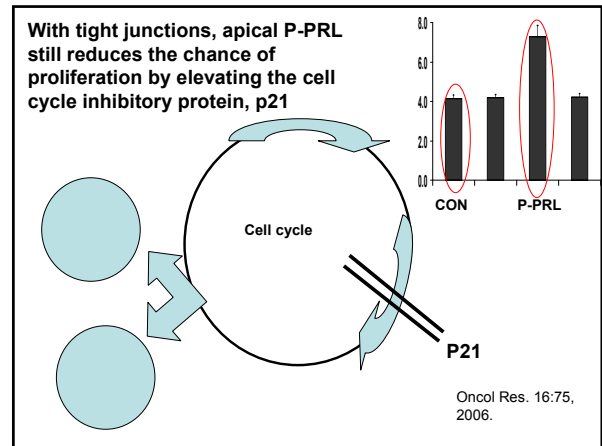
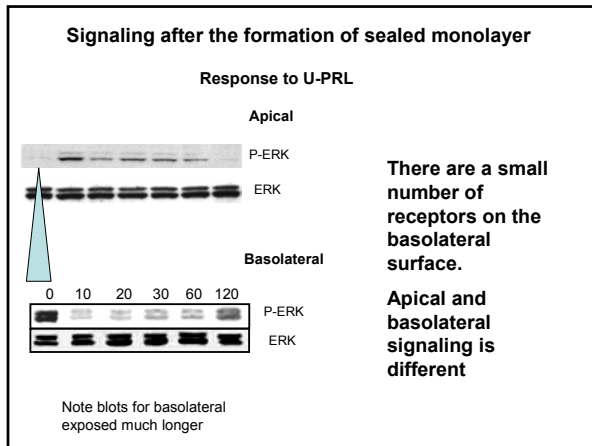
Transfection with different forms of the human receptor shows Short form 1b to promote junction formation



Once polarized, Prolactin receptors were only seen on the apical (milk) face

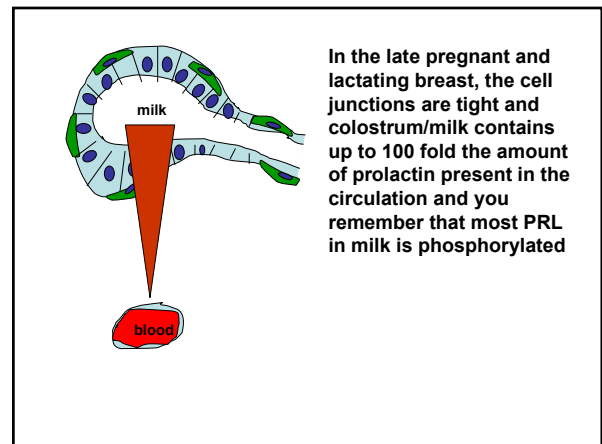


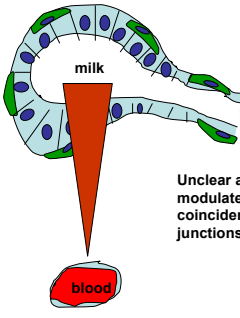
Could not detect any basolateral (blood face) staining for prolactin receptor



### With increasing cell-cell interactions

- P-PRL increases expression of Short PRL receptors and signaling through short receptors in turn increases cell-cell junctions and the two effects below
- P-PRL is the best stimulator of differentiated function, as assessed by  $\beta$ -casein expression
- Apical P-PRL continues to prevent cell proliferation, whereas U-PRL predisposes to cell proliferation
- As soon as junctions begin to form, the receptors are primarily on the apical side (regardless of type) and so the vast majority of the response to PRL is by PRL released by mammary cells, although it may not have been synthesized by those cells





**U-PRL from the circulation is taken up by the mammary epithelial cells and gets phosphorylated on its way across.**

Unclear as yet is how this phosphorylation is modulated. It is clear that it gets turned on coincidentally with the formation of tight junctions.

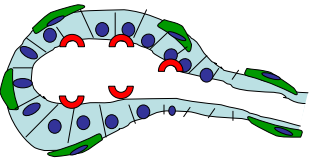
**Is there any evidence that the components of milk affect the mammary epithelial cells?**

### Apical human breast milk signaling in HC11 cells

0 5 15 30 min				0 5 15 60 120 180 min				
IP: α-stat5				P-ERK				
WB: α-pY				T-ERK				
α-Stat5								

**These signals look like a combination of U-PRL and P-PRL**

**They can be reduced by antibodies to PRL**



Whatever the stage of development in the model system, exposure to P-PRL inhibits cell proliferation, promotes junction formation and promotes cell differentiation. This is primarily by interaction with luminal receptors

**The P-PRL component of milk may be an important player in conferring protection against breast cancer afforded by pregnancy/lactation, possibly through the differentiation of mammary stem cells**

### Walker lab 2005-2007



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 Eric Ueda    Mary Lorenson    Wei Wu    Valencia Williams