

Butter and Buttermilk:
All you wanted to know, but were
afraid to ask....

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California Polytechnic State
University



Butter

R. Jimenez



What is Butter?

- Commercial butter is 80–82 percent milk fat, 16–17 percent water, and 1–2 percent milk solids other than fat (sometimes referred to as curd).
- It may contain salt , added directly to the butter in concentrations of 1 to 2 percent.
- Unsalted butter is often referred to as “sweet” butter. This should not be confused with “sweet cream” butter, which may or may not be salted.
- Reduced-fat, or “light,” butter usually contains about 40 percent milk fat.
- Butter also contains protein, calcium and phosphorous (about 1.2%) and fat-soluble vitamins A, D and E.

Fat content %	Milk fat products	Mixed fat products	Margarine products
80 – 95	Butter*	Blend	Margarine*
> 62 – < 80	Dairy spread	Blended spread	Fat spread
60 – 62	3/4 fat or reduced fat butter	3/4 fat or reduced fat blend	3/4 fat or reduced fat margarine
> 41 – < 60	Reduced fat dairy spread	Reduced fat blended spread	Reduced fat spread
39 – 41	1/2 or low fat butter	1/2 or low fat blend	1/2 or low fat margarine or Minarine*
< 39	Low fat dairy spread	Low fat blended spread	Low fat spread

* The following FAO/WHO individual standards currently apply to products in international trade and indicate the designations permitted:

A1 – Standard for Butter and Whey Butter

(A16 – Standard for Low Fat Dairy Spreads – draft)

Codex Standard 32–1981 for Margarine

Codex Standard 13–1981 for Minarine



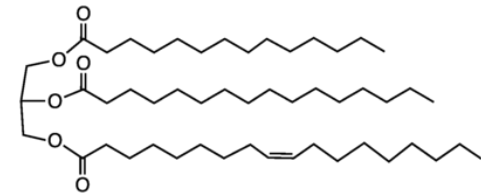
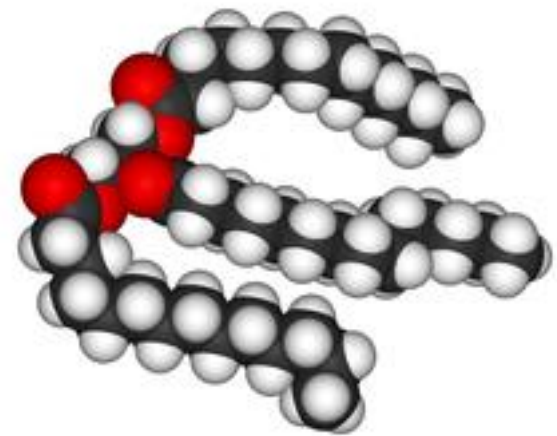
United States

- The definition and standards for butter in the U.S. was first set by congress in 1886. Current standards have not been changed since a statute to set the criterion was enacted into law on March 4, 1923. Butter grades are determined by classifying the flavor, then rating the body, color and salt characteristics. The resulting score translates into a grade B, A, or AA.

Why does it taste so good?

- Master chef Julia Child steadfastly clung to some ideas. She didn't mind the microwave; it sometimes comes in handy. But try to cut corners with butter, and she wouldn't stand for it. "If you're afraid of butter, as many people are nowadays," she said in one of her last television shows, "just put in cream!" she proclaimed, with a twinkle in her eye.



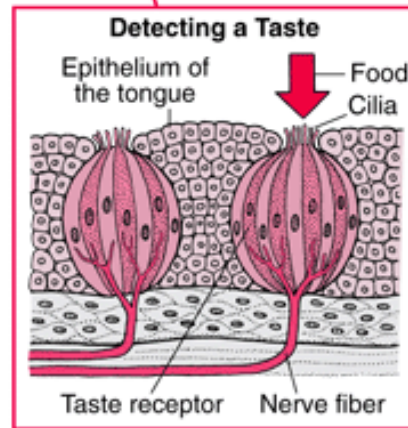
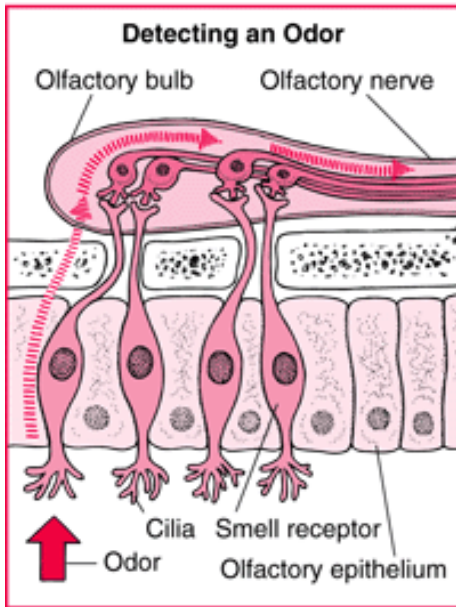
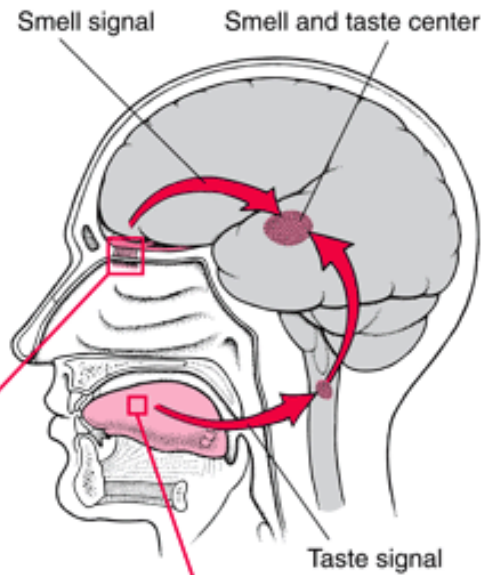


$$TG = 2^n$$

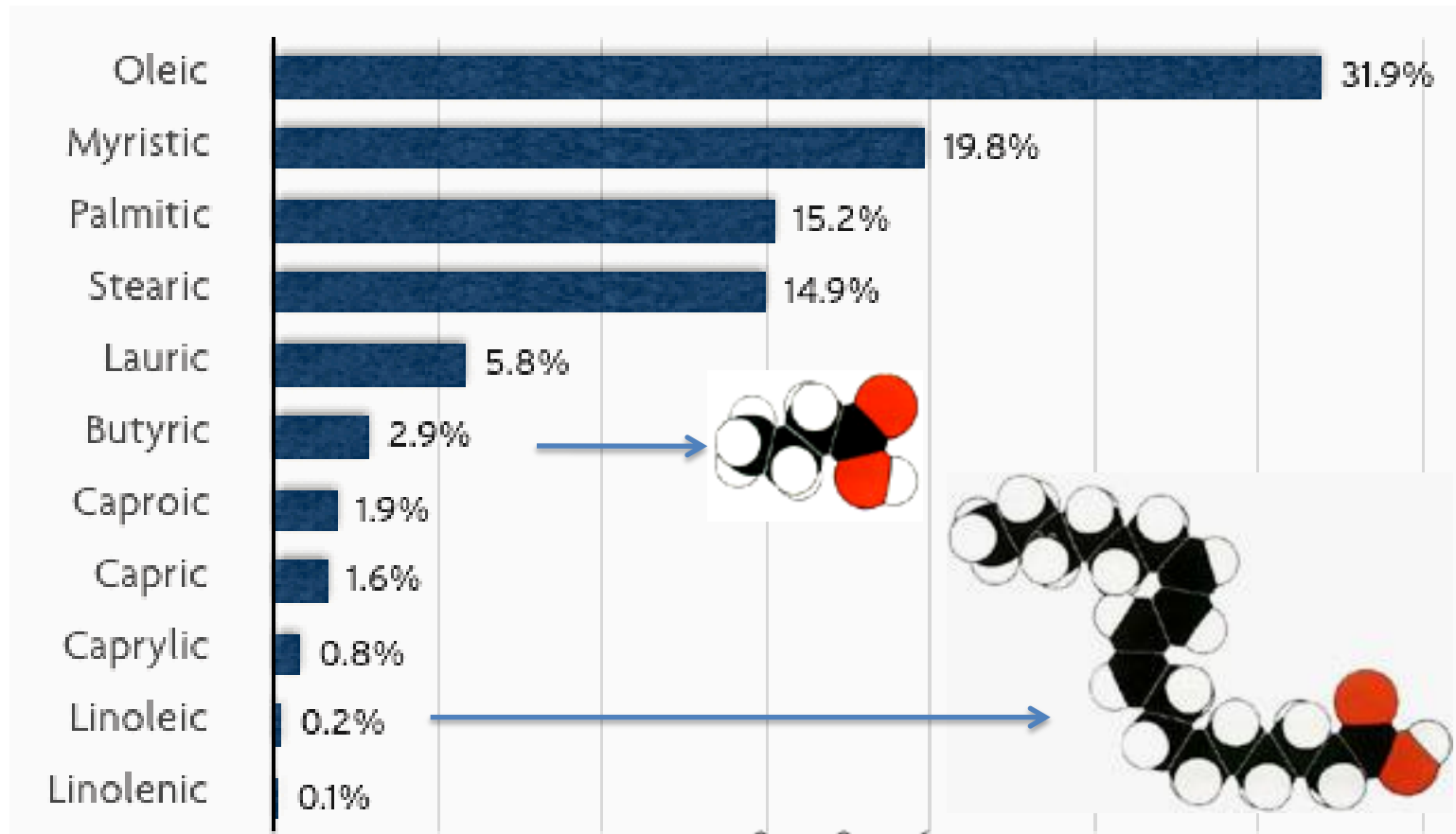
$n =$ number of FA

$$n=3 \quad TG=8$$

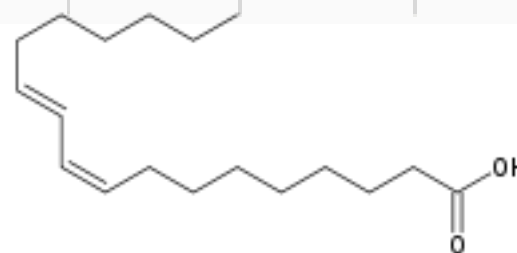
$$n=20 \quad TG=1,048,576$$



Fatty acid composition in Butter



c9, t11 conjugated linoleic acid



History and Geography

- In 3500 BC, the people of Sumer shook cream in a vertical churn. And butter was important enough to write about — records have been carved in stone. In the frieze below, to the right of the farm shed, temple staff milk a cow, and to the left they strain and stores the milk and make butter.



Limestone and shale mosaic frieze framed in copper from the Ninursag temple facade al-'Ubaid. ca. 2500 BCE.





Yak butter may be wrapped in cloth (left), or stored in a stitched yak stomach (right). A few times a year, they go to market to trade it for corn and other things they need

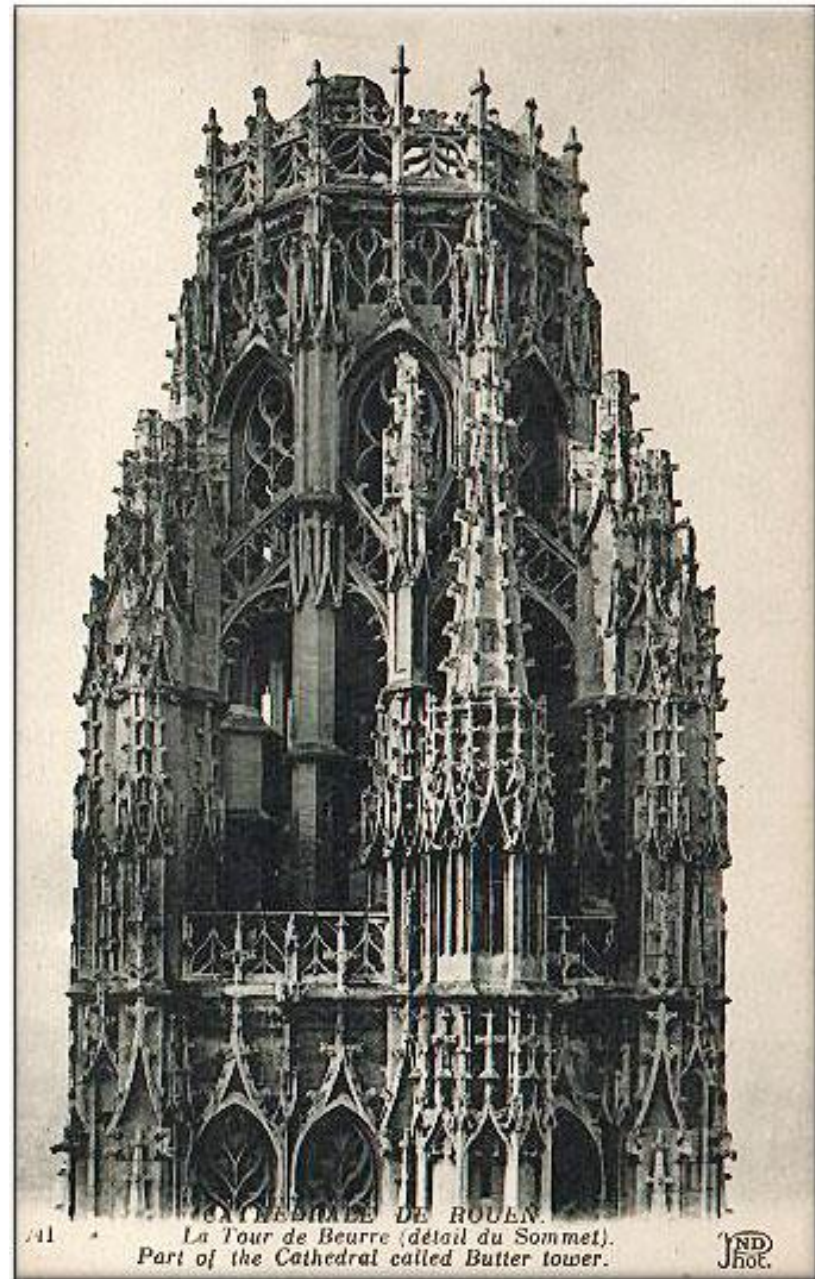


Yak butter is for sale in yellow packages in the country, as well as this downtown market in Lhasa.

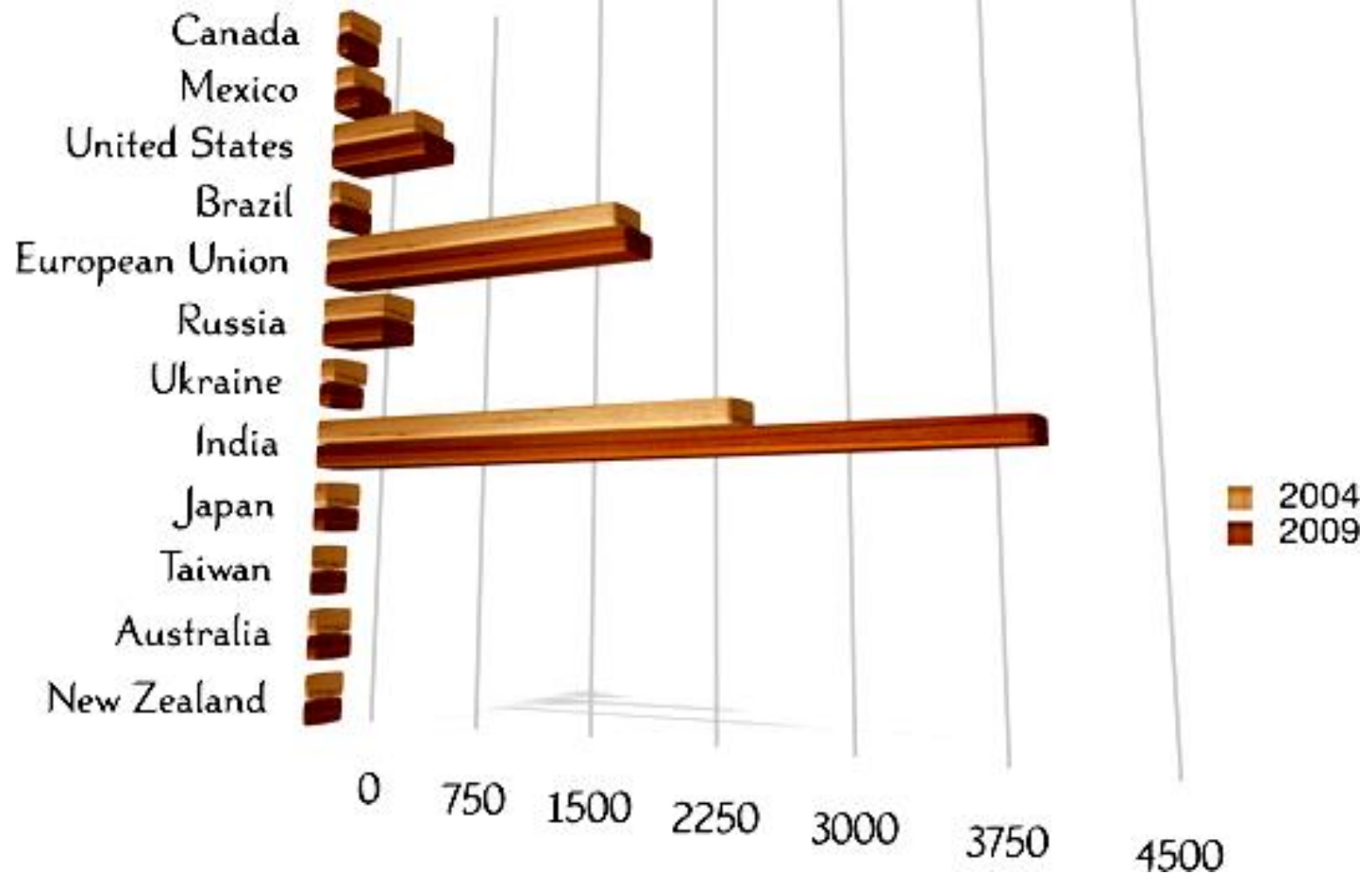


Inside the 1300 year old Jokhang Temple, a pilgrim lights a yak butter candle.

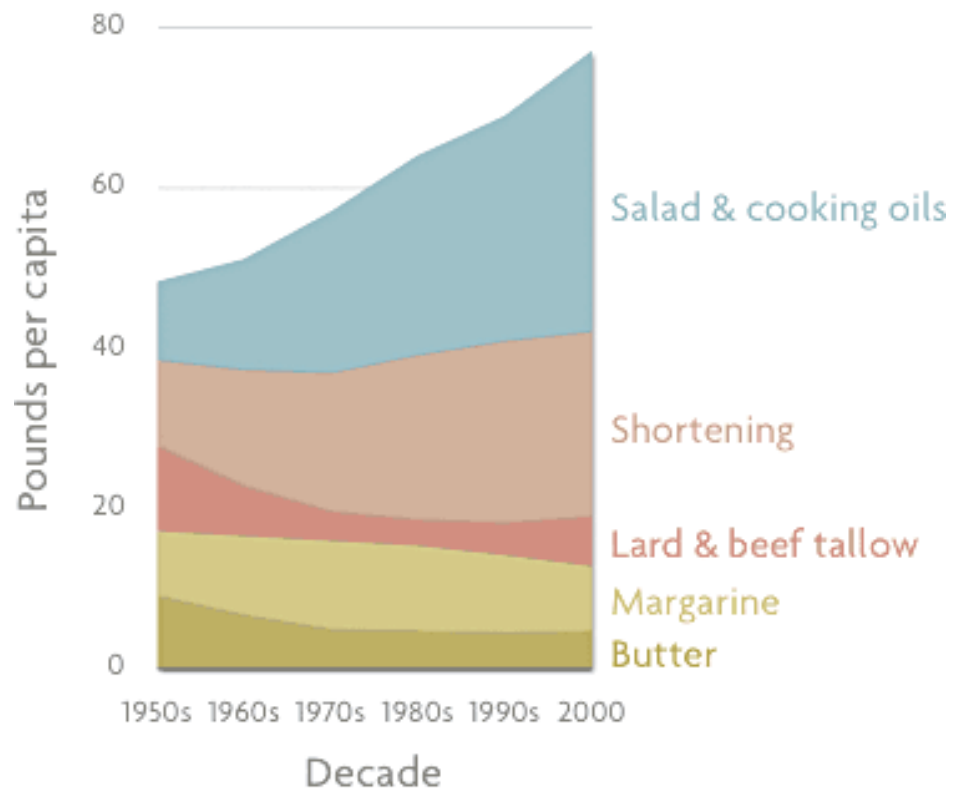
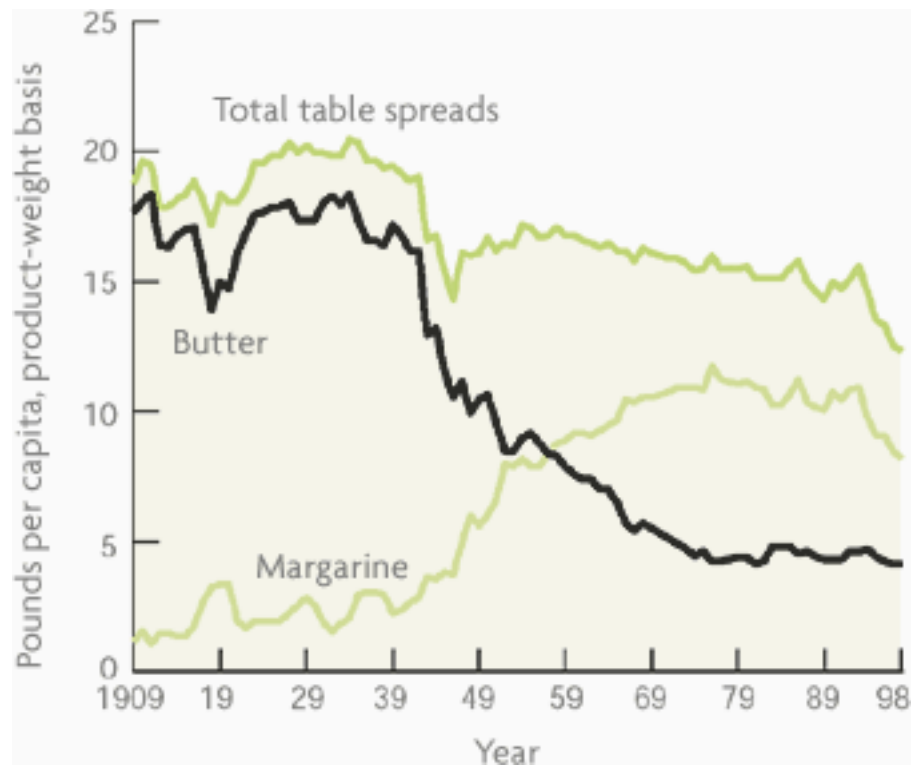
- The magnificent Butter Tower of Rouen Cathedral was built with money which the Church received from people who preferred to pay rather than forgo their daily butter



11
CATHÉDRALE DE ROUEN.
La Tour de Beurre (détail du Sommet).
Part of the Cathedral called Butter tower. JND Phot.



Every year, over 8 million metric tons of butter are consumed worldwide. The chart above shows how much butter is being eaten for selected countries. Americans have been eating less and less butter since the mid 20th century. This is a graph of total annual butter consumption, in 1,000 metric tons.



Lipids and Phospholipids

February 14, 2015

 Print This Post

Time Magazine: We Were Wrong About Saturated Fats

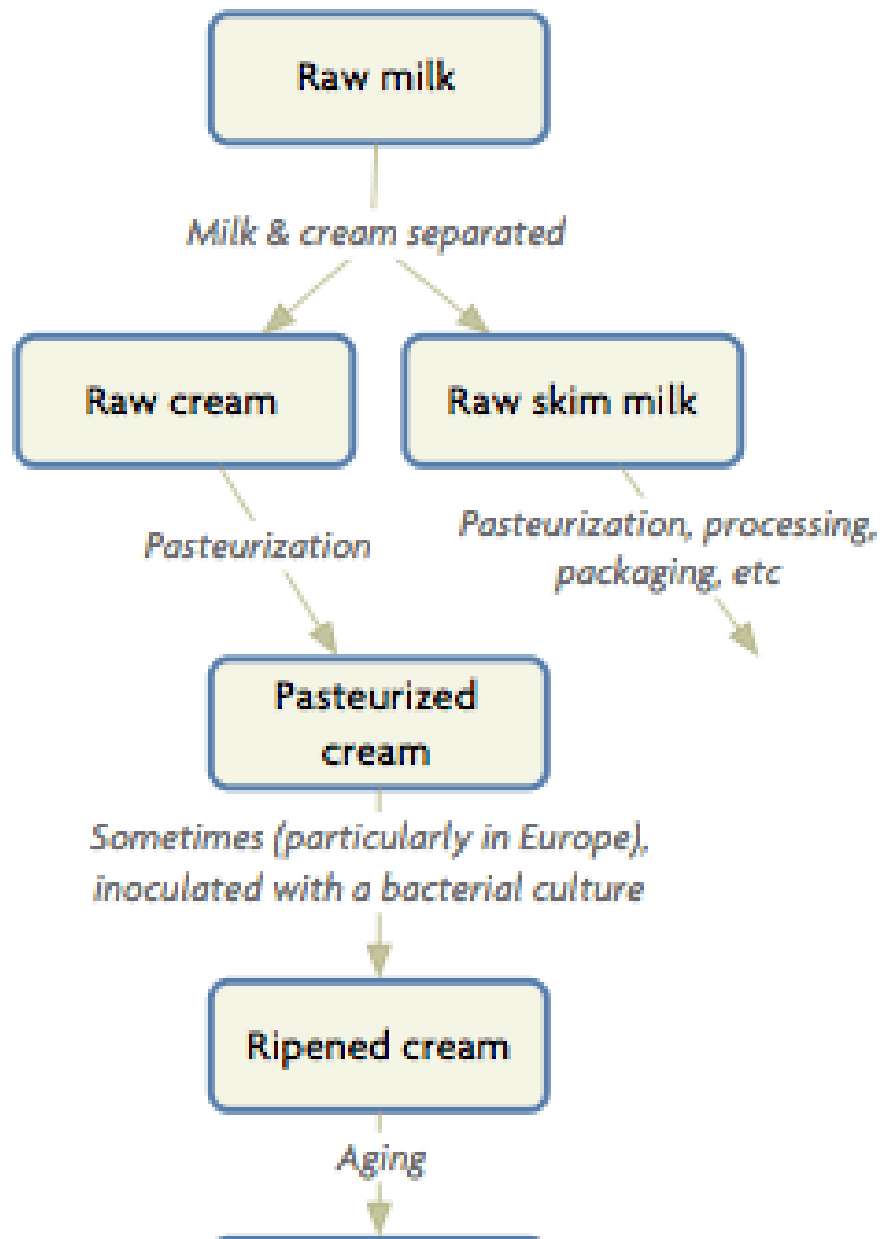


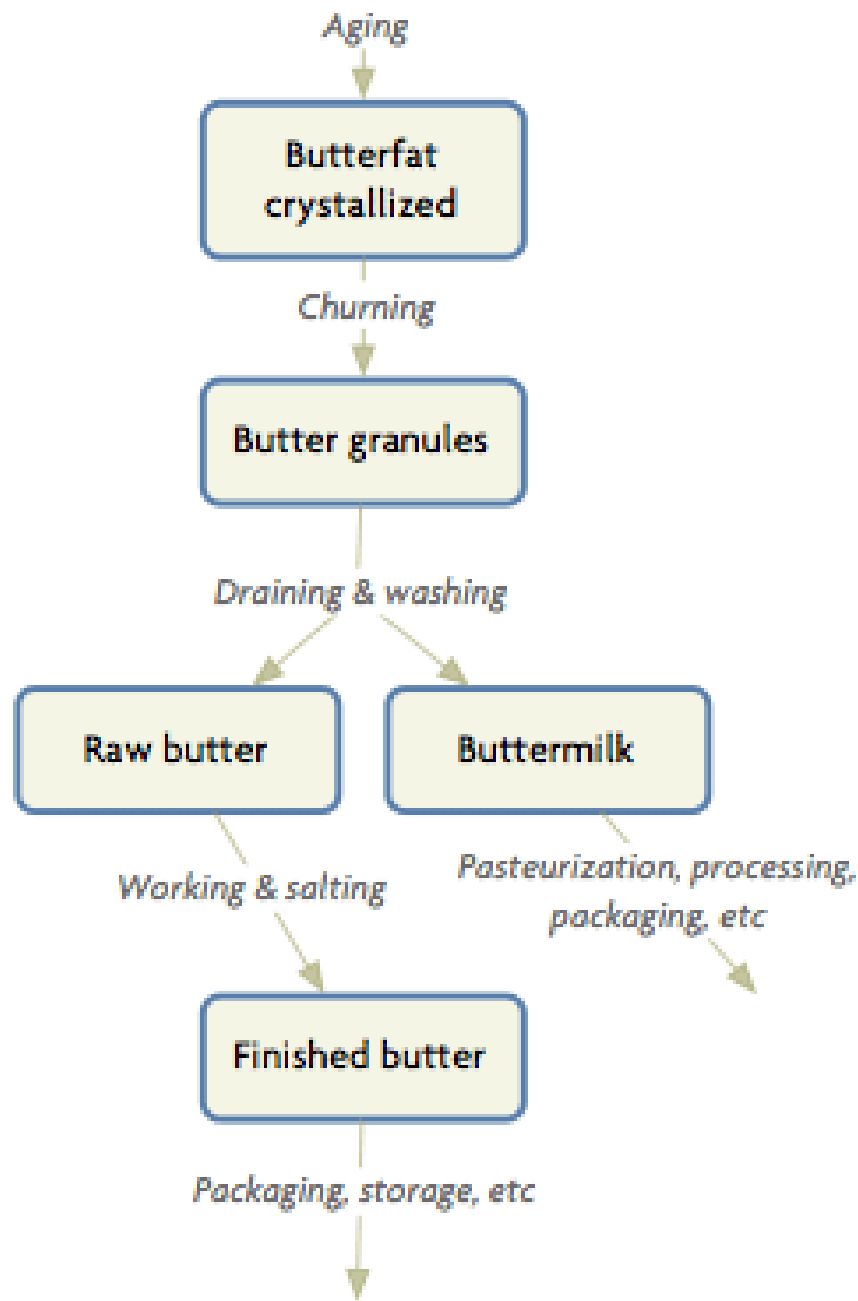
Ansel Keys on the cover of Time Magazine in 1961. He claimed that saturated fats in the diet clogged arteries and caused heart disease.



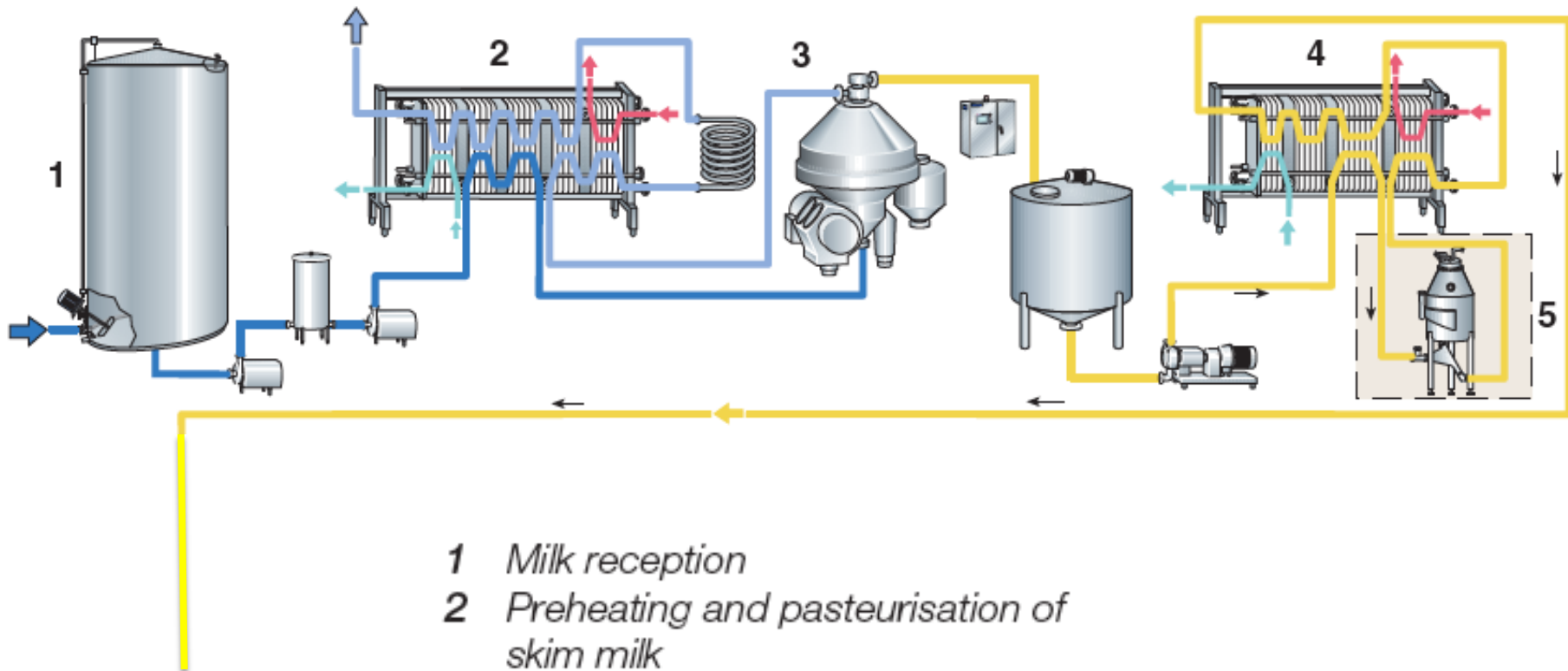
Time Magazine cover story in 2014. Scientists were wrong about saturated fats. They don't cause heart disease after all.

But Don't Expect More Mainstream Media to Follow this Repentance on Saturated Fats





Processing



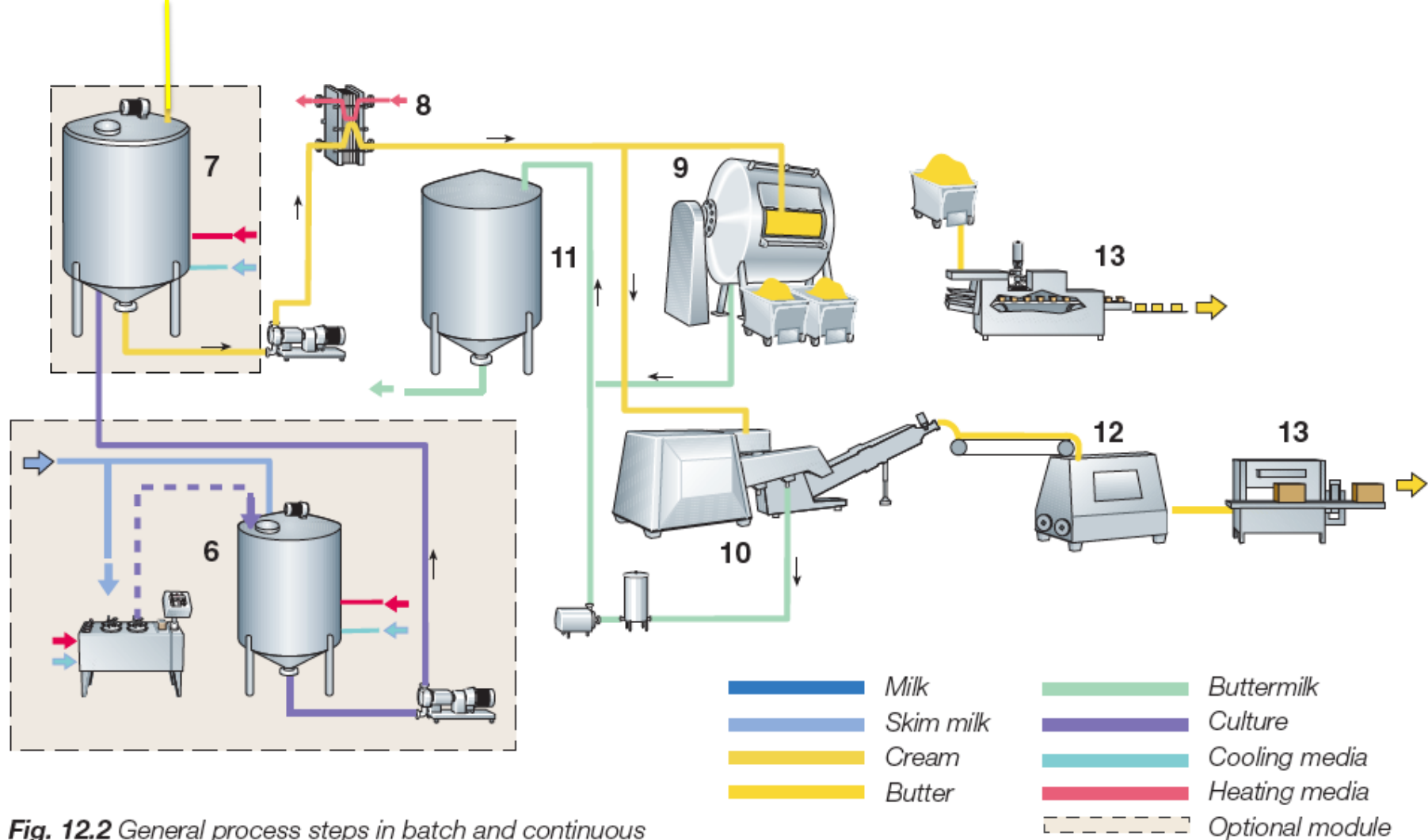


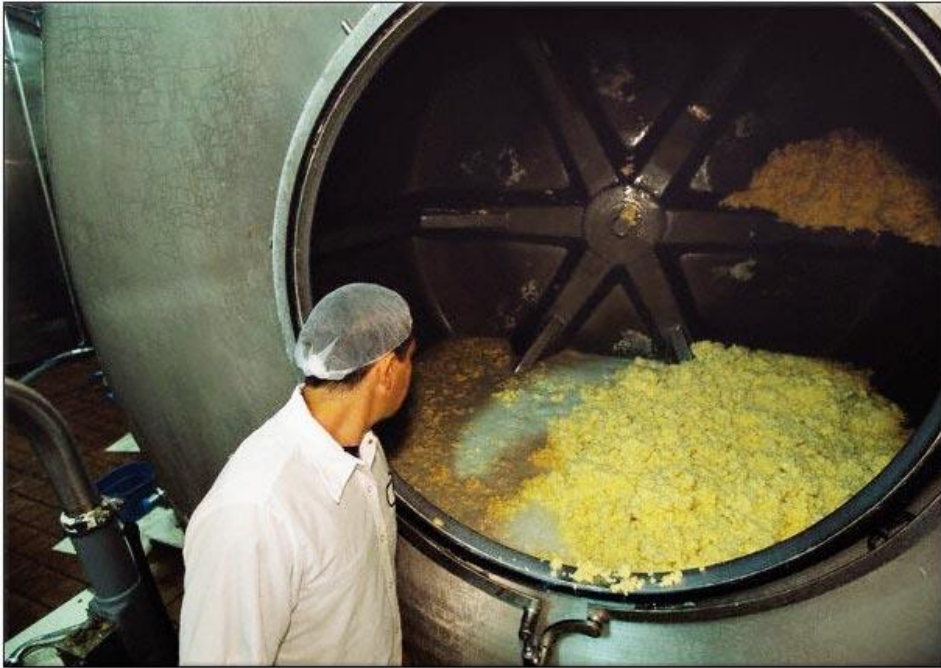
Fig. 12.2 General process steps in batch and continuous production of cultured butter.

- 6** Culture preparation, when used
- 7** Cream ripening and souring, when used
- 8** Temperature treatment
- 9** Churning/working, batch
- 10** Churning/working, continuous

1 Milk reception

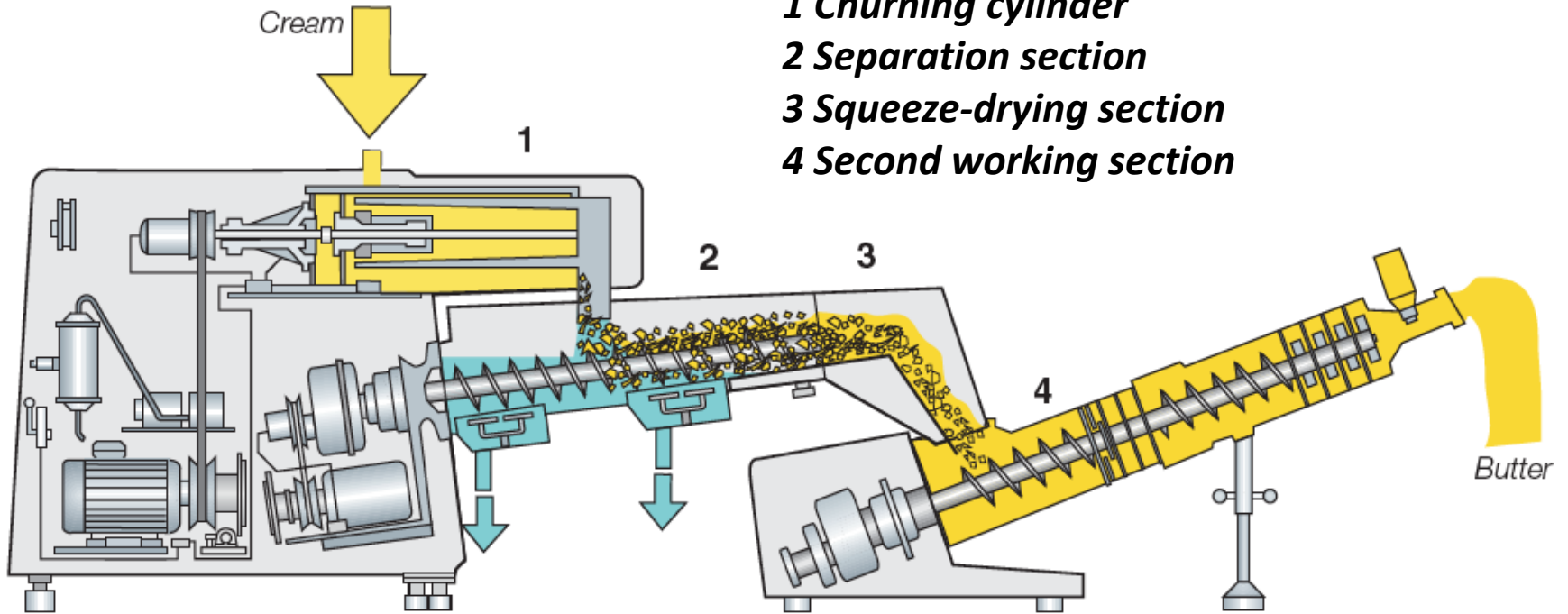
- 11** Buttermilk collection
- 12** Butter silo with screw conveyor
- 13** Packaging machines

Batch Butter Churn



Butter Churn

- 1 Churning cylinder**
- 2 Separation section**
- 3 Squeeze-drying section**
- 4 Second working section**

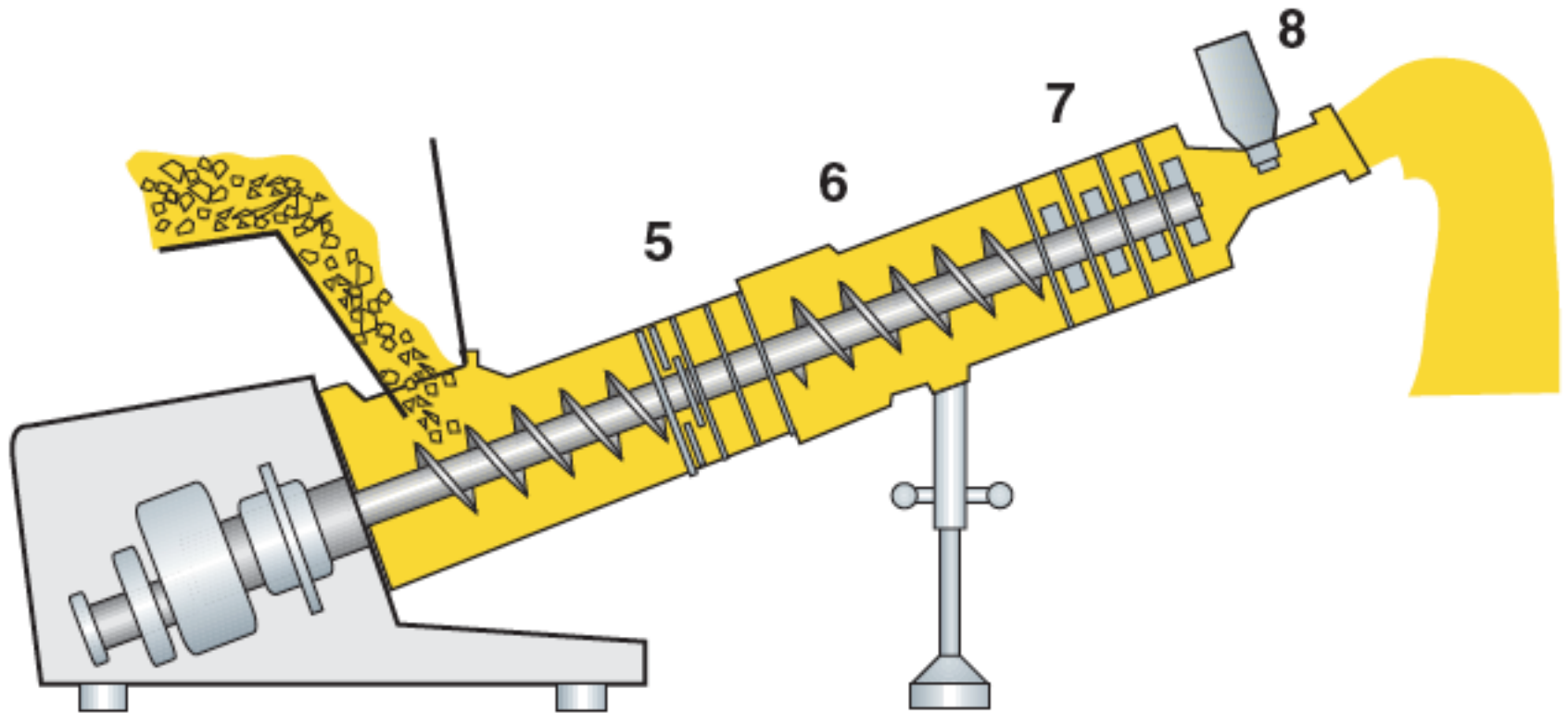


5 Injection section

6 Vacuum working section

7 Final working stage

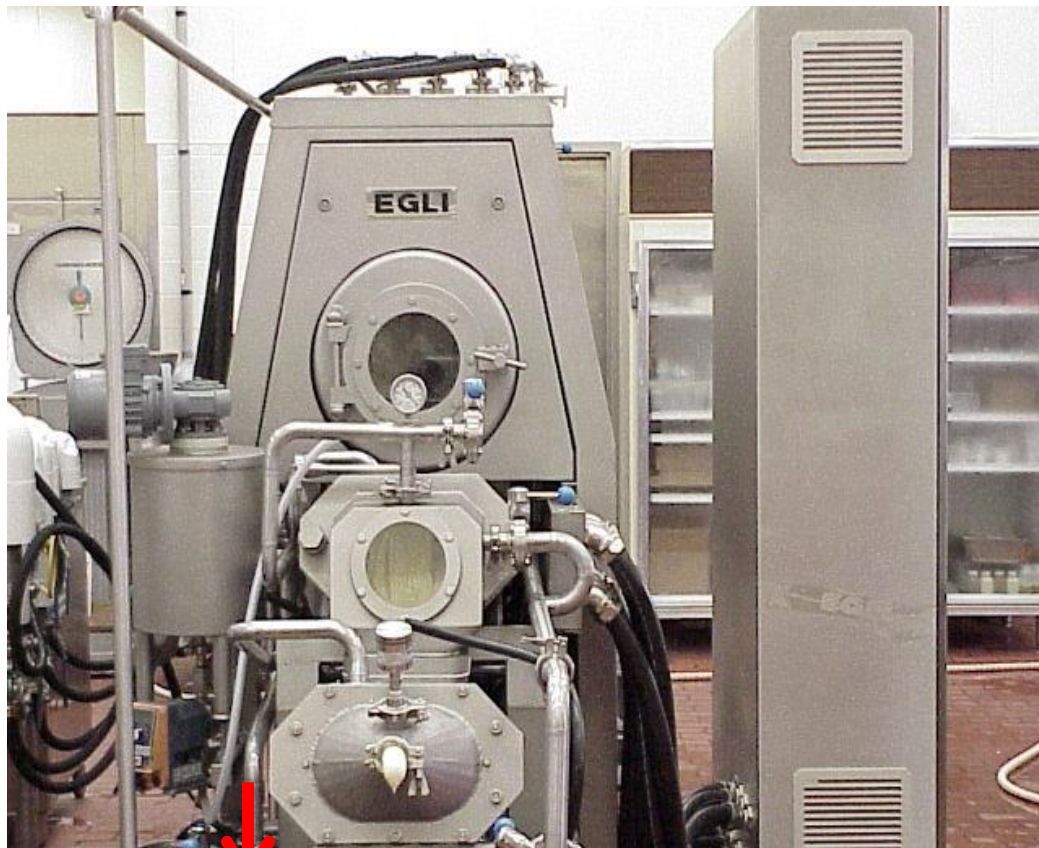
8 Moisture control unit



Whey cream



Egli Continuous Butter and Spreads System



Buttermilk



Continuous Butter Churning

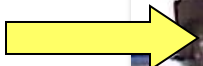


Buttermilk





Condensed
Buttermilk
Or
Retentate



Spray drier



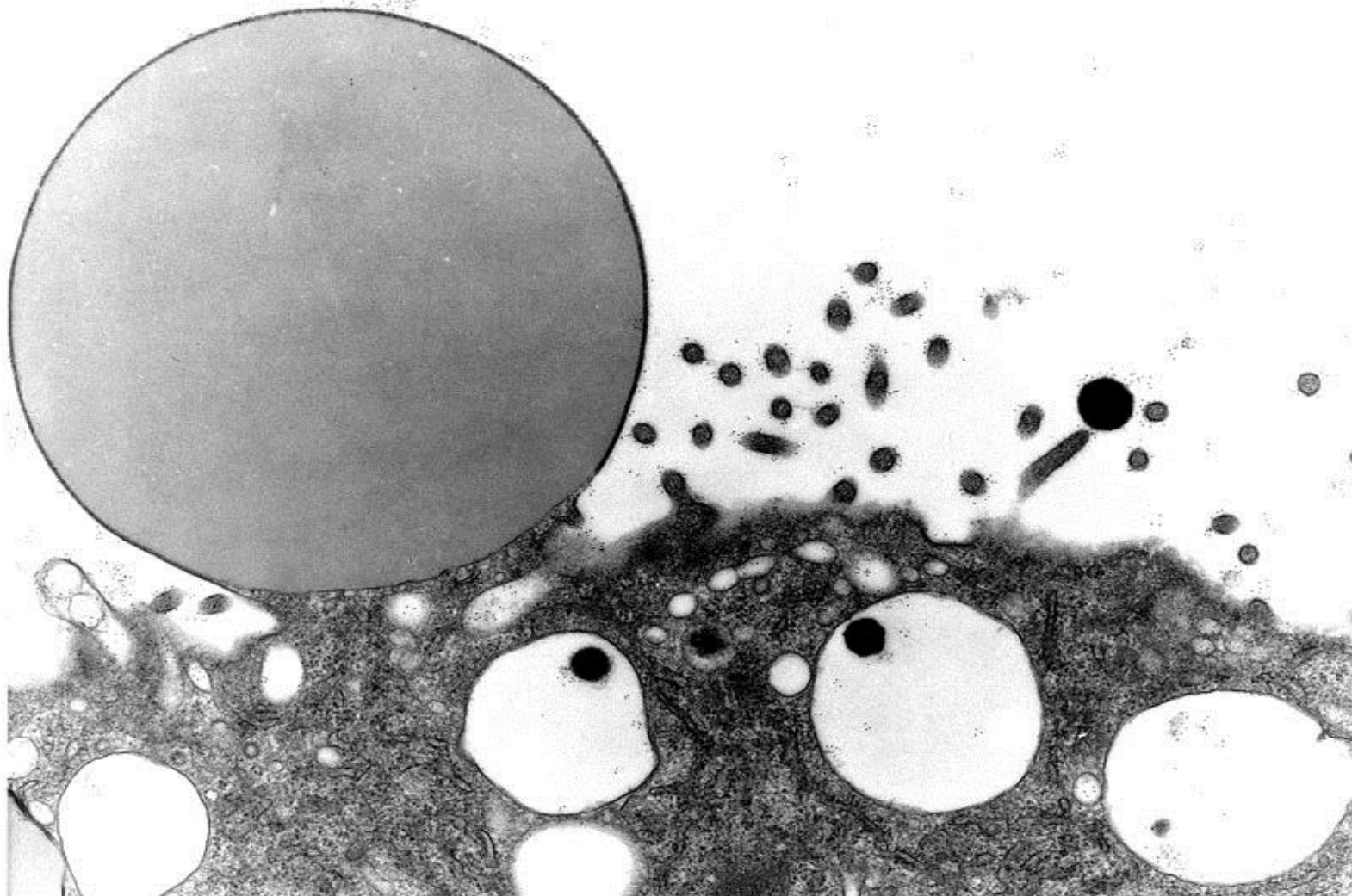
To assay

Products

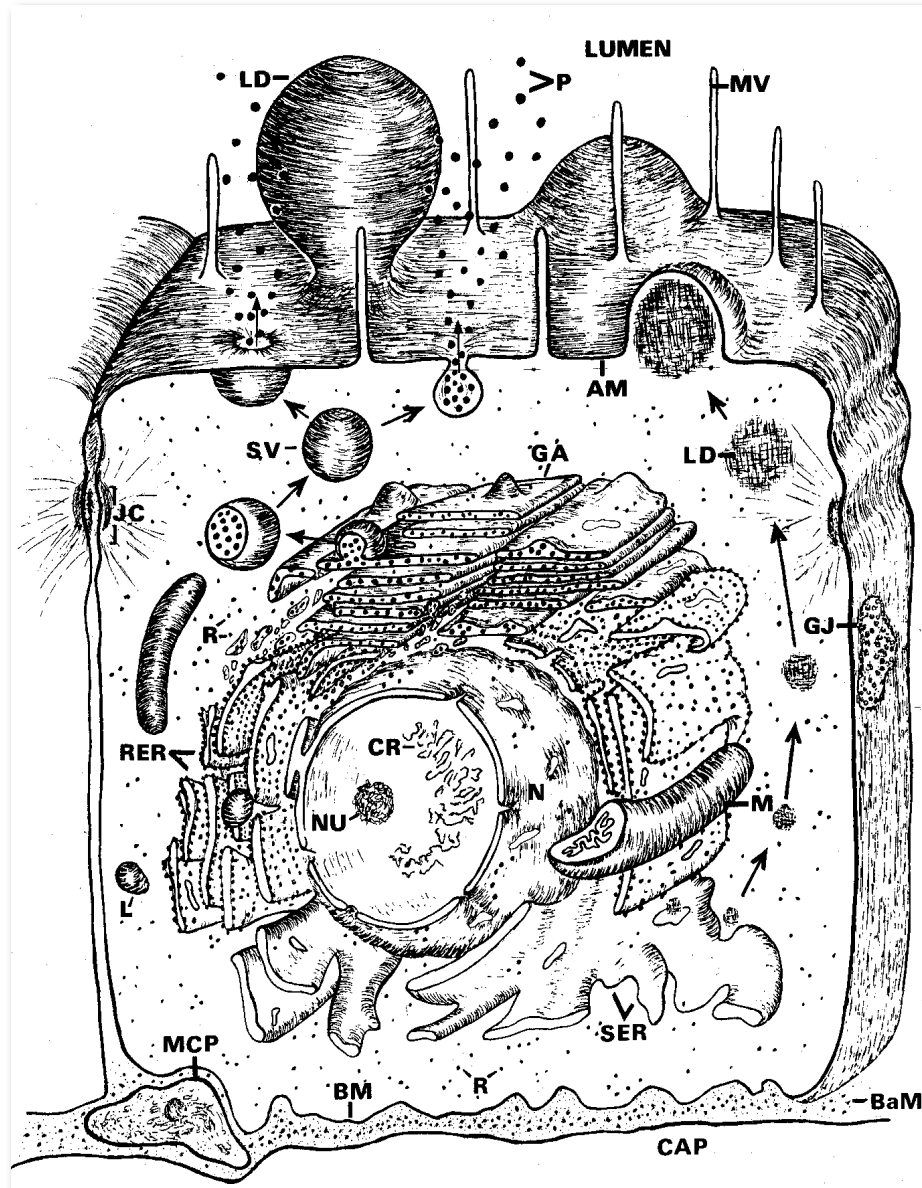
Challenge for Milk Scientist

- Recognize the natural biological function of the milk components
 - Such as their role and relevance in structure
- Isolate the important factors without destroying the ‘milieu’ in which they work
 - Understand the mechanism of action
- Design a process that preserves or even enhances the targeted biological function
 - Capitalize on innovation

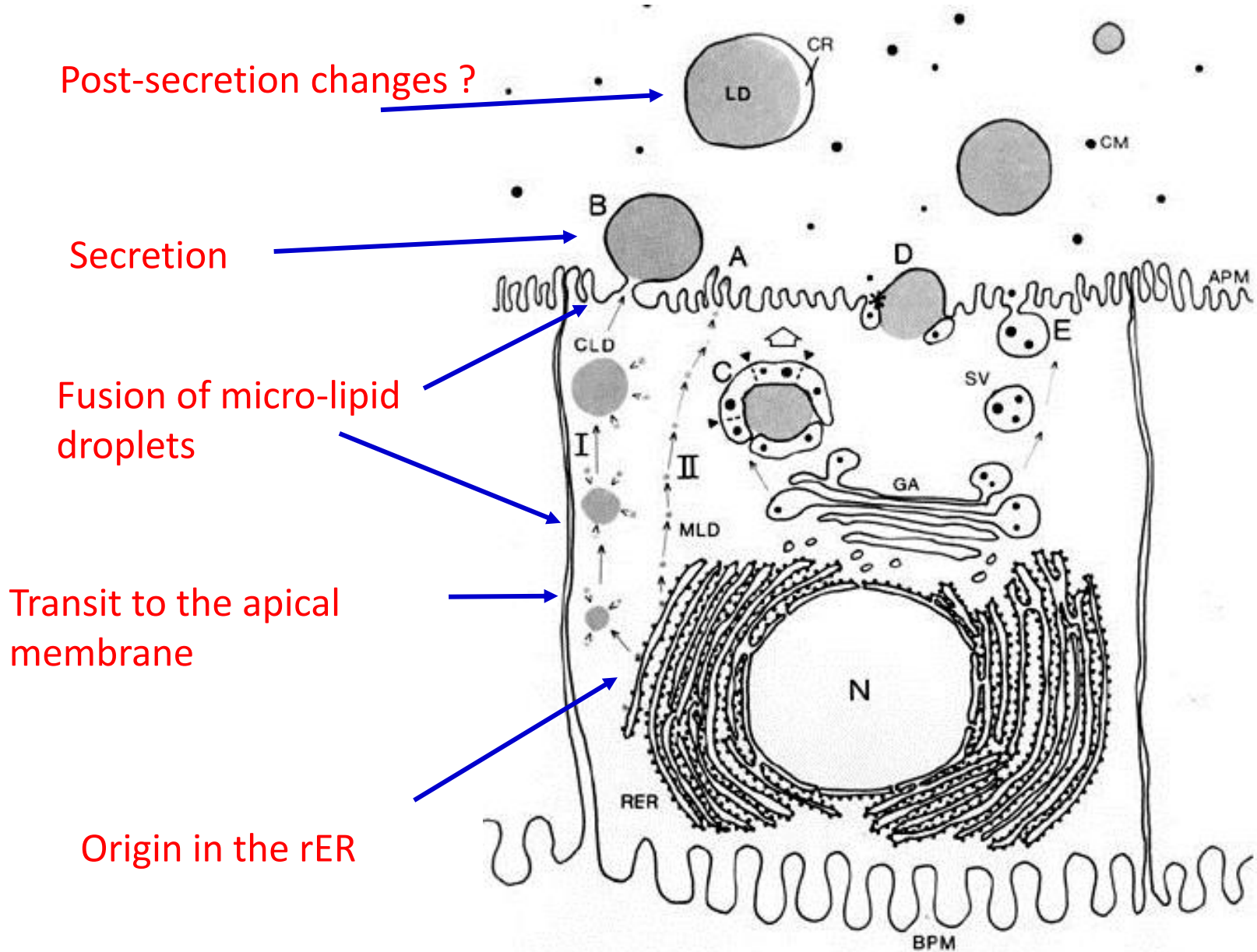
MFGM: Origin



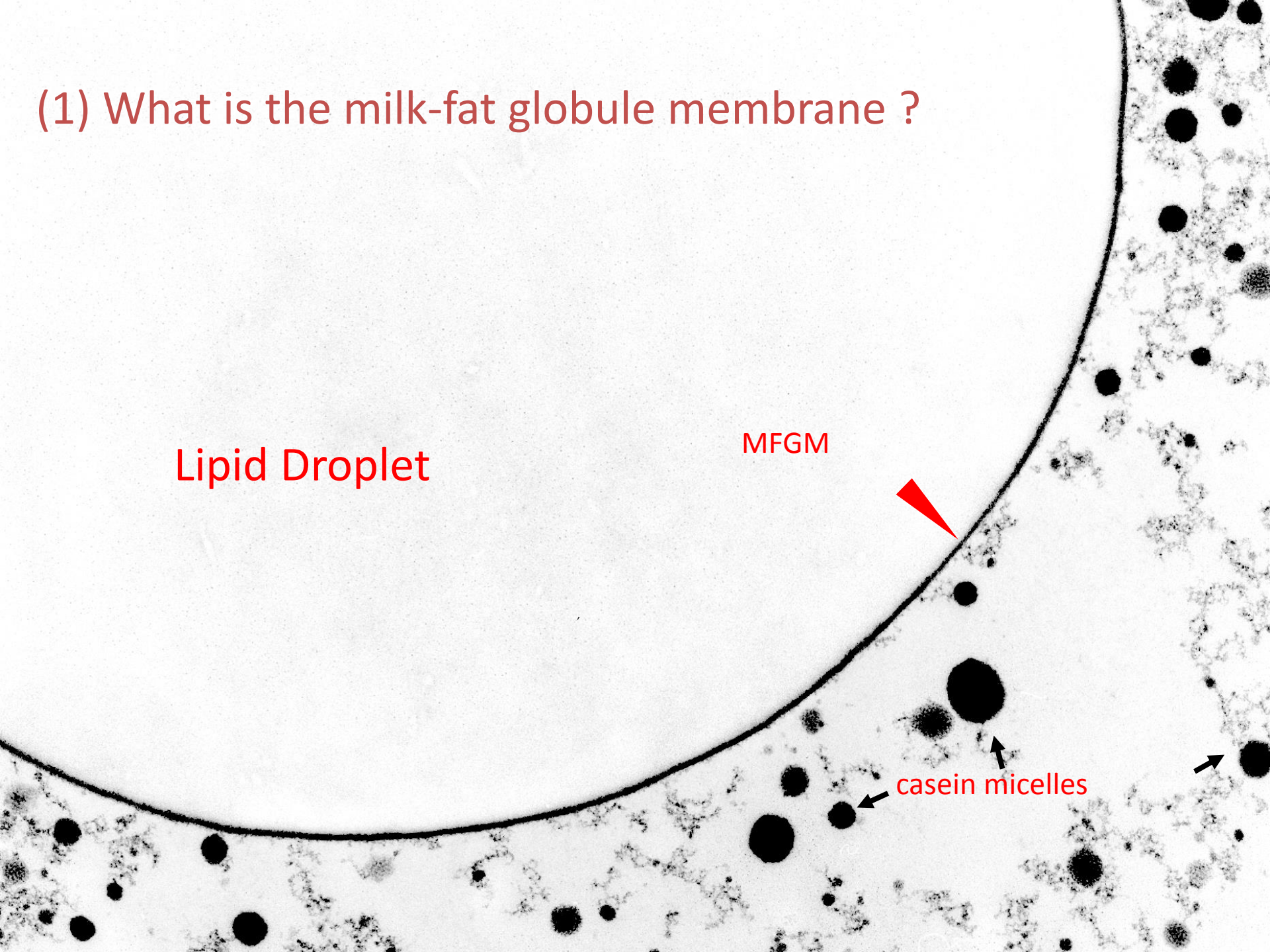
Mammary Epithelial Cell



How is the MFGM formed?



(1) What is the milk-fat globule membrane ?

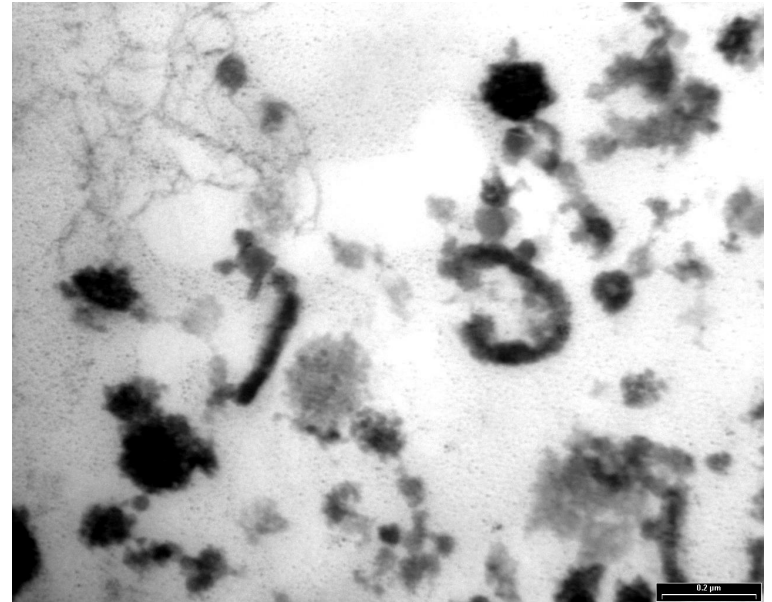
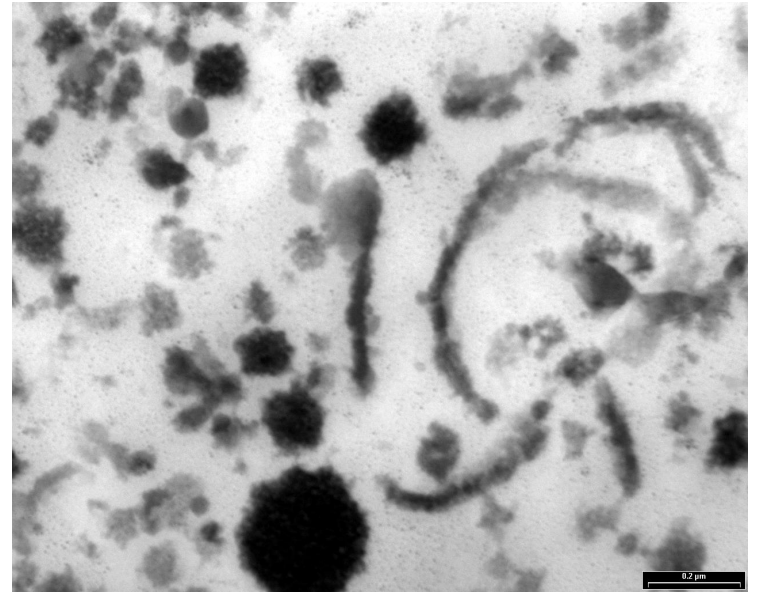
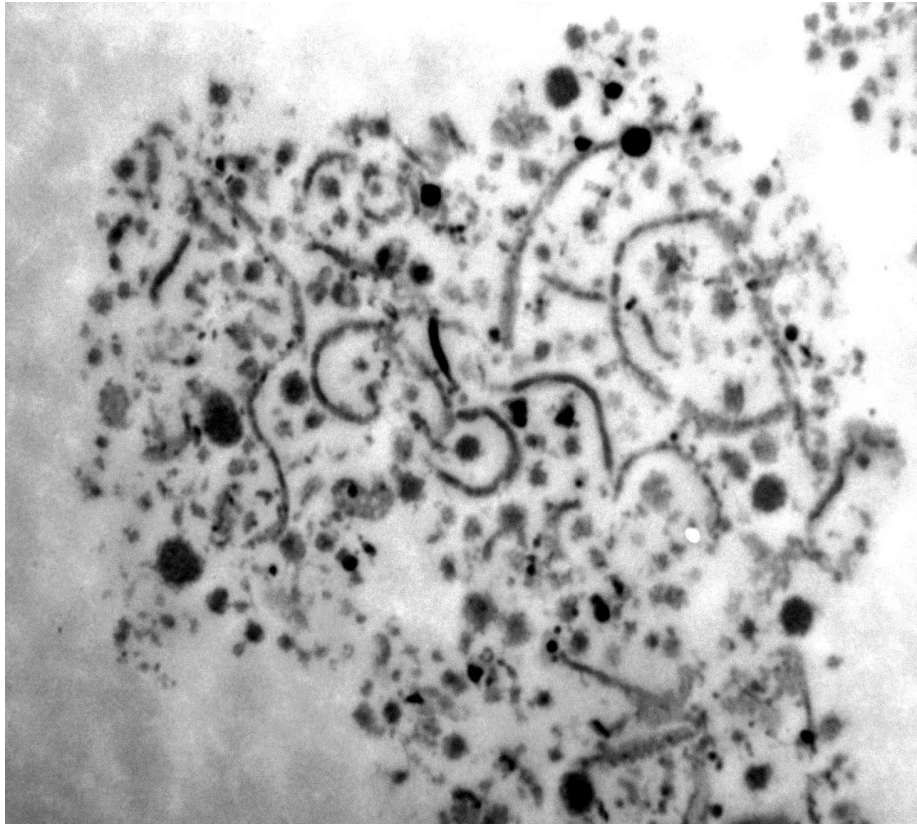


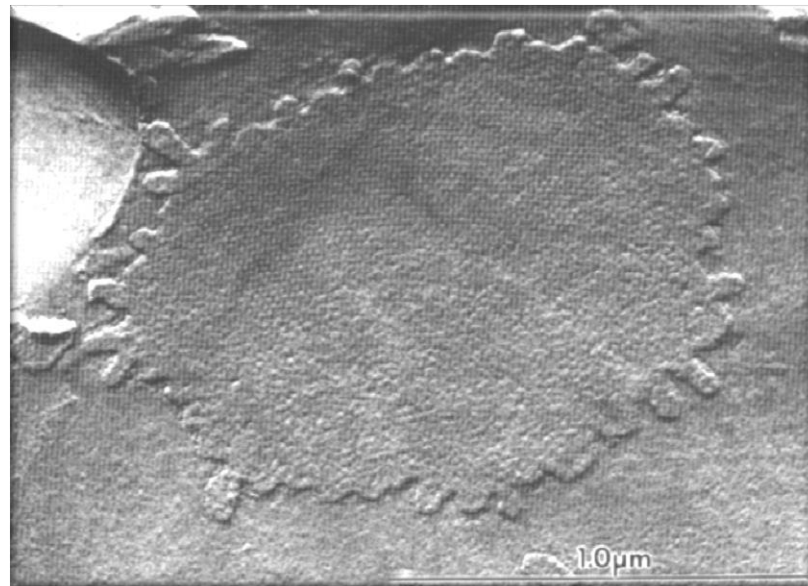
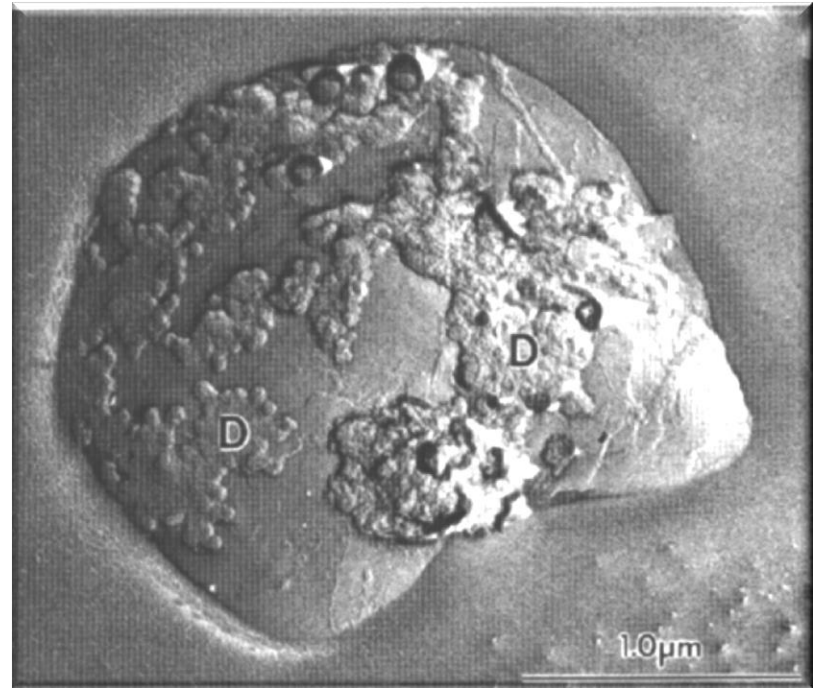
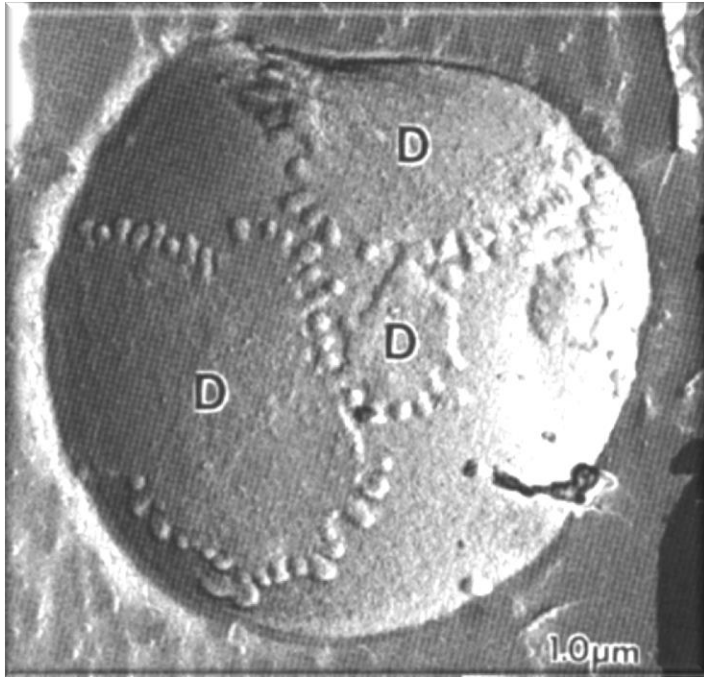
Lipid Droplet

MFGM

casein micelles

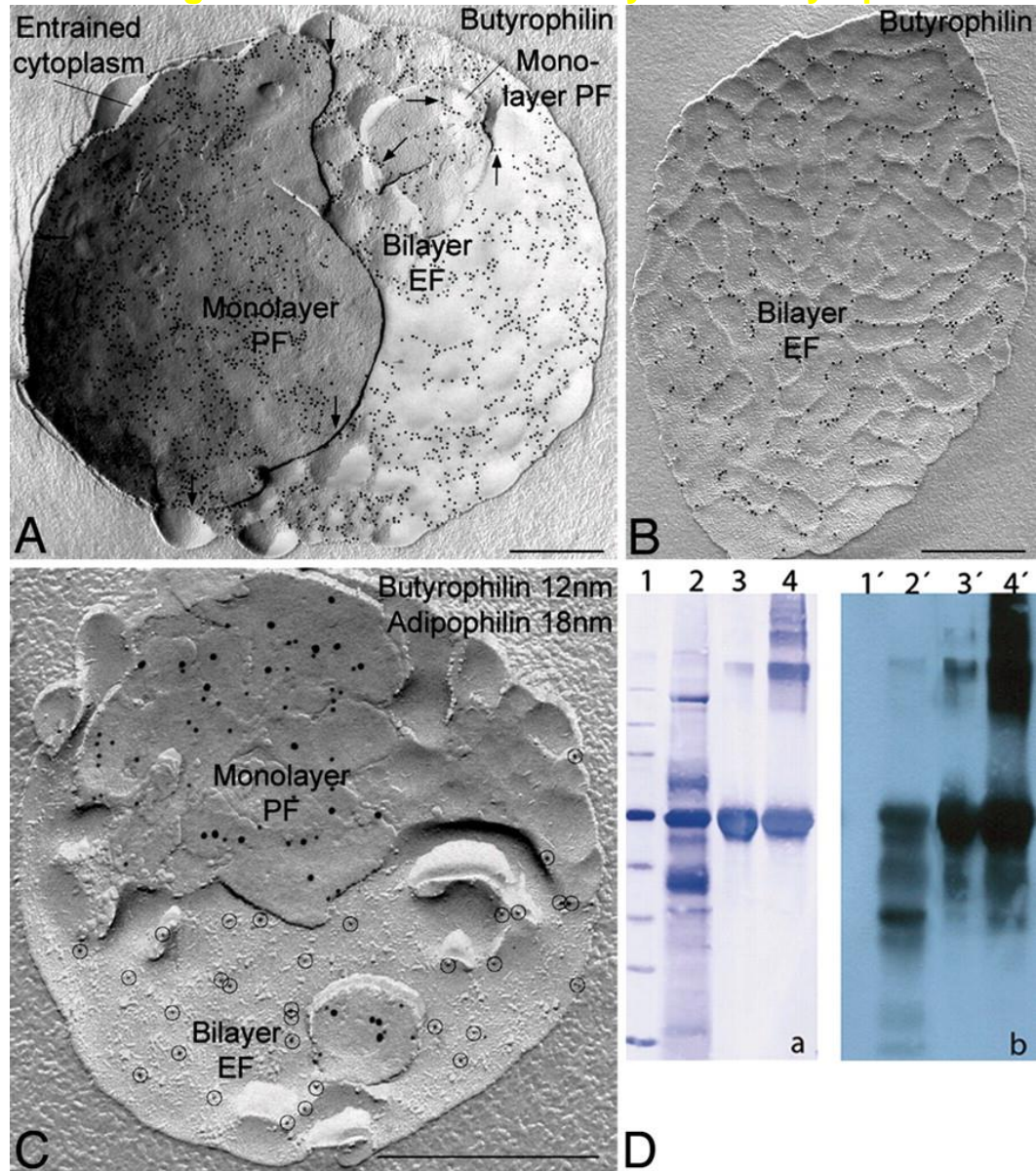
Transmission Electron Micrograph of Buttermilk





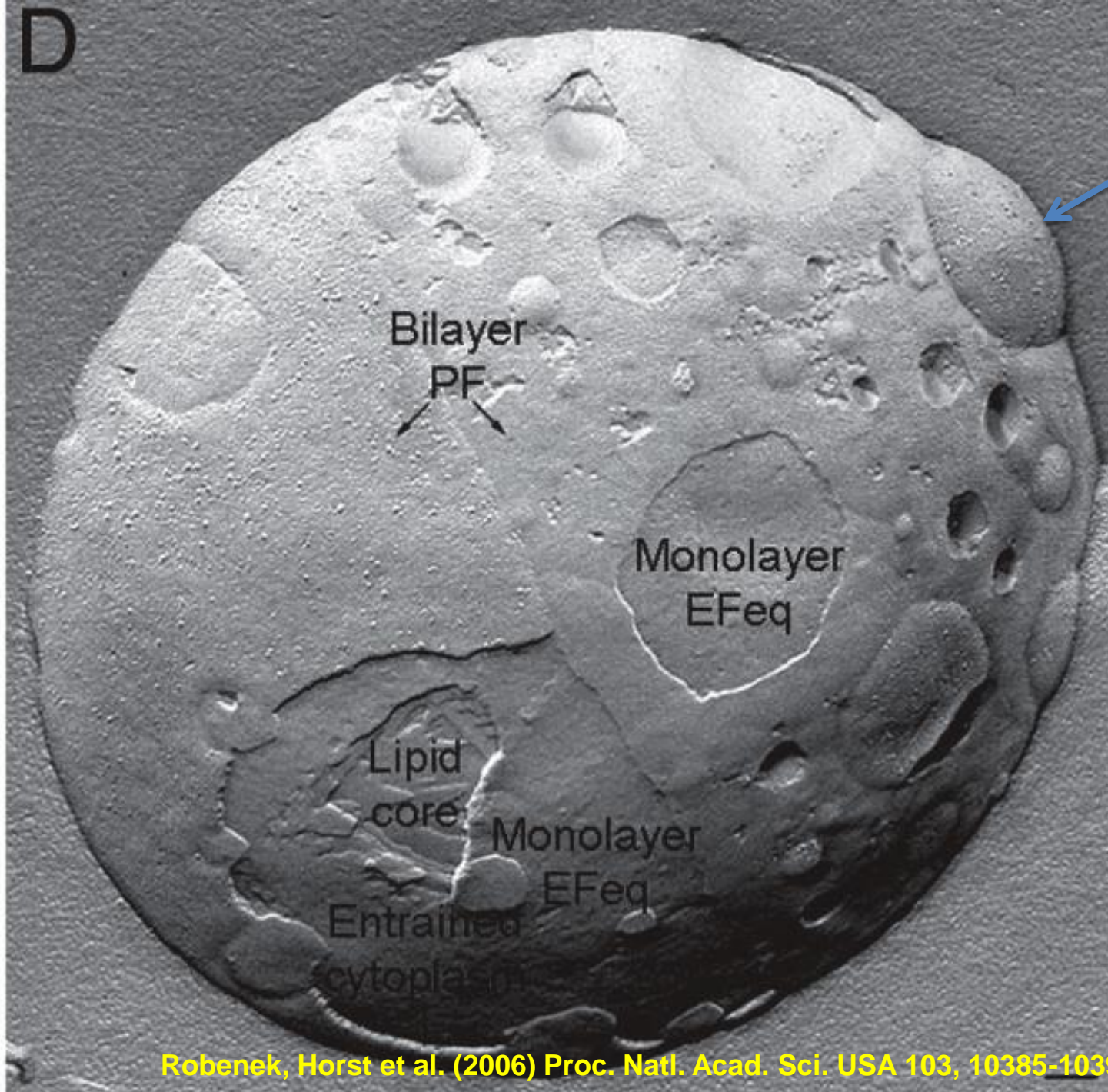
From 'Food Emulsions'
Larsson & Freberg 1990
Buckheim 1973...78

Fig. 3. Distribution and analysis of butyrophilin

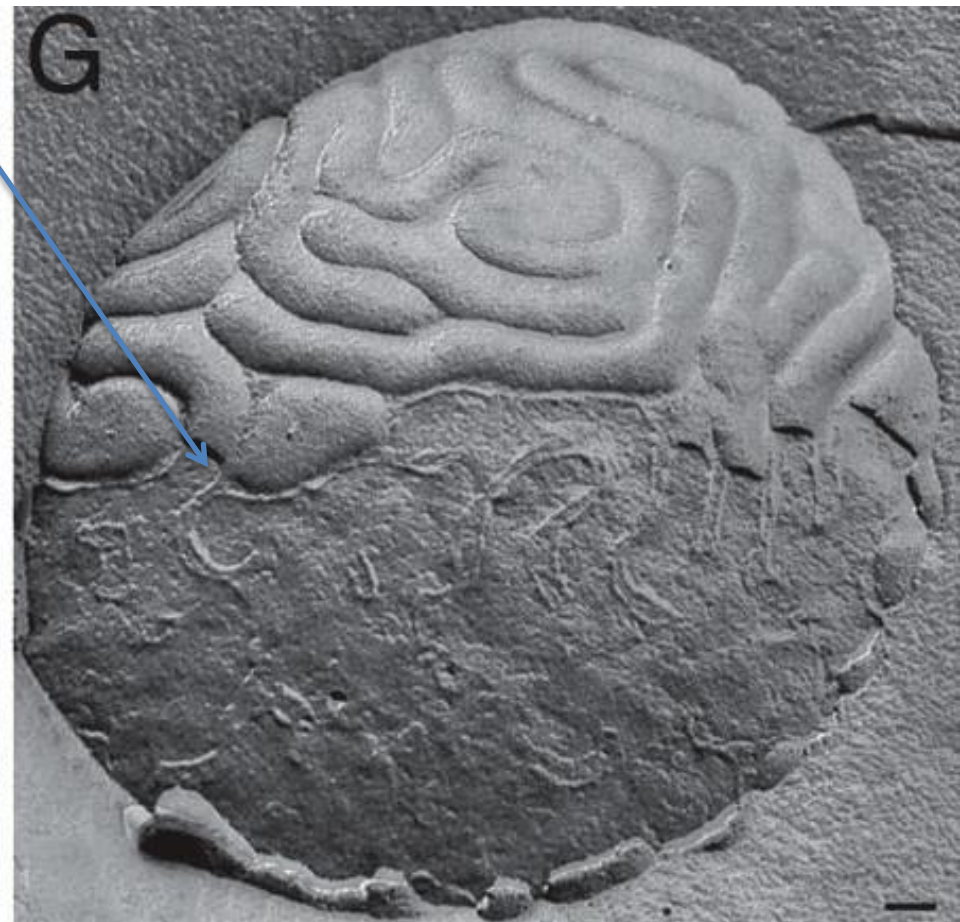
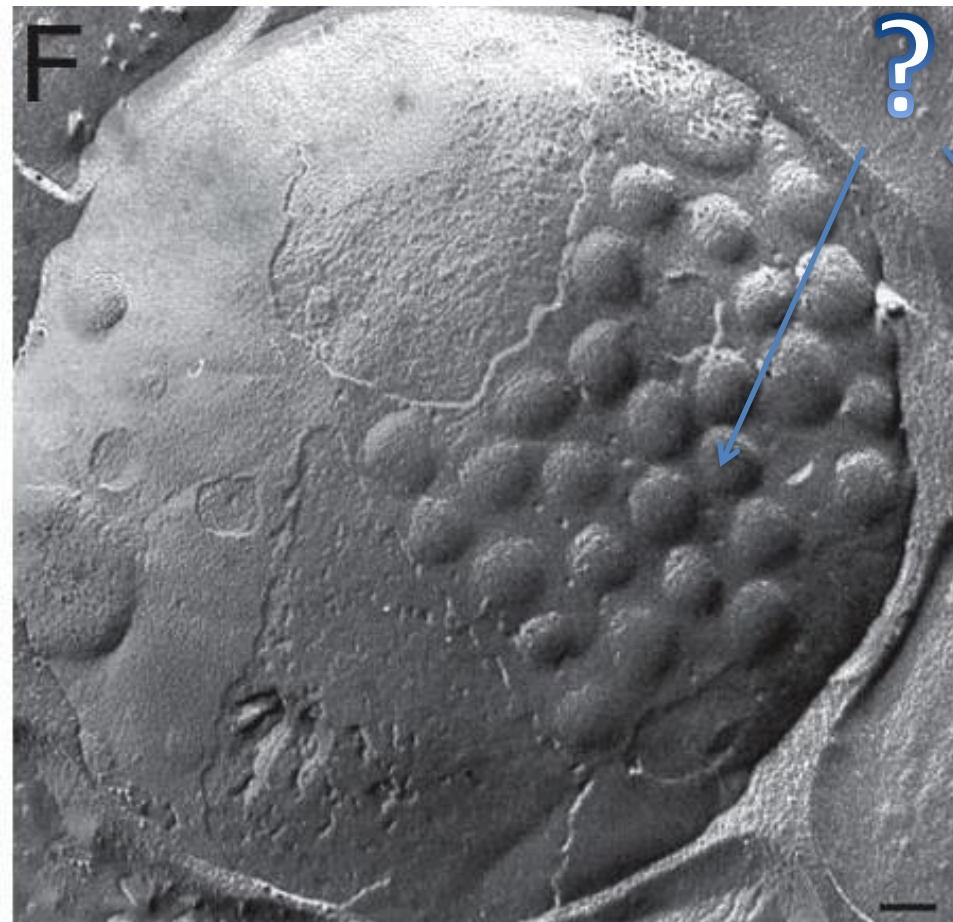


Robenek, Horst et al. (2006) Proc. Natl. Acad. Sci. USA 103, 10385-10390

D



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Proteins in the MFGM

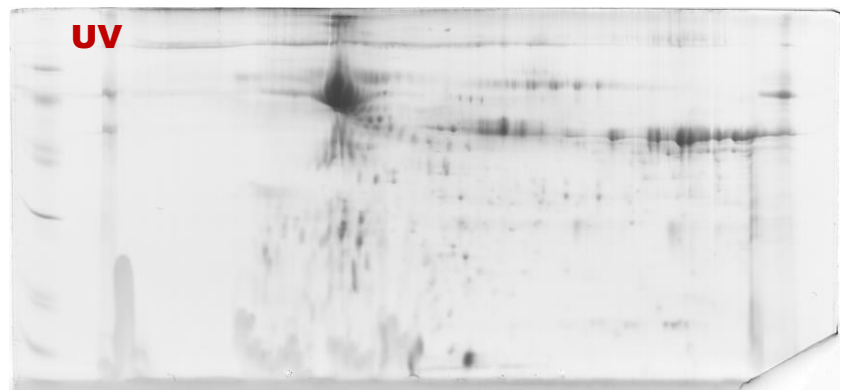
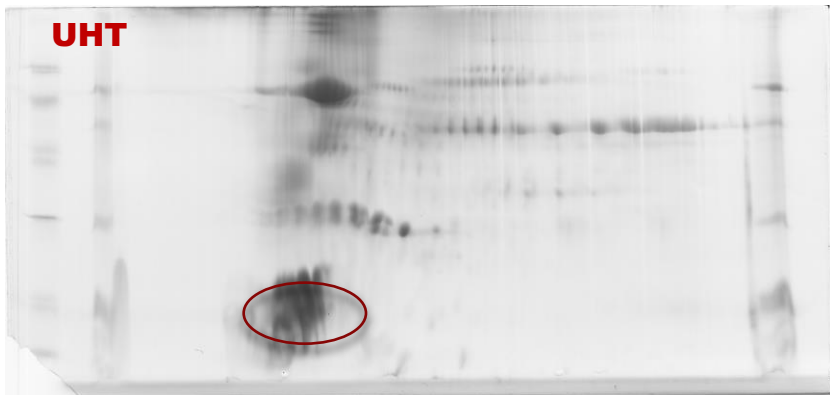
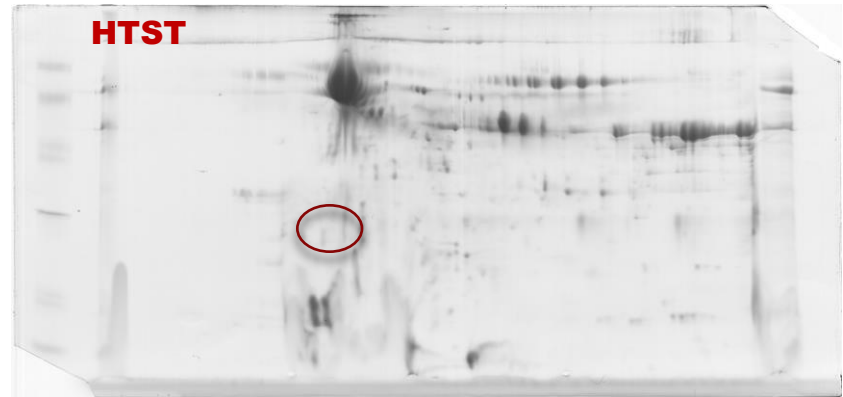
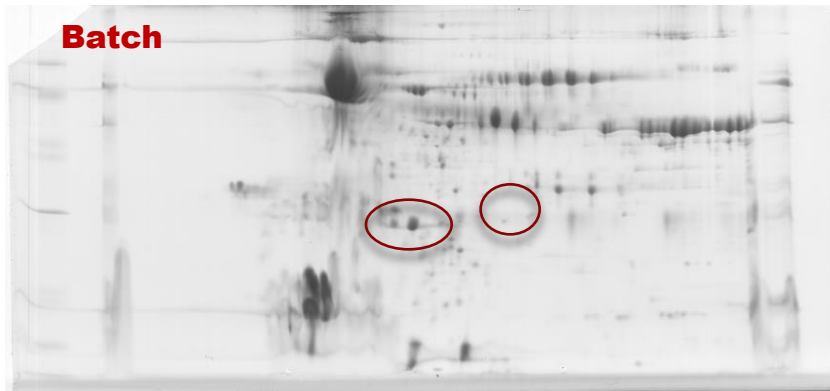
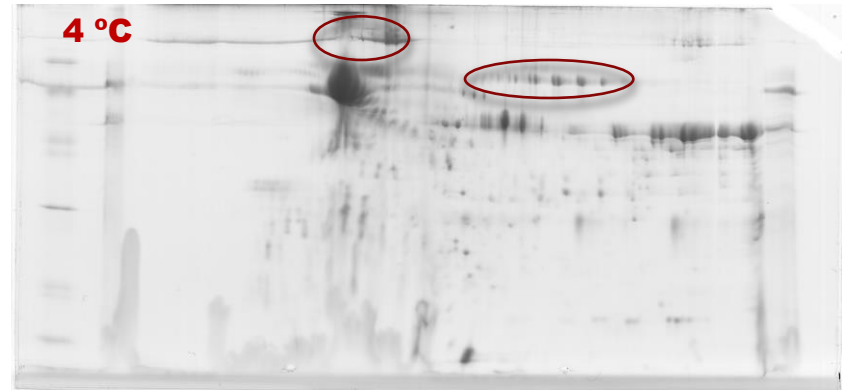
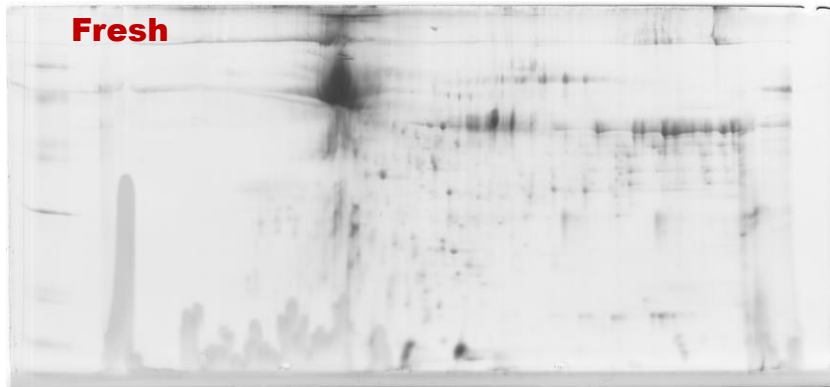
Table 1. Some proteins of bovine milk fat globule membrane (MFGM) as seen by SDS-PAGE.

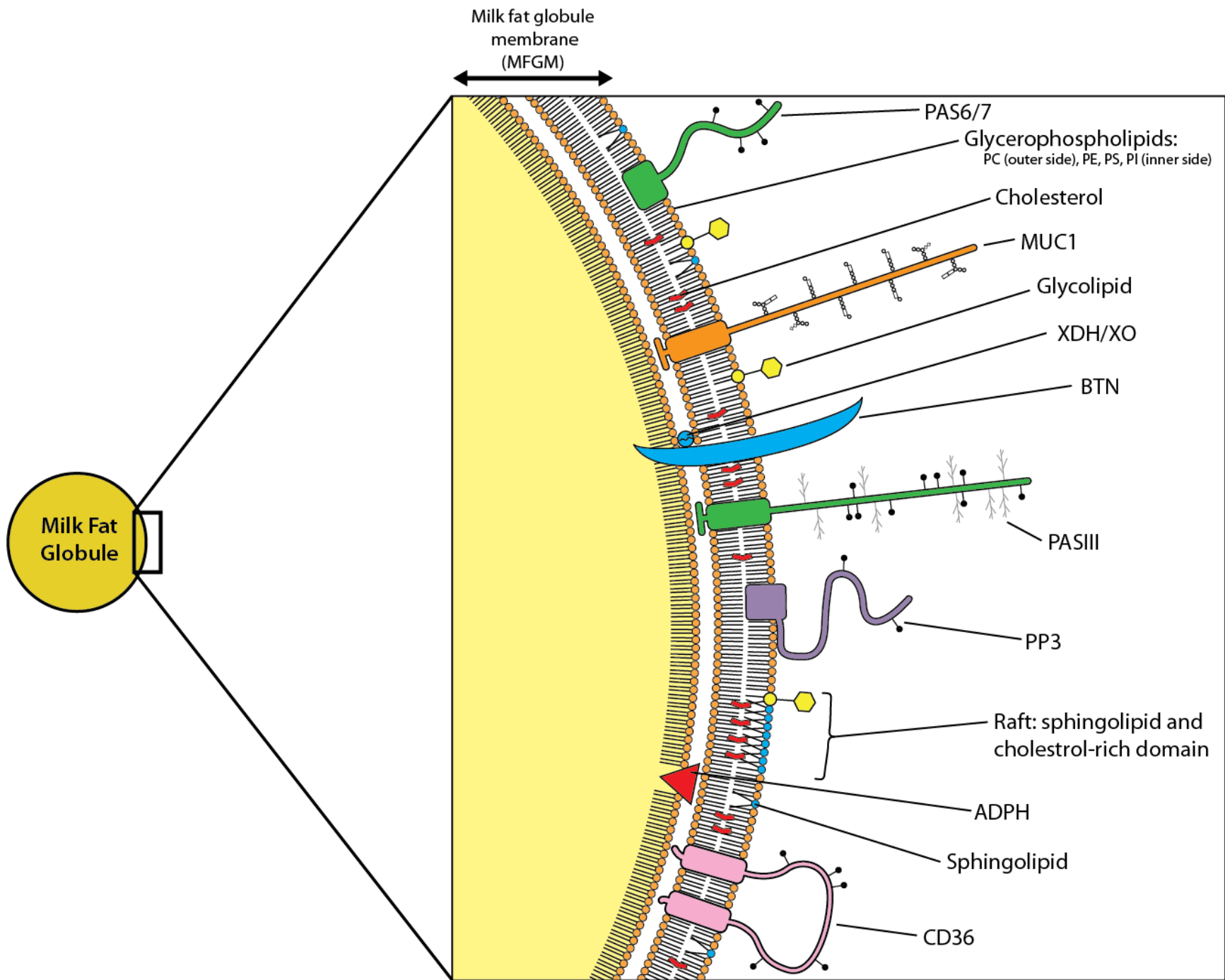
Protein	Type	Molecular weight, Da	Suggested role
Mucin I	Glycoprotein	>160,000	
Butyrophilin	Glycoprotein	66,000	Lipid secretion
Xanthine oxidase		150,000	Structural; lipid secretion
CD36	Glycoprotein	78,000	Fatty acid transporter
Fatty acid binding protein (FABP)		15,000	Fatty acid metabolism
BRCA1 ¹		210,000	Cancer suppressor
Focal adhesion kinase ¹ (FAK)		110,000	Signal transduction
Membrane-associated protein tyrosine kinase ¹ (cellular proto-oncogene c-src)		55,000	Signal transduction
Mitogen-activated protein kinase ² (MAPK)		42,000	Signal transduction
Caveolin ¹		27,000	Signal transduction

Bioactive Components in MFGM

Component	Health benefit
BRCA1	Inhibition of breast cancer
BRCA2	Inhibition of breast cancer
Fatty acid binding protein (FABP)	Cell growth inhibitor
Beta-glucuronidase inhibitor	Inhibition of colon cancer
FABP as selenium carrier	Anticancer factor
<i>Helicobacter pylori</i> inhibitor	Prevention of gastric diseases
Cholesterolemia-lowering factor	Anticholesterolemic
Butyrophilin	Suppression of multiple sclerosis
Vitamin E and carotenoids	Antioxidants
Vitamin B2	
Xanthine oxidase	Bactericidal agent
Phospholipids	Inhibition of colon cancer Anticholesterolemic Suppression of gastrointestinal pathogens Anti-Alzheimer, antidepressant Antistress
Phosphoproteins	Source of organic phosphorus and Ca-phosphate

Two-Dimensional Gels





Sequence variation among mammals



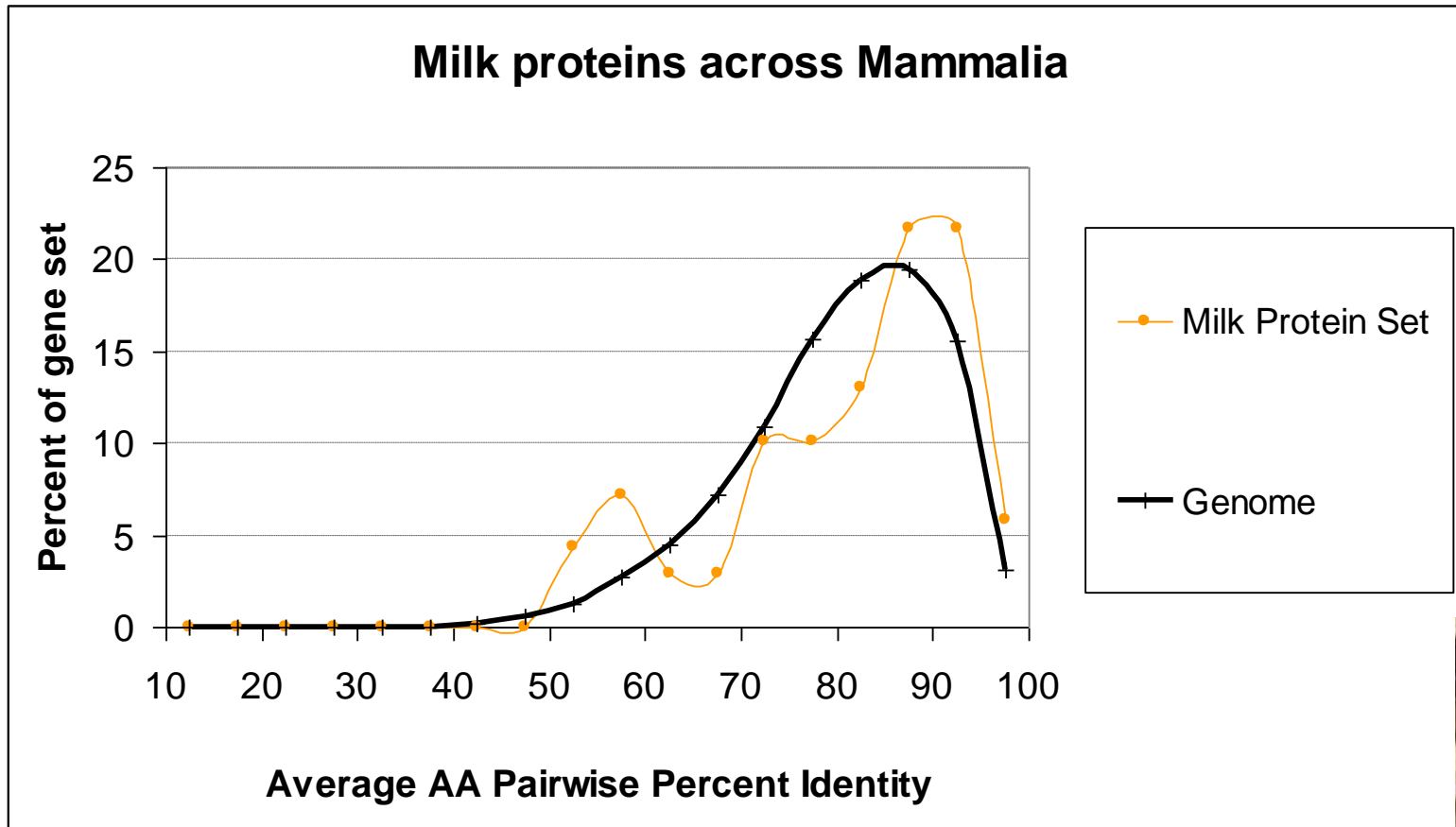
Align amino acid sequences



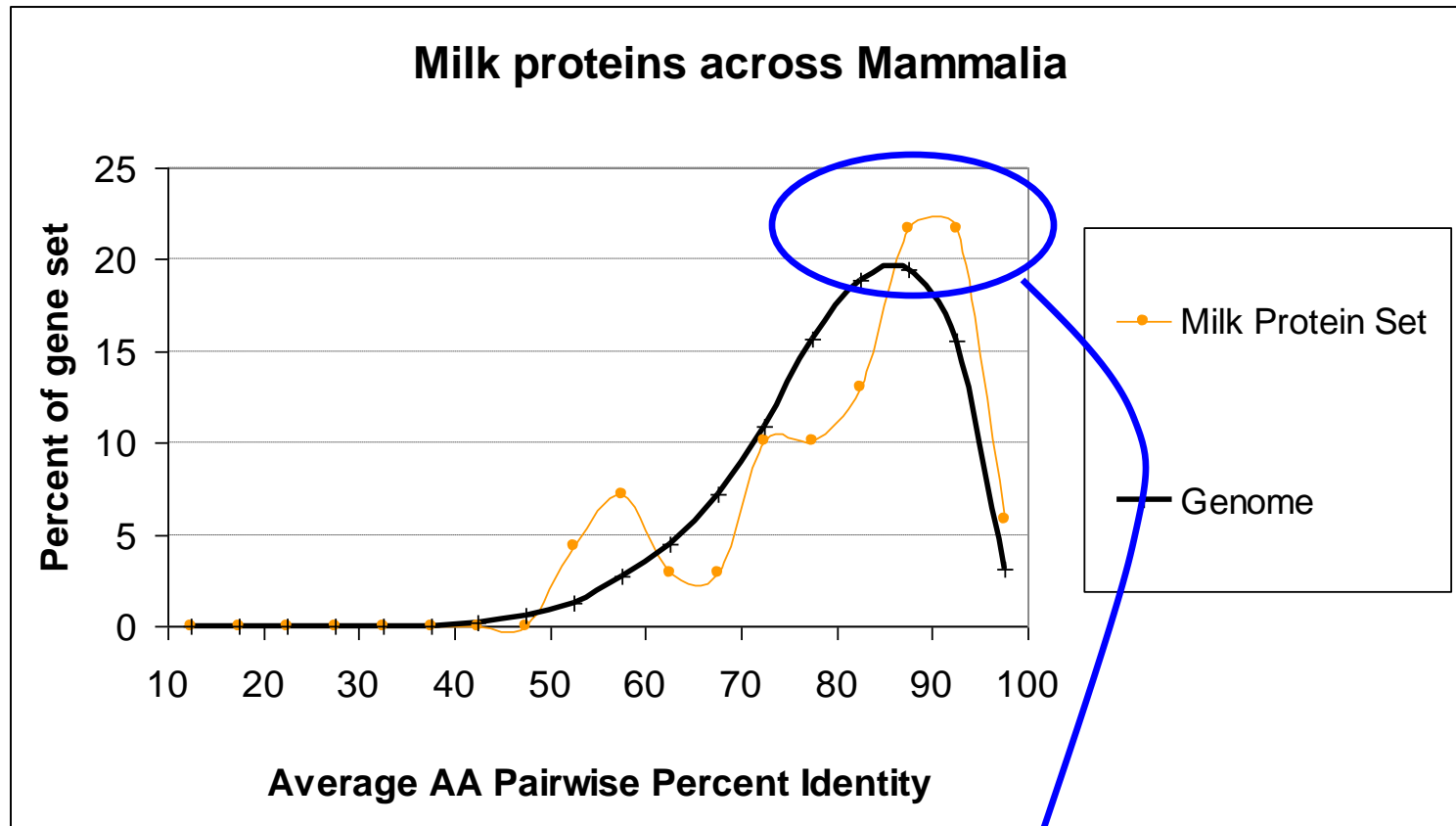
Compute average of all 21 pairwise identities



Pairwise % identity of milk proteins



Milk proteins across species

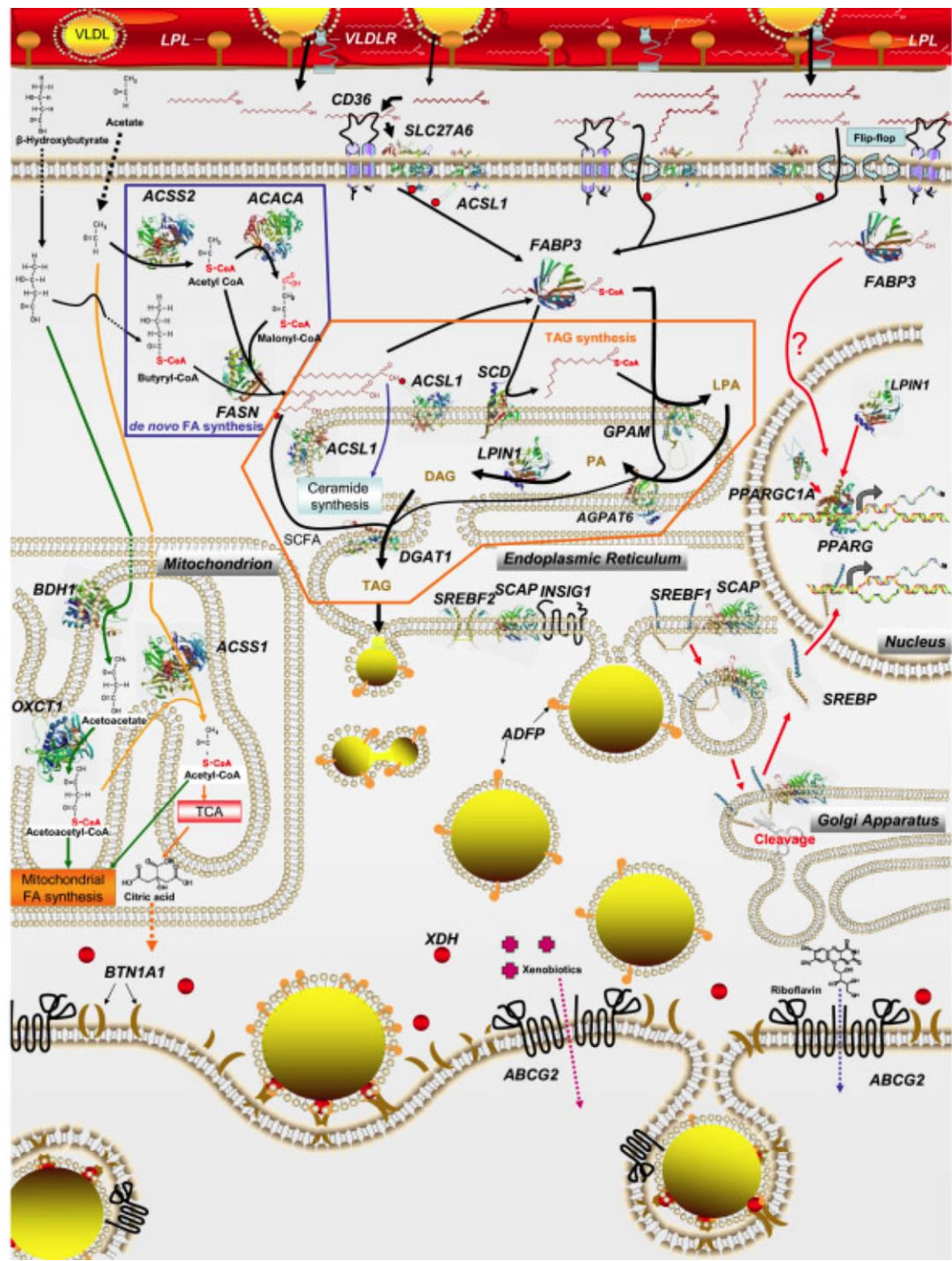


Which milk proteins are encoded by these highly conserved genes?

Which milk proteins are conserved?

Protein Name	GLEAN ID	Gene Symbol	Average PID
Rab 11A	GLEAN_20537	RAB11A	100
GTP binding protein G I G S G T beta subunit 1	GLEAN_21827	GBB1	100
GTP binding regulatory protein beta 2 chain	GLEAN_22534	GNB2	100
Rho C	GLEAN_13128	RHOC	99.8
Rab 11B	GLEAN_03051	RAB11B	99.6
Rap 1b	GLEAN_10112	RAP1B	99.6
GTP binding protein Sara	GLEAN_16602	SAR1A	99.5
Rab 3A	GLEAN_02763	RAB3A	99.5
histone 2, H2ab	GLEAN_23583	HIST2H2AB	99.2
SAR1B protein	GLEAN_08536	SAR1B	98.9
14-3-3 protein beta alpha	GLEAN_04527	YWHAB	98.9
Leucine-rich repeat containing protein 8	GLEAN_26477	LRC8A	98.8
Rab 18	GLEAN_21462	RAB18	98.6
Rho GDP-dissociation	GLEAN_11194	ARHGDI A	98.5
Rab 5C	GLEAN_17381	RAB5C	98.5
AD158	GLEAN_08550	LRC8C	98.1
Rab 3C	GLEAN_20950	RAB3C	98.0

Metabolic pathway for MFGM production



Taken from Bionaz *et al.*, 2008

Highly conserved milk proteins

- Constituents of the milk fat globule
- Associated with GO terms “GTPase activity”, “GTP binding”, “intracellular protein transport”, “secretory pathway”
- Highly conserved milk proteins likely **involved in secretion of milk fat globules**

The “foundation” of mammalian milk is the transfer of LIPID from mother to offspring.

Phospholipids in milk

Table 1. Polar lipid content (g/100 g of fat) and phospholipids composition (percentage of total phospholipids (PLs)) of liquid milk.

Reference	Polar lipids	PE	PI	PS	PC	SM	Note
[21]	0.69	38.6	-	-	32.2	29.2	¹
[22]	0.36	32.3	9.3	10.5	27.3	20.5	
[23]		26.9	13.7	4.1	27.5	27.7	
[24]	0.96	33.2	5.2	9.3	27.4	25.1	
[25]	0.7	46.4	5.3	7.4	21.1	19.8	
[17]		32.6	7.6	5.3	33.2	21.3	
[26]		36.4	7.6	6.5	32.1	17.3	
[19]	0.25–0.30	26.8	13.6	16.1	22	21.6	
[27]	0.48	28.5	14.1	-	32.7	23	^{1,2}
[28]	0.36	38.5	6.5	7.7	25.9	21.4	
[12]		26.4	3.4	2	42.8	25.5	
[29]	0.69	36.9	6.1	6.3	27	23.7	
[30]		72.3	1.4	11.5	8	7.9	
[13]		33.8	3.9	10.6	30.5	21.2	
[31]	0.65–0.89	34.2	7.7	8.6	45.5	4.1	¹

¹ the total amount of polar lipids was calculated considering an average fat content of cow milk of 3.5%.

² The percentage of PI includes also PS.

Table 2. Content of main PLs in milk fat of different mammalian species. Values are expressed as percentage of total PLs, applying the same calculation reported for Table 1.

Reference	Species	PE	PI	PS	PC	SM	Note
[23]	buffalo	24,5	19,7	6,6	24,3	24,9	
[28]	goat	31.7	6.3	8.3	28.5	25.2	
	ewe	34.4	4.4	5.2	28.6	27.4	
[32]	human	21.3	16.4		19	43.3	¹
[30]	donkey	60.2	2.4	11.2	17.3	8.8	
[13]	mare	24.3	8.5	10.6	27.8	28.9	
	human	21.7	4.5	9.6	29	35.2	
	camel	34.3	4.9	10.5	22.1	28.1	

¹ The percentage of PI includes also PS.

Table 3. Polar lipid content (g/100 g of fat) and phospholipids composition (percentage of total PLs) in milk fat of dairy products and by-products of butter-making process.

Reference	Matrix	Polar lipids	PE	PI	PS	PC	SM
[22]	cream	0.86	42.7	6.8	7.2	14.6	28.6
	butter	0.2	31	11.9	15.3	24.7	17.1
	buttermilk	4.49	33.5	2.4	10.3	35.5	18.3
[33]	cow cream	0.17					
	cow buttermilk	0.17	38.7	9.3	9.1	23.9	18.9
	cow butter serum	0.88	27.2	10.8	7.2	29.8	24.9
	goat cream	0.2					
	goat buttermilk	0.19	35.2	9.8	9.9	24.8	20.3
	goat butter serum	1.01	27.1	11.7	8.2	26.2	26.8
[34]	cream		26.7	7.5	11.7	26.5	20.8
[35]	cream	5.65	17.7	15.4	11.3	33.7	21.8
	butter	5.31	17.7	15.8	11.5	33.3	21.8
	buttermilk	12.4	17	7.1	8.1	46.1	21.7
[12]	buttermilk		8.4	8.2	4.6	51.2	27.6

Not Just Fat: The Structure and Function of the Lipid Droplet

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Article

Phospholipid Architecture of the Bovine Milk Fat Globule Membrane Using Giant Unilamellar Vesicles as a Model

Haotian Zheng, Rafael Jimenez-Flores, Derek E. Gragson, and David W. Everett

J. Agric. Food Chem., Just Accepted Manuscript • DOI: 10.1021/jf500093p • Publication Date (Web): 18 Mar 2014

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Technological Properties

- Emulsification
- Texture development
- Flavor carriers
- Liposome formation

Milk fat globule membrane isolated from buttermilk or whey cream and their lipid components inhibit infectivity of rotavirus in vitro

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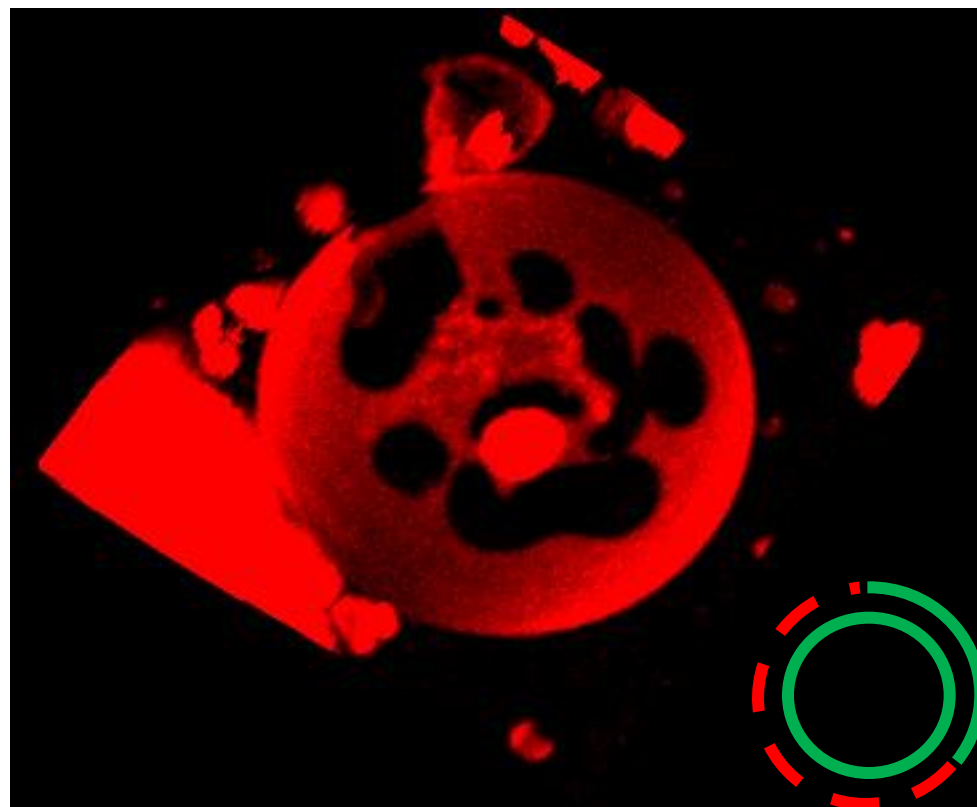
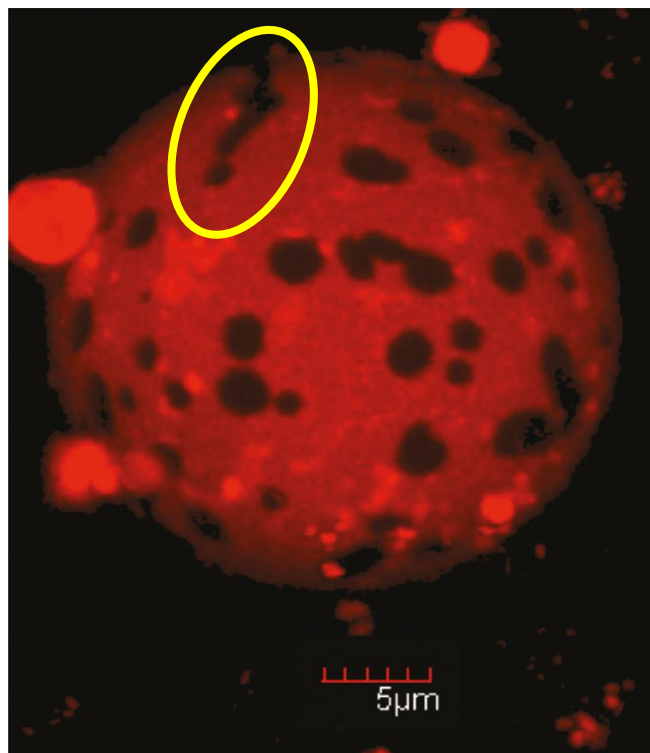
Phospholipid Architecture of the Bovine Milk Fat Globule Membrane Using Giant Unilamellar Vesicles as a Model

Haotian Zheng, Rafael Jimenez-Flores, Derek E. Gragson, and David W. Everett

J. Agric. Food Chem., Just Accepted Manuscript • DOI: 10.1021/jf500093p • Publication Date (Web): 18 Mar 2014

Downloaded from <http://pubs.acs.org> on March 24, 2014

Synthesis of GUV under PL ratios equal to those found in cow's milk



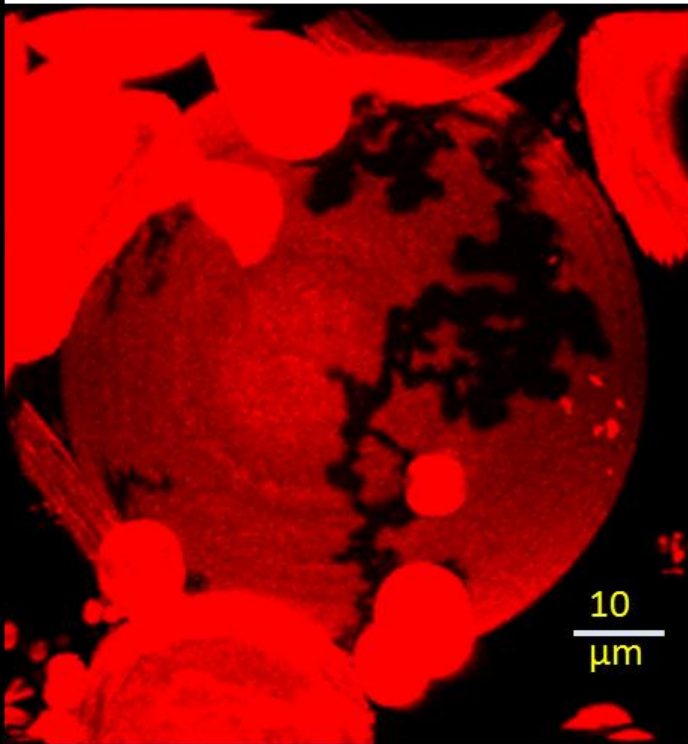
Gallier, et al, 2010, Using Confocal Laser Scanning Microscopy To Probe the Milk Fat Globule Membrane and Associated Proteins

Zheng et al. 2014

PC and SM role in formation of liquid domains in GUVs

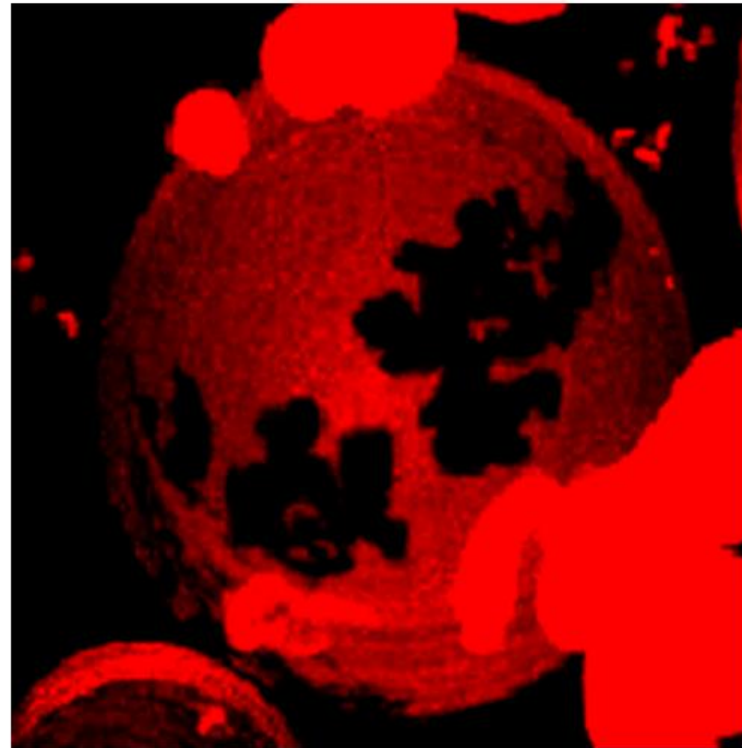
Milk SM/DOPE

3/7



Milk SM/DOPE

7/3



Biological and Health Effects

Review

Phospholipids in Milk Fat: Composition, Biological and Technological Significance, and Analytical Strategies

Giovanna Contarini * and Milena Povolo

Consiglio per la Ricerca e la Sperimentazione in Agricoltura–Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, Via A. Lombardo, 11-26900 Lodi, Italy;
E-Mail: milena.povolo@entecra.it

Int. J. Mol. Sci. **2013**, *14*, 2808-2831; doi:10.3390/ijms14022808

Biological Activity and Health Effects

- *Cardiovascular Diseases*
- *Inflammation and Gastrointestinal Infections*
- *Stress Conditions*
- *Cancer*
- *Cholesterol Absorption*
- *Nervous System Myelination and Neurological Development*
- *Lipid digestion regulation (obesity prevention)*

Methods:

0 Hr

- Culture KRTs to 50% confluency
- Add 0.1% Sphingomyelin solution

24 Hr

- Expose to 40mJ/cm²
- Use 302nm UV lamp

48 Hr

- Stain for P21, Nuclear DNA
- Image using confocal microscope
- Image analysis

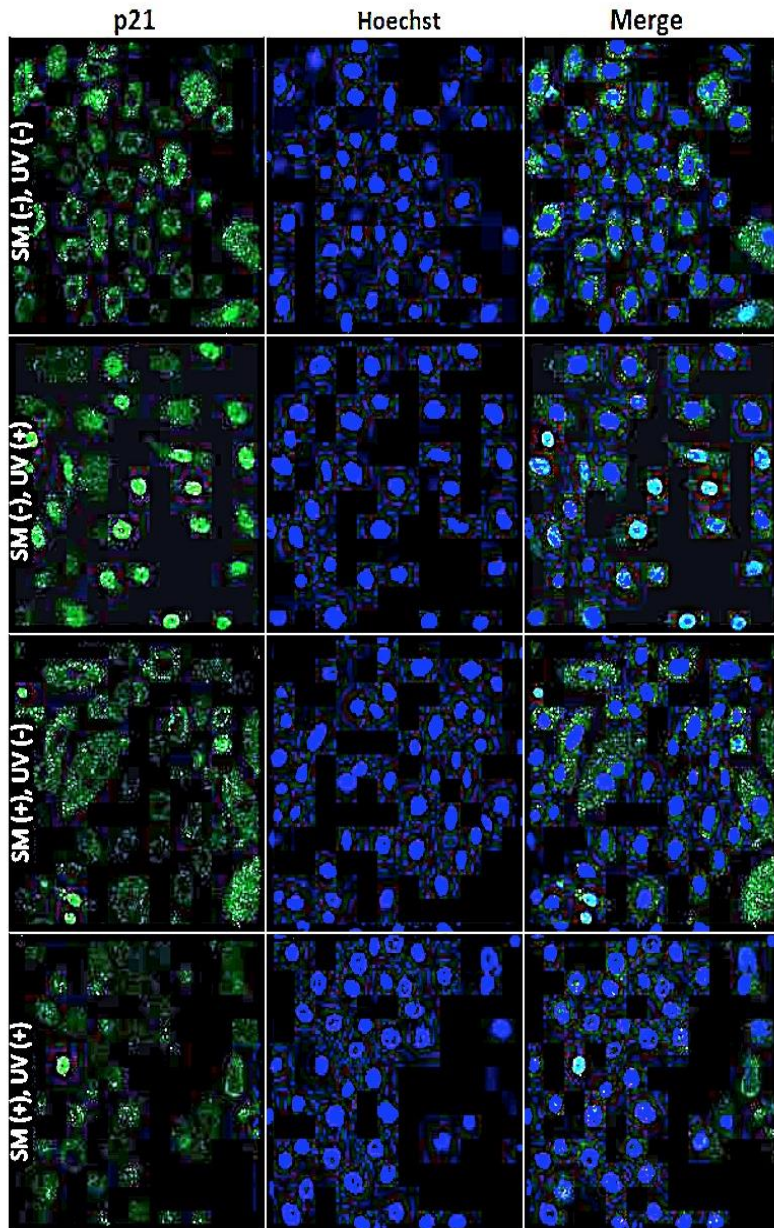


Figure 5.4 Representative confocal images of p21 expression (green) and keratinocyte nuclei (blue) of the different treatment groups.

Table 5.1 Mean Nuclear p21 Intensities and Standard Errors

Treatment Group	Mean Nuclear p21 Intensity (0-255)	Standard Error
SM (-) UV (-)	5.431	0.69
SM (-) UV (+)	17.63	0.722
SM (+) UV (-)	8.443	0.69
SM (+) UV (+)	8.537	0.69

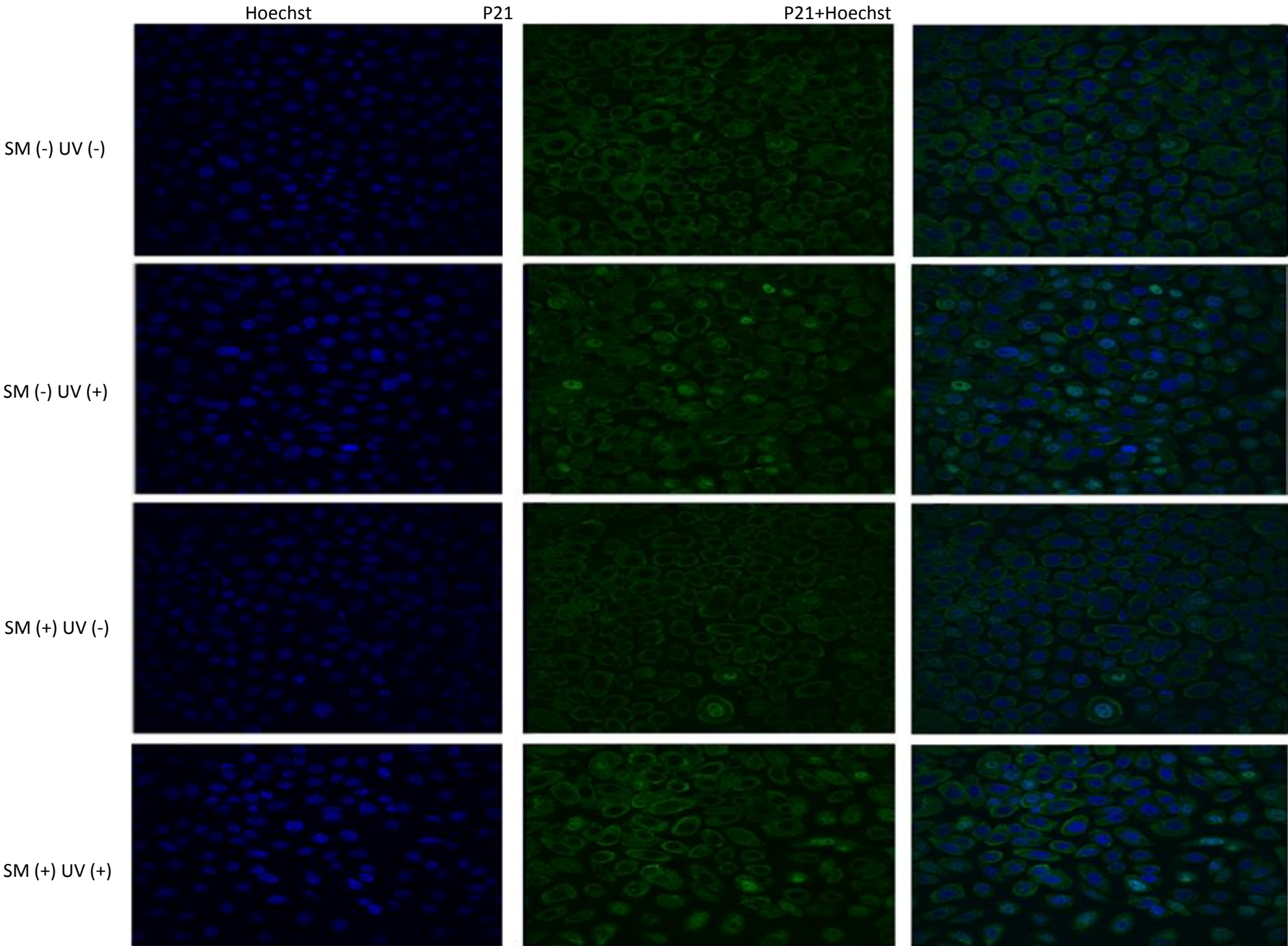


Figure 4. Representative P21 and Nuclear Images

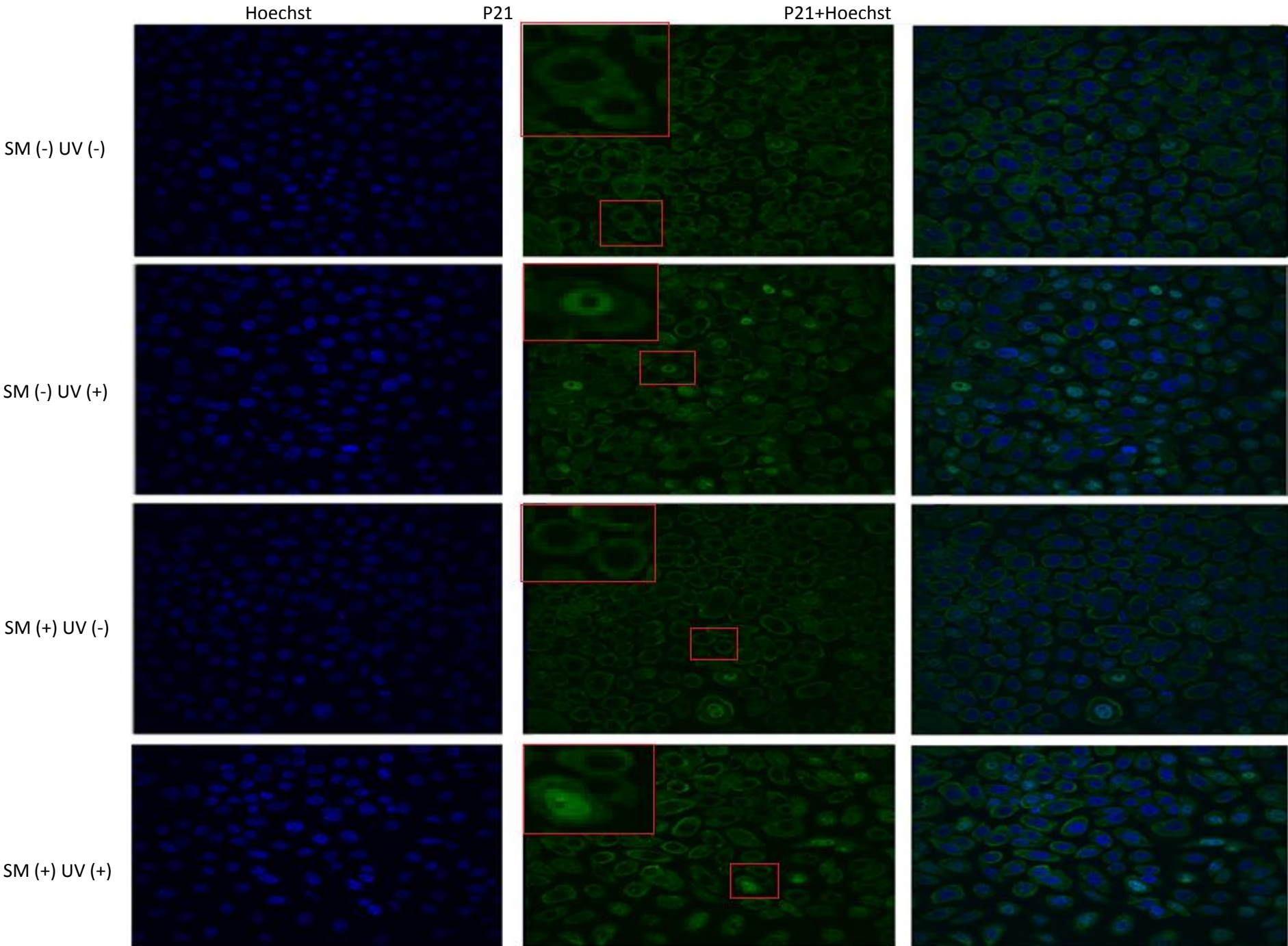
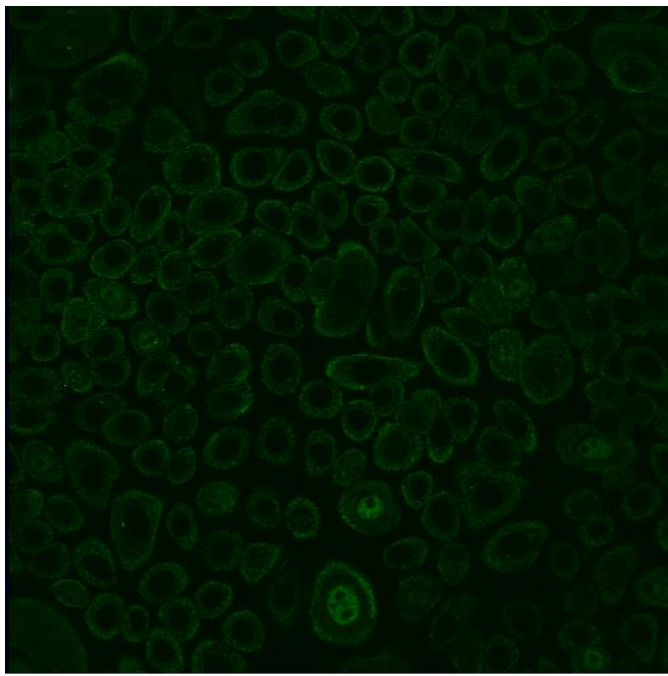
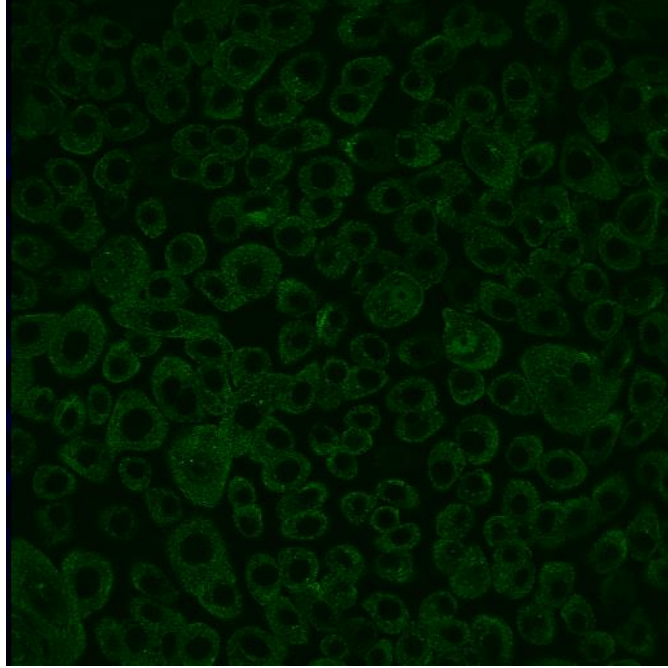


Figure 4. Representative P21 and Nuclear Images

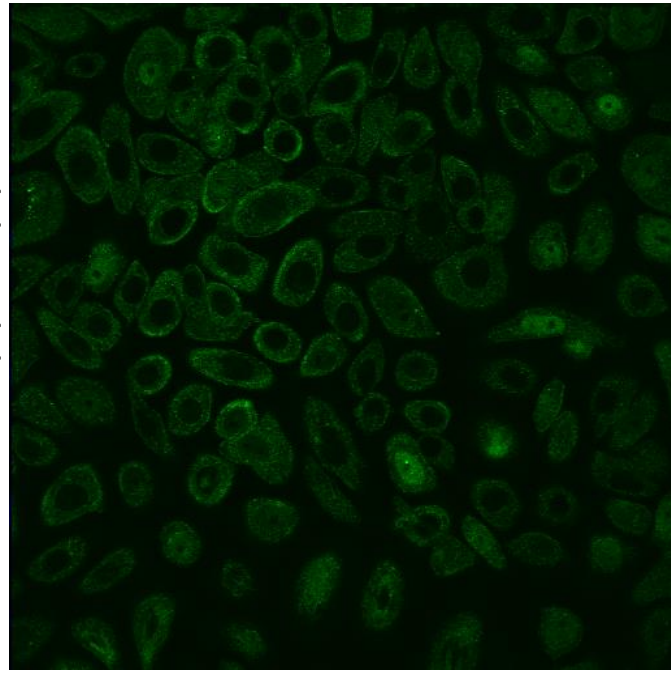
SM (+) UV (-)



Control



SM (+) UV (+)



SM (-) UV (+)

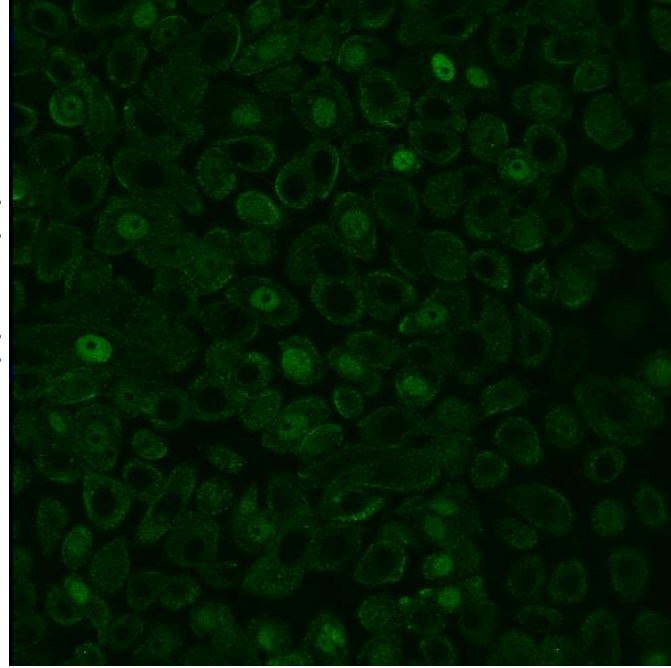


Figure 5. Representative P21 Images

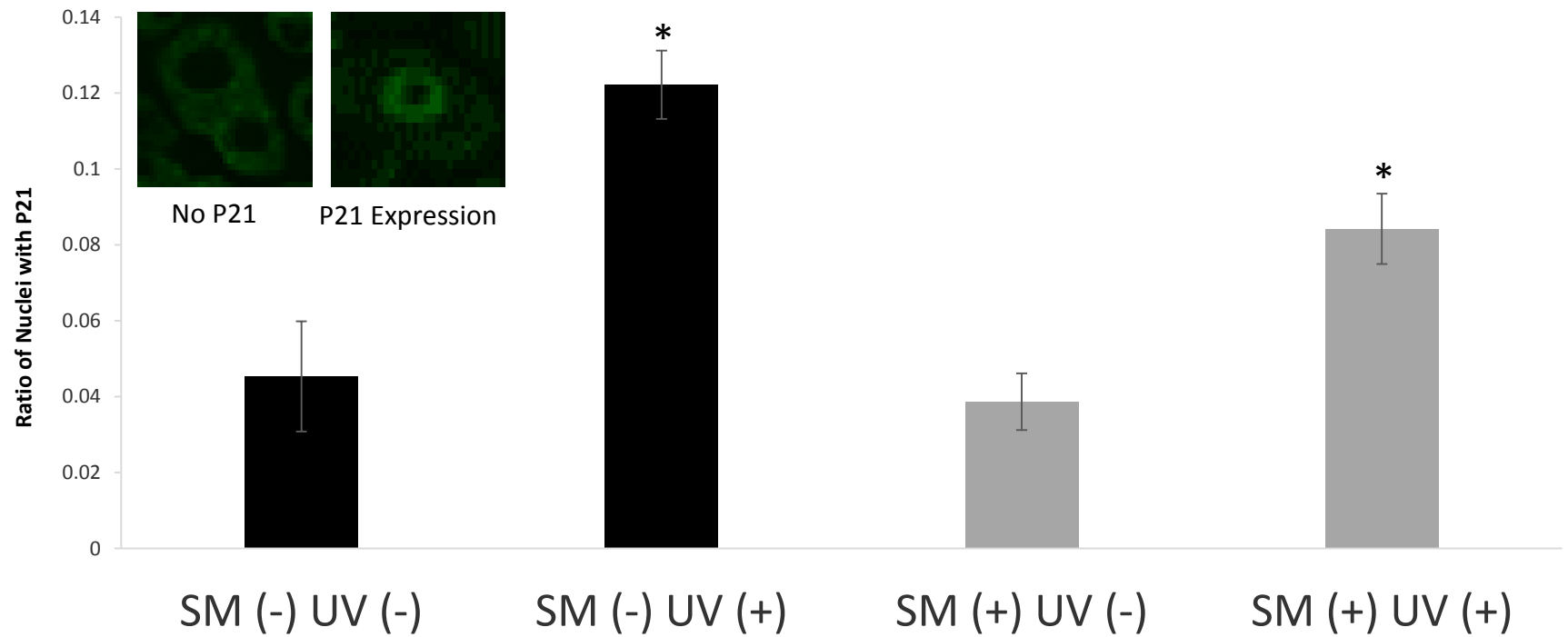


Figure 6. P21 Nuclear Ratio. * Indicates Significant Results

- Nuclear Intensity of p21:
 - Increased in the presence of UV exposure
 - ☐ Indicates DNA damage is present following UV

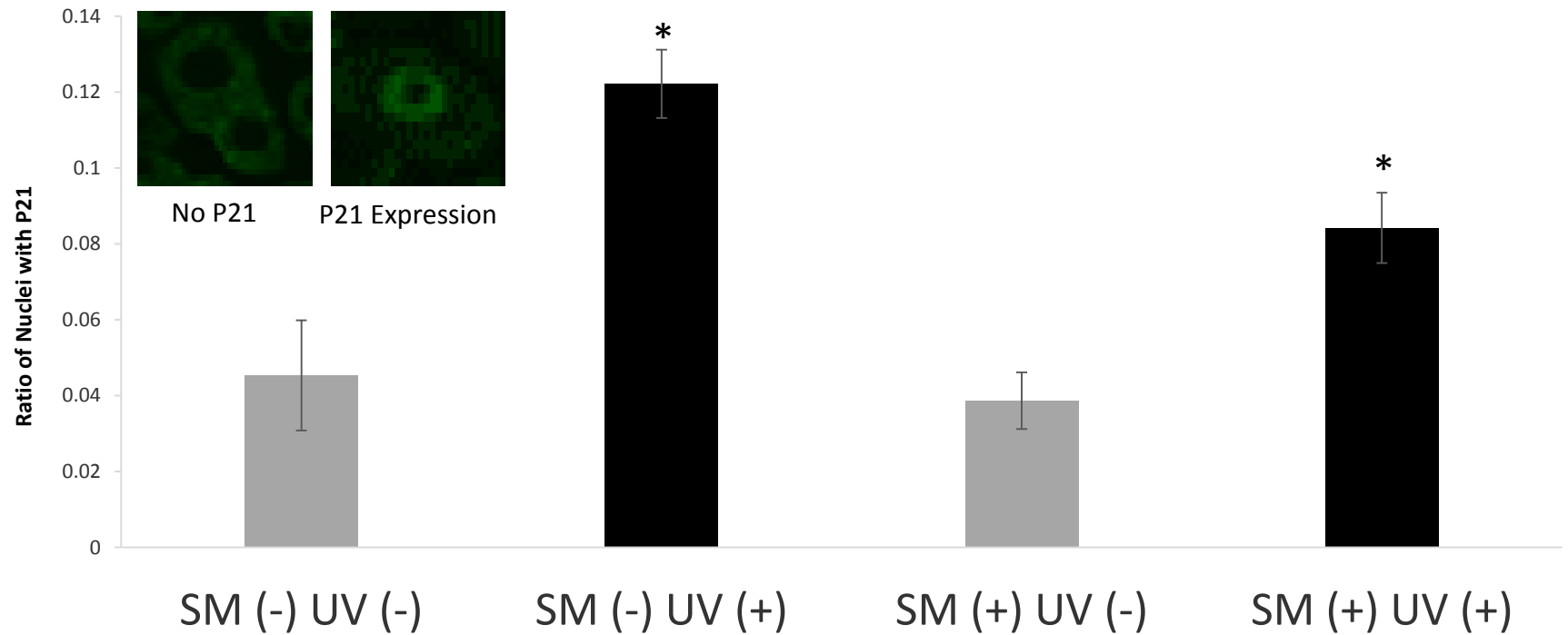


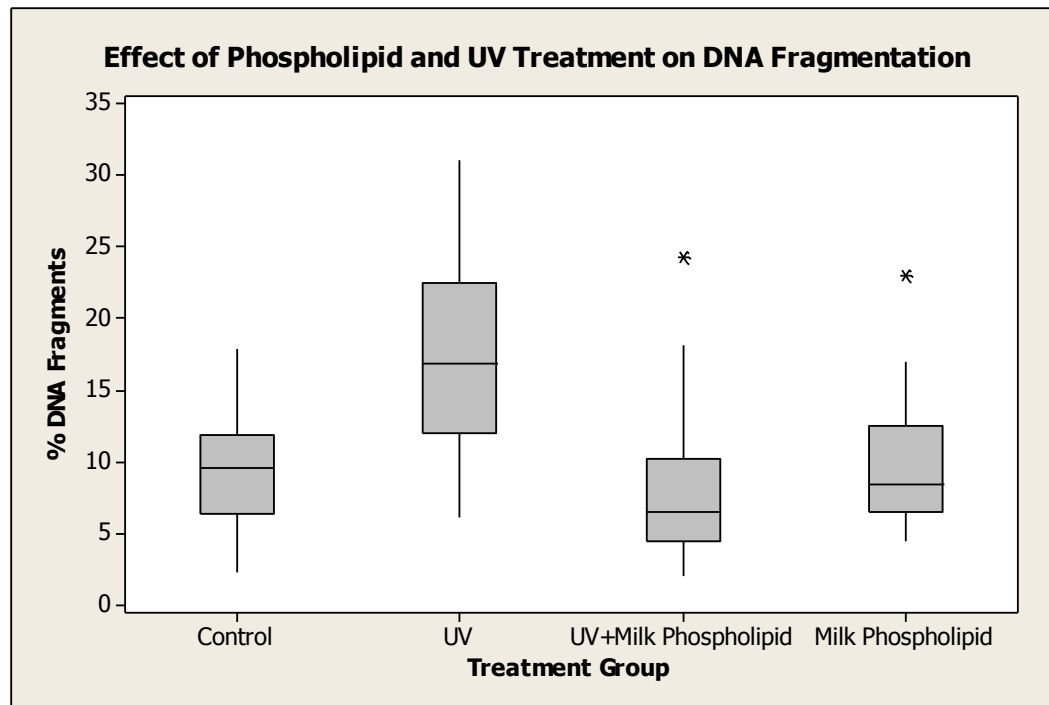
Figure 6. P21 Nuclear Ratio. * Indicates Significant Results

- Nuclear Intensity of p21:
 - Increased in the presence of UV exposure
 - Indicates DNA damage is present following UV
 - Cells exposed to UV had less p21 expression with SM
 - SM may impart photoprotective properties to skin
 - Could support SM as a preventative cancer treatment

Results

■ % DNA fragment count

- UV sample group had significantly higher fragment count (p-value = 0.005)

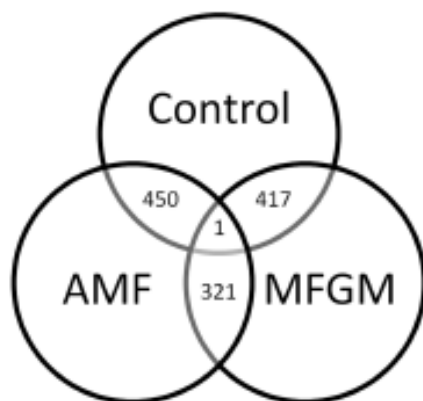


Dietary Milk Fat Globule Membrane Reduces the Incidence of Aberrant Crypt Foci in Fischer-344 Rats

DALLIN R. SNOW,[†] RAFAEL JIMENEZ-FLORES,[‡] ROBERT E. WARD,^{†,§} JESSE CAMBELL,[†]
MICHAEL J. YOUNG,[†] ILKA NEMERE,[†] AND KORRY J. HINTZE^{*,†,§}

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[§]These authors contributed equally to this work



“Our results support the hypothesis that diets containing MFGM are protective against colon cancer (epithelial cells) in Fischer-344 rats, perhaps because of MFGM’s high polar lipid content, namely, sphingomyelin”.

Figure 3. Venn diagram of differentially regulated genes in the mucosa between dietary treatments. Out of 21,792 genes, a total of 417, 450, and 321 genes were differentially regulated with significance in the mucosa between the MFGM vs control, AMF vs control, and MFGM vs AMF dietary treatments, respectively. See Supplementary Table 1 in the Supporting Information for a listing of the most differentially regulated genes between treatments.

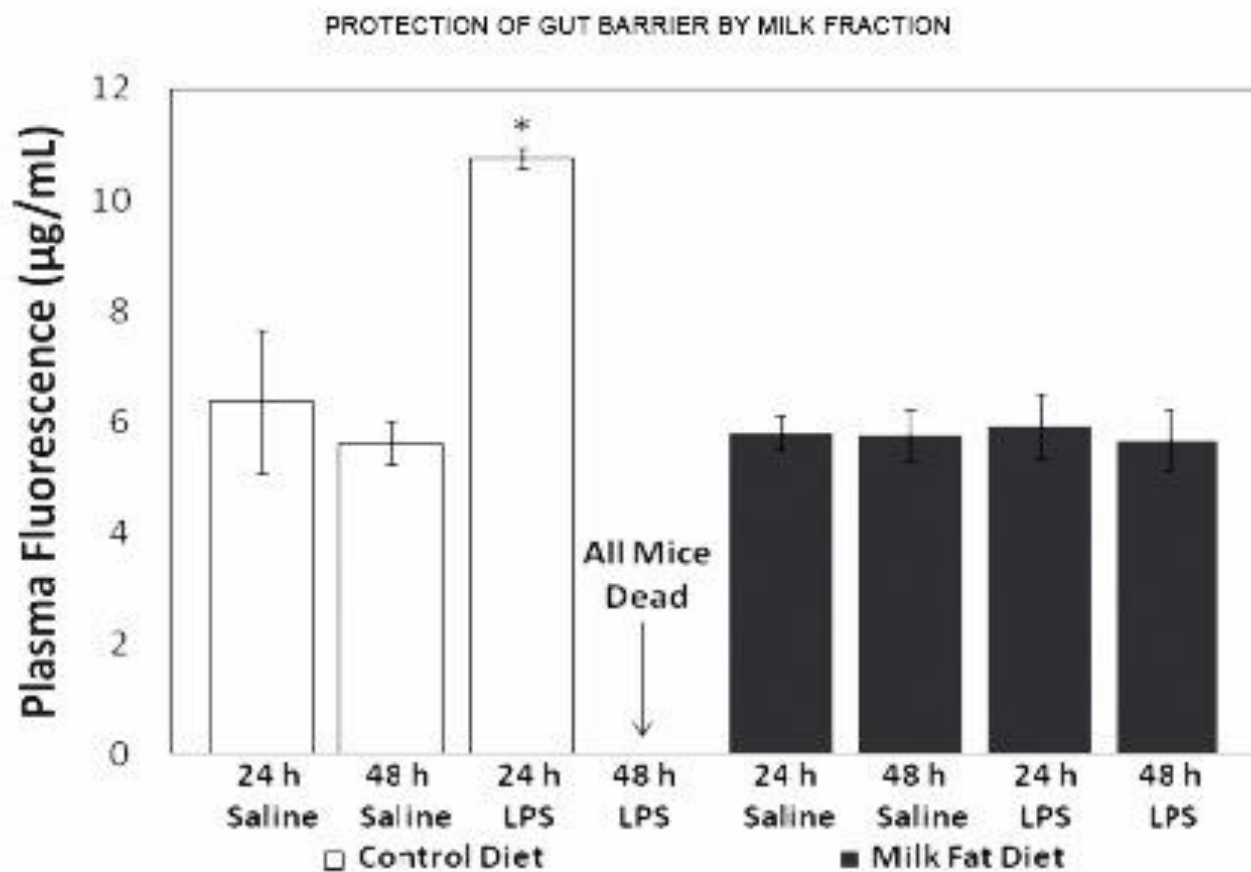
Membrane-rich milk fat diet provides protection against gastrointestinal leakiness in mice treated with lipopolysaccharide

D. R. Snow,* R. E. Ward,*¹ A. Olsen,† R. Jimenez-Flores,‡ and K. J. Hintze*^{1,2}

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Thank you.
Questions?



RJF group January 2014