EAAP Bratislava 2012

Secretion of water into milk: specific constituent or unregulated diluent?

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Overview

- In addition to nutrition and immunological protection, milk provides an essential hydration function

- Water content of milk varies widely between species, and there is potential benefit to be gained from manipulating water content

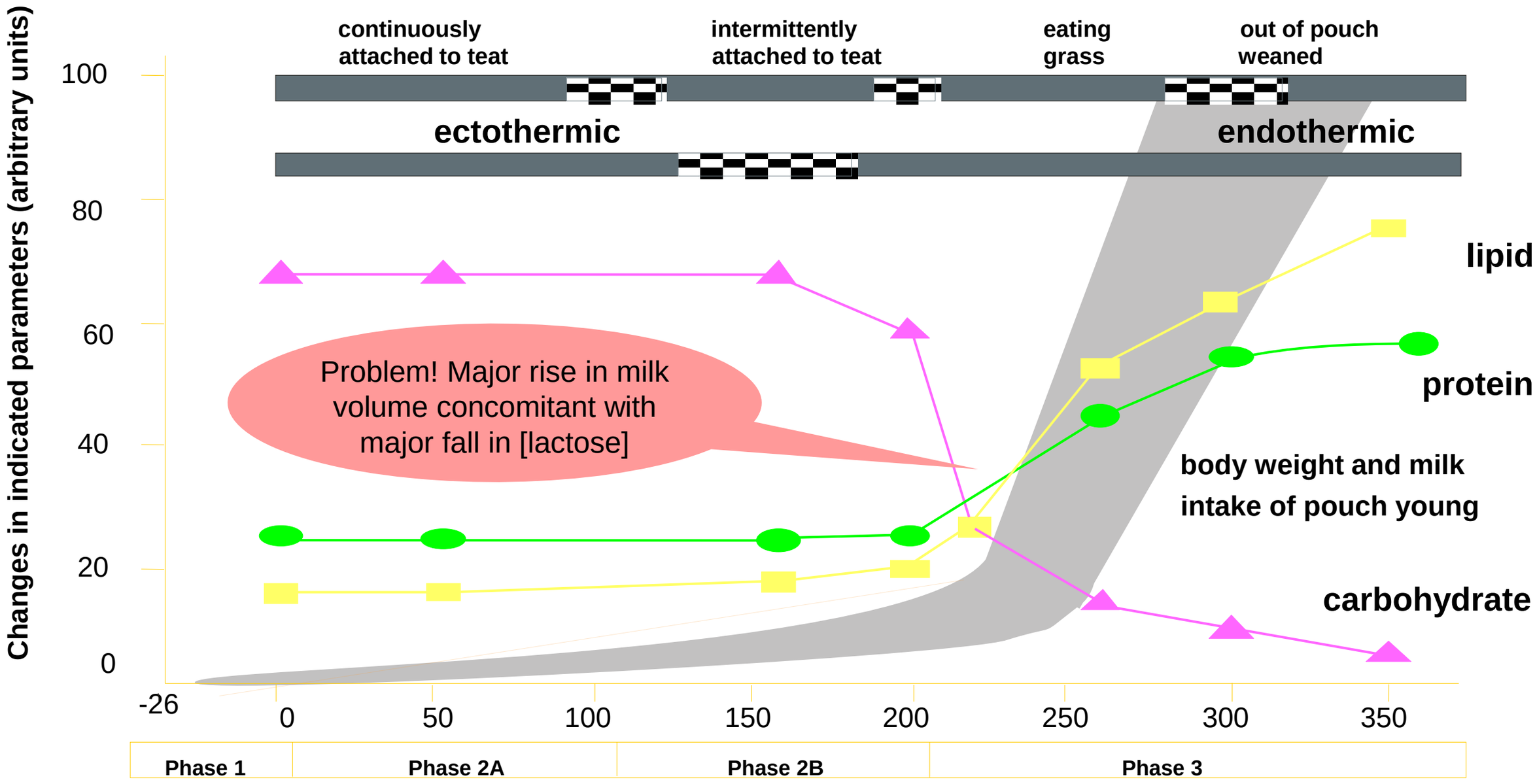
- The mechanism linking lactose, ion and water secretion has been recognized for many years (but may not be universal)

- There remains one problem: lipid membranes are relatively impermeable to water

- Water channels (primarily aquaporins) have not been extensively studied in the mammary gland

Why manipulate composition?

Changes in milk composition during the tammar wallaby lactation cycle

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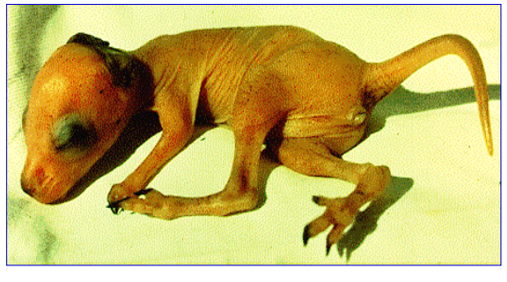

Day 70


Day 185


Day 220

Water secretion into milk

- **Matsuzaki *et al* (2005)** Expression and immunolocalisation of water-channel aquaporins in the rat and mouse mammary gland. *Histochem. Cell Biol.* **123** 501-512

- **Mobasheri *et al* (2011)** Cellular localization of aquaporins along the secretory pathway of the lactating bovine mammary gland: An immunohistochemical study. *Acta Histochem.* **113** 137-149

Matsuzaki *et al* 2005

- Because saliva, sweat and tears are rapidly secreted.....rapid bulk water flow across the apical membrane is necessary

- In comparison, milk is secreted gradually....it seems that rapid bulk water transfer is unnecessary in the apical membrane of the mammary gland

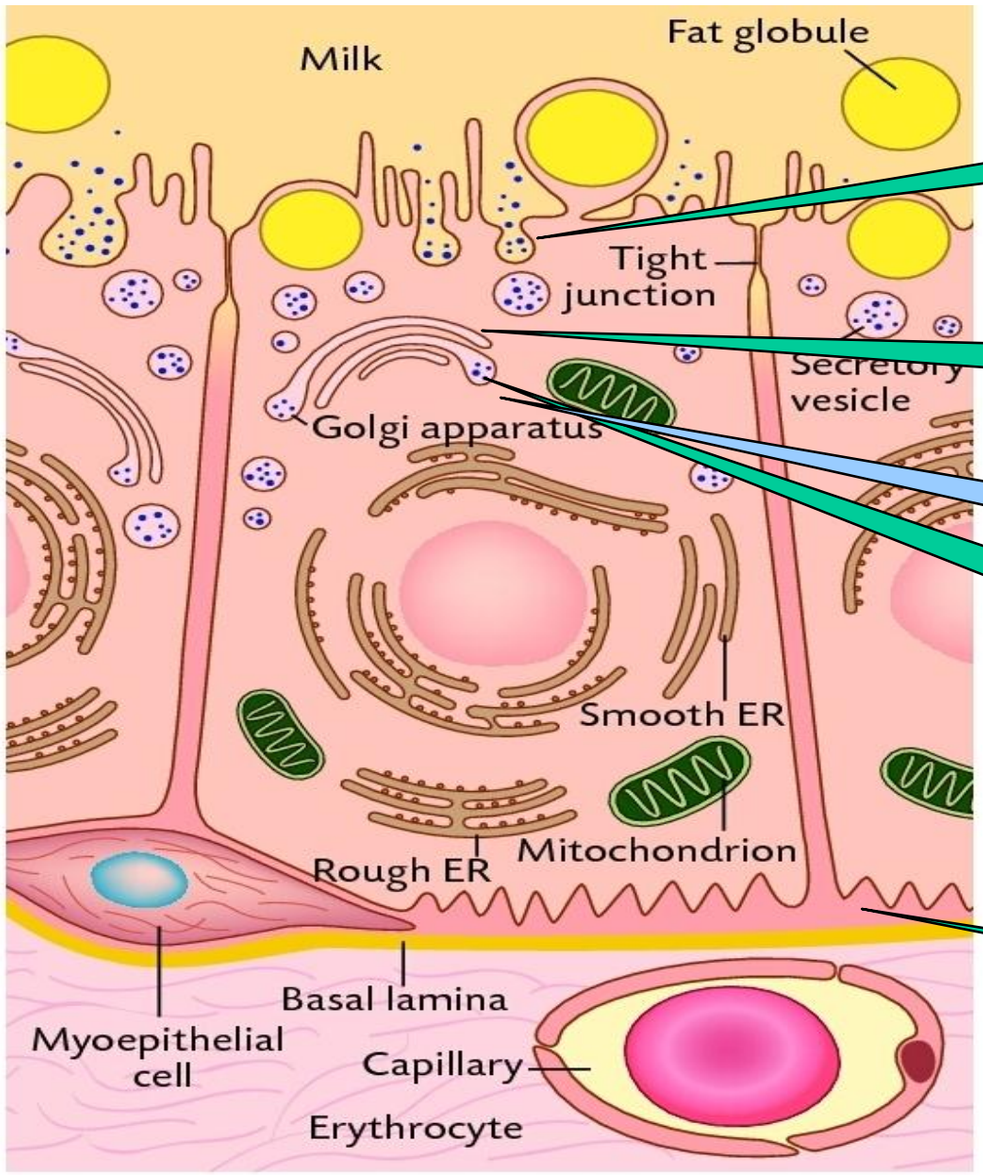
Really?

Mobasheri *et al* 2011

- Aquaporins... may be participants in the control of milk water content by diluting the sugar protein and lipid content of milk to an isotonic solution as it descends through the teat duct system

Really?

Secretion of lactose and water



Exocytosis of golgi apparatus/secretory granules

Accumulation of glucose and galactose, lactose synthesis. Osmotic drive for water.

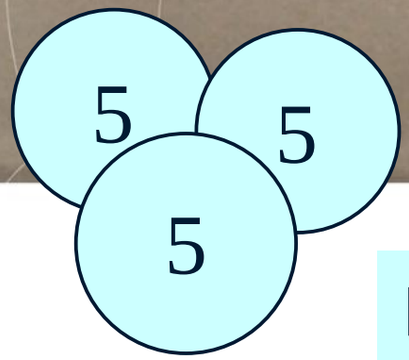
Protein trafficking through golgi and packaging into secretory vesicles

Trafficking of intact glucose to Golgi

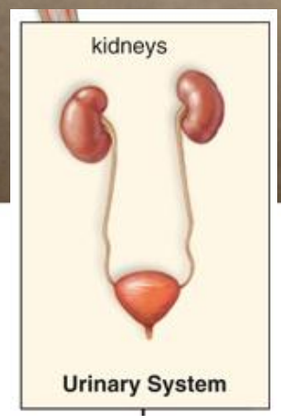
Insulin-insensitive uptake of glucose. Glut 1?

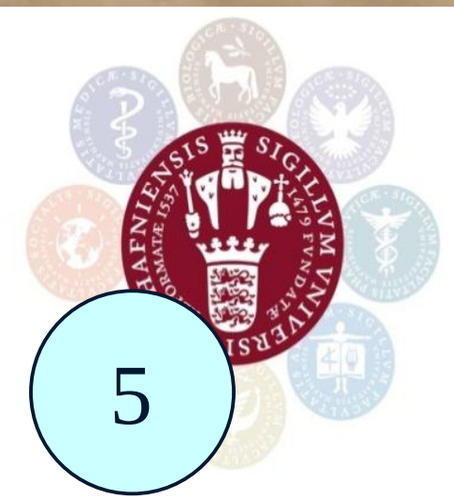
Water flux requires strict regulation

Bovine water fluxes

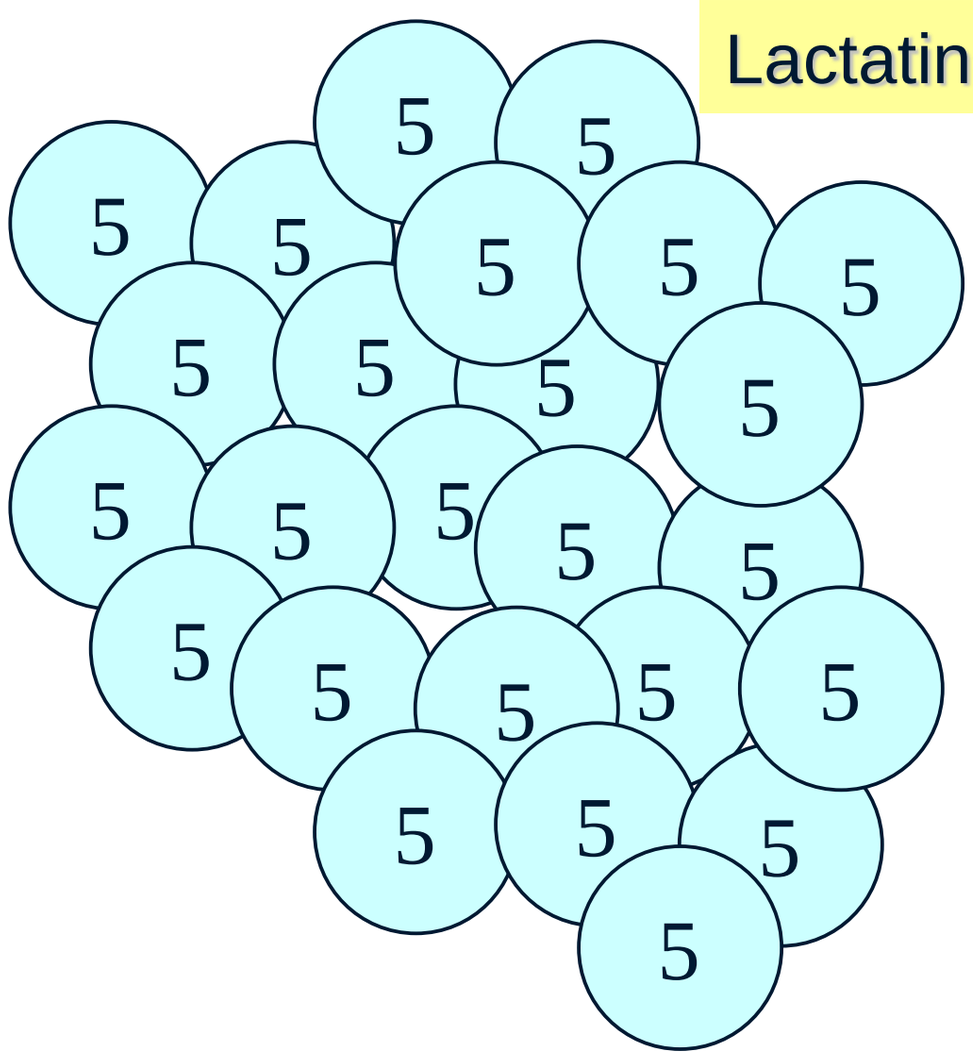


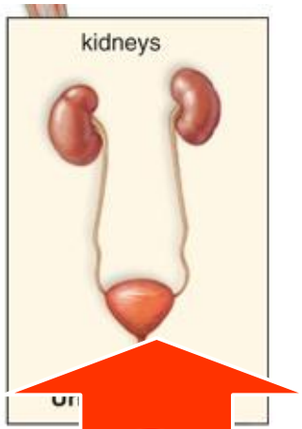
Non-lactating cow





Lactating cow





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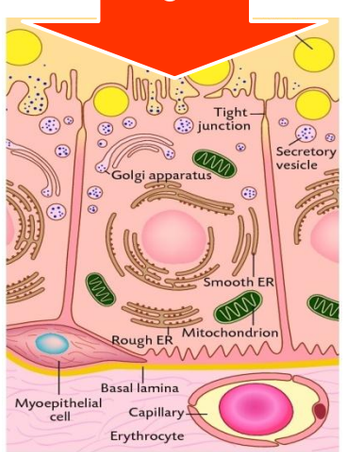
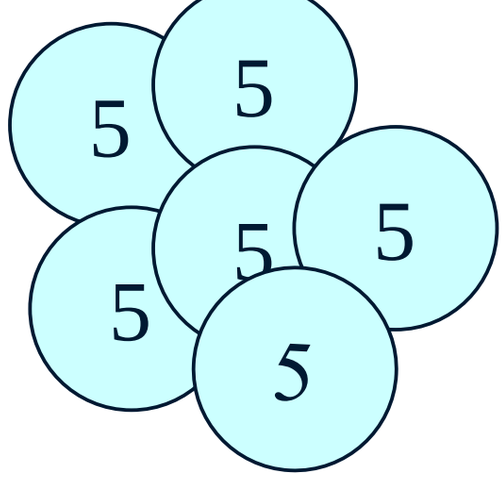
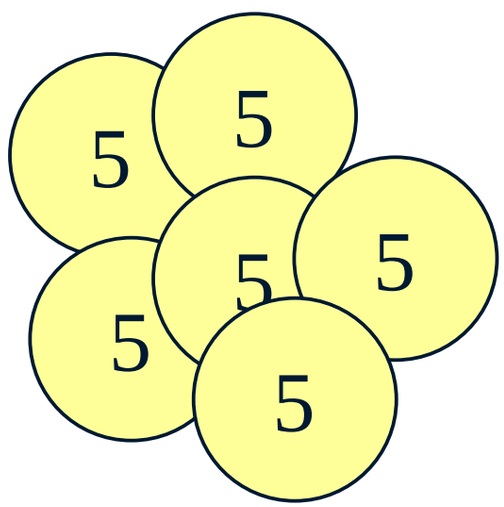
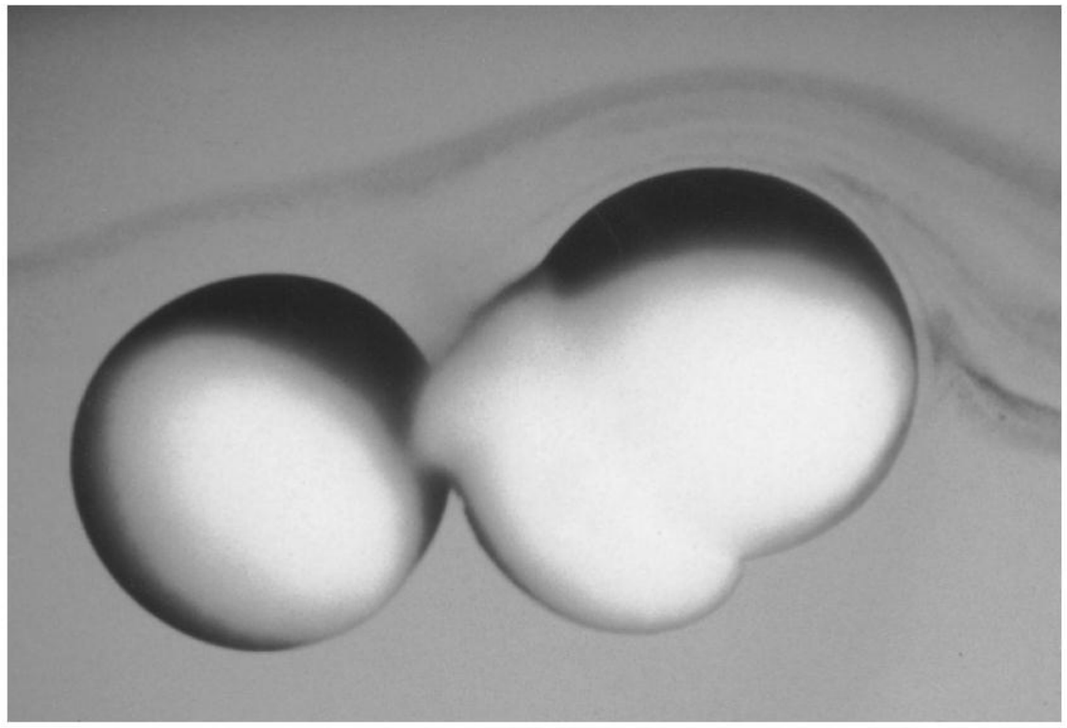




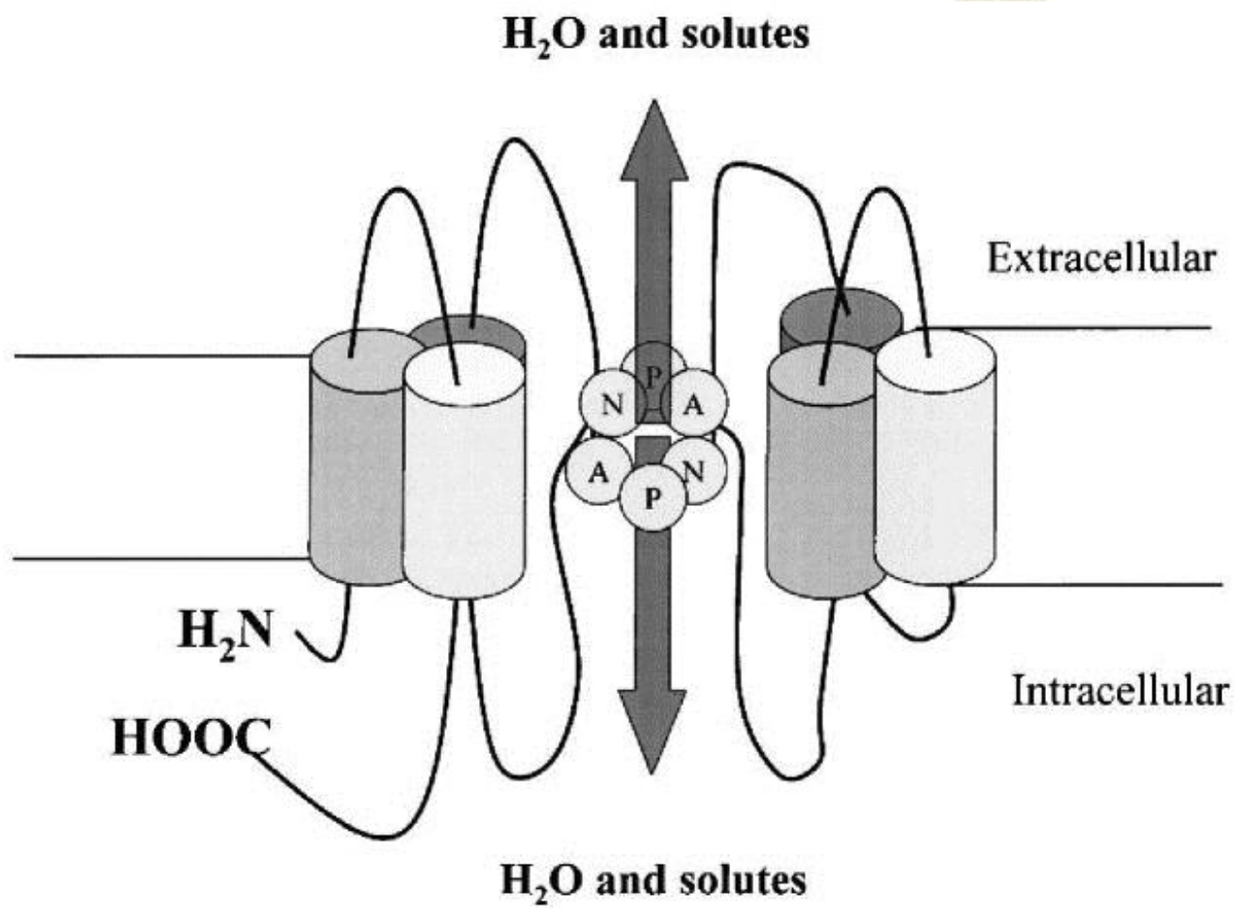


Aquaporins



AQP1 (CHIP28) expressed in *Xenopus laevis* oocyte (right)

After Agre and Parker, 1991



The "hourglass" AQP structure. Symmetrical repeats of 3 membrane spanning domains fold such that conserved NPA motifs form a neutral central pore

Aquaporins

- Aquaporins (AQPs) are small molecular weight (26 kDa) pore-forming membrane proteins, members of the MIP family, that facilitate water transport down an osmotic gradient

- There are at least 13 mammalian AQP, AQP0 to AQP12

- Three classes of AQP transport water only, water and small solutes including glycerol or water and ammonium

- Physiological roles have been demonstrated in numerous tissues, extending beyond water flux (eg NO flux and hence vasodilation, interaction with claudins and occludins to regulate Tight Junction function)

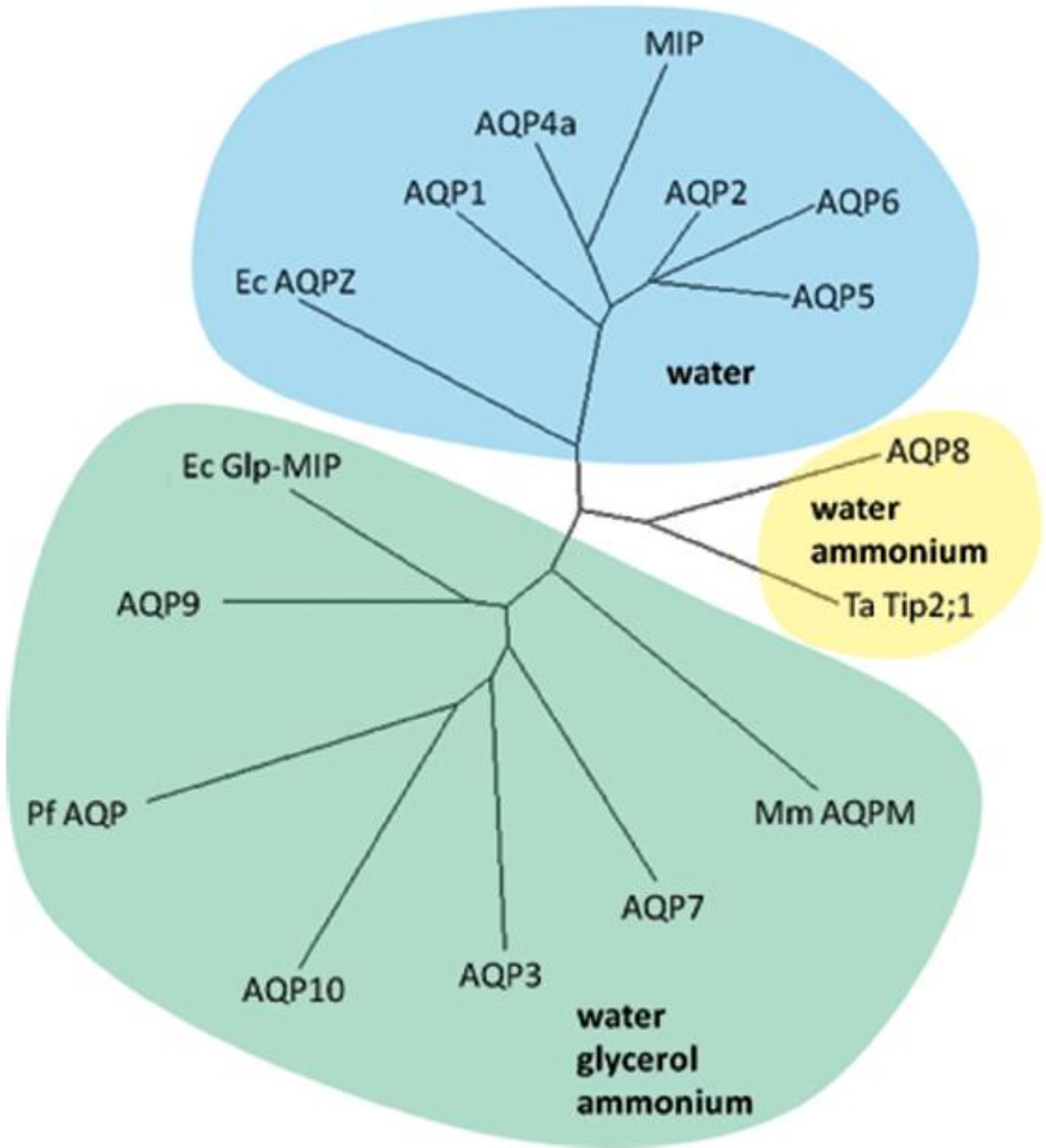
- Numerous examples of developmental regulation of expression, relatively few examples of permeability regulation

- Swelling of secretory granules in salivary glands represents a possible role involving an AQP located on an intracellular (Golgi) membrane

- Evidence for a role in mitochondrial function is equivocal

Aquaporins

Ammonia and Urea Permeability of Mammalian Aquaporins



Aquaporins

Table 1 Permeability and tissue distribution of aquaporins AQP

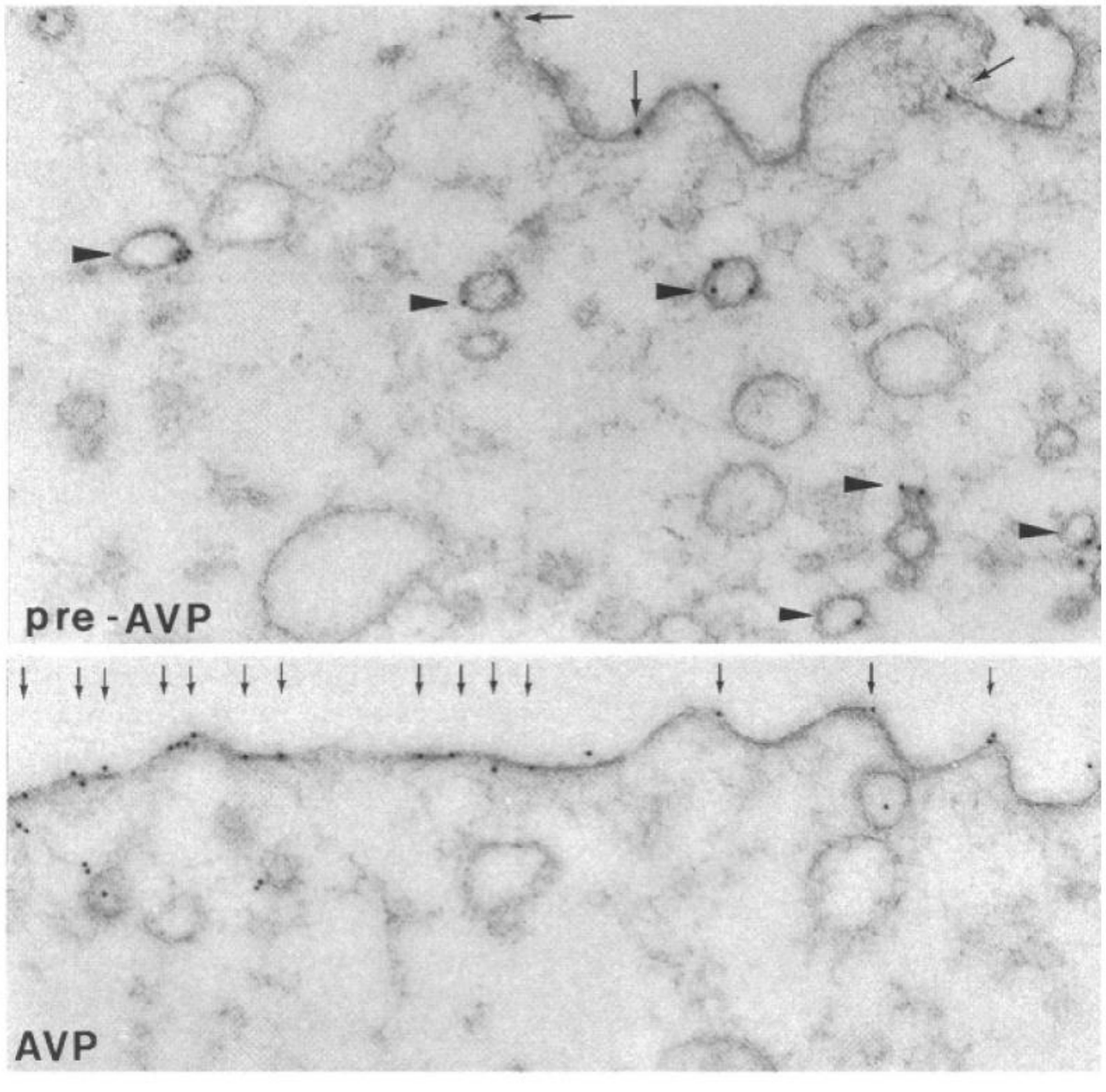
	H_2O	Ammonia	Urea	Glycerol	Tissue or cells
AQP0	(+)	ND	−	−	Eye lens fibers
AQP1	+	−	−	−	Erythrocyte, endothelia, choroid plexus, epithelium, corpus ciliare, kidney proximal tubules, etc
AQP2	+	−	−	−	Kidney collecting duct
AQP3	+	+	(+)	+	Colon, epidermis, airway epithelium, kidney collecting duct, eye
AQP4	+	−	−	−	Astroglial in spinal cord and brain, skeletal muscle
AQP5	+	−	−	−	Alveolar epithelium, glandular epithelia, corneal epithelium
AQP6	(+)	ND	(+)	(+)	Intercalated cells in kidney, organelles
AQP7	+	+	+	+	Adipose tissue, kidney proximal tubule, testis
AQP8	+	+	−	−	Liver, testis, kidney, pancreas
AQP9	(+)	+	+	+	Liver, testis, brain, leucocytes
AQP10	(+)	ND	+	+	Small intestine (intracellular)
AQPZ	+	ND	−	−	Bacteria, *Escherichia coli*
AQPM	+	ND	−	+	*Methanothermobacter marburgenesis*
PfAQP	+	+	+	+	Malaria, *plasmodium falciparum*
TaTIP2;1	+	+	−	−	Wheat, *Tritium aestivum*
GlpF	−	ND	−	+	Bacteria, *Escherichia coli*

Physiological functions of AQPs

Water transport

Altered sub-cellular localization of AQP2 in kidney collecting duct in response to arginine-vasopressin

Similar mechanisms have been demonstrated in bile duct cholangiocytes in response to secretin (AQP1) and in selected salivary glands in response to acetylcholine (AQP5), etc



Physiological functions of AQPs

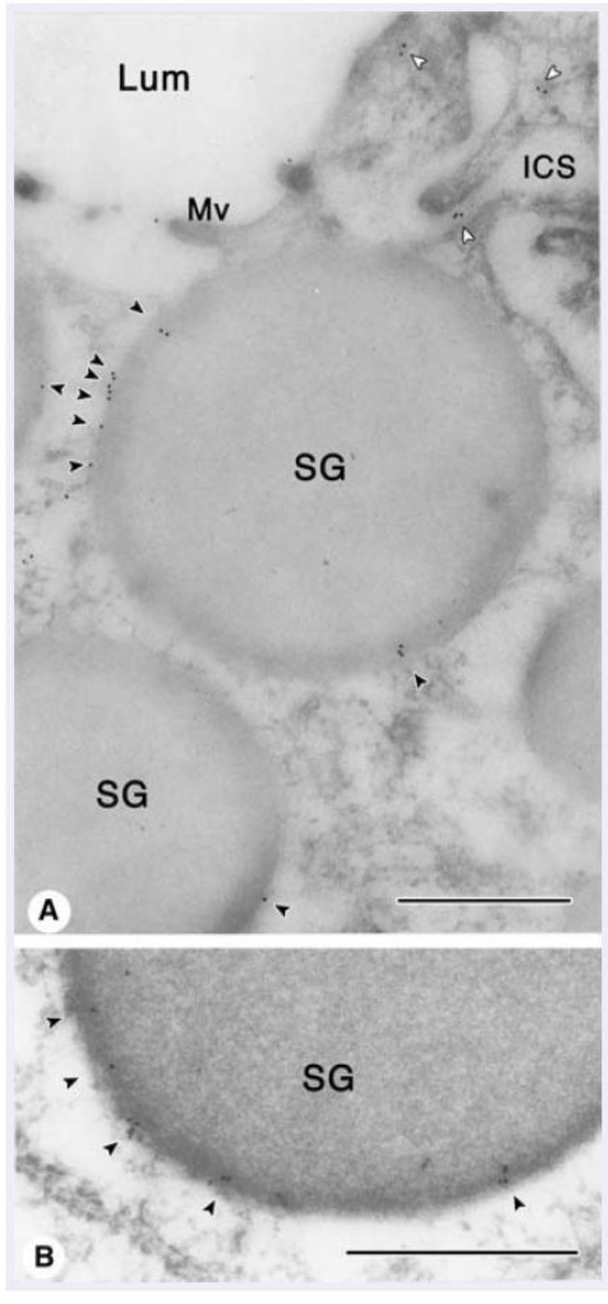
Not associated with water transport

- Cell-cell adhesion, AQP0, lens fibre cells
- Cell migration, AQP1, mouse tumor models
- Maintenance of blood brain barrier, AQP4
- Maintenance of GI tract epithelial integrity, AQP3
- Skin hydration, elasticity, wound healing, AQP3
- Adipose tissue metabolism, AQP7
- Liver gluconeogenesis, AQP9

Physiological functions of AQPs

Protein secretion, swelling of secretory granules

AQP6, rat parotid secretory granules



Sugiya *et al* 2008 Role of aquaporins and regulation of secretory vesicles volume in cell secretion. *J. Cell Mol. Med.* **12** 1486-1494

Physiological functions of AQPs

Protein secretion, fusion at apical porosomes

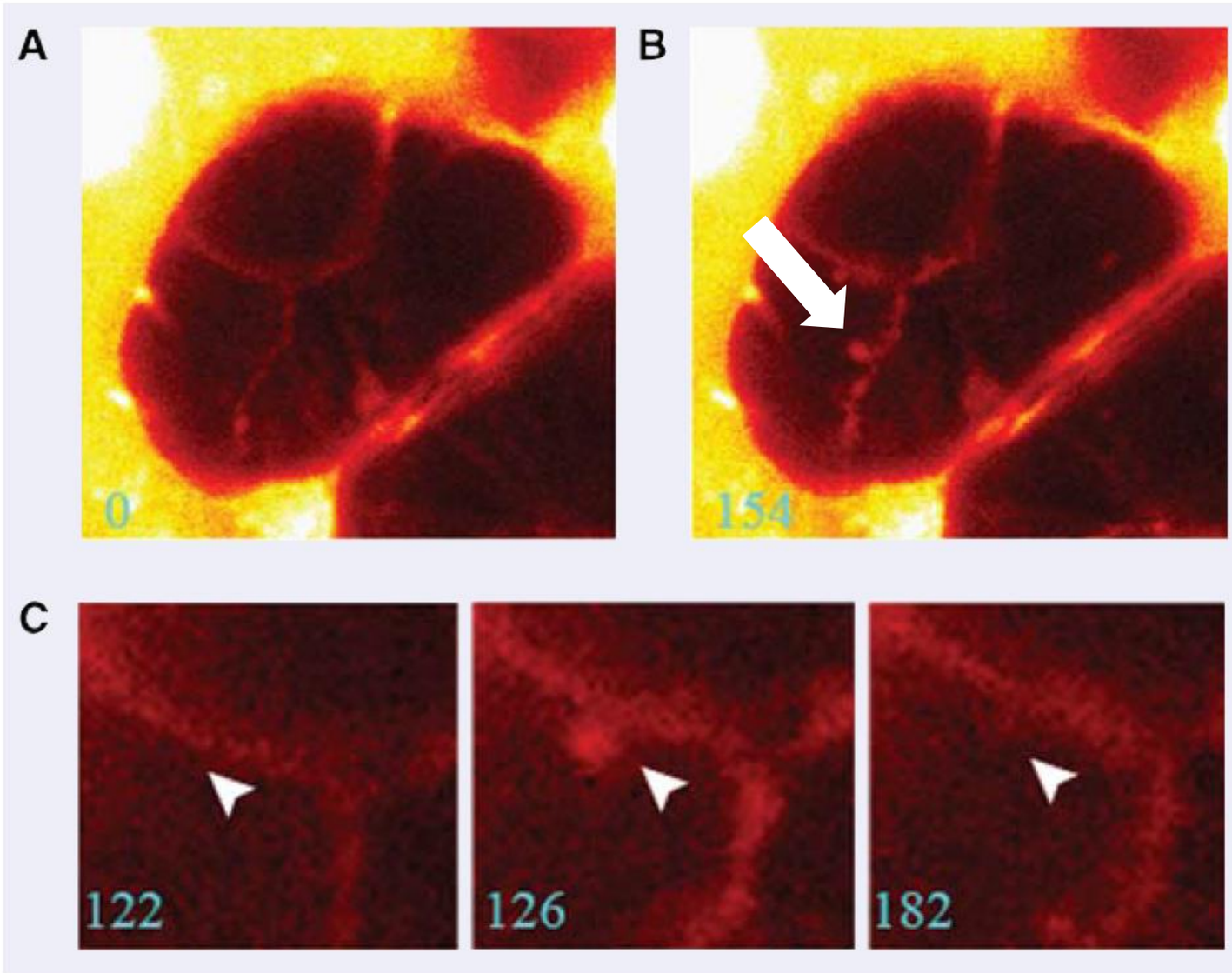
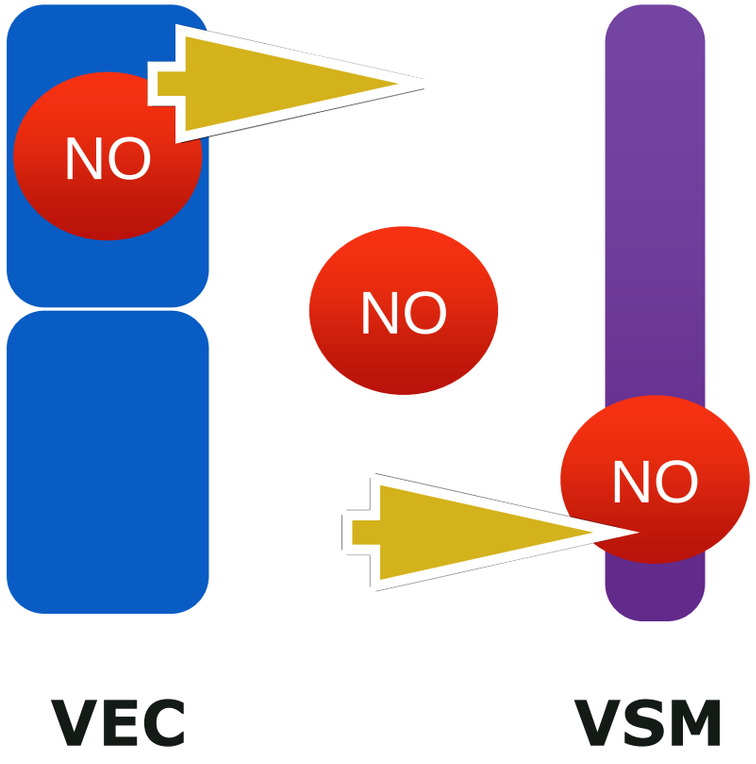


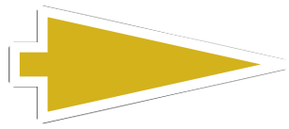
Fig. 2 Cell secretion provoked by isoproterenol in rat parotid acinar cells. In sulforhodamine B (SRB) fluorescence image, there was no docked-granule profile before stimulation (**A**). When the cells were stimulated by isoproterenol for 154 sec., docked-granule profiles appeared at apical region (**B**). During stimulation with isoproterenol (122–182 sec.), a docked-granule profile formed and subsequently disappeared as indicated by an arrowhead (**C**).

Physiological functions of AQPs

Nitric oxide transport and vasodilation

- **Herrera, M & Garvin, JL (2007).** Novel role of AQP1 in NO-dependent vasorelaxation. ***Am. J. Physiol. Renal Physiol.* 292** F1443-F1451





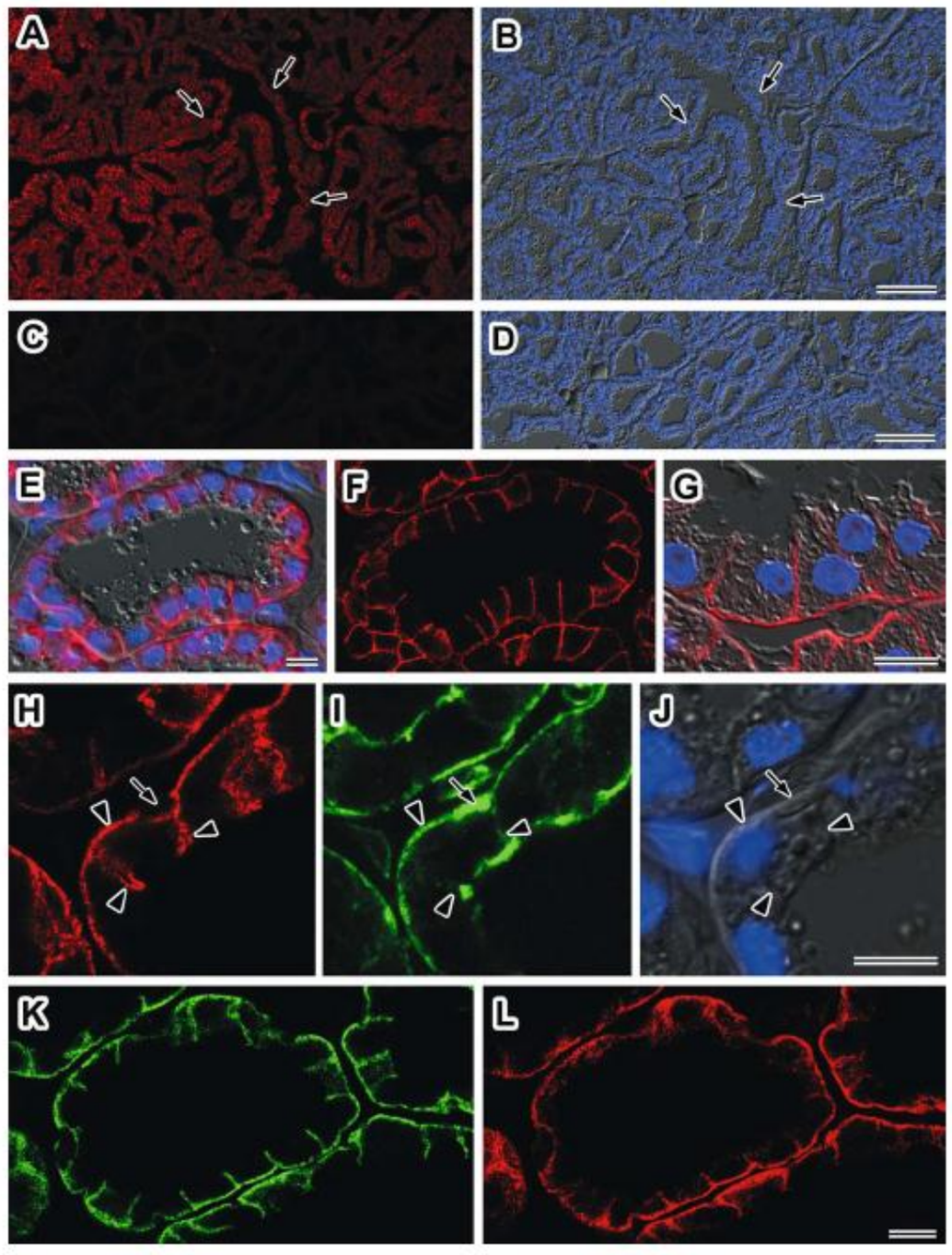
AQP1

AQP1-null mice have impaired vasodilation in response to acetylcholine

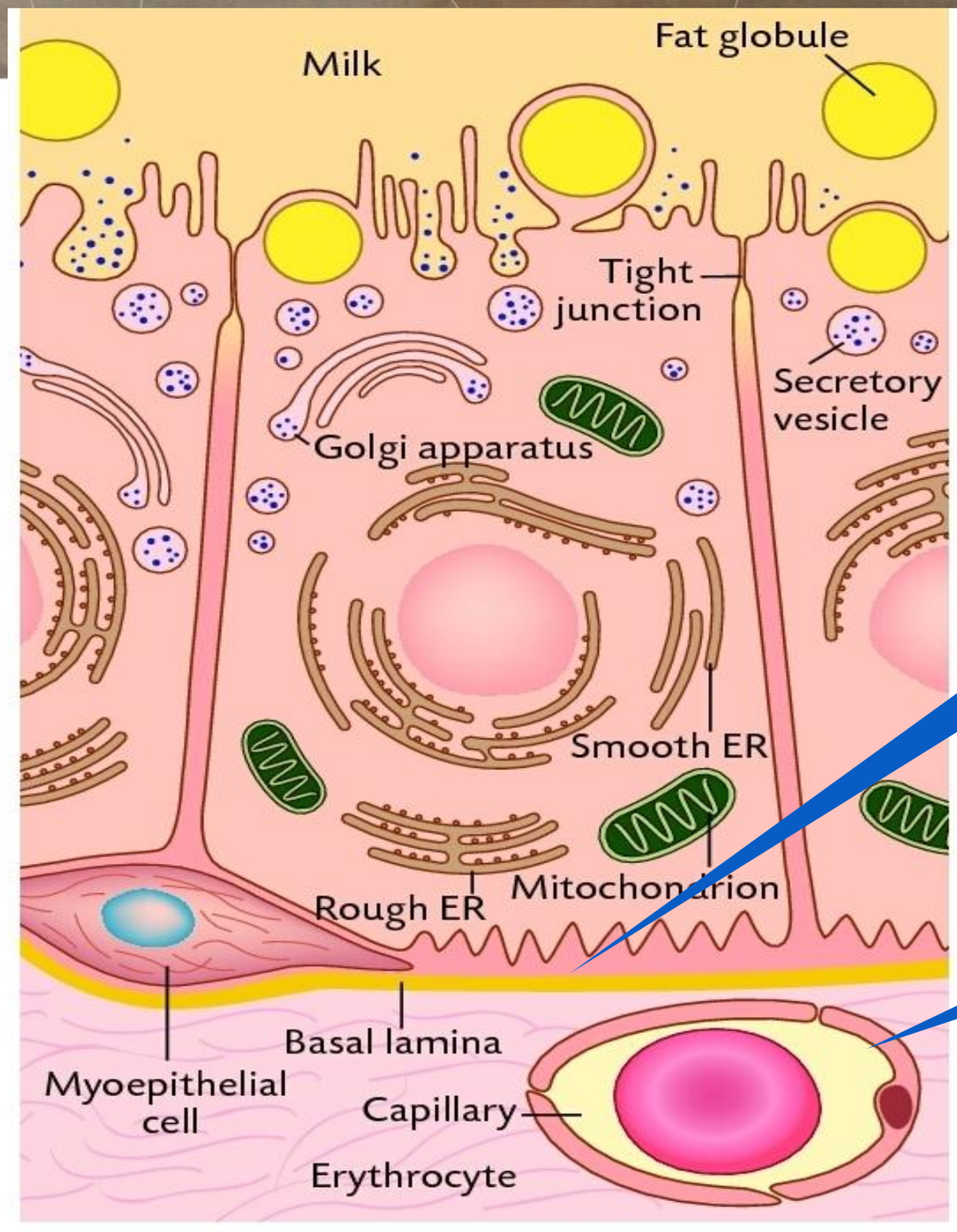
Back to the mammary gland

Human lactation is important!
www.colact.net

Matsuzaki *et al* 2005



Matsuzaki *et al* 2005



AQP3

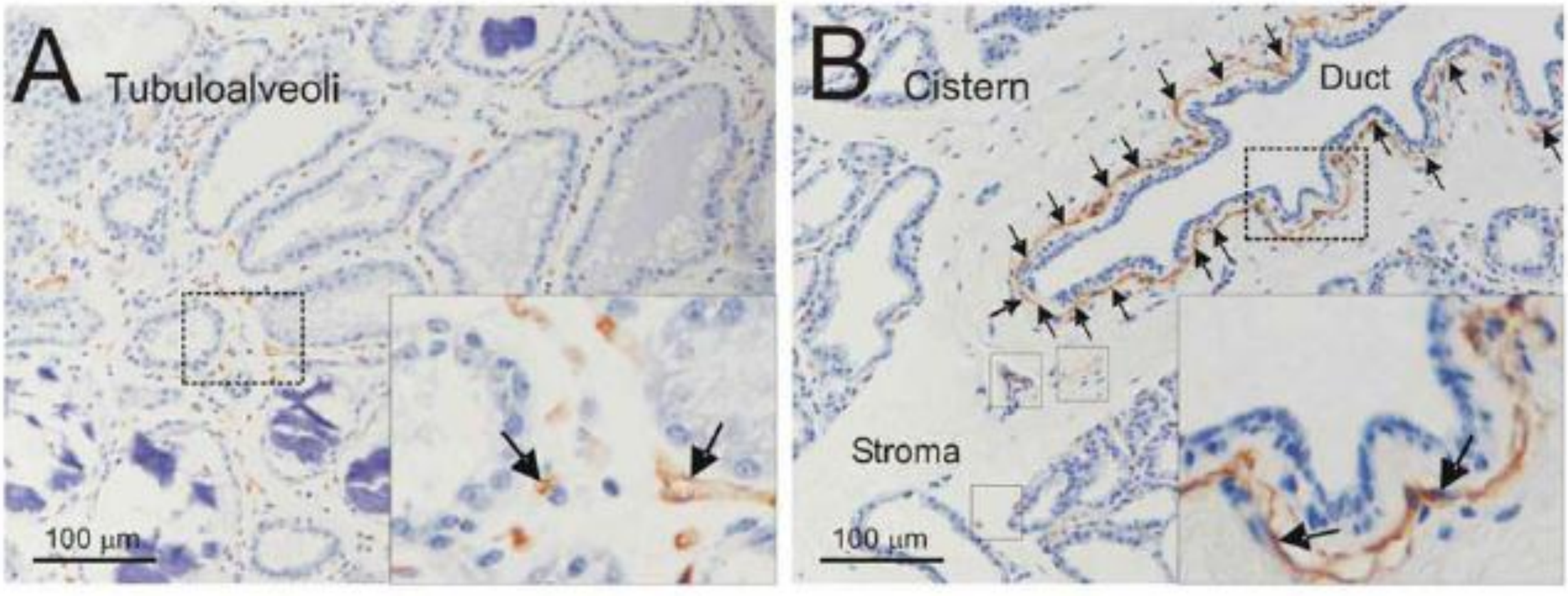
AQP5

AQP1

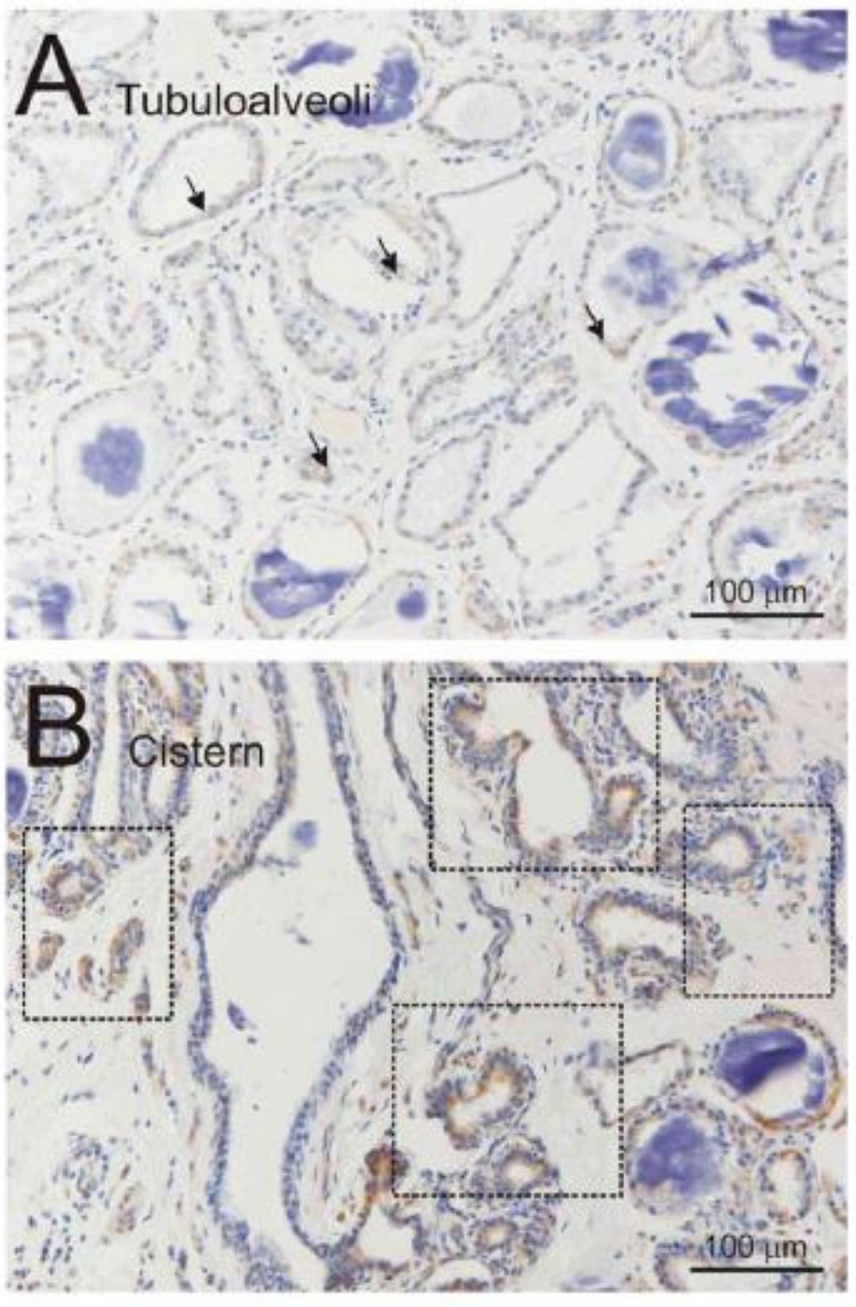
Evidence of developmental regulation

Mobasheri *et al* 2011

AQP1



Mobasheri *et al* 2011



Mobasheri *et al* 2011

Table 2
Table summarizing the immunolocalisation of AQP proteins in the lactating bovine mammary gland.

AQP Protein	Comments on immunolocalisation
AQP1	Present in capillary endothelia (microvessels) in the deep mammary gland, cistern, teat, and adipose tissue AQP1 was also present in myoepithelial cells underlying cistern and teat duct epithelia
AQP2	Not detected in any portion of the bovine mammary gland
AQP3	Present in selected epithelial cells in teat, cistern and acini (tubuloalveoli) Also detected in teat smooth muscle bundles (data are not shown)
AQP4	Diffuse immunopositivity in selected epithelial cells in teat, cistern and acini Apically localized in selected cells in cistern and teat duct epithelia Low immunopositivity in teat smooth muscle bundles
AQP5	Weak but prominent immunopositivity in acini. Prominent immunopositivity in small cistern ducts
AQP6	No immunopositivity in any part of the bovine mammary gland
AQP7	Weak immunopositivity in epithelial cells in the teat and secretory acini Also present in adipocytes, teat ducts and smooth muscle bundles within the mammary gland and the teat
AQP9	No immunopositivity in any epithelial, stromal or endothelial structure in the bovine mammary gland. Only detected in leukocytes within the mammary gland (data are not shown)

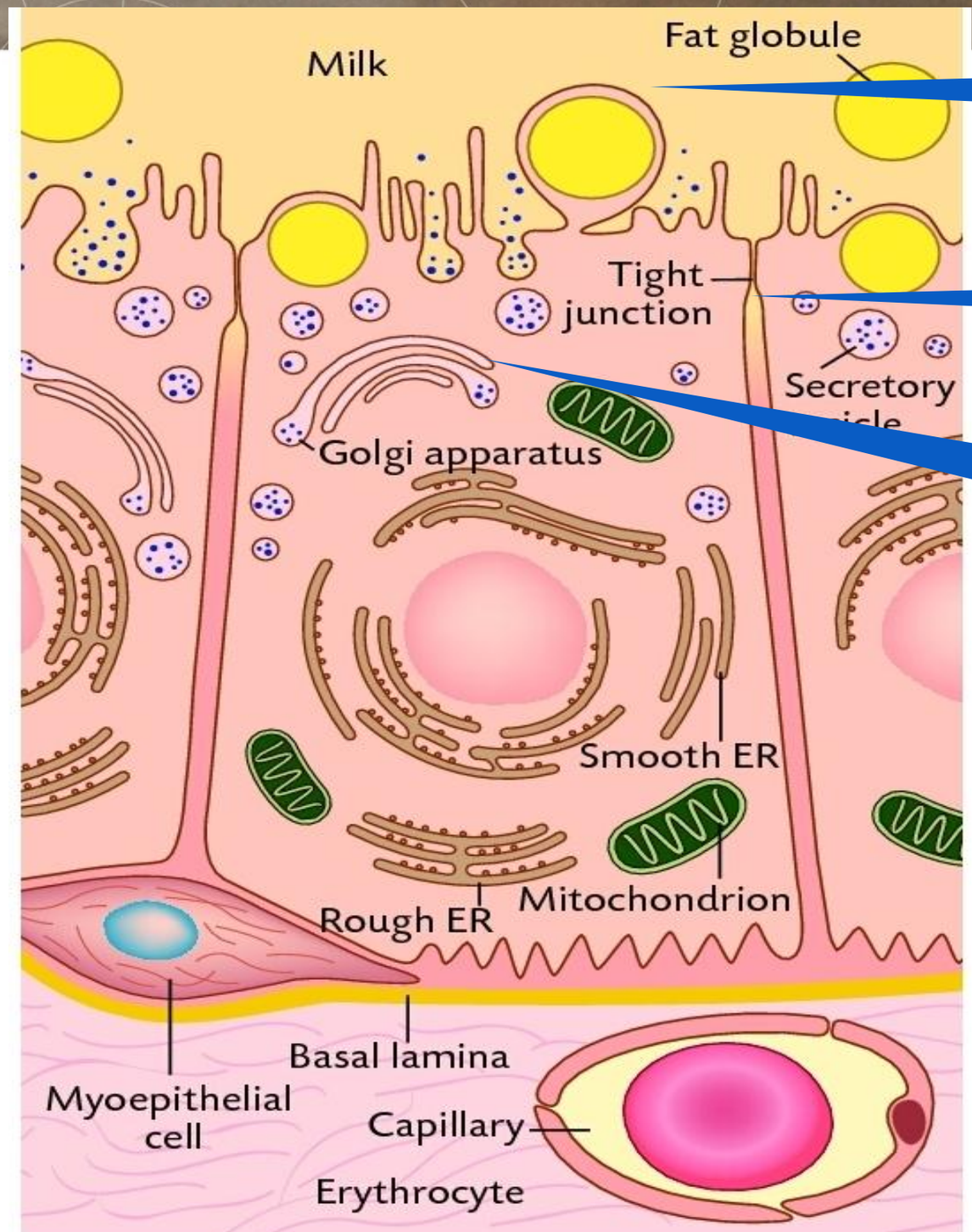
AQP at Copenhagen

- Sasan Nazemi, PhD student. Main project involved with murine model of intramammary inflammation and tumorigenesis.

- Mette Rhabek, PhD student. Main project involved with uterine AQP expression using rat endocrine stimulation model.

- Dan Klærke. Membrane physiologist, international reputation in K channels.

Are there AQP on the mammary Golgi membrane?



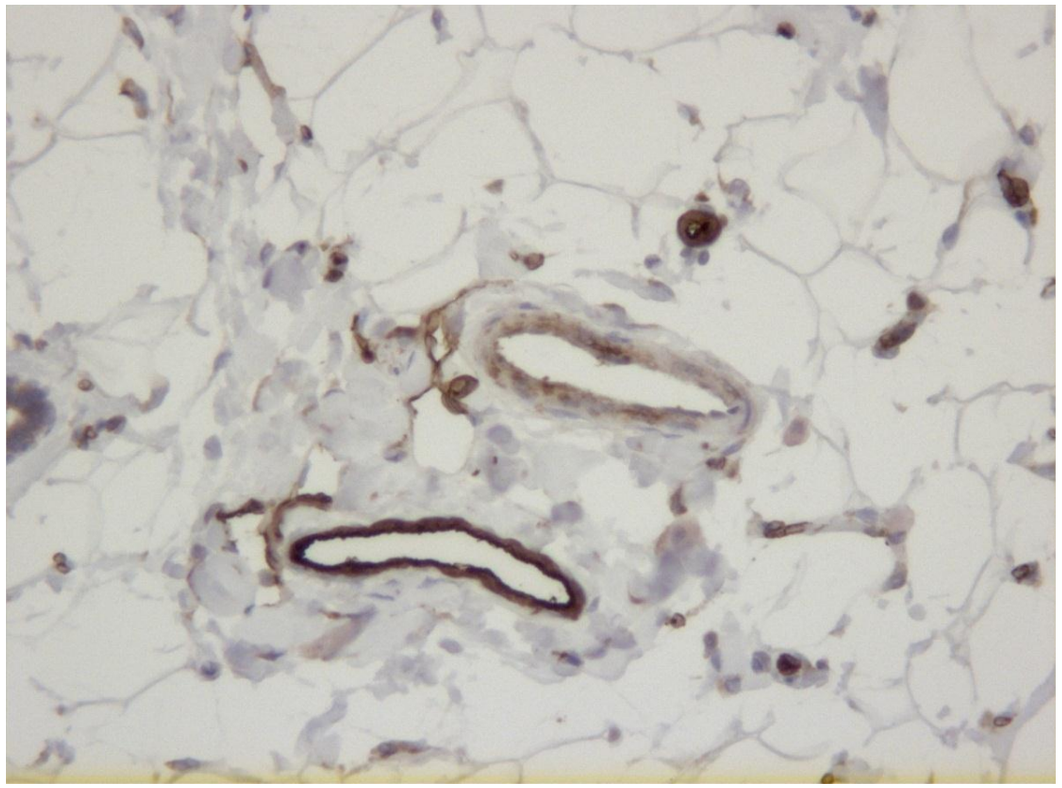
AQP?

AQP?

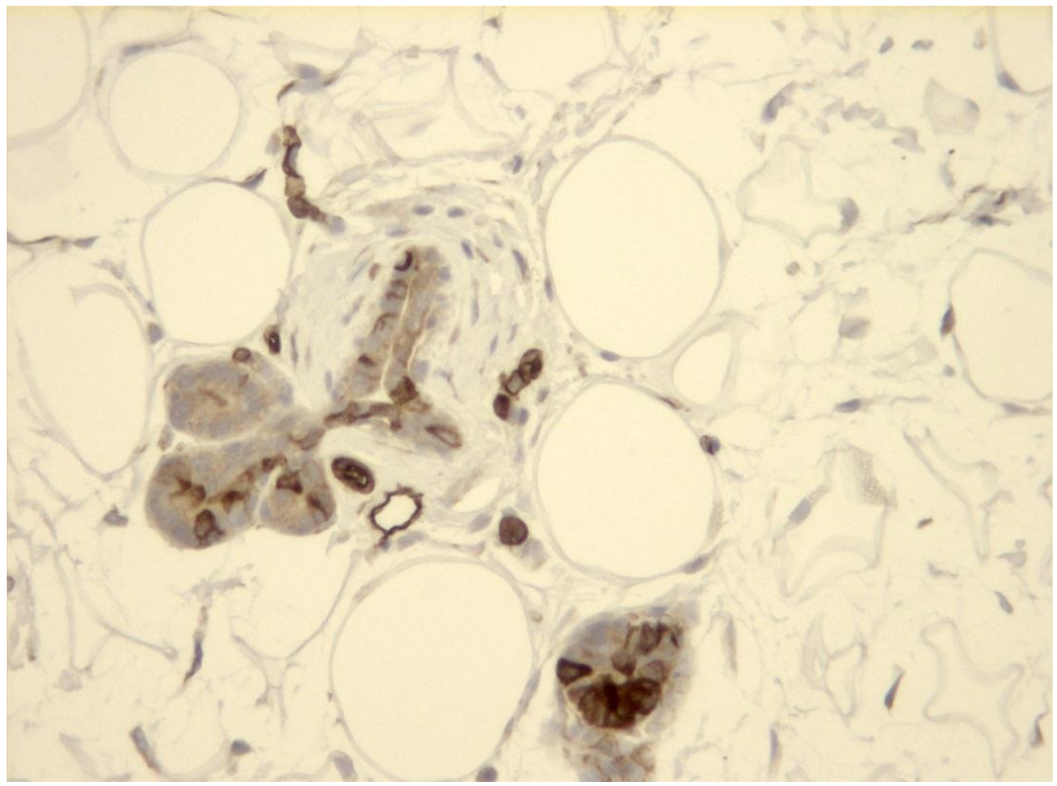
AQP?

If so, are they developmentally regulated?

AQP1 in hormone-stimulated rat mammary tissue

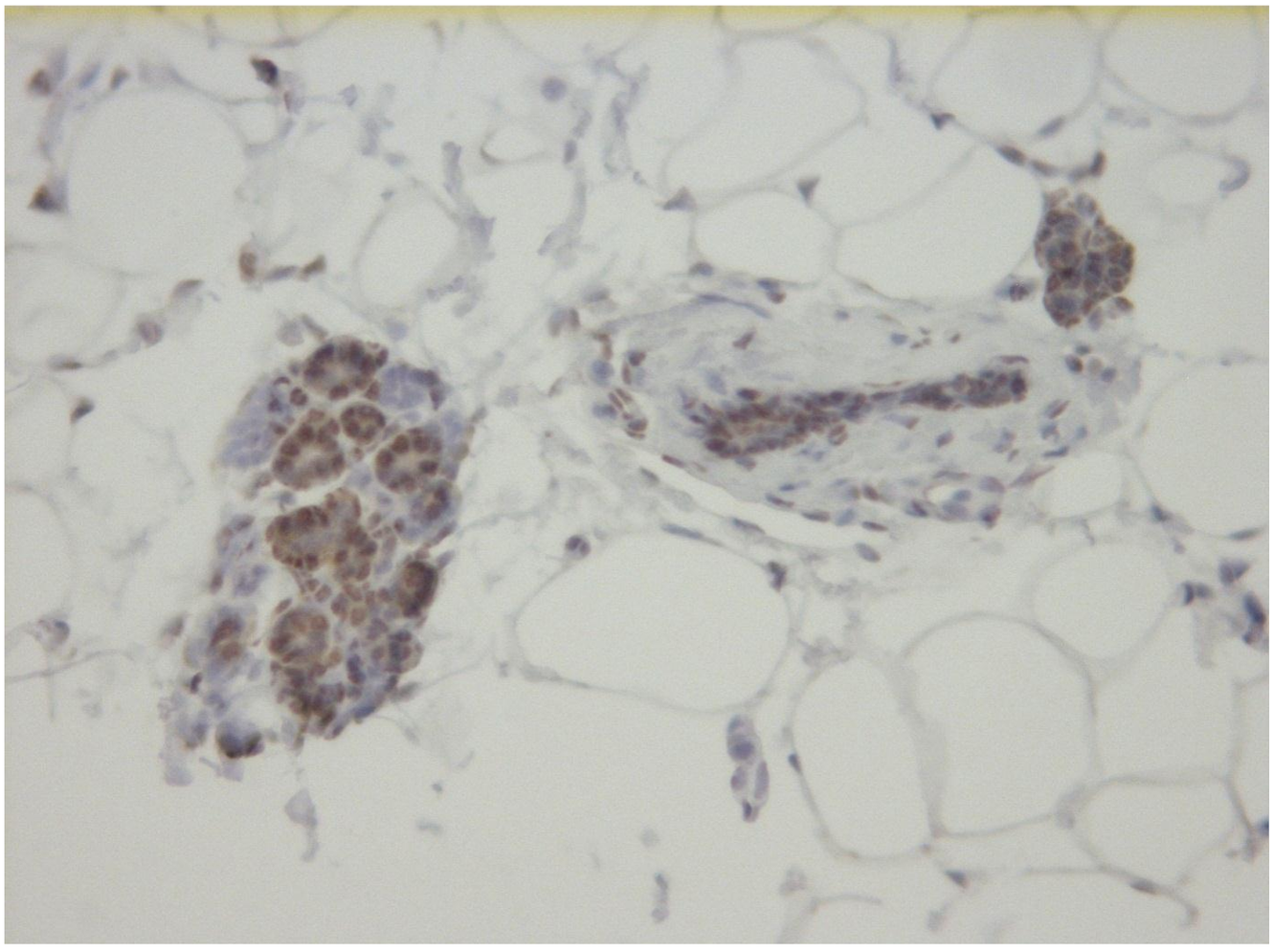


Placebo



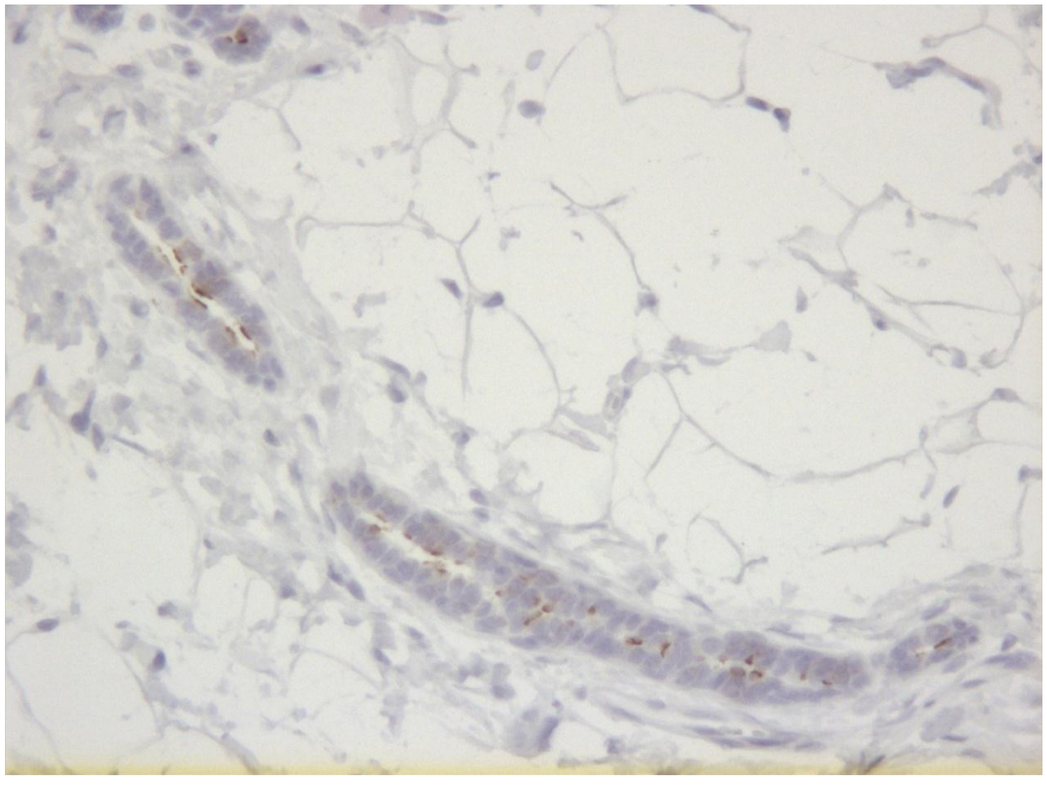
Estrogen

AQP3 in hormone-stimulated rat mammary tissue



Progesterone

AQP5 in hormone-stimulated rat mammary tissue



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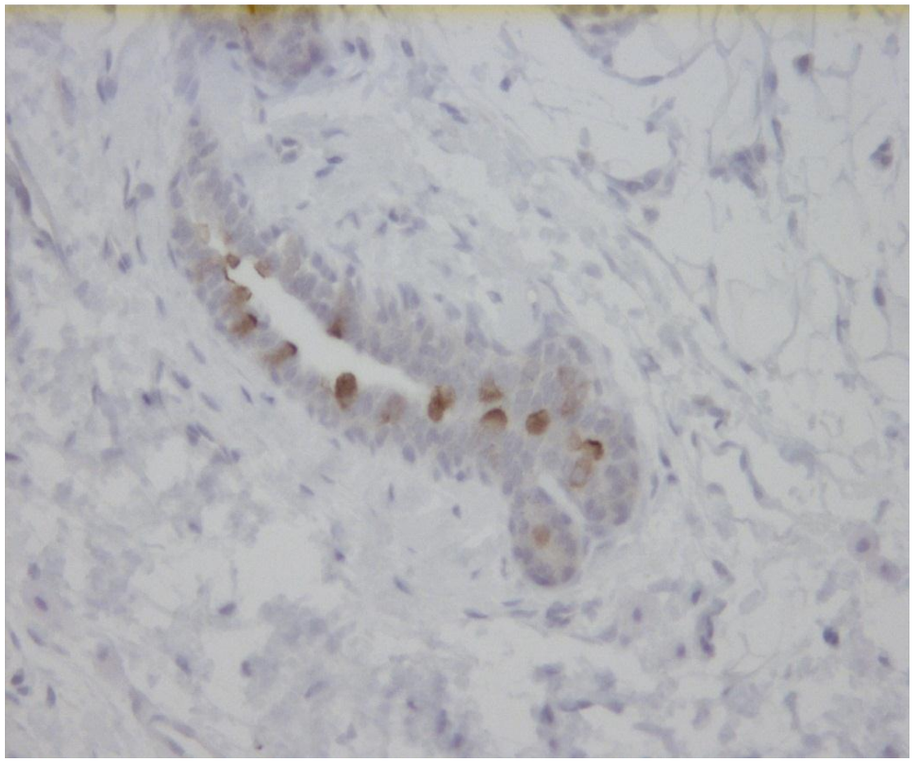


Estrogen

AQP in hormone-stimulated rat mg

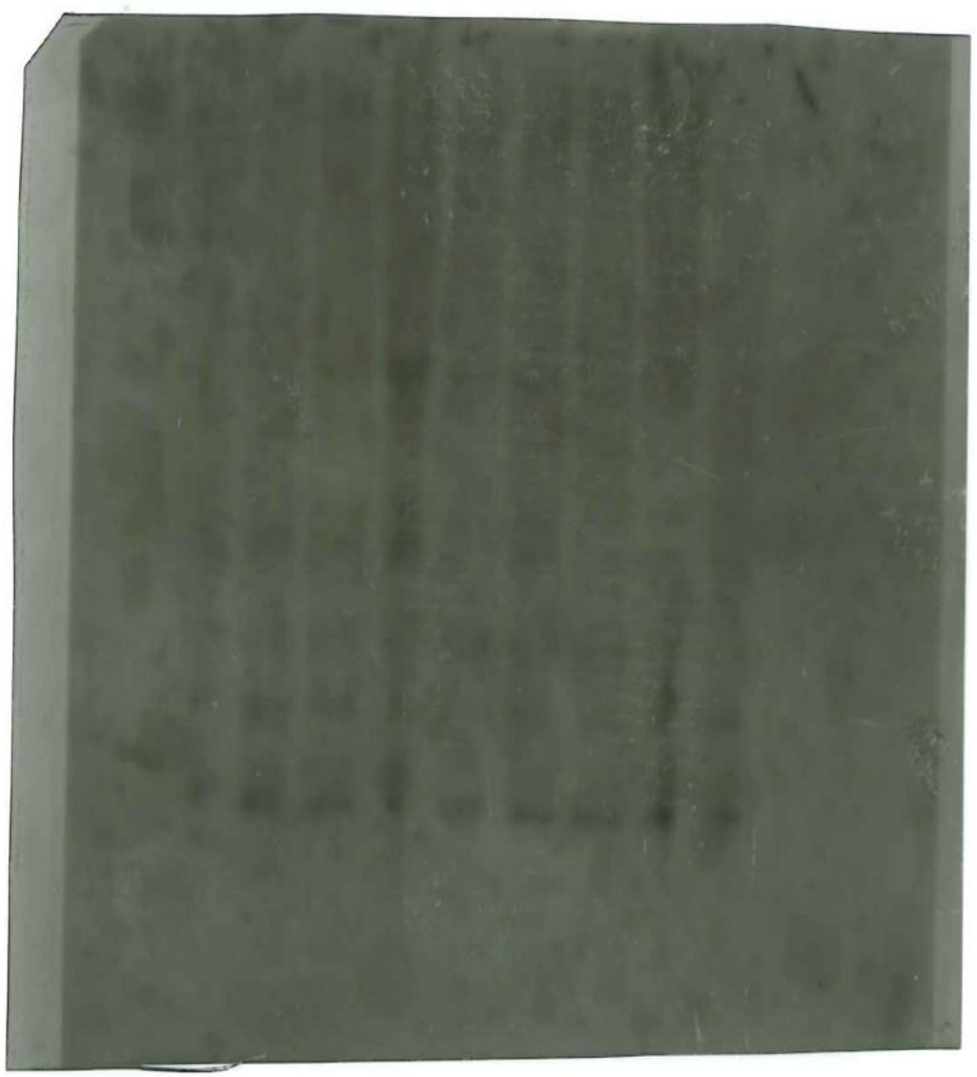
- Immunohistochemical evidence of AQP1, 3 and 5 expression

- AQP5 *possibly* associated with apical membrane

- No evidence of developmental regulation

- The approach has allowed for methodological development, but is *not* recommended

AQP in normal rat mammary gland



- Normal rat mg is now available
- Rapid detection methodologies are being developed
- We have some evidence of developmentally regulated but variable AQP expression
- Example is AQP1, 2x pregnant then 3x early lactation, 3x late lactation and 1 more pregnant
- We hope to have data for purified Golgi membrane soon
- It may ultimately be possible to purify appropriate membranes from milk
- Proteomics approaches are also available

Take home message

- There is an indisputable link between lactose synthesis in the Golgi vesicle and water secretion into milk

- It is likely but not proven that various AQP are involved in water fluxes within the mammary secretory cell, including from cytosol into Golgi vesicle

- This has implications for regulation of milk secretion and milk protein secretion

Thank you for your attention

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