Markers of proliferation and differentiation in human keratinocyte models

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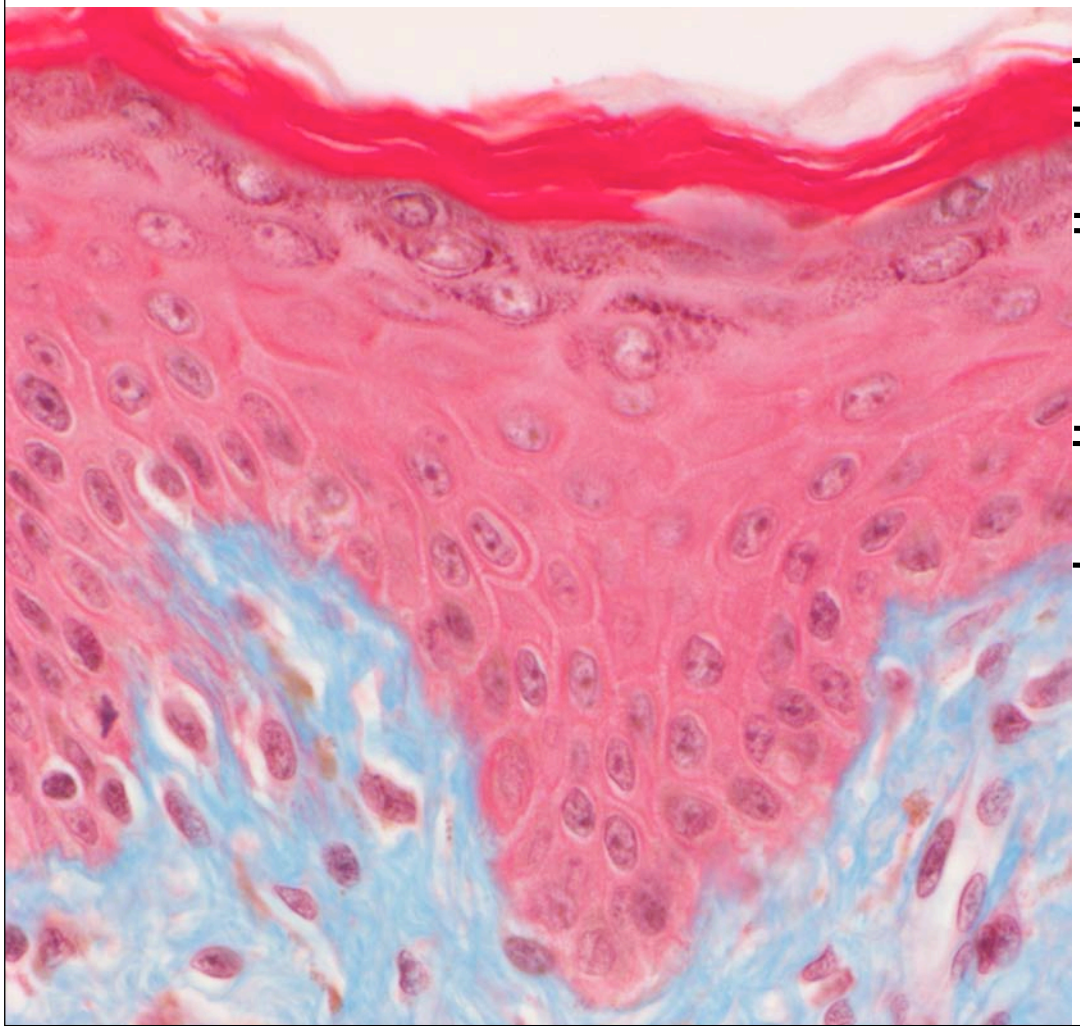
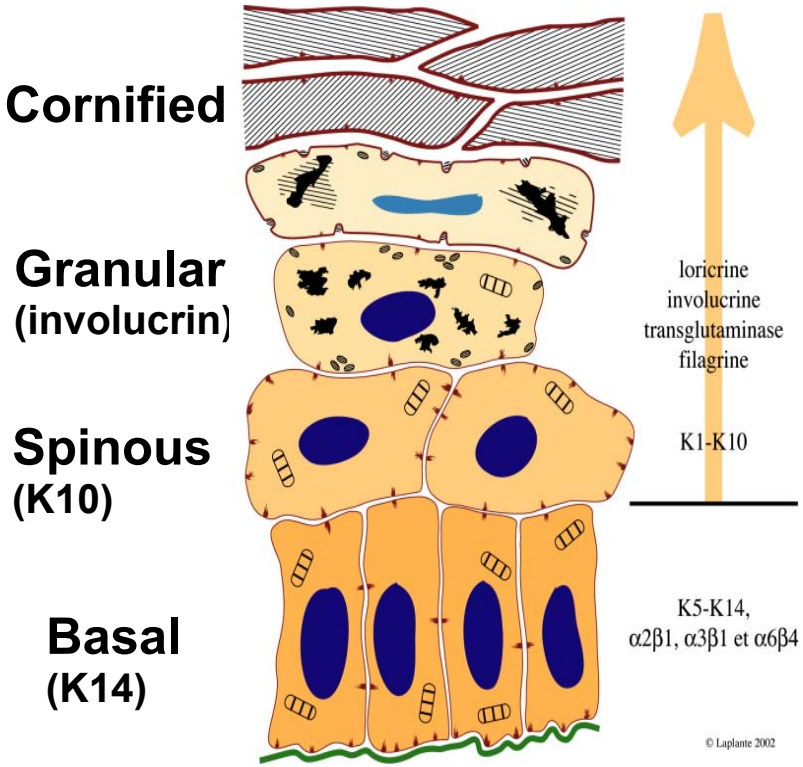
Namur, Belgium

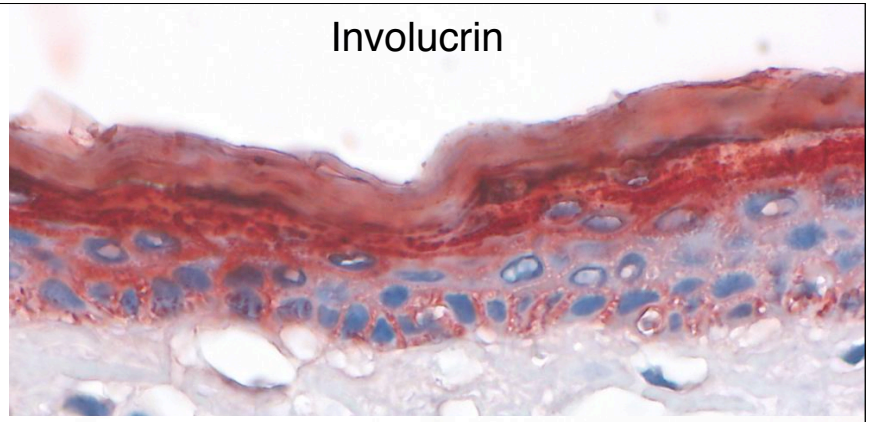
Alicante, October 17, 2008

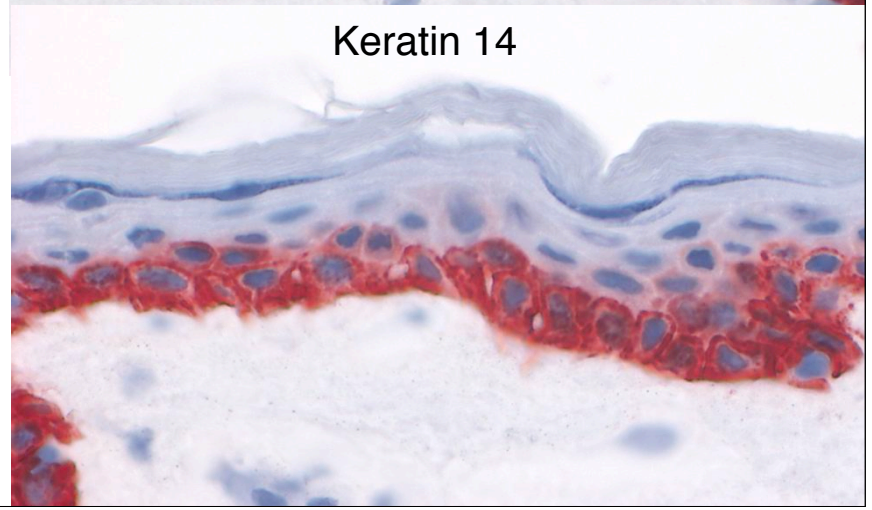
Homeostasis of the epidermis



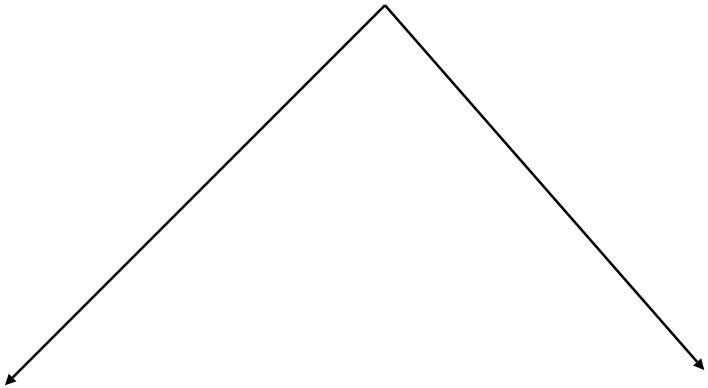
Epidermal proliferation and differentiation markers







Epidermal cell cultures



- 1/ Cultures of keratinocytes immersed in the growth liquid medium
- 2/ Cultures of keratinocytes grown on filter at the Air-Liquid Interface

Content

- Human epidermal tissue and differentiation

Part 1 :

- Monolayer cultures of human keratinocytes
- Epidermal differentiation in keratinocyte monolayers

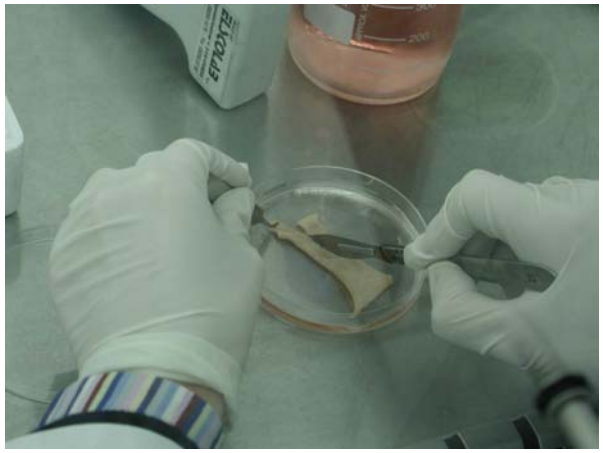
Part 2 :

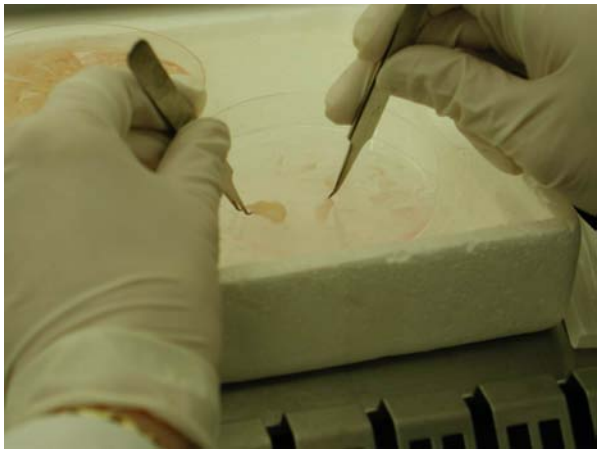
- The Reconstructed Human Epidermis (RHE) and its differentiation
- Tissue response within the RHE

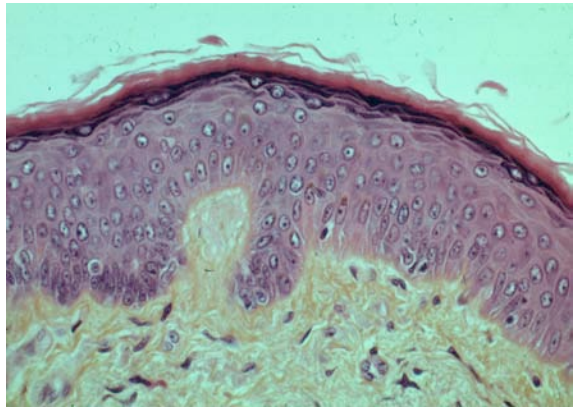
Conclusions

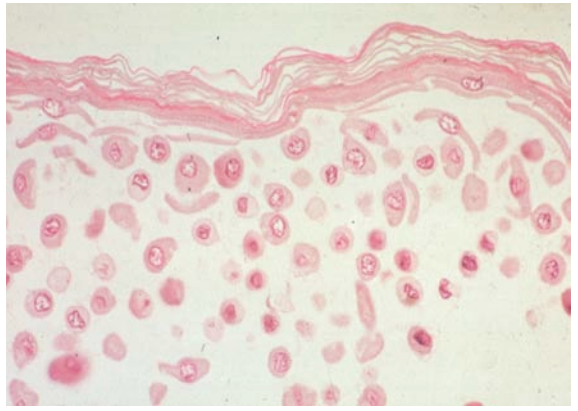
Part 1 :
Cultures of human keratinocytes as

Human epidermal keratinocytes in cell culture



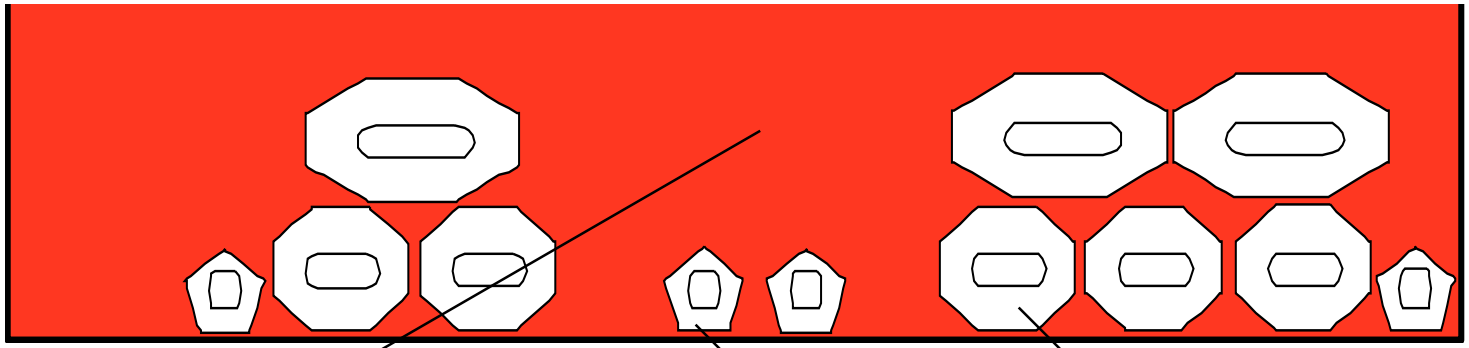






Culture of epidermal keratinocytes

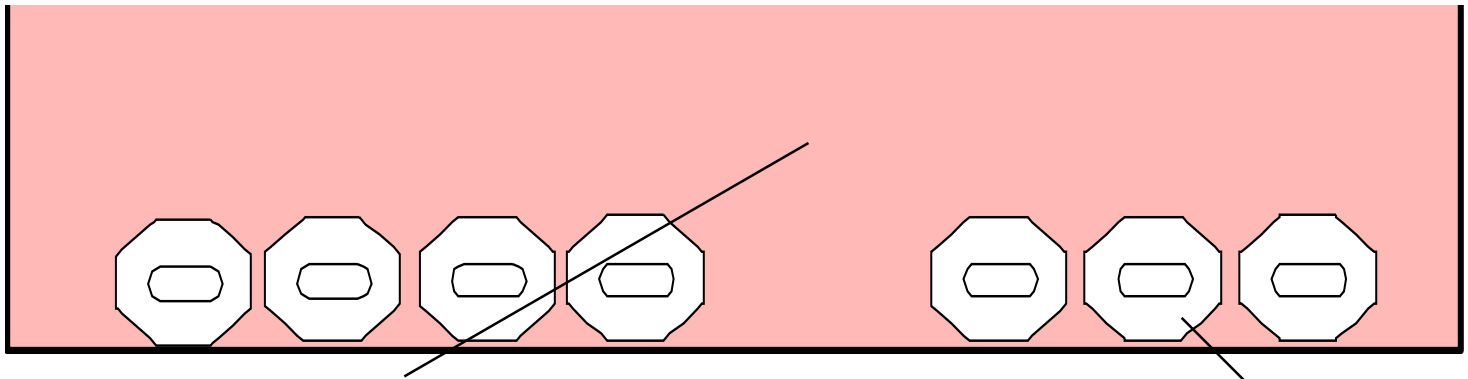
- Rheinwald and Green, 1975



Culture medium:
DMEM + Ham-F12
EGF
Insulin
Fetal calf serum
High Ca^{2+}

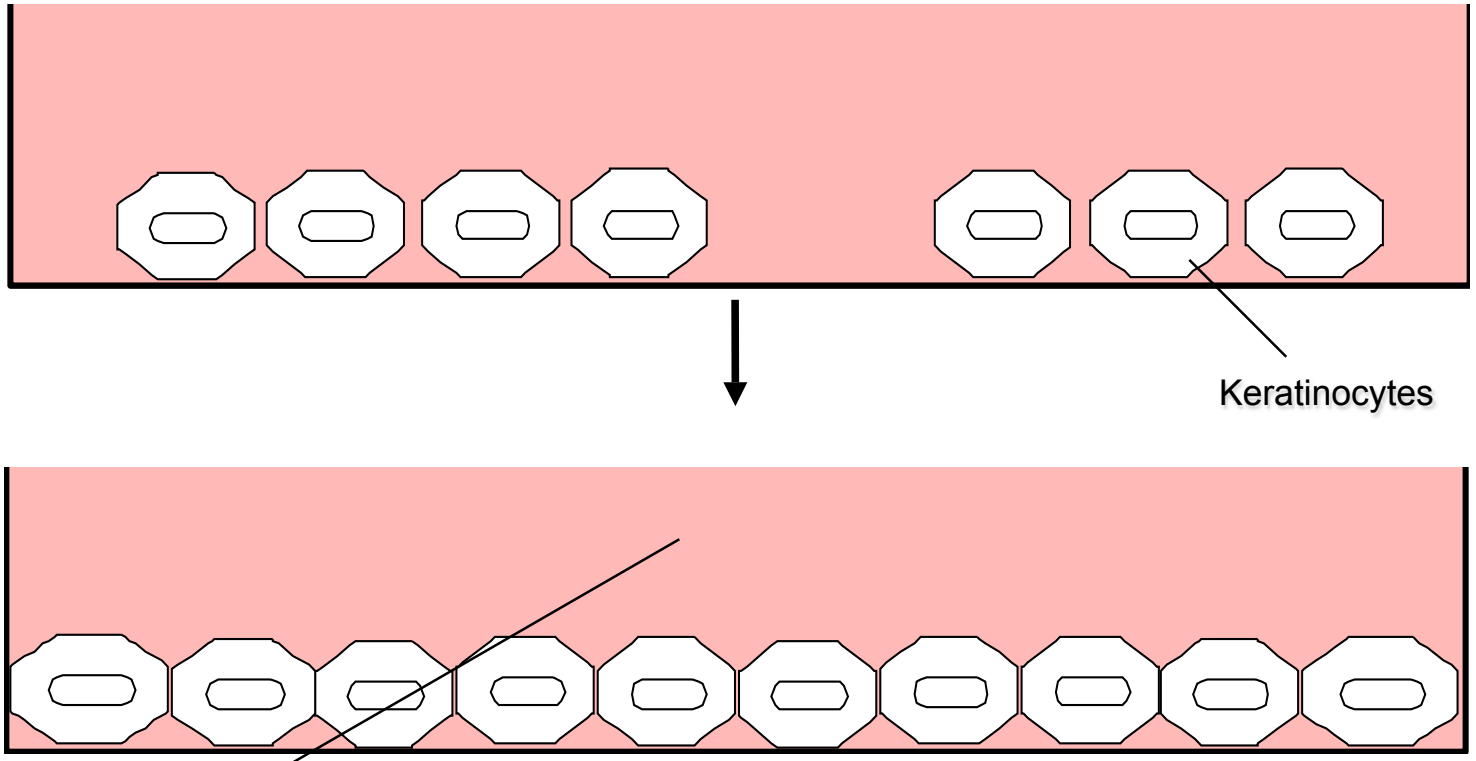
Serum-free culture of keratinocytes

- Boyce & Ham, 1983
- Wille, Pittelkow, Shipley & Scott, 1984 at the Mayo Clinic, Rochester MN



Culture in autocrine conditions

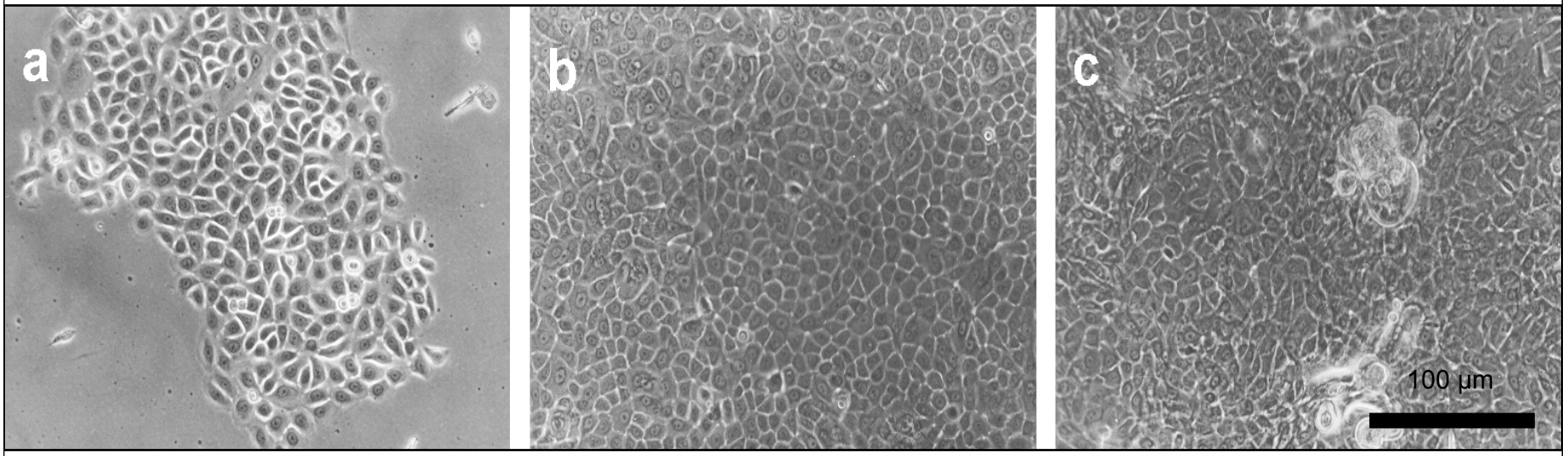
- Cook, Pittelkow & Shipley, 1991



Epidermal differentiation in keratinocyte monolayers

Do serum-free cultures of keratinocytes demonstrate the ability of this cell type to differentiate in vitro ?

Epidermal differentiation in keratinocyte monolayers



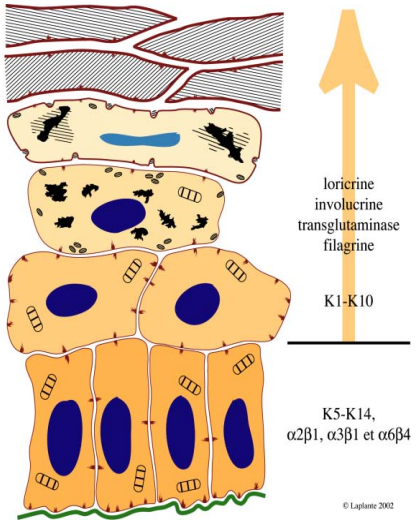
subconfluence → confluence → postconfluence

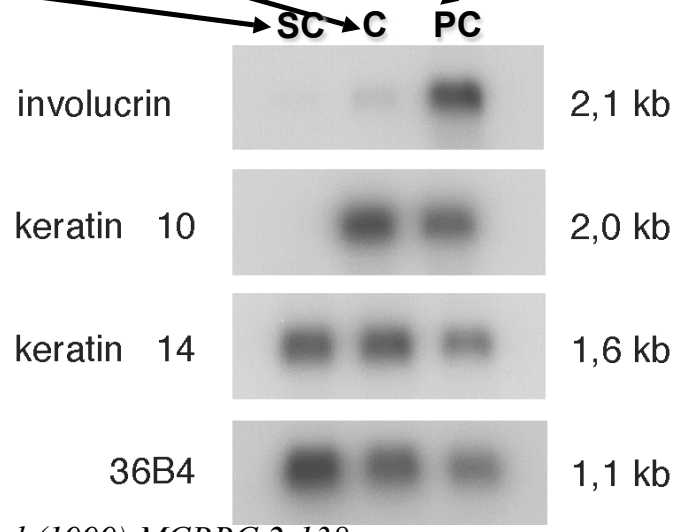
Cornified

Granular
(involucrin)

Spinous
(K10)

Basal
(K14)





Poumay & Pittelkow (1995) JID 104:271

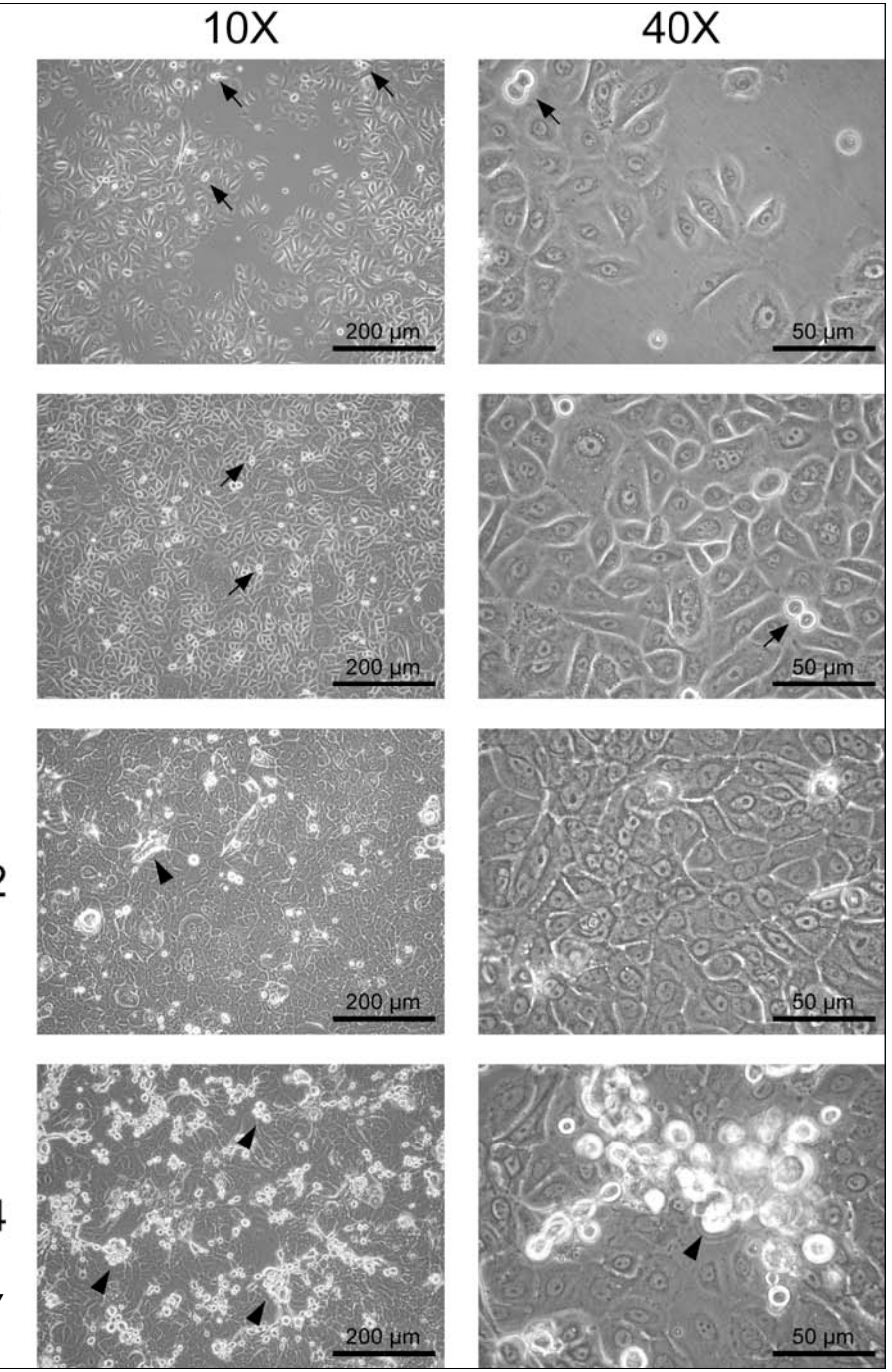
Poumay et al (1999) MCBRC 2:138

Epidermal differentiation in keratinocyte monolayers

- **Choice of house-keeping gene(s)**
- Effect of cell density
- Effect of cholesterol depletion
- Analysis of retinoids

Epidermal differentiation in keratinocyte

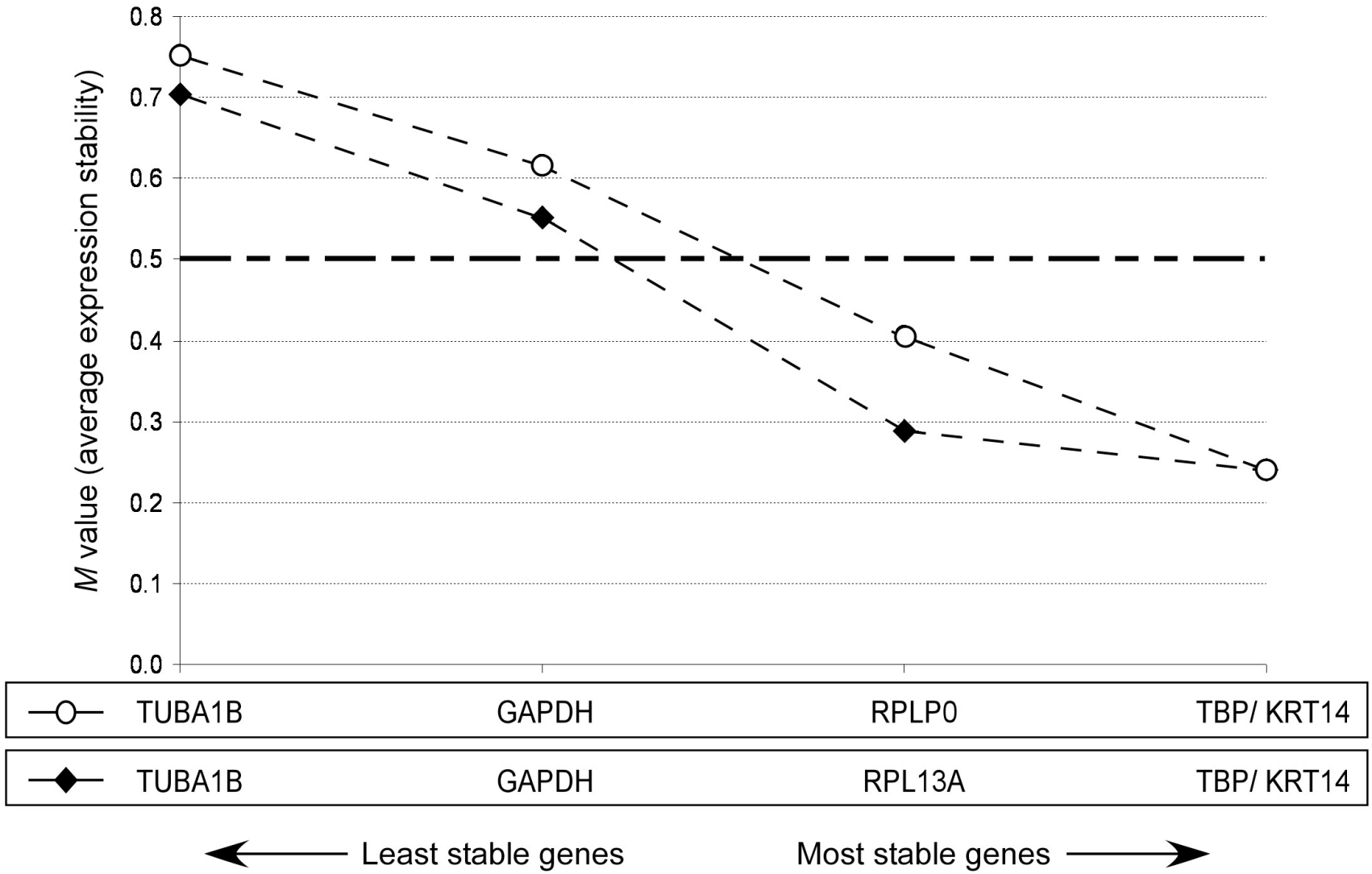
Average expression stability in 4 strains of human epidermal keratinocytes at 4 different cell densities



Minner & Poumay (2008) JID 128: doi:10.1038/jid.2008.247

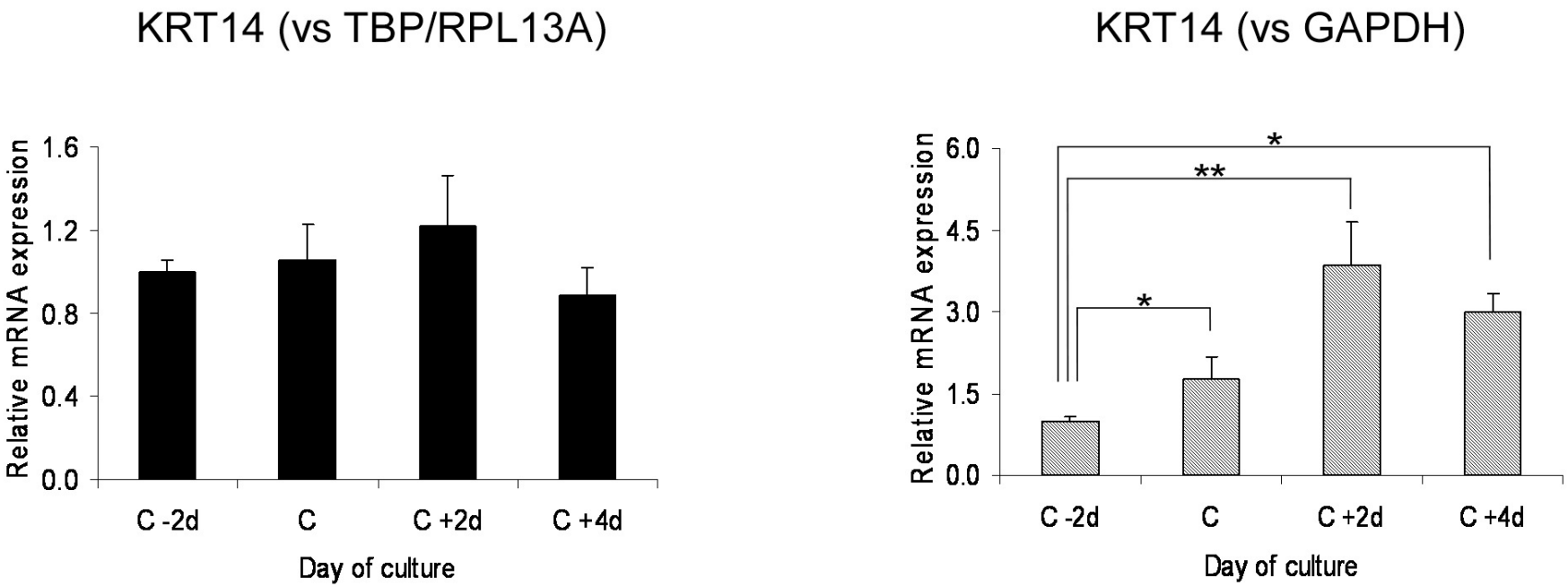
Average expression stability (GeNorm Software)

(Vandesompele *et al.*, 2002)



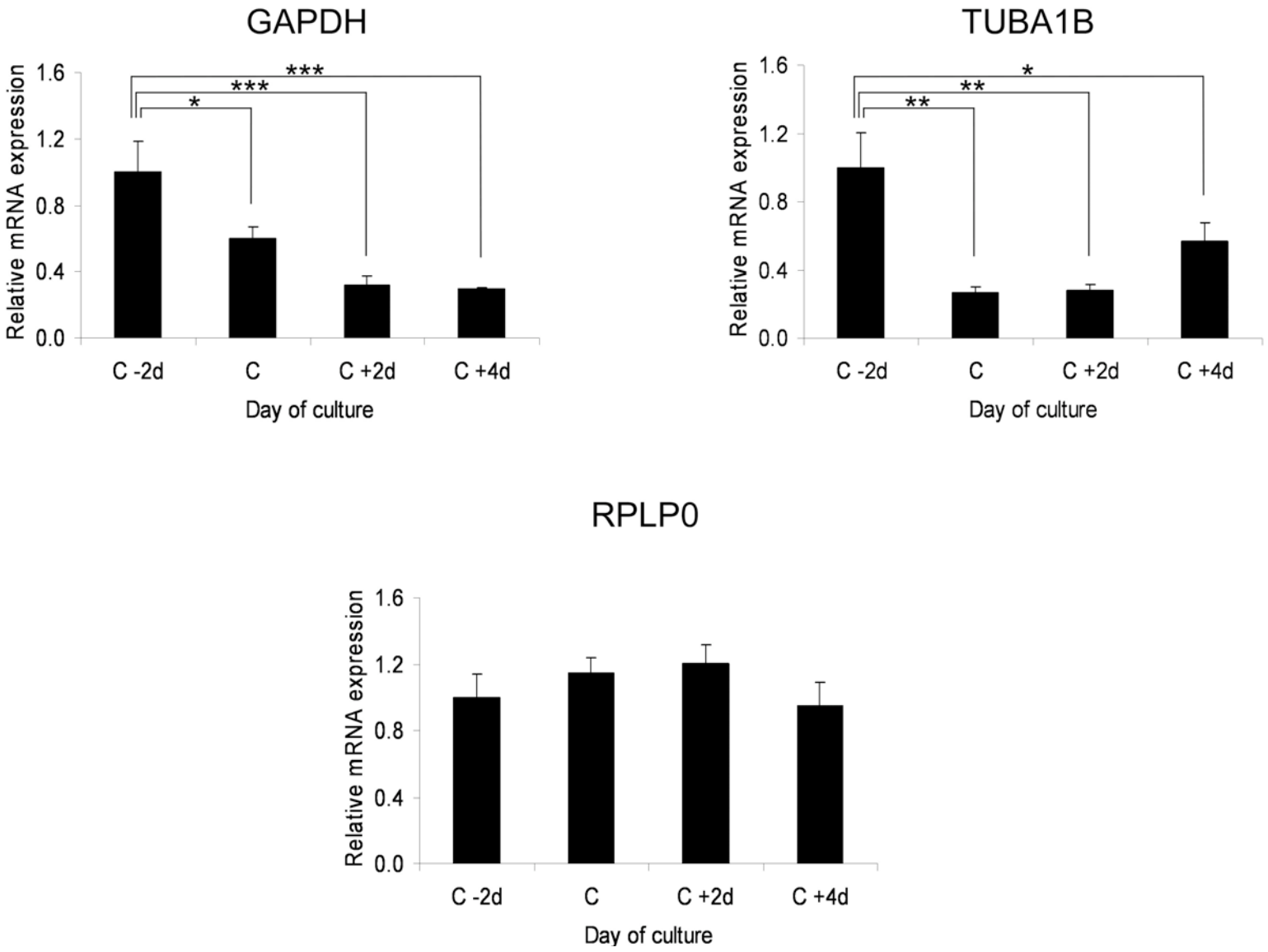
Minner & Poumay (2008) JID 128: doi:10.1038/jid.2008.247

Average expression of keratin 14



Minner & Poumay (2008) JID 128: doi:10.1038/jid.2008.247

Average expression of markers

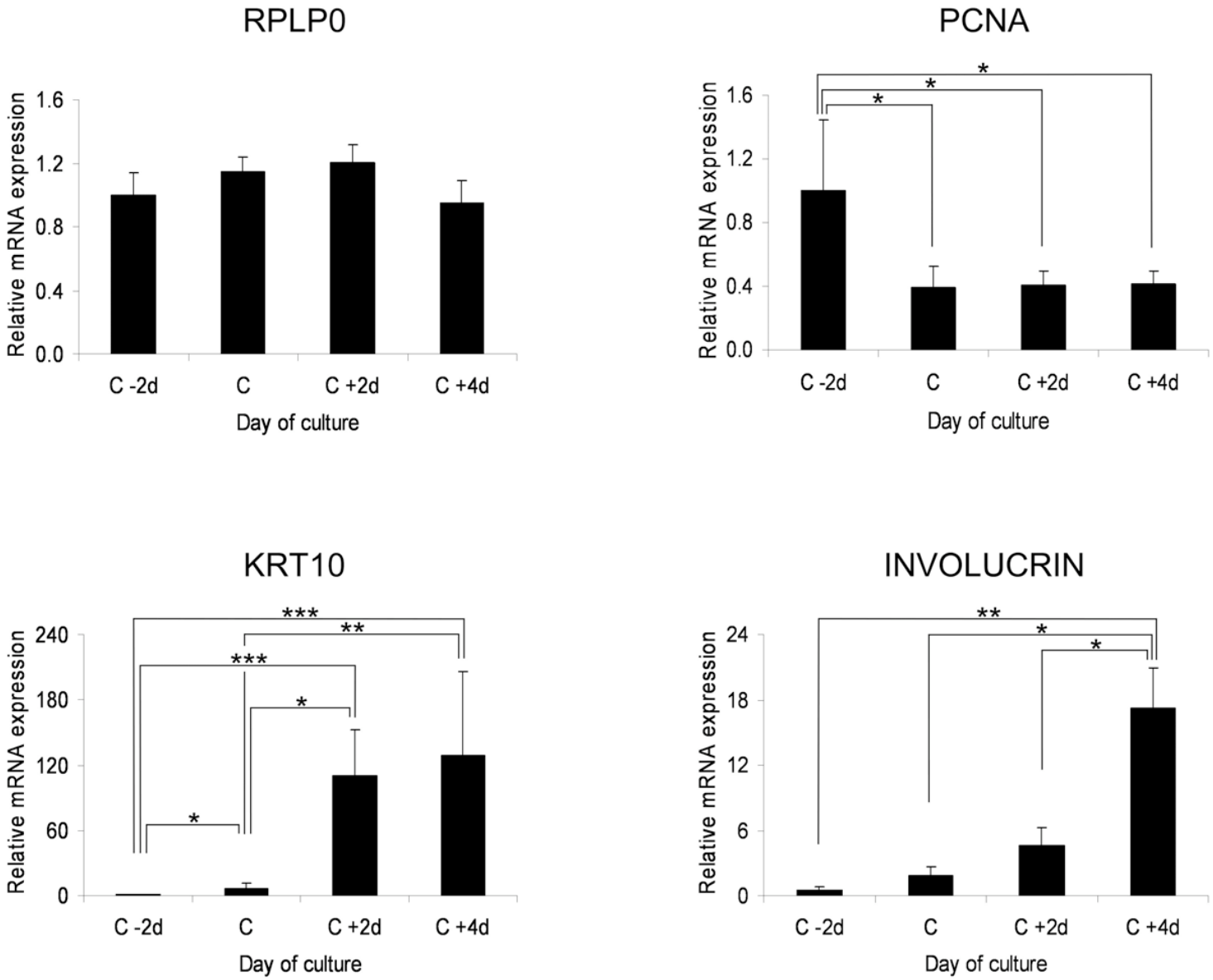


Minner & Poumay (2008) JID 128: doi:10.1038/jid.2008.247

Epidermal differentiation in keratinocyte monolayers

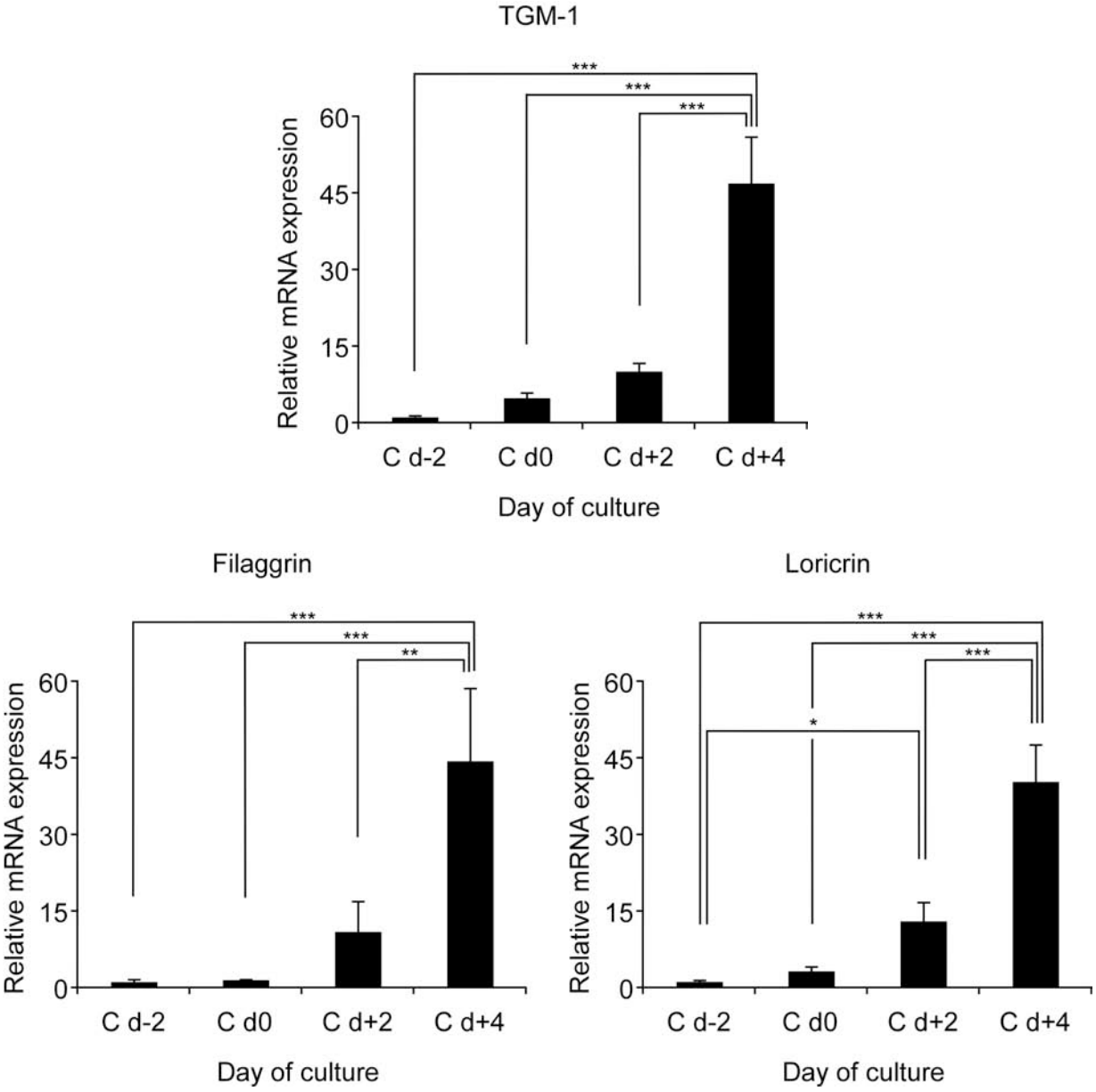
- Choice of house-keeping gene(s)
- **Effect of cell density**
- Effect of cholesterol depletion
- Analysis of retinoids

Average expression of markers



Minner & Poumay (2008) JID 128: doi:10.1038/jid.2008.247

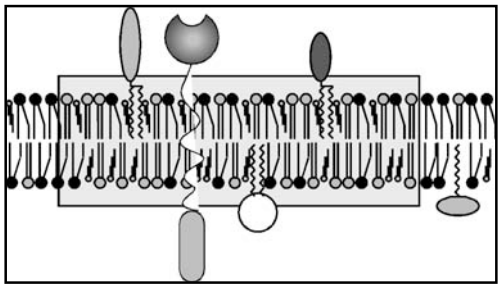
Average expression of markers



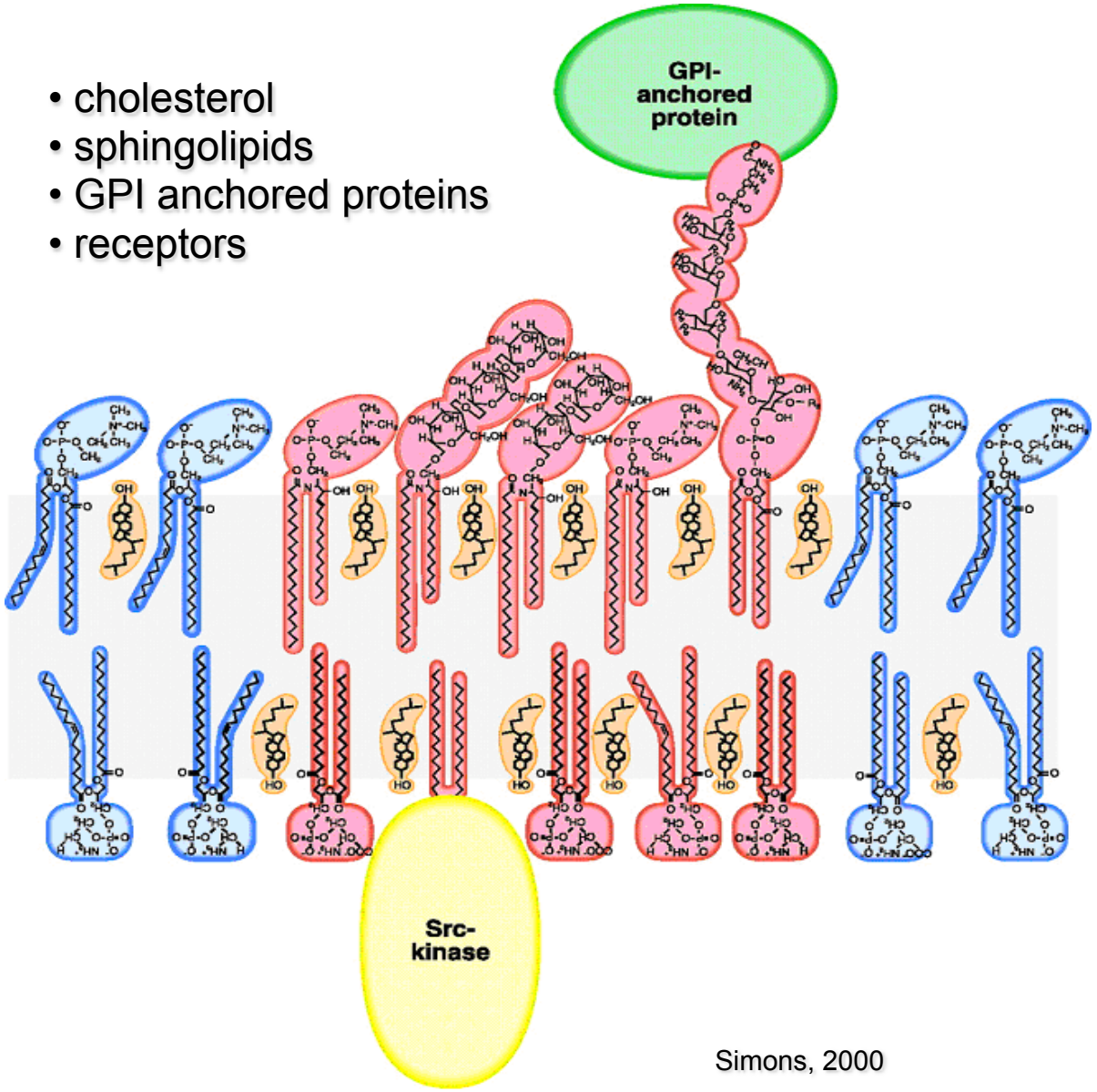
Epidermal differentiation in keratinocyte monolayers

- Choice of house-keeping gene(s)
- Effect of cell density
- **Effect of cholesterol depletion**
- Analysis of retinoids

Lipid rafts and cholesterol



- cholesterol
- sphingolipids
- GPI anchored proteins
- receptors



Simons, 2000

Methyl-β-cyclodextrin (MβCD)

Keratinocytes in culture

↓

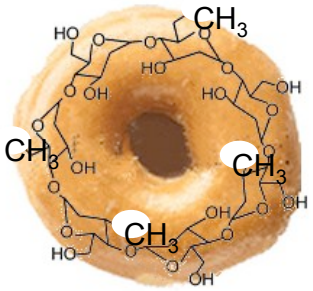
Incubation with methyl-β-cyclodextrin

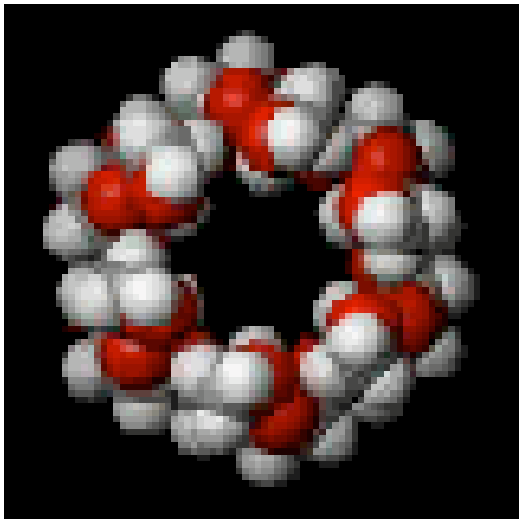
↓

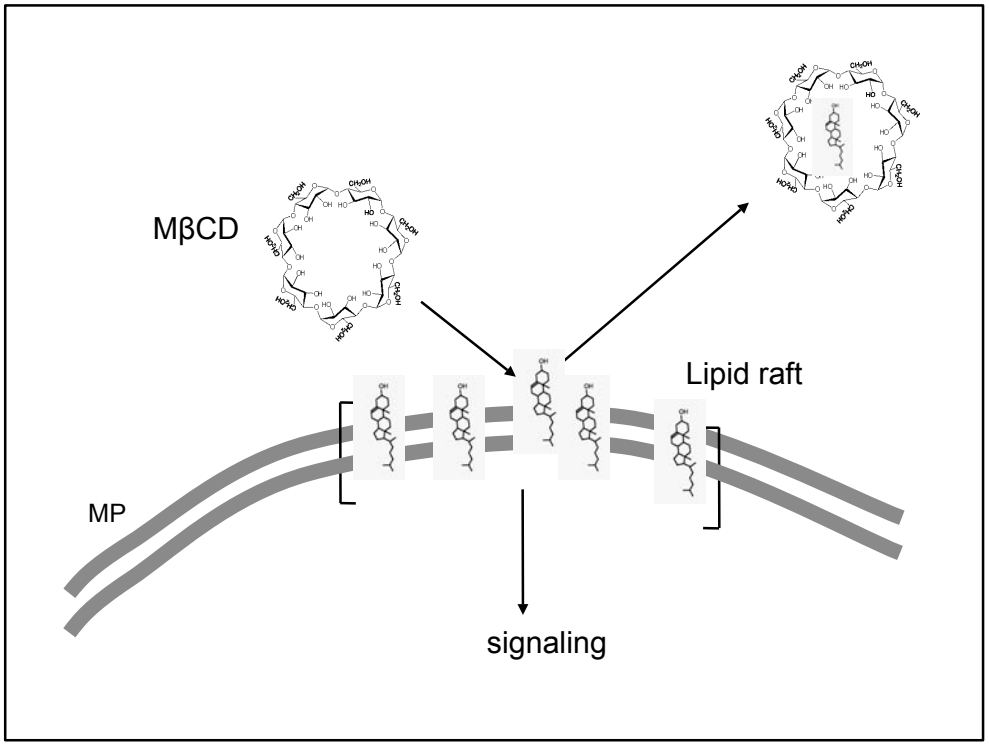
Depletion of cholesterol

↓

Disruption of lipid rafts in keratinocytes as a model of cell stress

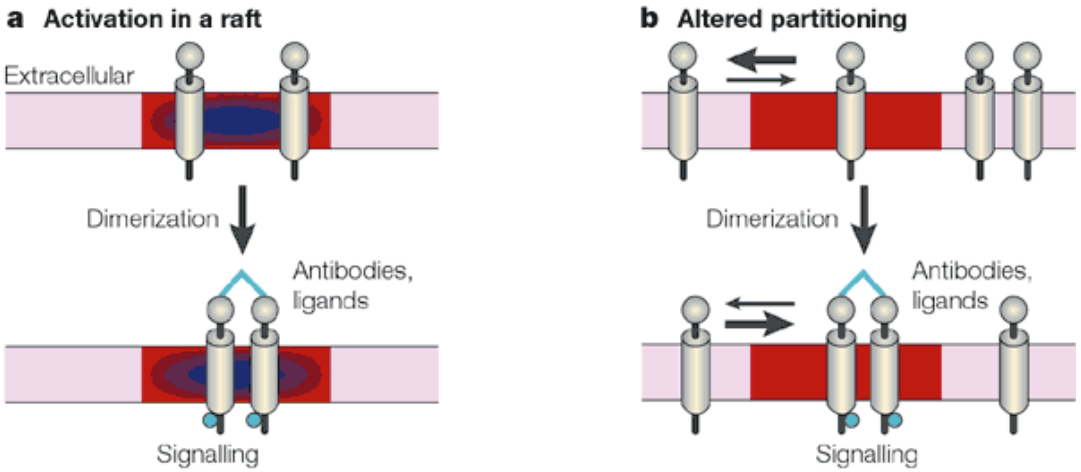






Cell signaling and Lipid rafts

Signaling platform
(reviewed by Simons & Toomre, 2000)



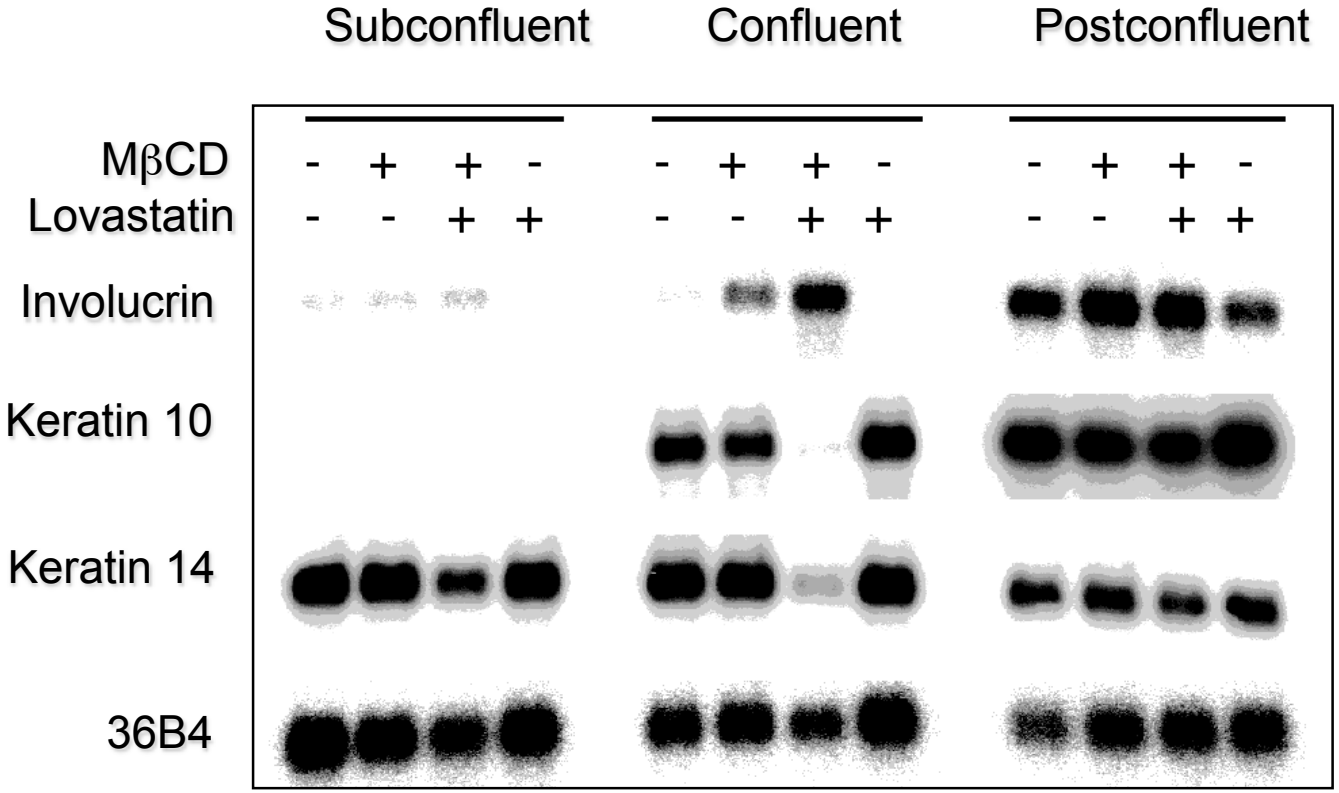
EGF receptor

(Chen & Resh, 2001, 2002; Pike & Casey, 2002; Roepstorff et al., 2002; Ringerike et al., 2002; Westover et al., 2003)

MAPK p38

(Hossain et al., 2002; Iwabuchi & Nagaoka, 2002; Tuluc et al., 2003)

Effect of a depletion of cholesterol on the expression of differentiation markers by keratinocytes cultured at different cell densities

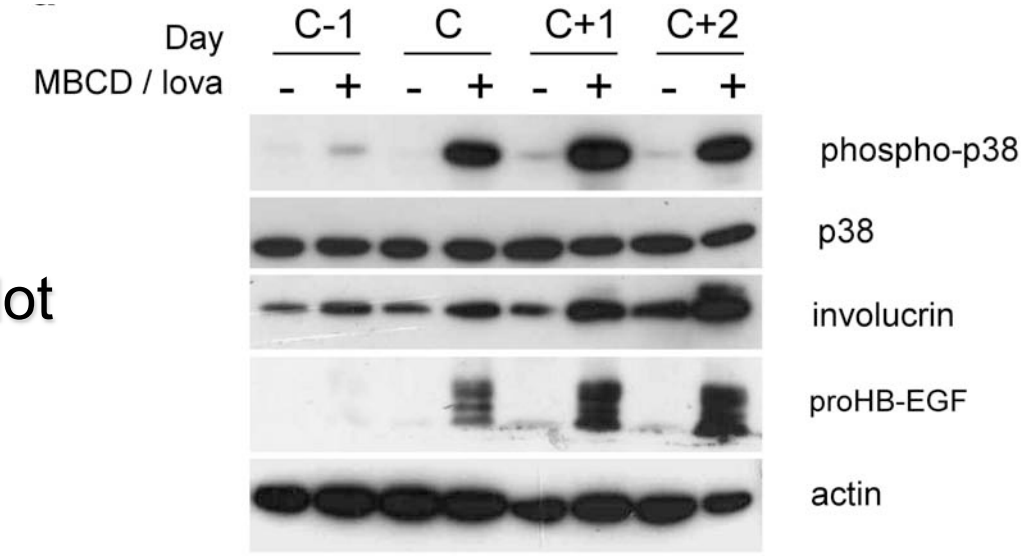


Accelerated Expression of Involucrin
Delayed Expression of Keratin 10

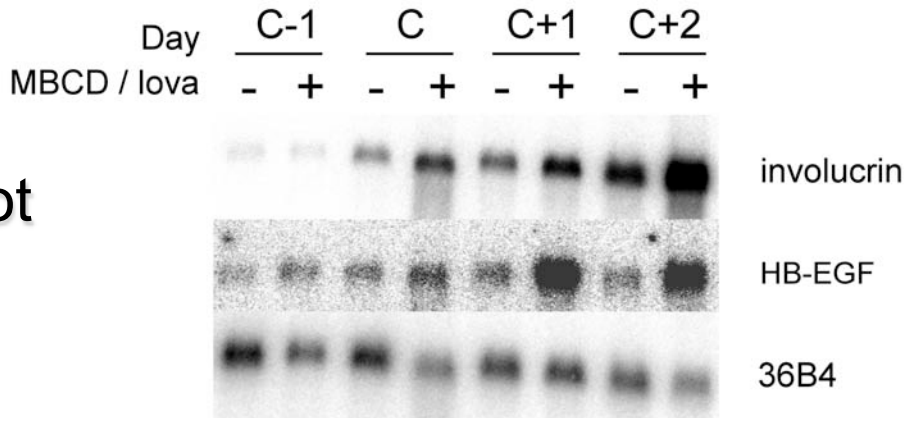
Jans et al (2004) JID 123:564

p38 activation by lipid raft disruption induces HB-EGF and involucrin expression

Western blot

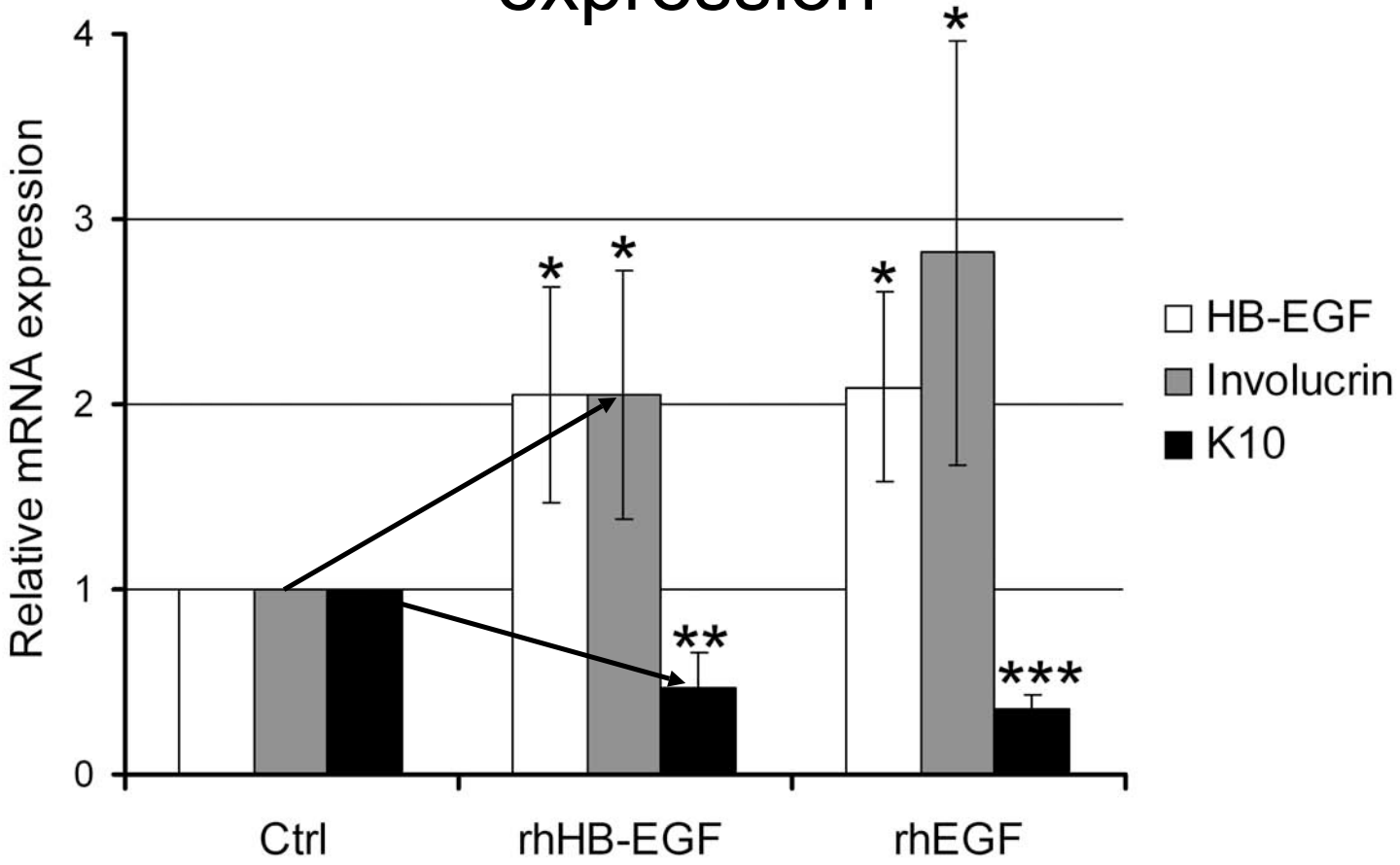


Northern blot



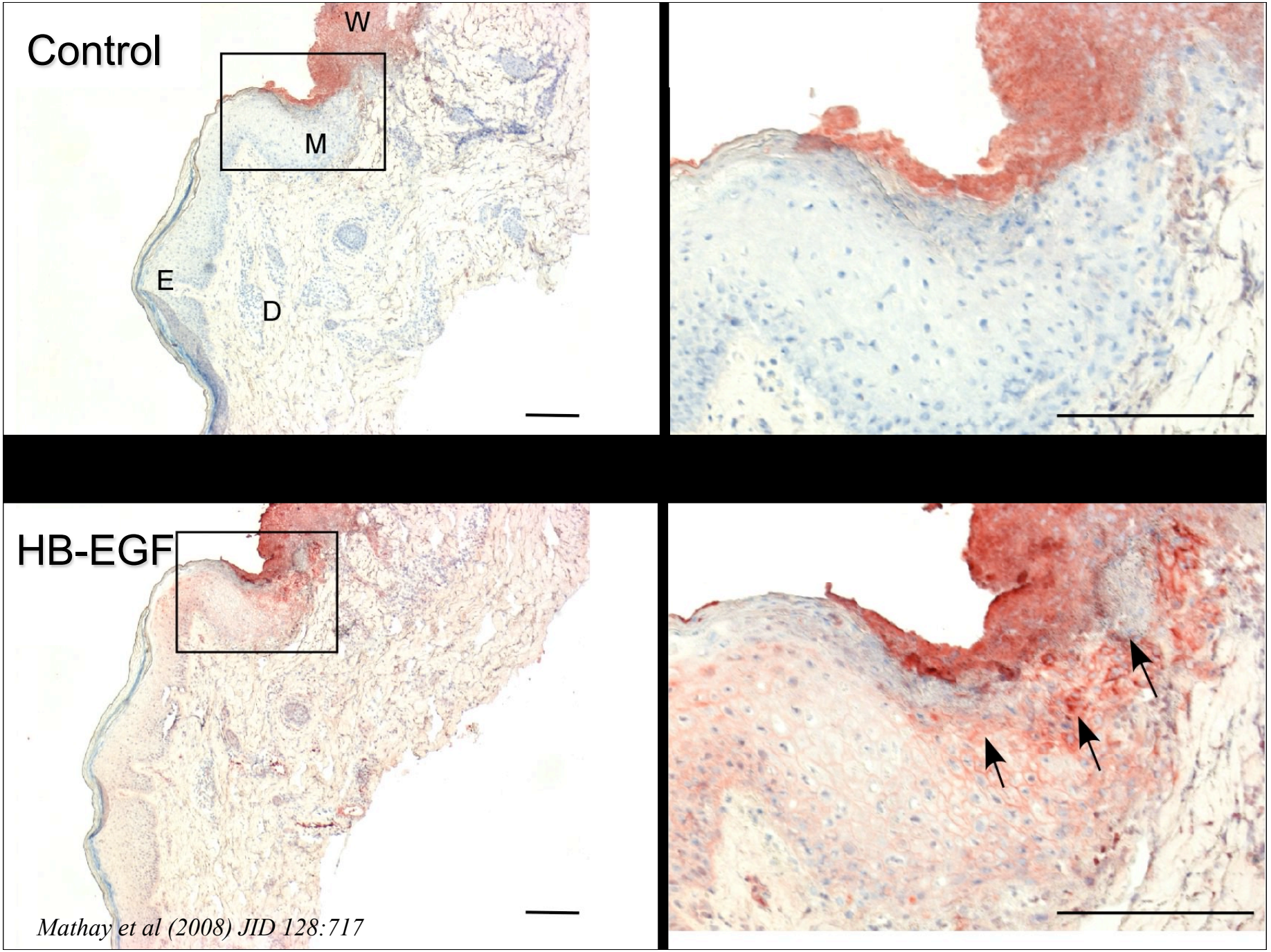
Mathay et al (2008) JID 128:717

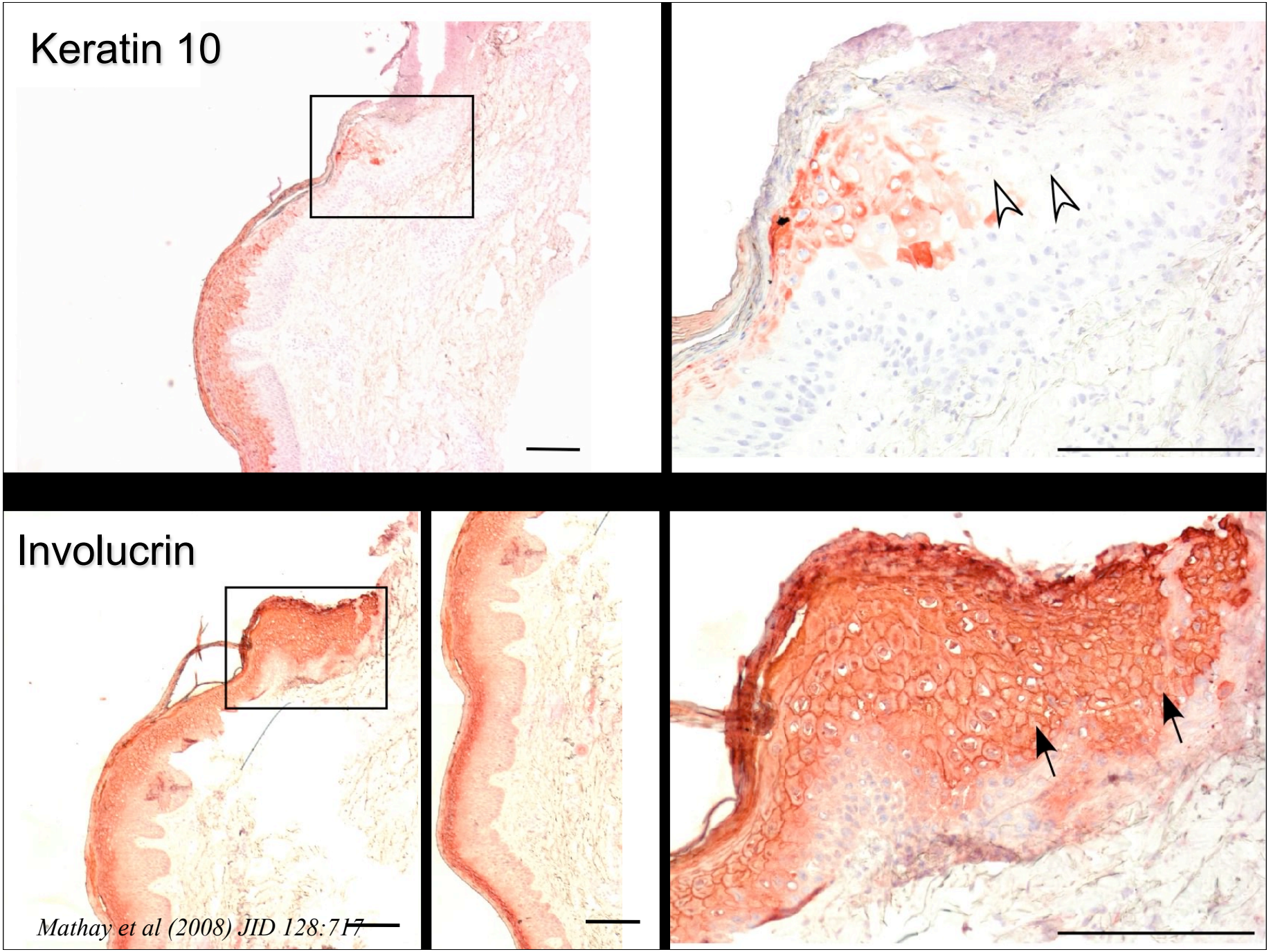
HB-EGF alters Involucrin and Keratin 10 expression



Accelerated Expression of Involucrin
Delayed Expression of Keratin 10

Mathay et al (2008) JID 128:717

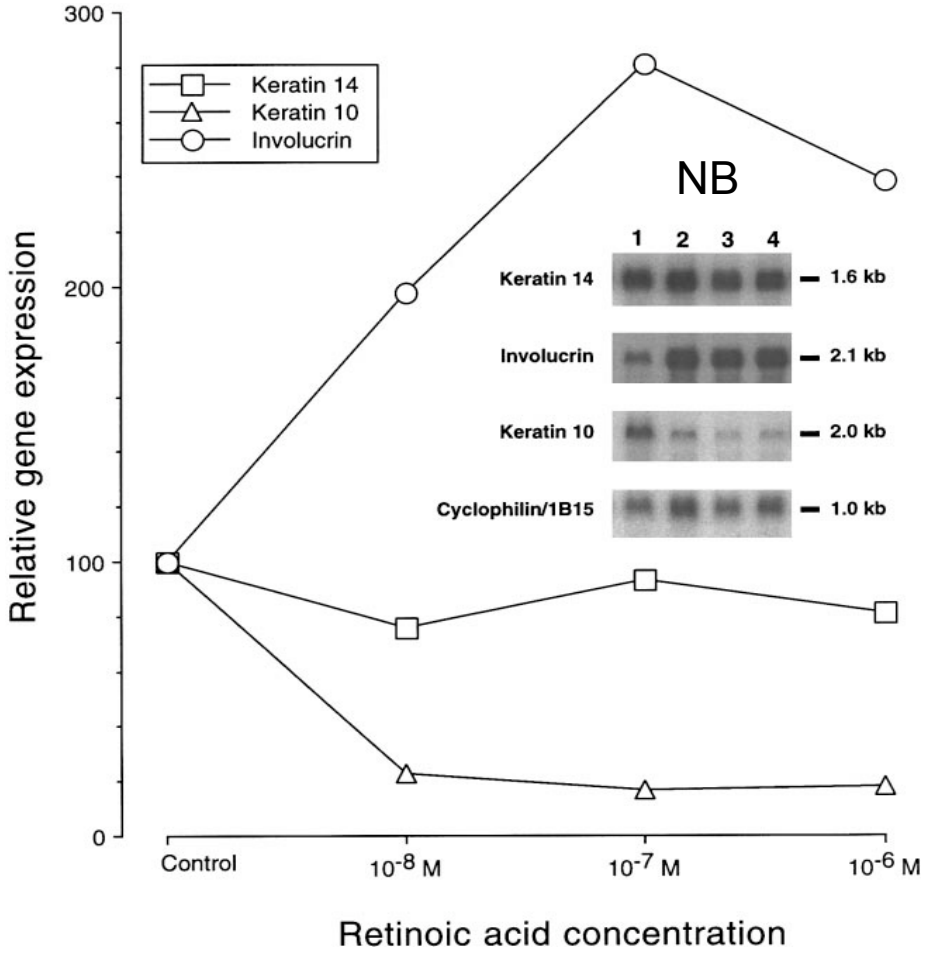

Control
W
M
E
D
HB-EGF
Mathay et al (2008) JID 128:717


Keratin 10
Involucrin
Mathay et al (2008) JID 128:717

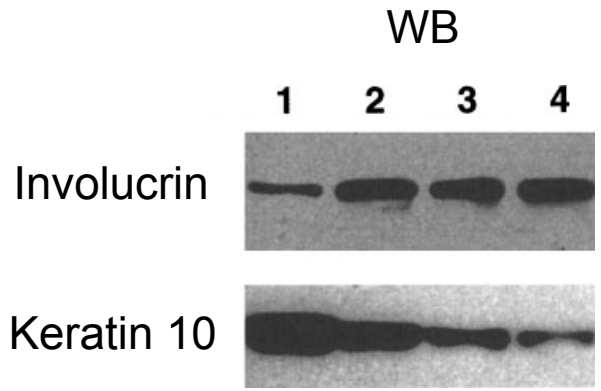
Epidermal differentiation in keratinocyte monolayers

- Choice of house-keeping gene(s)
- Effect of cell density
- Effect of cholesterol depletion
- **Analysis of retinoids**

Retinoic acid and R115866



Poumay et al (1999) MCBRC 2:138



<u>Degradation of retinoic acid</u>

Retinoic Acid (RA)

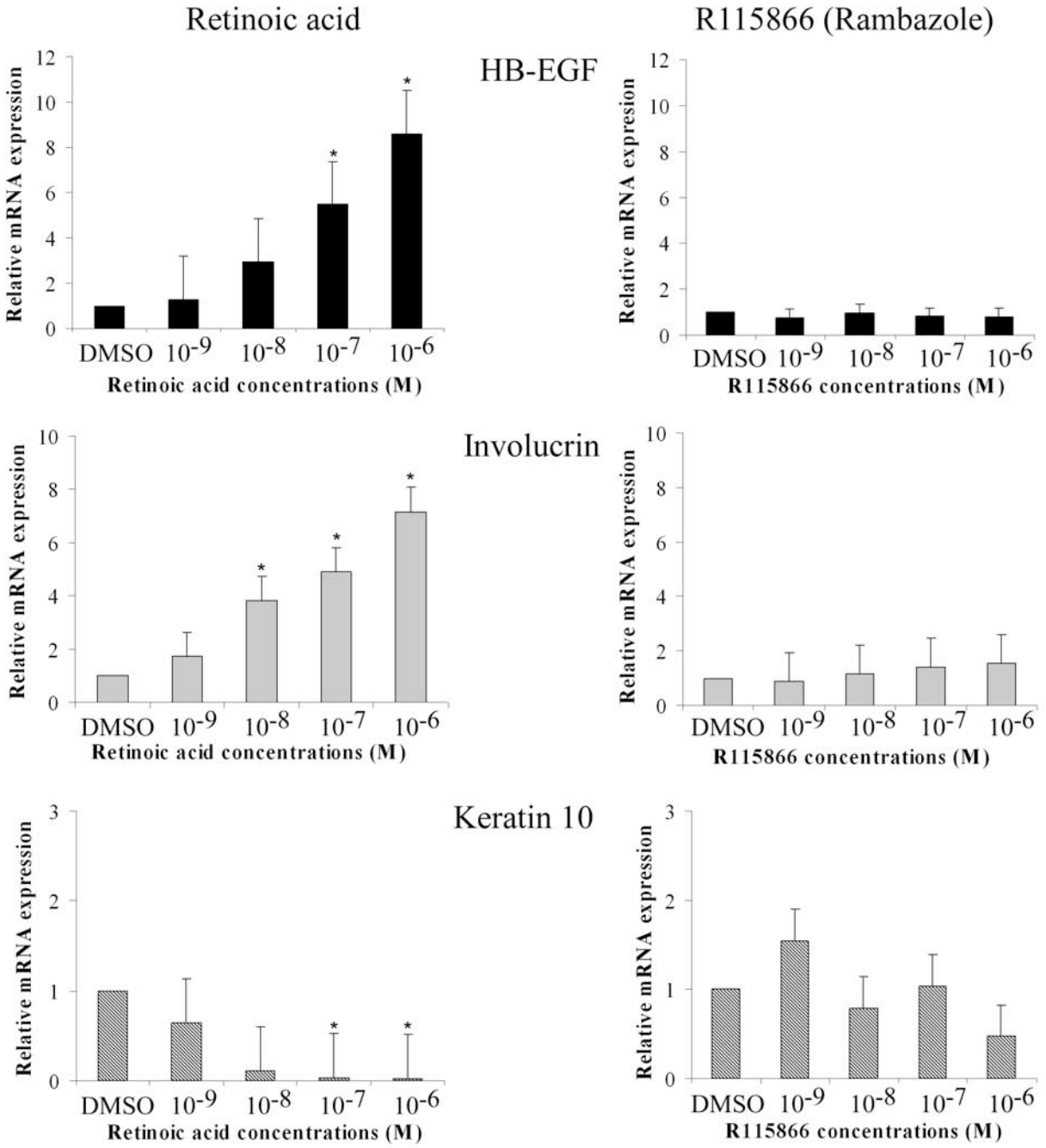
4-hydroxy-RA

4-keto-RA

CYP26

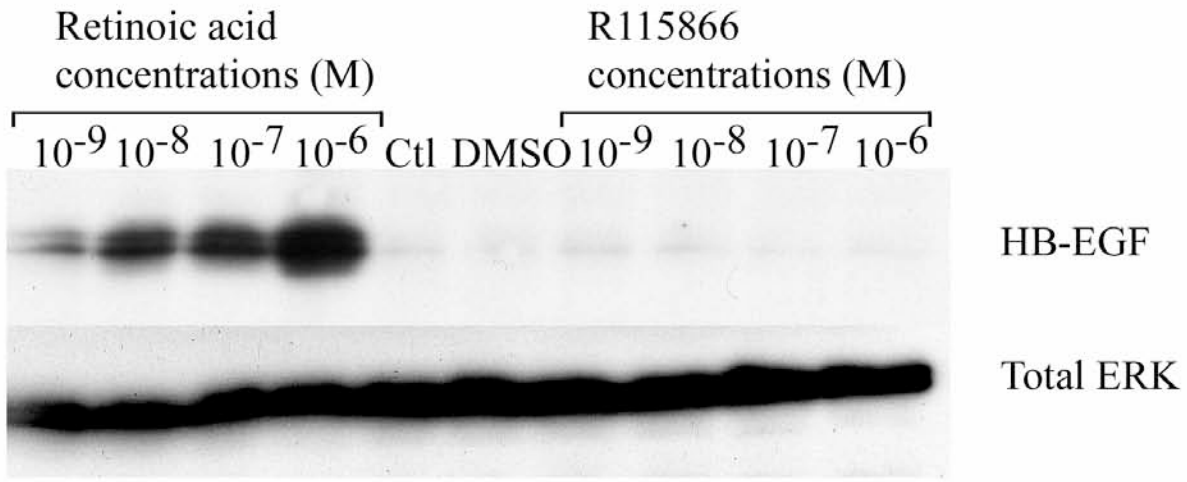
R115866

R115866 alone does not alter keratinocyte differentiation

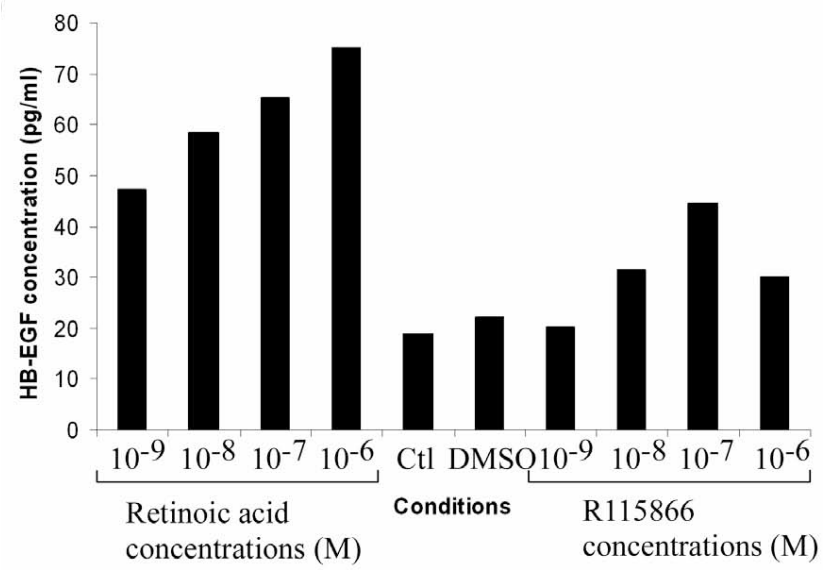


Giltaire et al (2008) BJD in press

Western blot

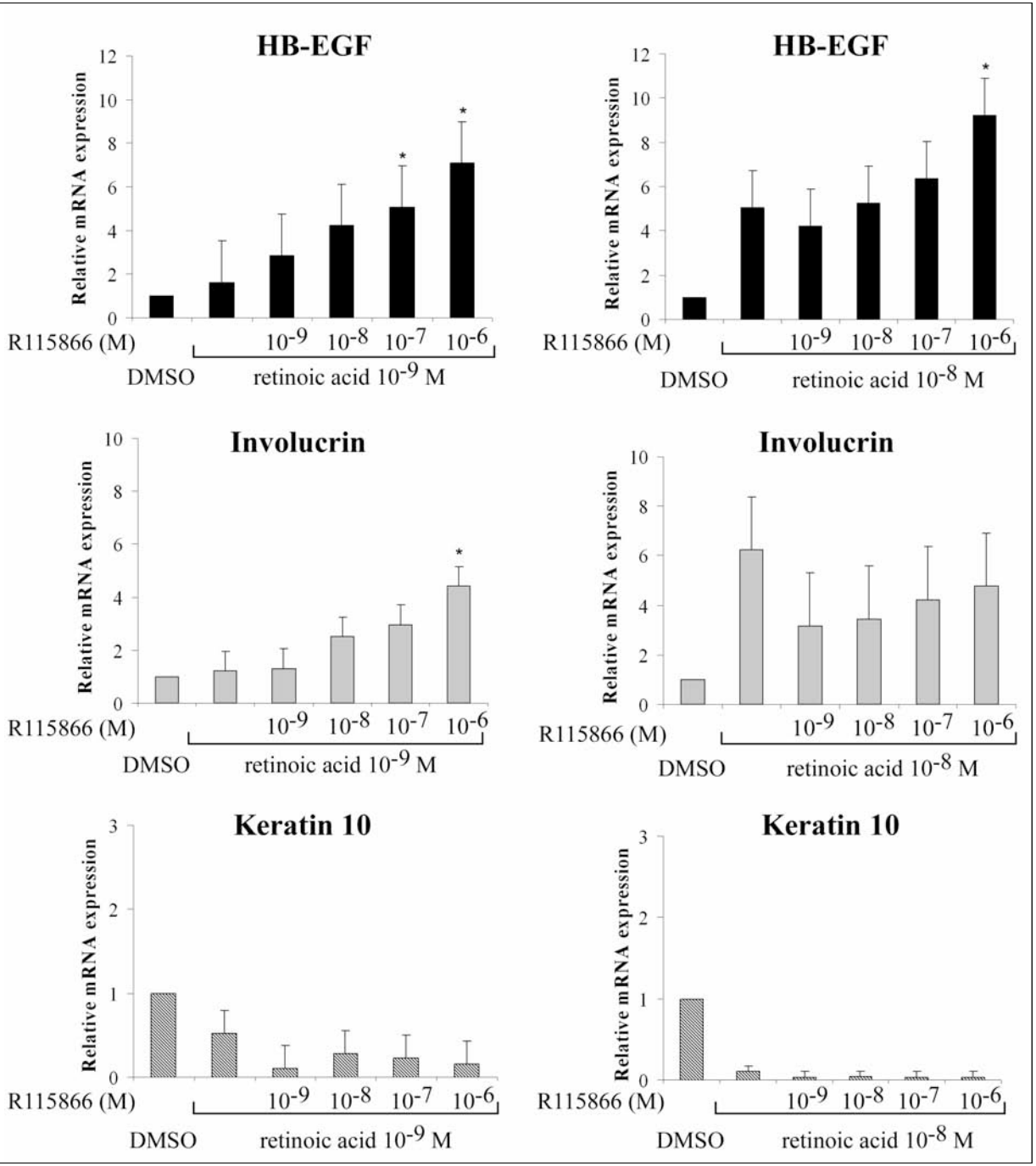


ELISA



Giltaire et al (2008) BJD in press

R115866 potentiates Retinoic Acid to alter keratinocyte differentiation at very low concentrations



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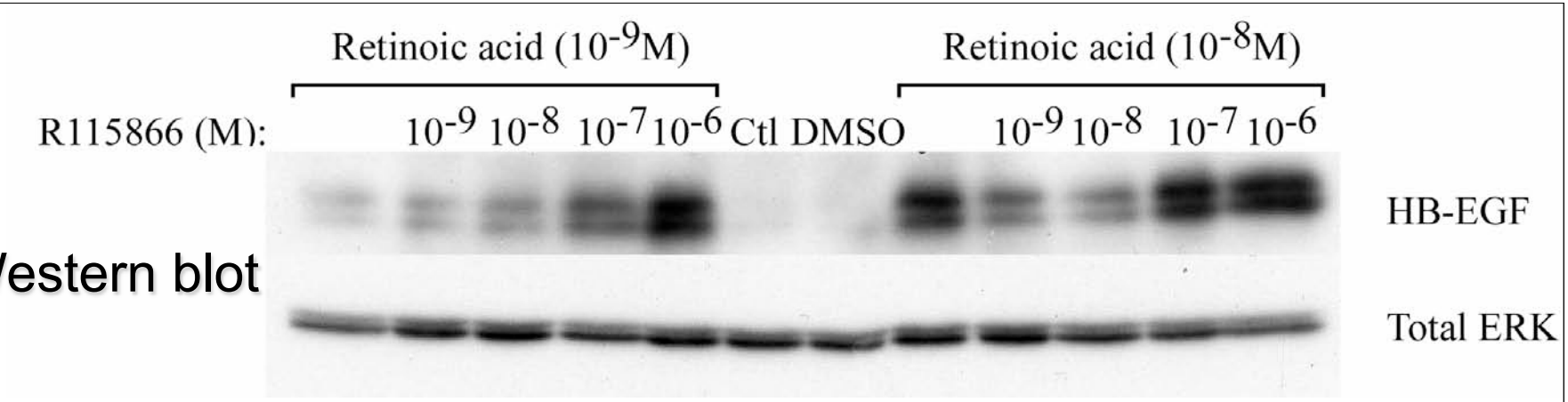
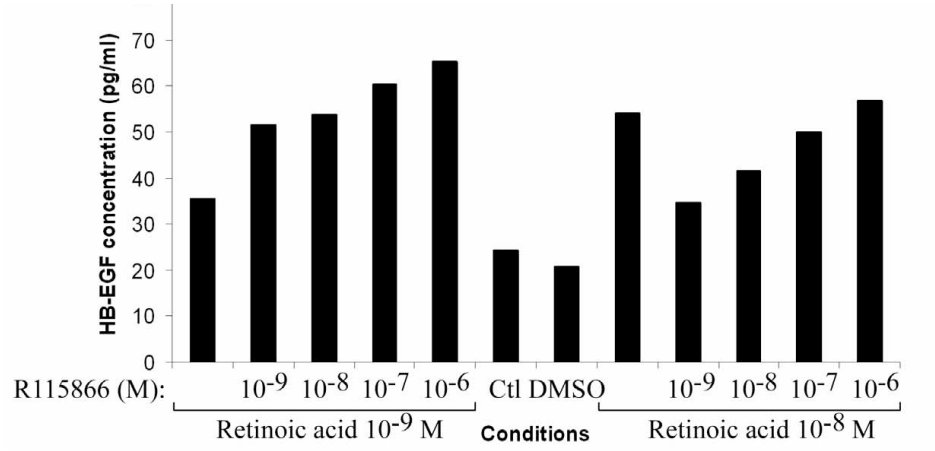


ELISA



Giltaire et al (2008) BJD in press

Summary on monolayers

- Easy to produce
- Can proliferate up to confluence without addition of growth factors
- House-keeping genes must be tested in experimental conditions
- Detection of markers in relevant conditions
- Adequate for the study of alterations (stress, pharmacology) of epidermal differentiation

Part 2 :
The Reconstructed Human

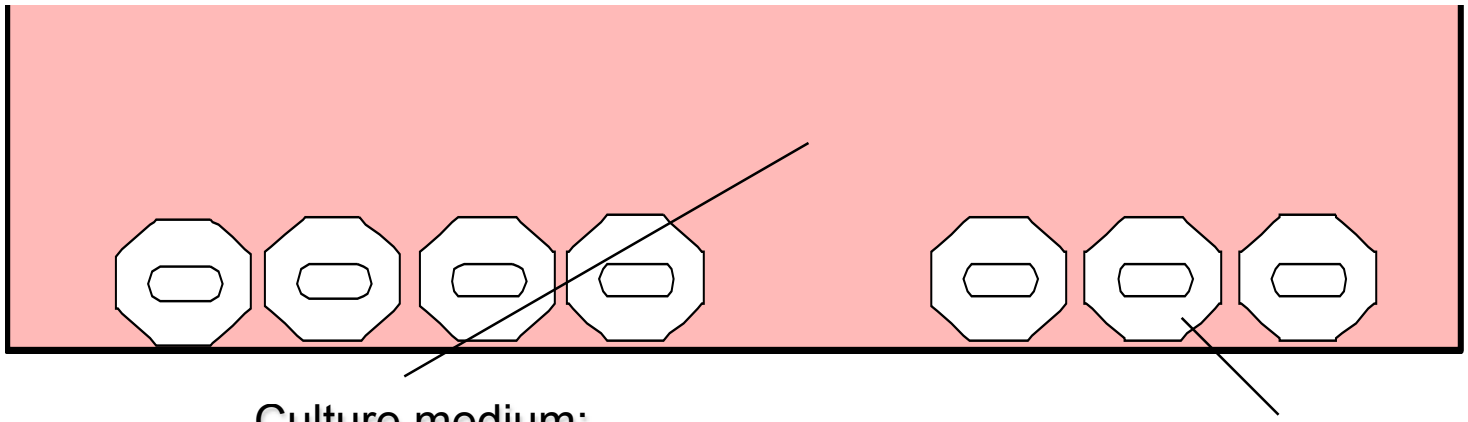
- Most published models with serum and collagen : inadequate for the interpretation of data regarding cell release

><

- Serum-free, collagen-free conditions : a simple model, adequate for easier interpretation of data regarding cell release

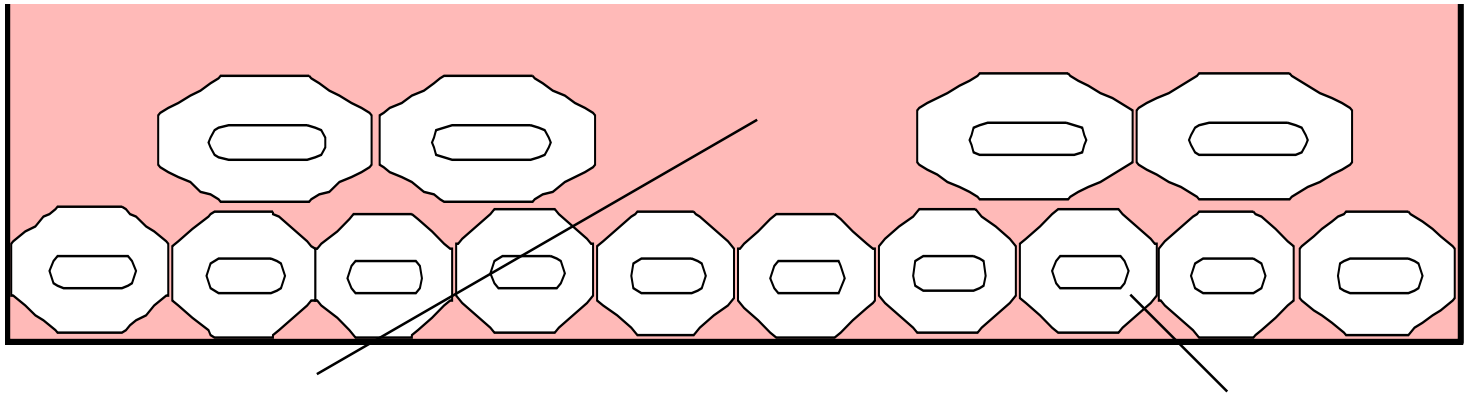
Serum-free culture of keratinocytes

- Boyce & Ham, 1983
- Wille, Pittelkow, Shipley & Scott, 1984



Stratification of serum-free cultures

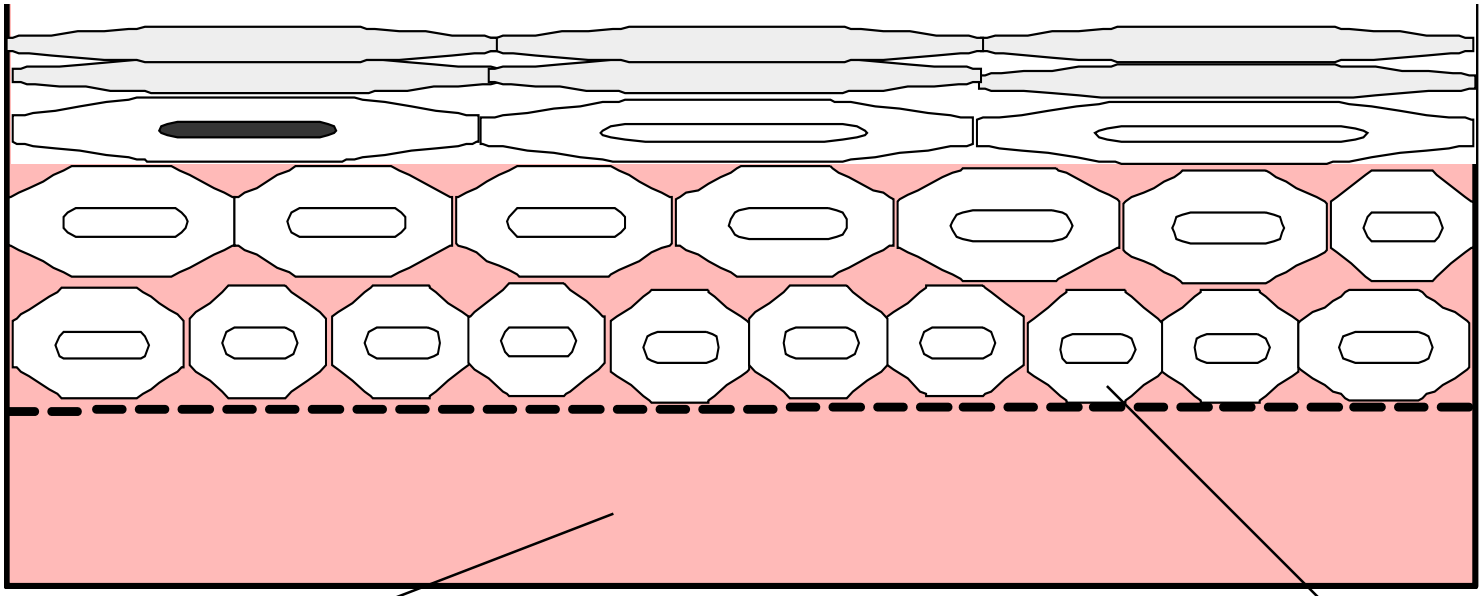
- Pittelkow & Scott, 1986



Culture medium:
MCDB-153
EGF
Insulin
Bovine pituitary extract + serum
Ca^{2+}>1.5 mM

Reconstruction of the epidermis

- Rosdy & Clauss, 1990

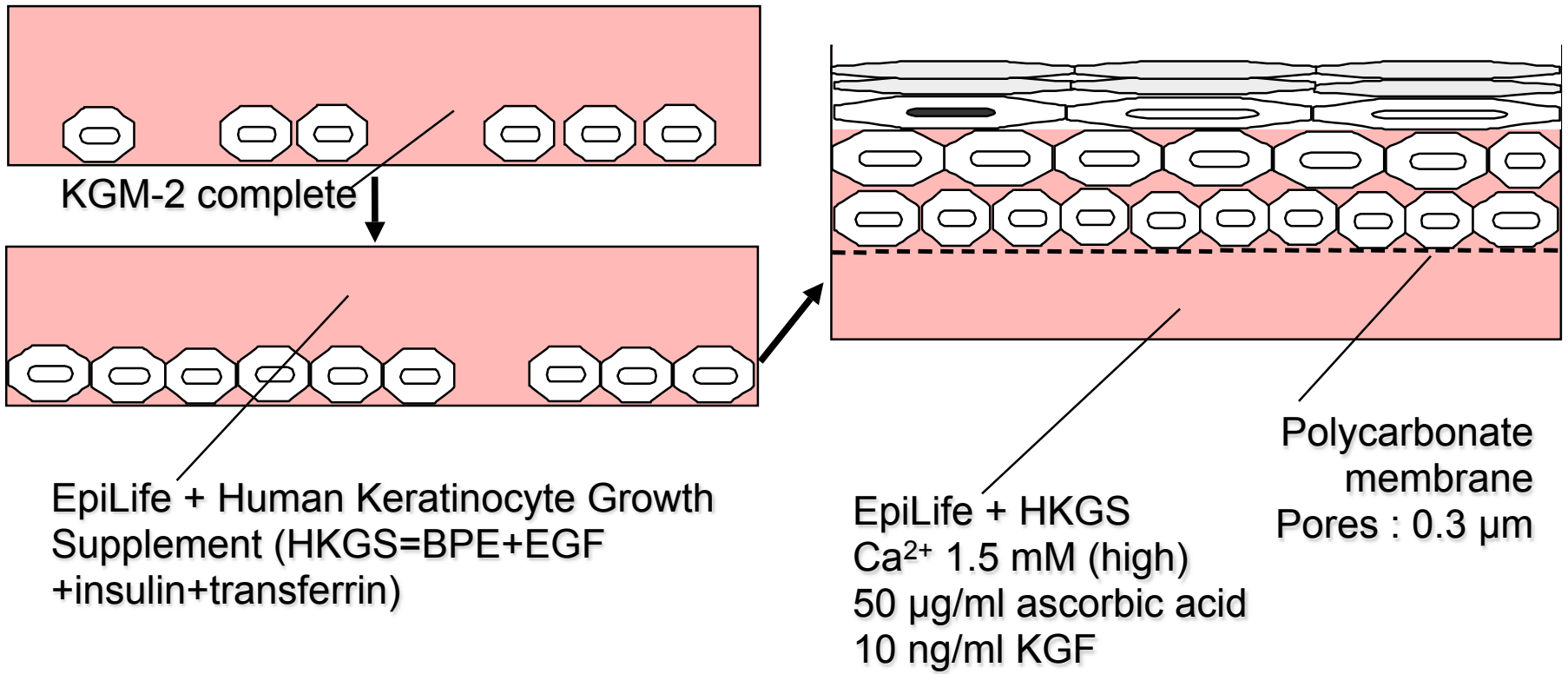


Culture medium:
MCDB-153
EGF + Insulin
low Ca^{2+}, then 1.15 mM (high)

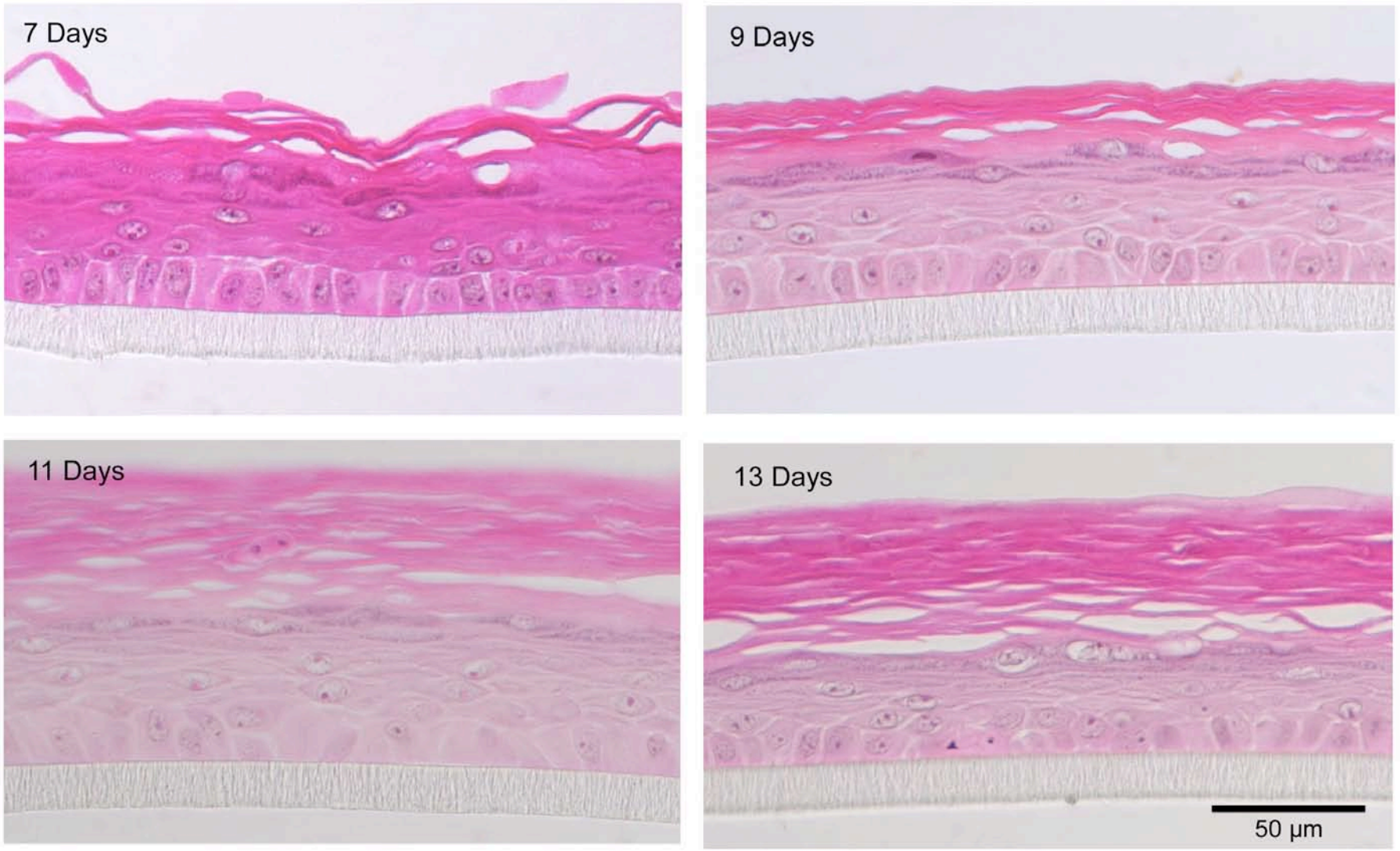
Keratinocytes
seeded at high cell density

Reconstruction of the epidermis

- Poumay et al., 2004



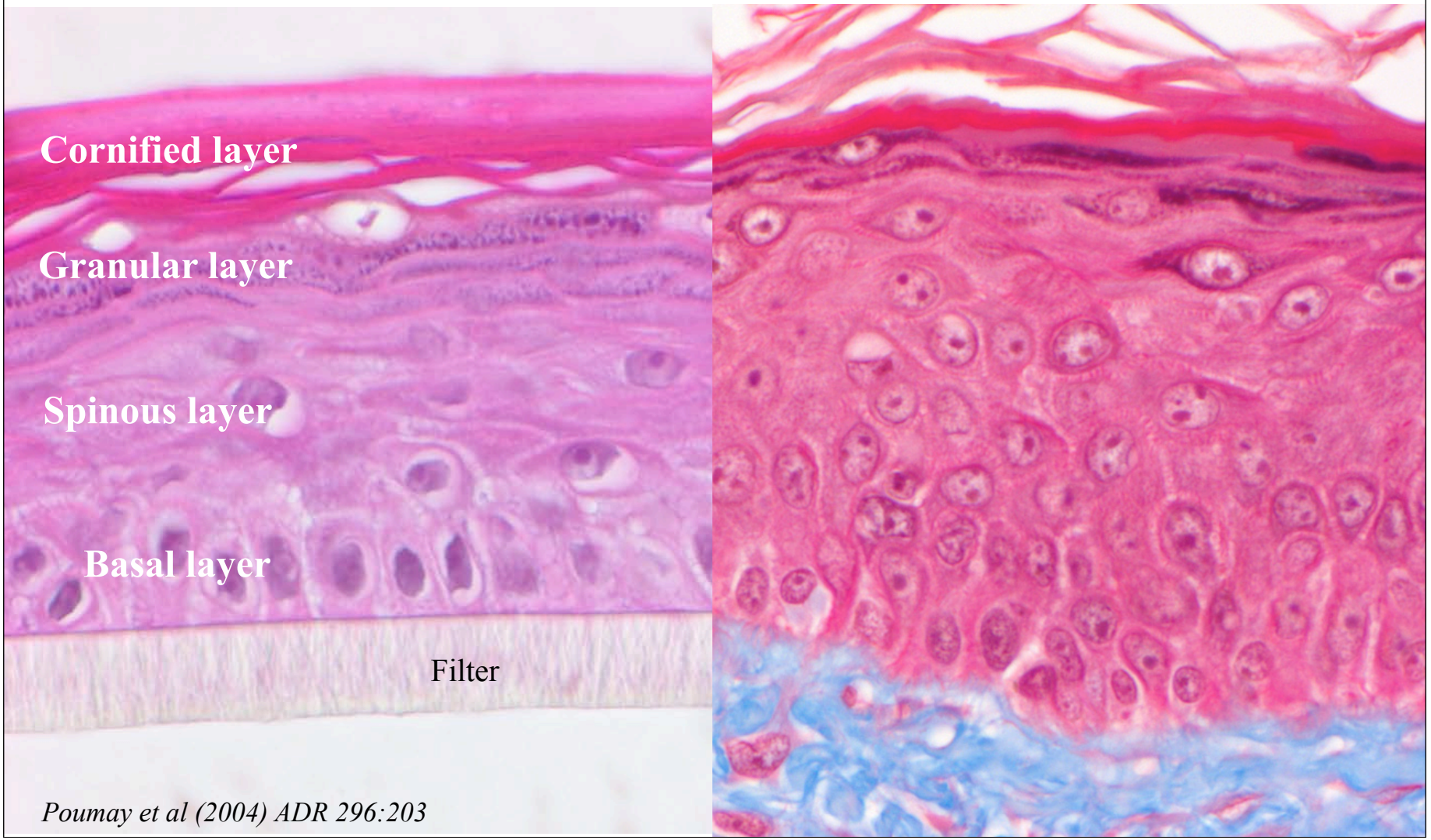
Reconstruction of the epidermis



Reconstructed and native epidermis

Poumay et al (2004) ADR 296:203

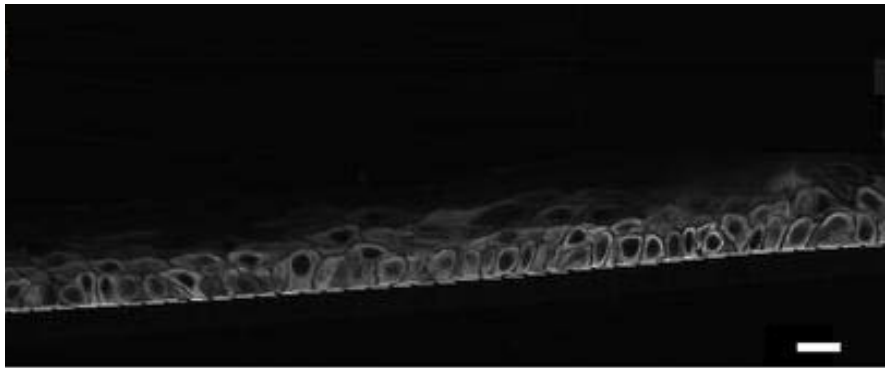
Reconstructed and native epidermis



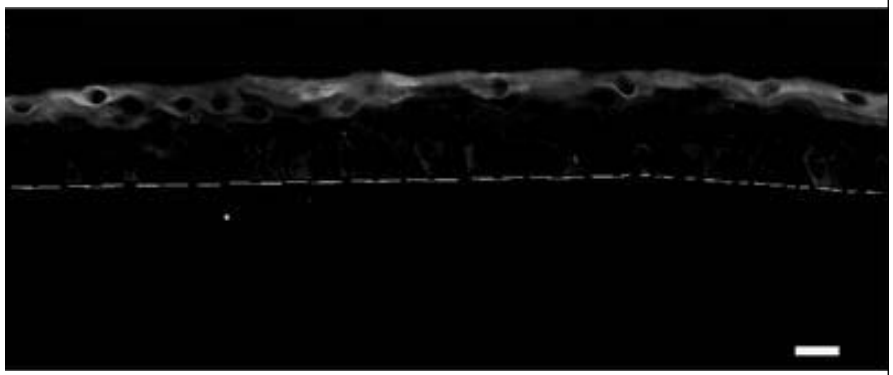
Poumay et al (2004) ADR 296:203

Immunofluorescence of epidermal markers

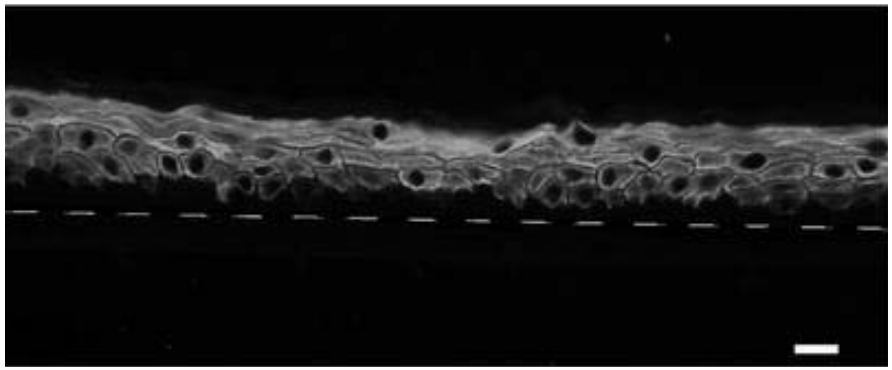
Keratin 14



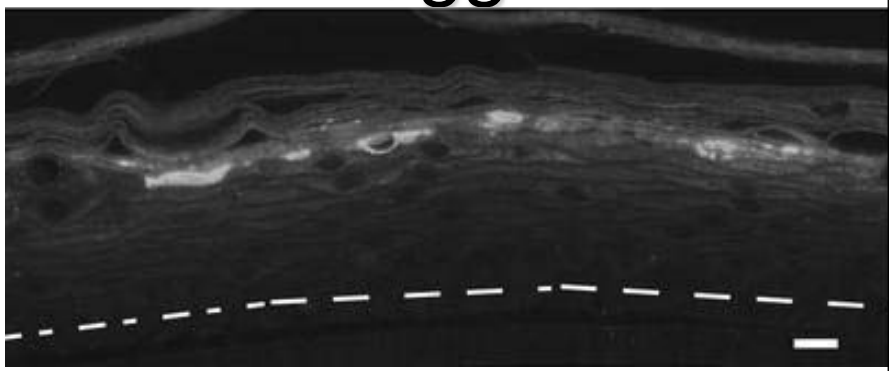
Involucrin



Keratin 10

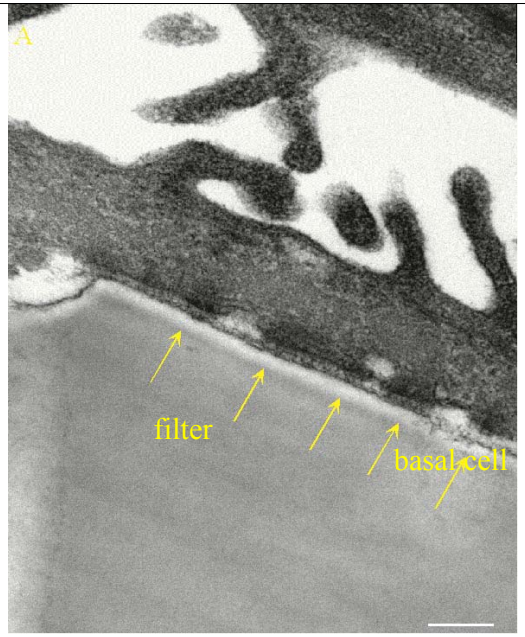


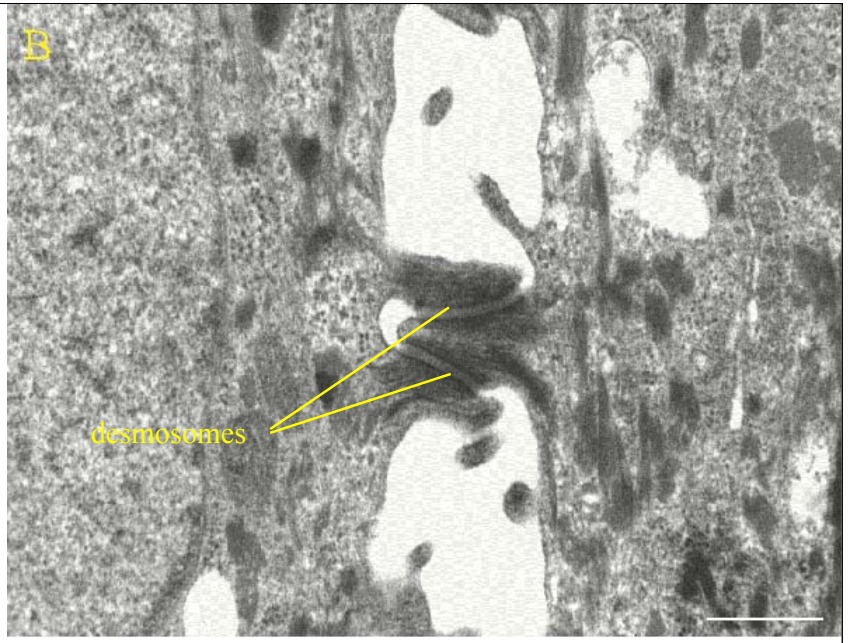
Filaggrin

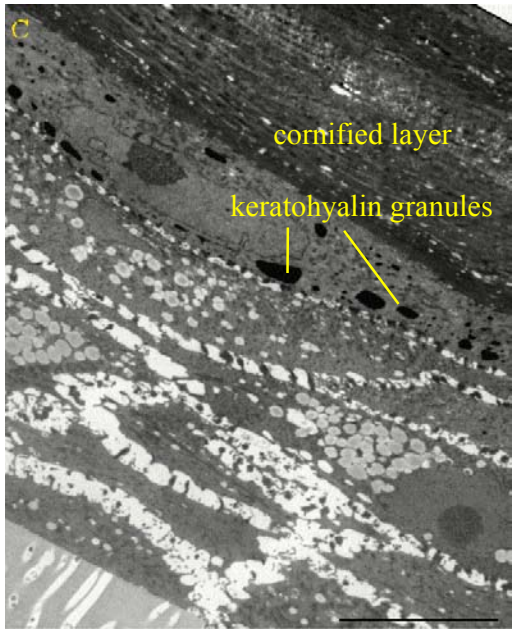


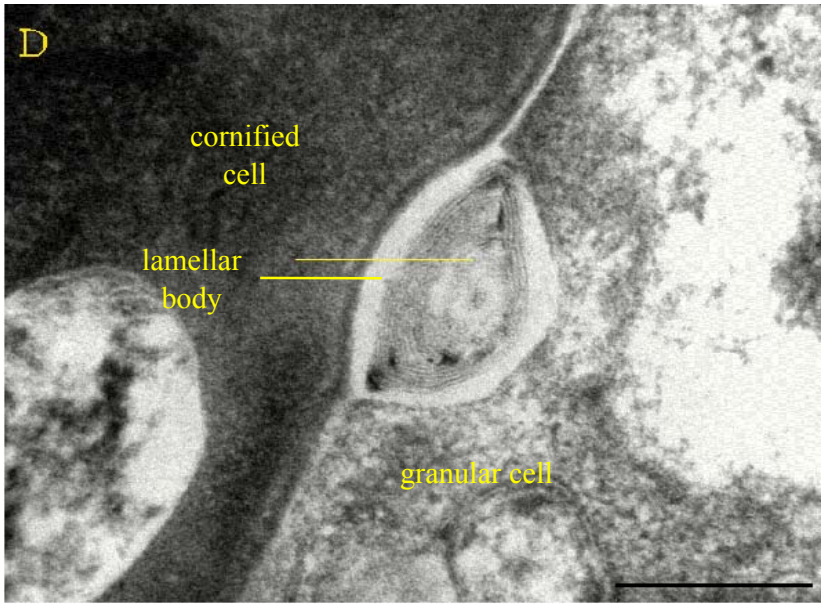
Poumay et al (2004) ADR 296:203

TEM







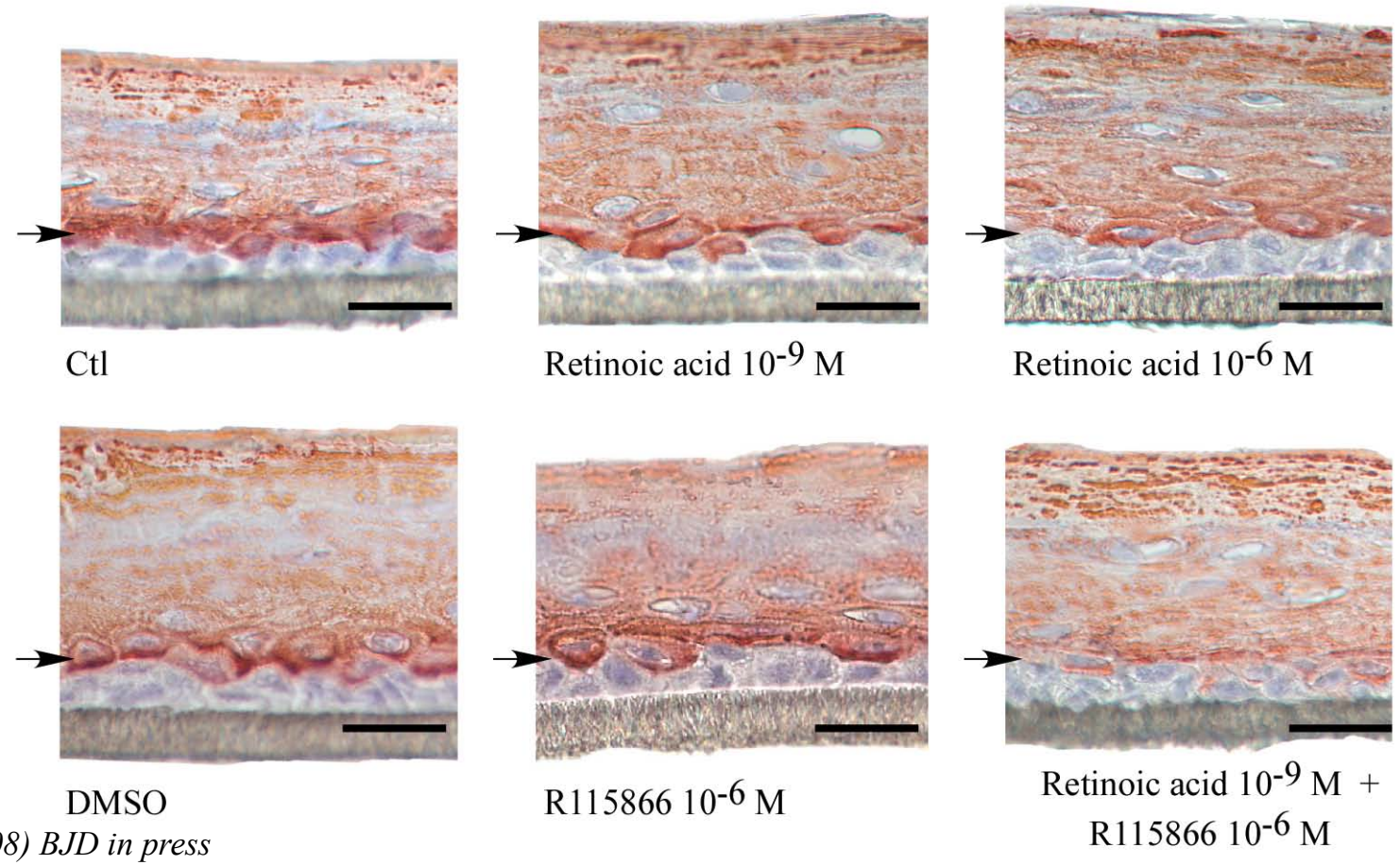


Poumay et al (2004) ADR 296:203

Tissue Response within the RHE

Analysis of tissue response

- Analysis of Keratin 10 expression in response to Retinoic Acid and R115866



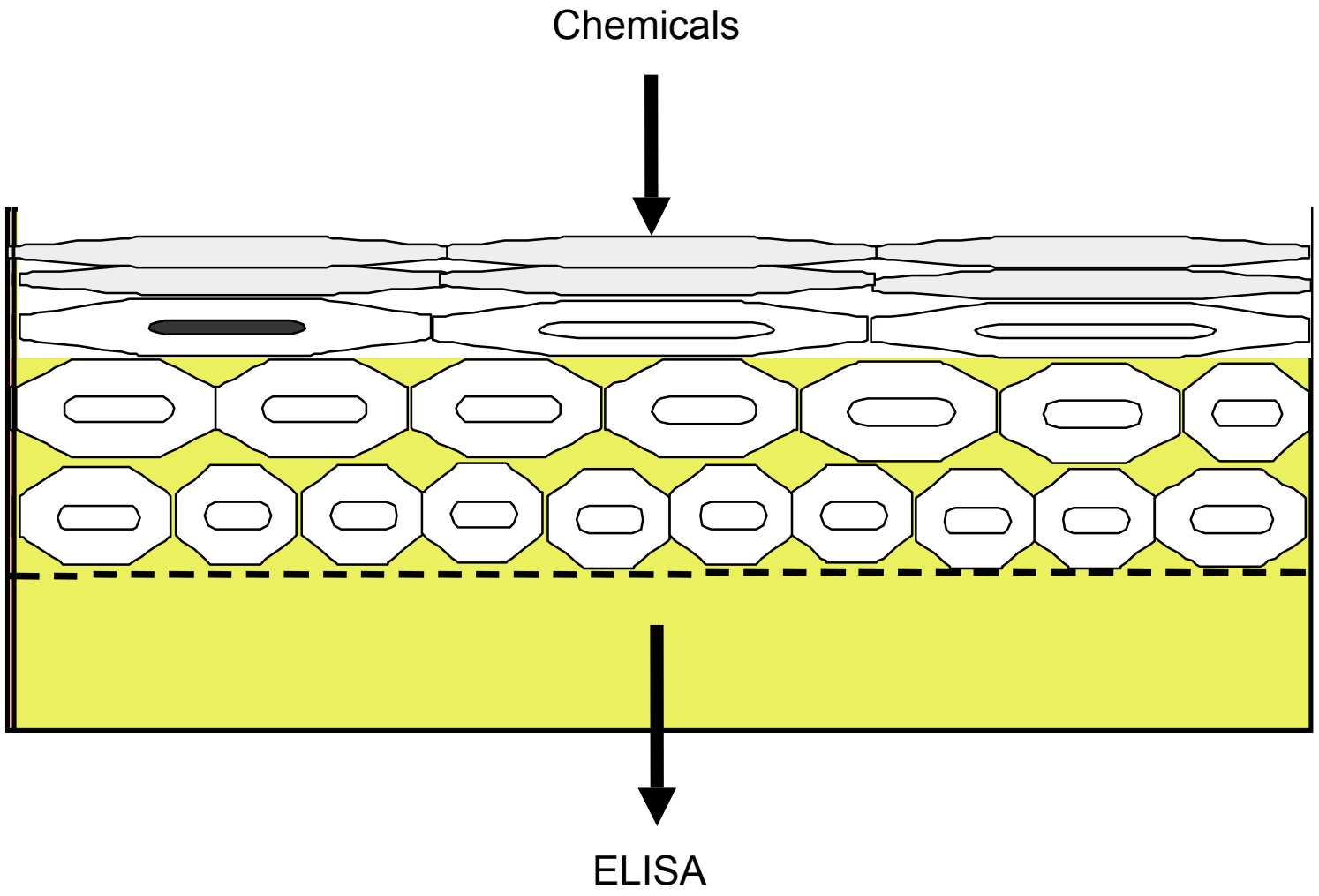
Giltaire et al (2008) BJD in press

Analysis of tissue response

- The RHE is being used in order to evaluate the release of interleukins during its response to irritants or sensitizers
  - IL-1α secretion is due to an unconventional mechanism
  - IL-8 secretion is due to exocytosis stimulated by various triggering events linked to stress-reponse

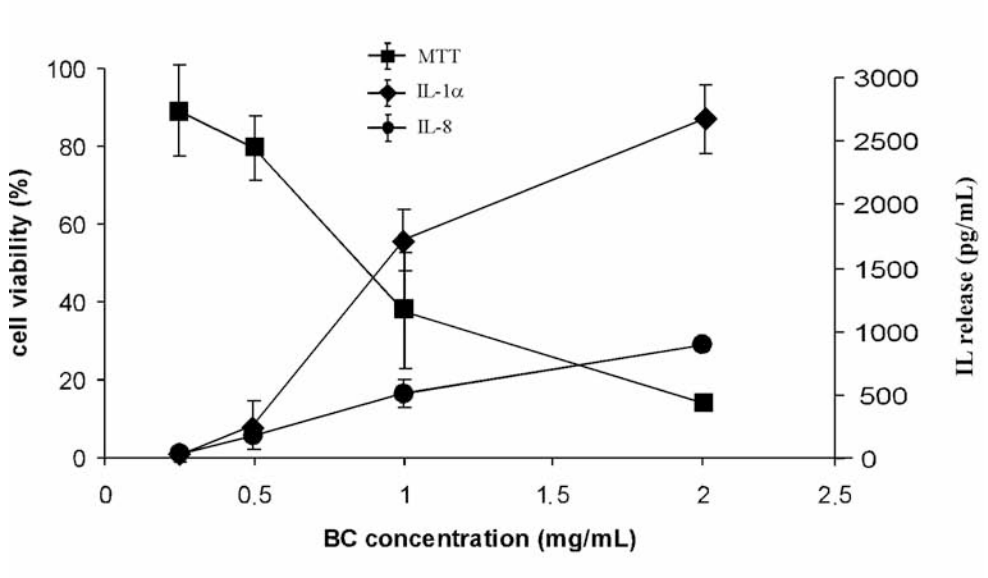
Release of Interleukins

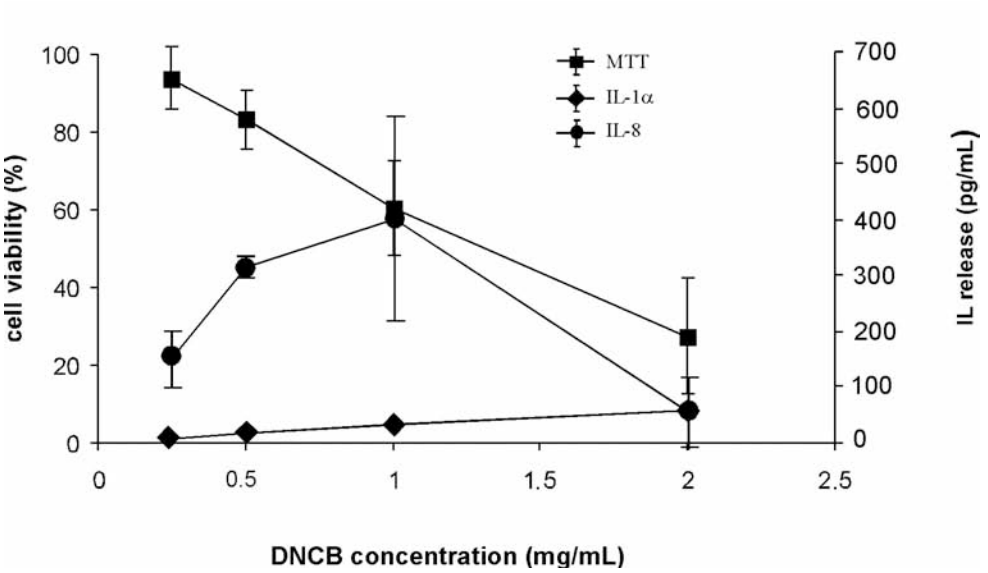
- Interleukin-1α or -8



Cell viability, IL-1α and IL-8 release

after Benzalkonium Chloride (BC) and Dinitrochlorobenzene (DNCB)





Coquette et al (2003) TIV 17:311

Poumay et al (2004) ADR 296:203

Analysis of tissue response

Data from keratinocyte monolayers are transposed to RHE :

- The role of signaling intermediates and
- The activation of particular (stress-response) signaling pathways are currently under investigation

See the work of L.-M. Koeper *And the work of Aurélie Frankart*

Summary on RHE

- More difficult and more expensive to produce
- Require addition of growth factors in culture medium
- Detection of markers in relevant layers
- Available for studies of :
  - Markers of proliferation and differentiation
  - Cellular release
  - Signaling

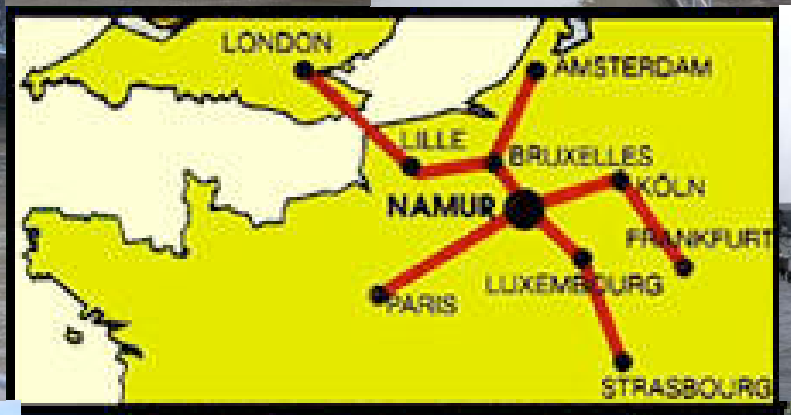
Future of the RHE

- Produced by different manufacturers (including home-made production), the RHE is becoming available for more numerous tissue and cell biology studies (Portland OR, Cleveland OH, Baltimore MD, Newcastle UK, Düsseldorf GE, Brussels BE, Italy, Brazil,…)
- Those studies will bring more information about the model and allow unlimited refinements in the analysis of the tissue

Conclusions

LabCeTi (Cell and Tissue Laboratory) - Namur

namur.be



Pictures: Lucie Poumay © 2008