Differential regulation of the sodium-ascorbic acid co-transporters SVCT1 and SVCT2 expression in glutathione depleted CaCo-2 cells as assessed by functional analaysis and rt-PCR

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VITAMIN C

1. Essential to human physiology because humans cannot synthesize it from glucose.

2. Potent antioxidant present in all cells at elevated concentrations.

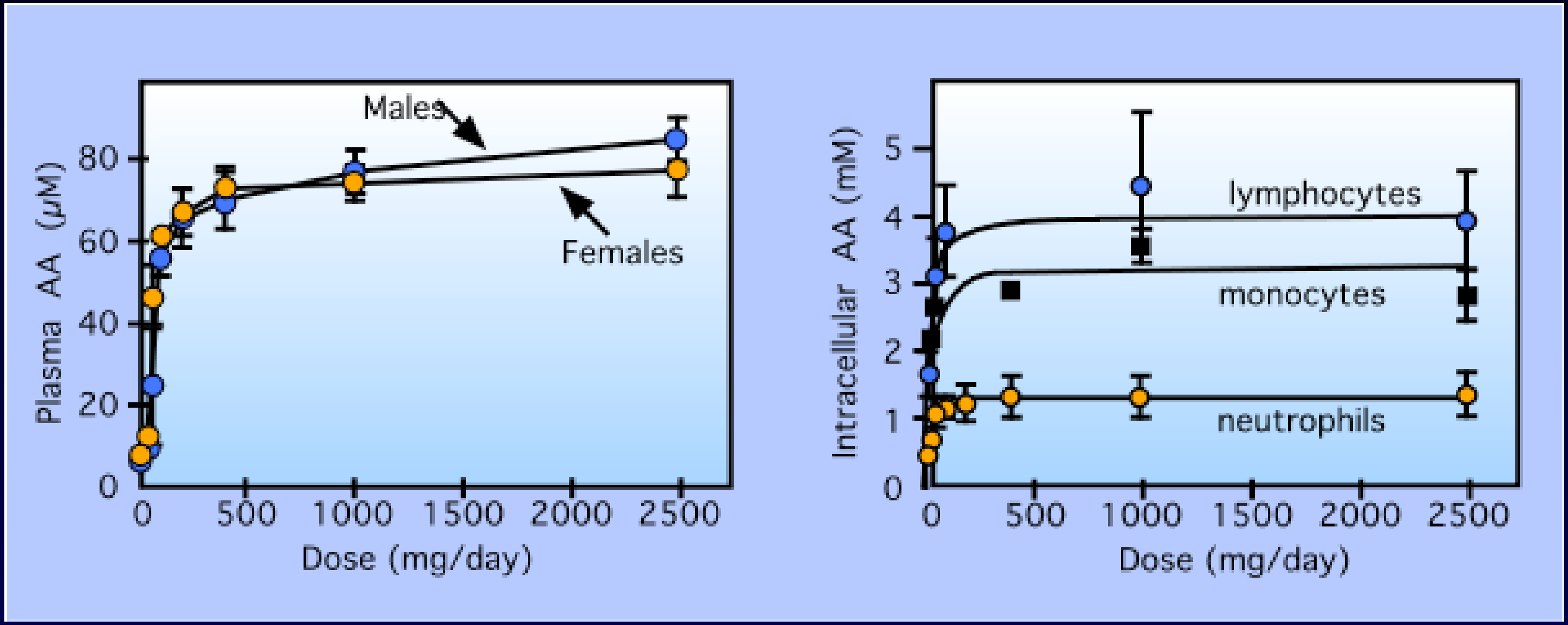
3. Protects cells from oxidative stress.

4. Dietary deficiency associated to increased risk of heart disease.

5. Its relationship to cancer prevention is highly controversial.

6. Supplementation with mega-dosis of vitamin C, although apparently common, has no scientific support.

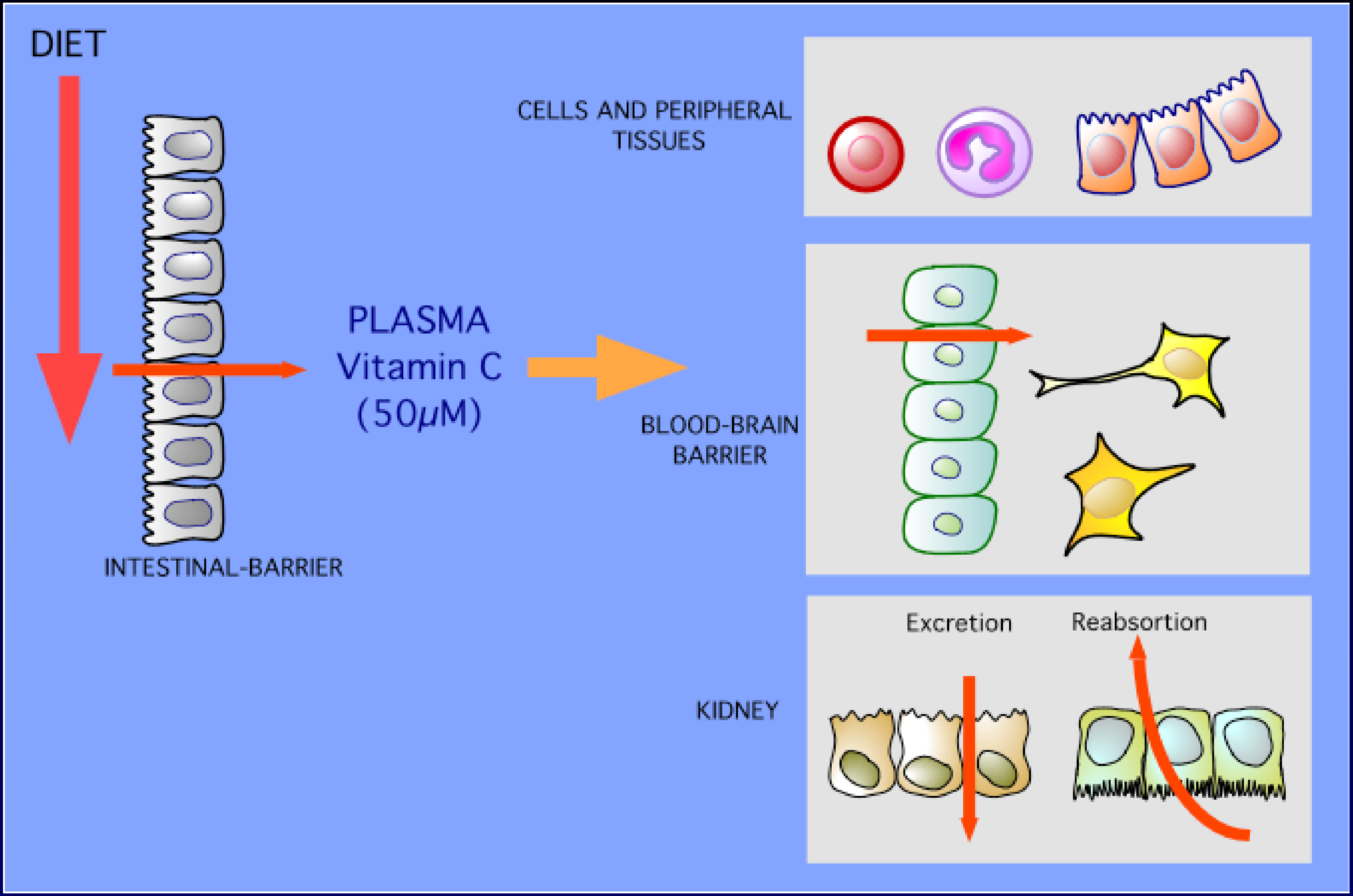
Vitamin C supplementation in humans



Vitamin C content in cells and tissues in humans

Tissue	Concentration (mM)
Plasma	0,045 ± 0,022
Eritrocytes	0,043 ± 0,016
Granulocytes	1,2 ± 0,3
Mononuclear leucocytes	3,8 - 5,0
Platelets	1,9
Tyroid	0,1
Testes	0,2
Squeletal muscle	0,2 - 0,3
Lungs	0,4
Cardiac muscle	0,3 - 0,9
Kidneys	0,3 - 0,9
Spleen	0,6 - 0,9
Pancreas	0,6 - 0,9
Liver	0,6 - 1,0
Brain	0,8 - 0,9
Cornea	1,6 - 2,0
Adrenals	1,9 - 2,5

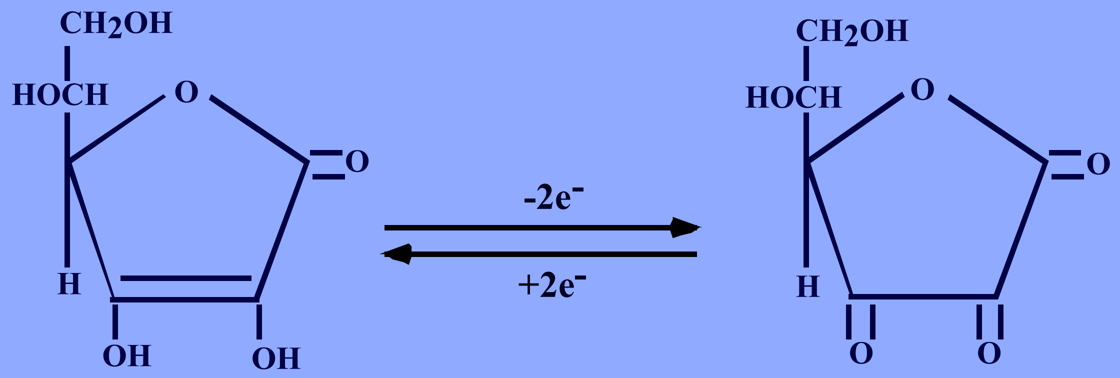
Acquisition and biodisponibility of vitamin C in humans



Vitamin C exists in two biologically active forms

REDUCED VITAMIN C
Acido L-asc—rbico
(AA)

OXIDIZED VITAMIN C
Acido deshidroasc—rbico
(DHA)



Vitamin C transport is mediated by two families of transporters with absolute specificity for reduced or oxidized vitamin C

The sodium-ascorbate co-transporters (SVCTs) show absolute specificity for the transport of reduced vitamin C:

SVCT1

SVCT2

Tsukaguchi et al. (1999). Nature 399:70-75

The glucose transporters show absolute specifcity for the transport of oxidized vitamin C (GLUTs):

GLUT1

GLUT2

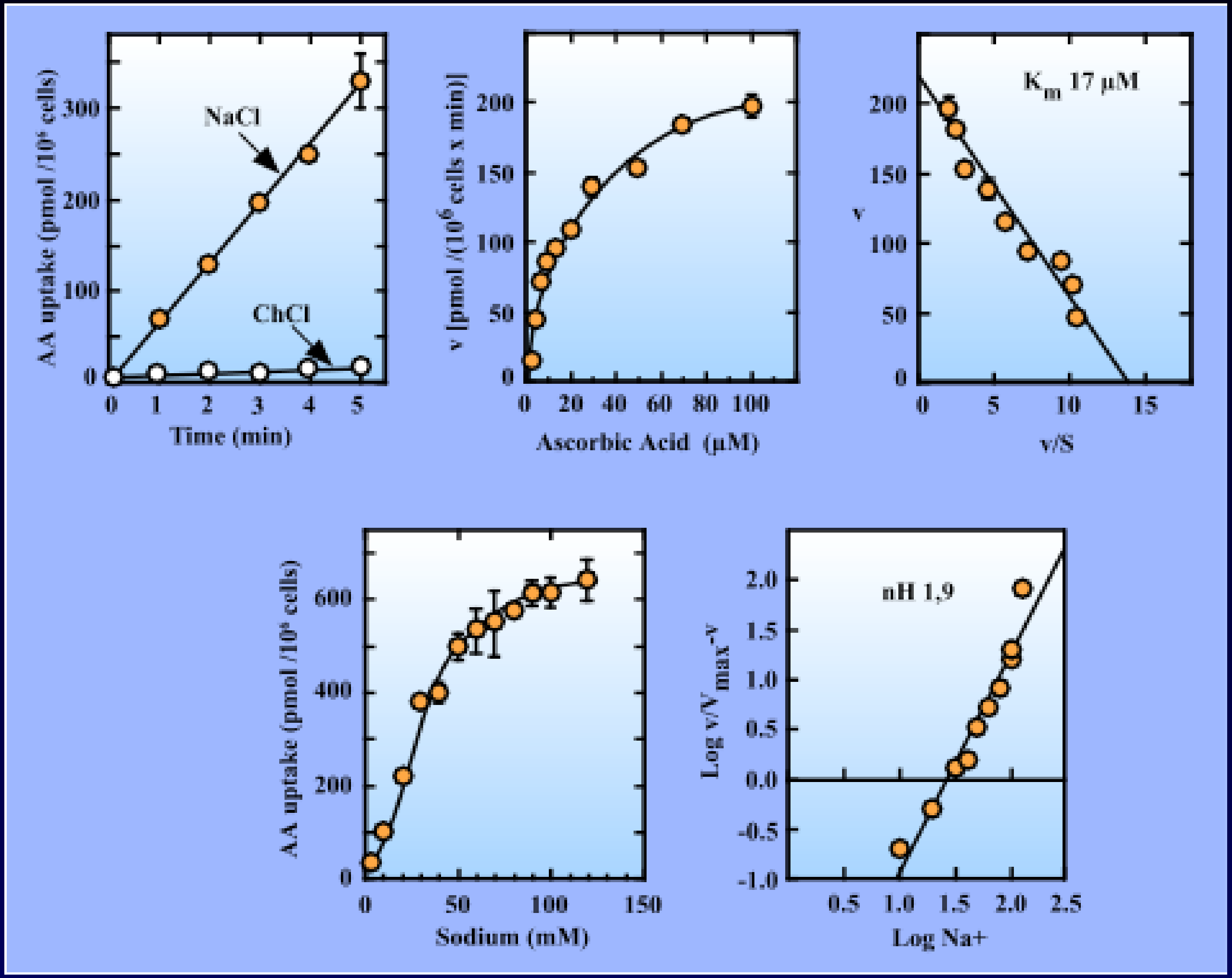
GLUT3

GLUT4

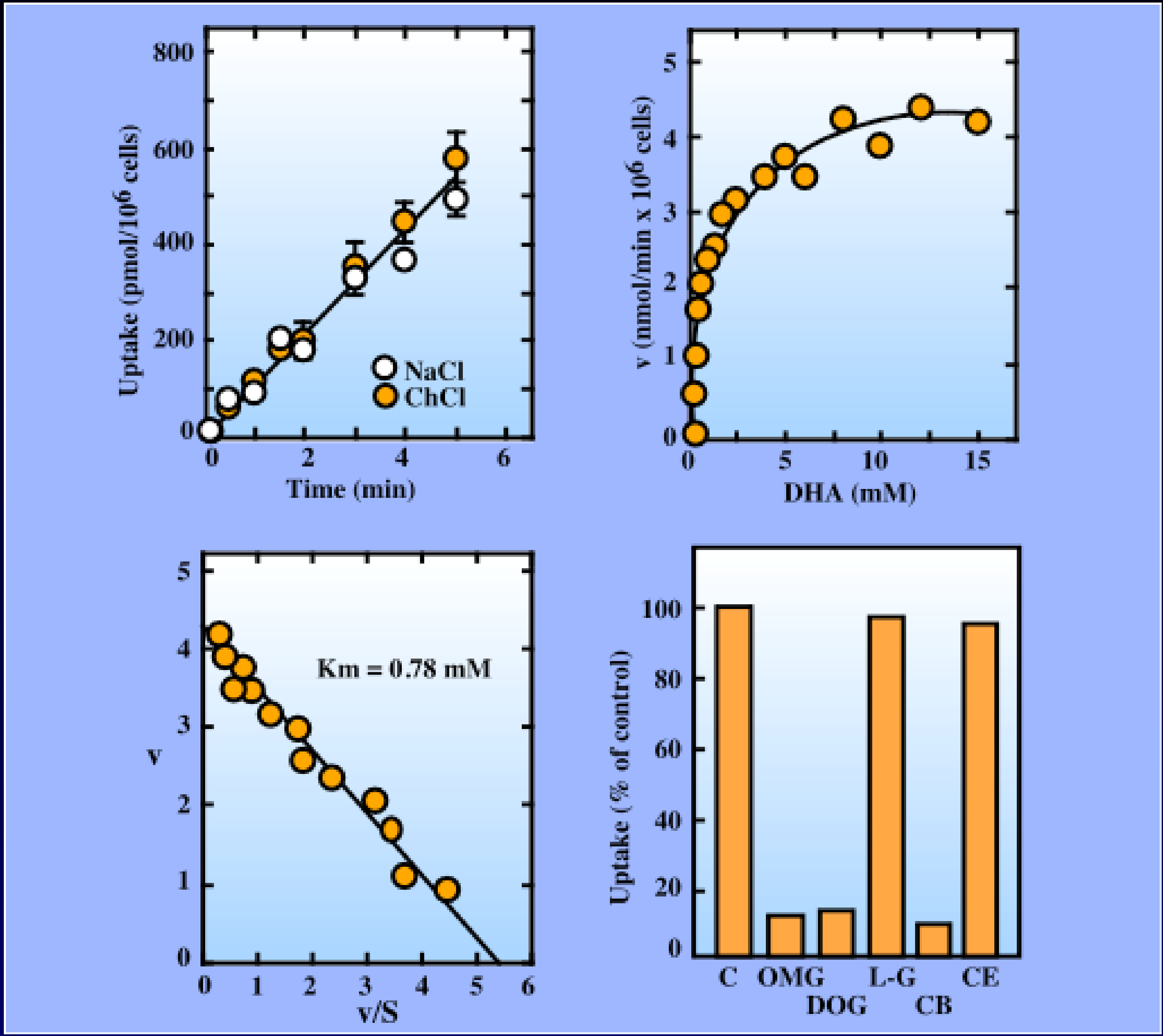
GLUT6

Vera et al. (1993) Nature 364:79-82

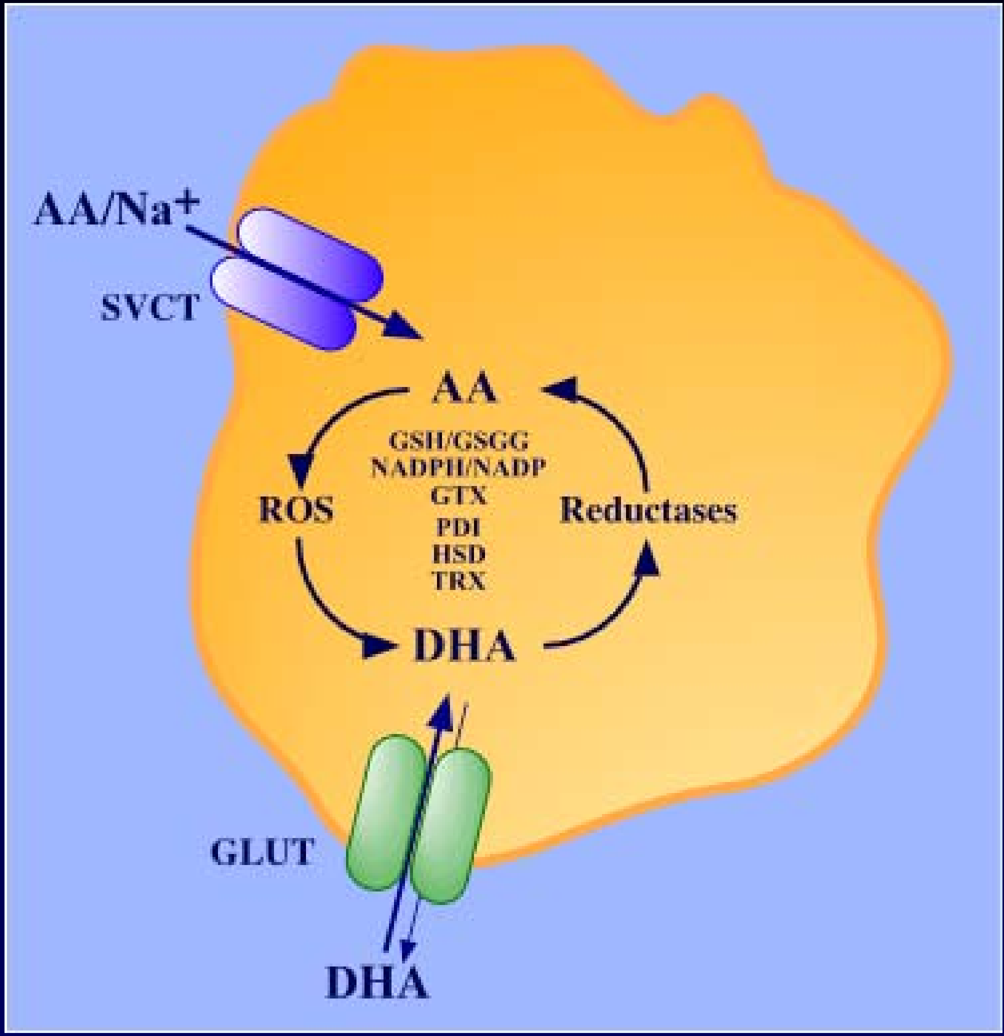
Transport of vitamin C mediated by the SVCTs is sodium-dependent



Vitamin C transport by the GLUTs is sodium-independent



Transport, accumulation and recycling of vitamin C



Cell models currently used to study vitamin C metabolism

PRIMARY CELL CULTURES

Normal human prostate epithelial cells
Normal human breast epithelial cells
Human neutrophils
Human erythrocytes
Human umbilical vein endothelial cells
Human tonsil endothelial cells
Rat hepatocytes
Rat erythrocytes

ESTABLISHED CELL LINES

Human leukemia cells (HL-60)
Human breast cancer cells (MCF-7, MDA-468)
Human prostate cancer cells (LNCaP, PC-3, DU-145)
Human endothelial cells (ECV-304)
Human astrocytes
Human hepatoma cells
Human melanoma cells
Rat hepatoma cells

BARRIER FORMING IMMORTALIZED CELLS

Human brain endothelial cells (HBMEC, HCEC)
Human colon cancer cells (CaCo-2)
Opossum renal cells (OK)

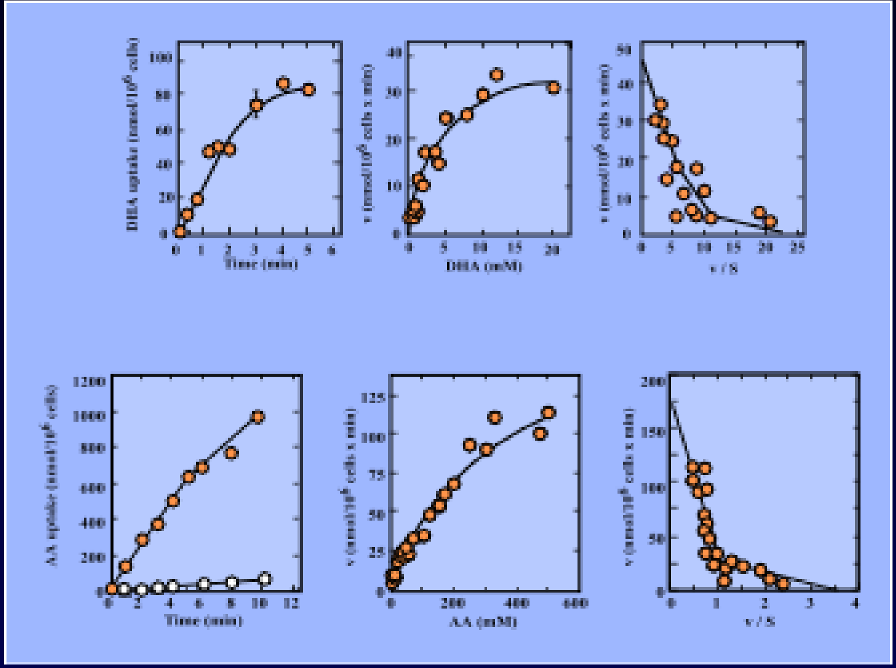
CaCo-2 cells as a model system to study the intestinal transport of vitamin C

In vitro:

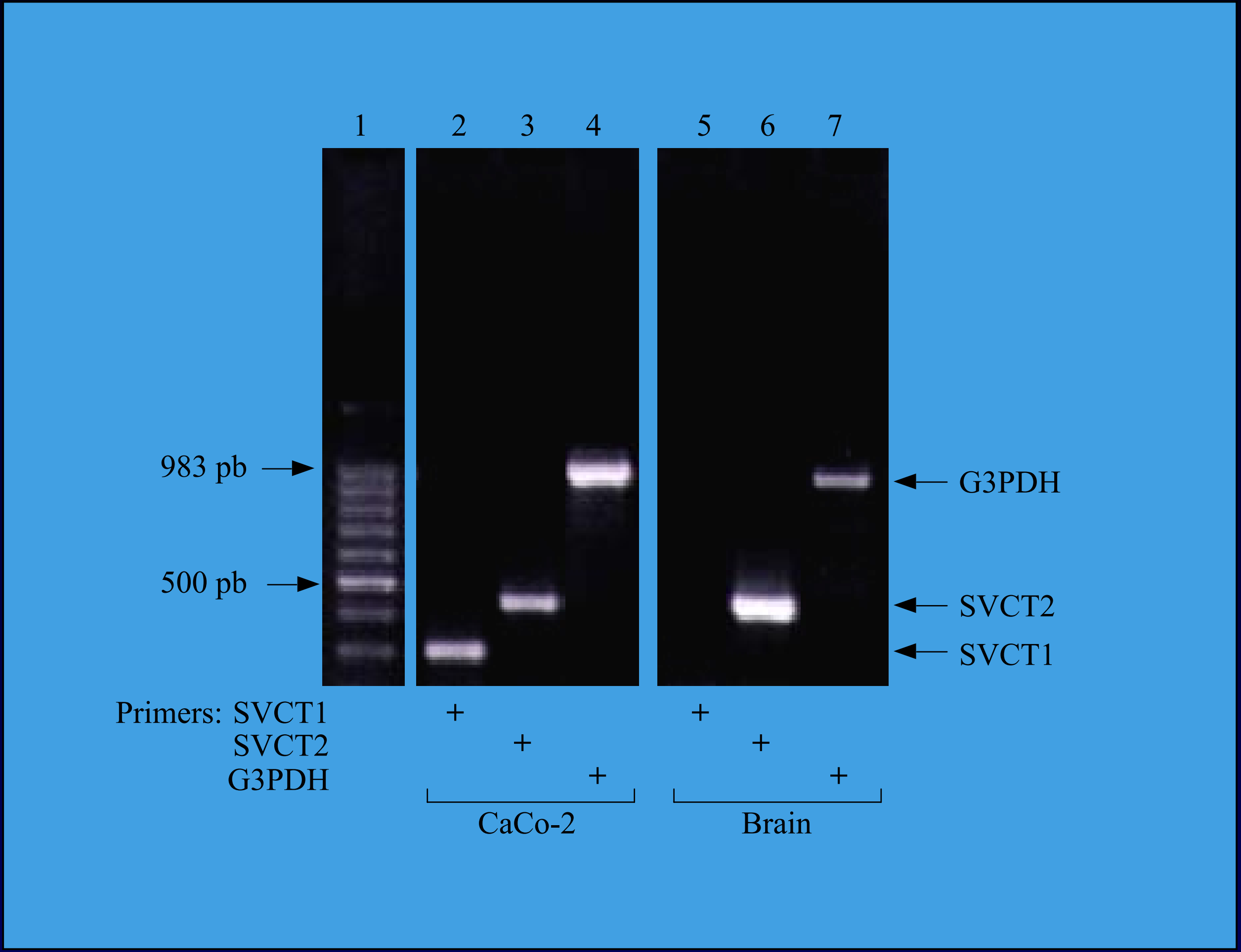
- **Differentiate into enterocyte-like cells.**

2. **Form higly impermeble barriers when cultured in bi-cameral systems.**

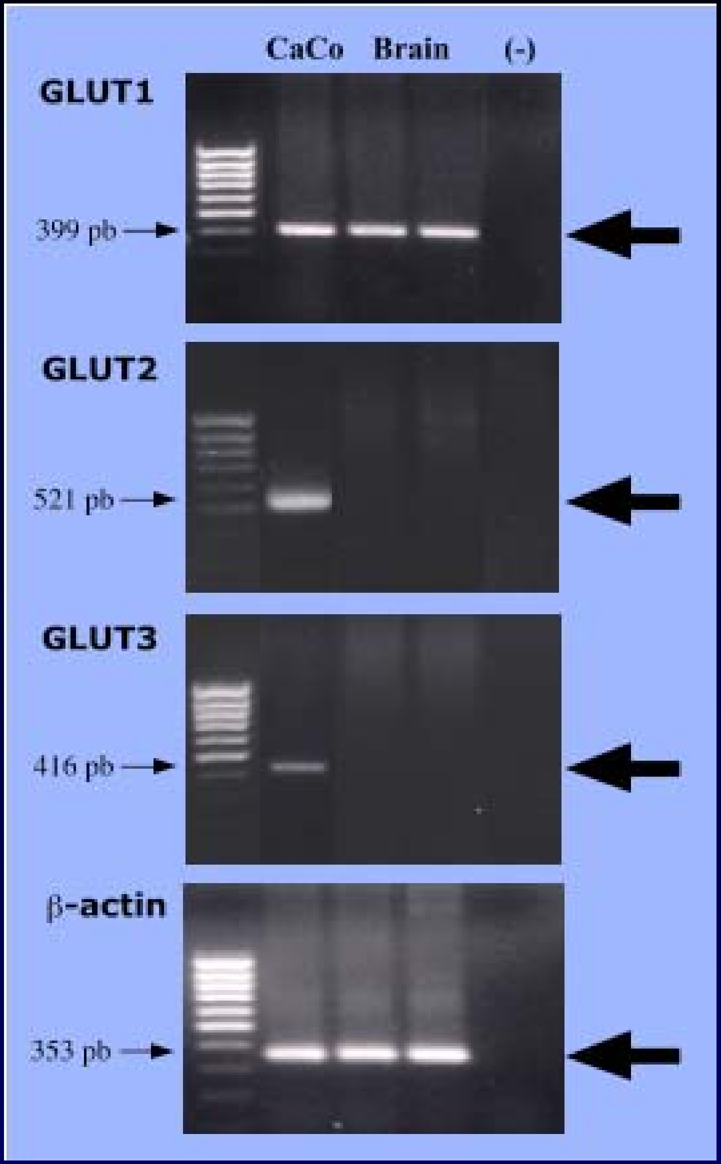
CaCo-2 cells transport both forms of vitamin C (reduced and oxidized)



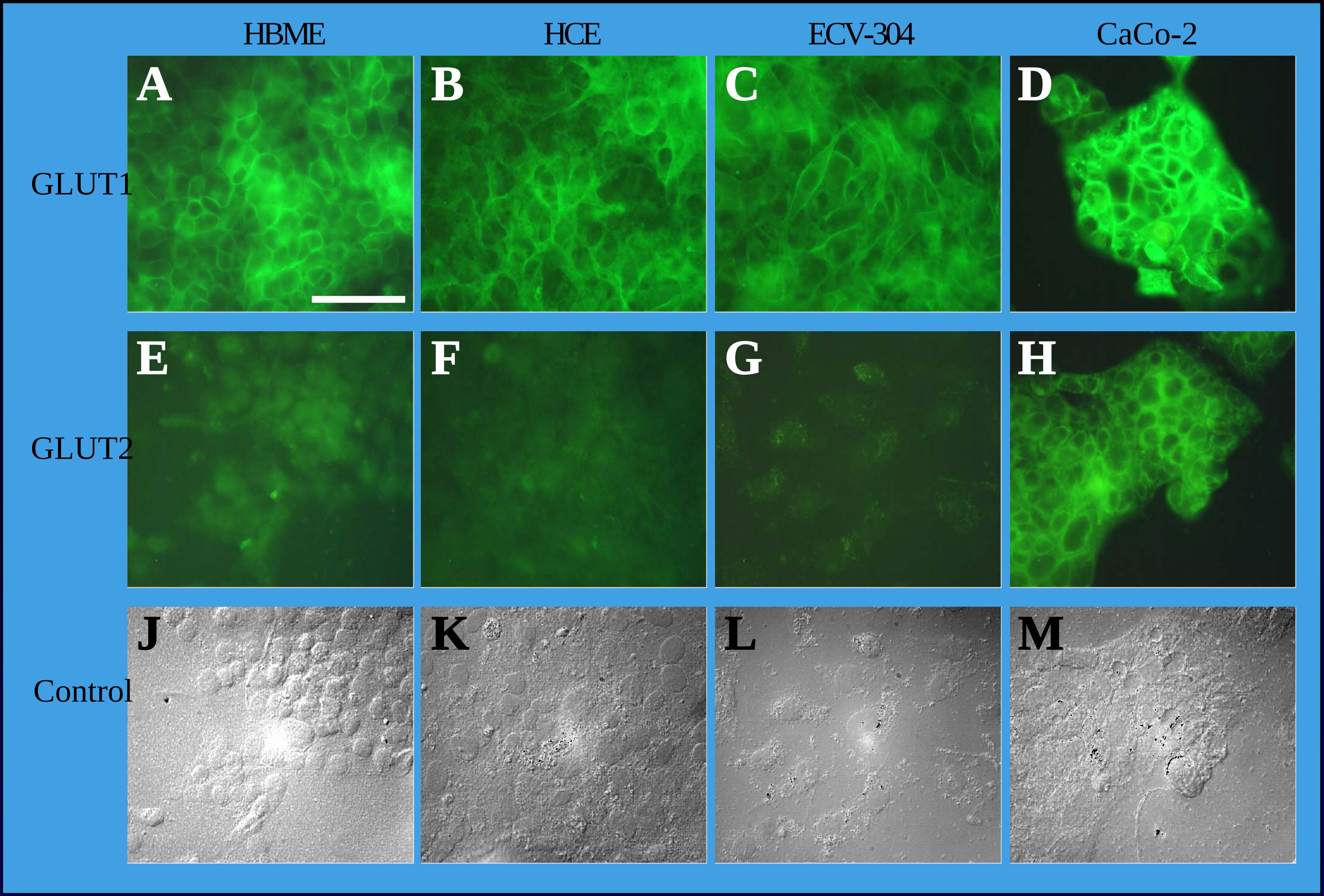
RT-PCR of SVCT1 and SVCT2 in CaCo-2 cells



RT-PCR of GLUTs in CaCo-2 cells

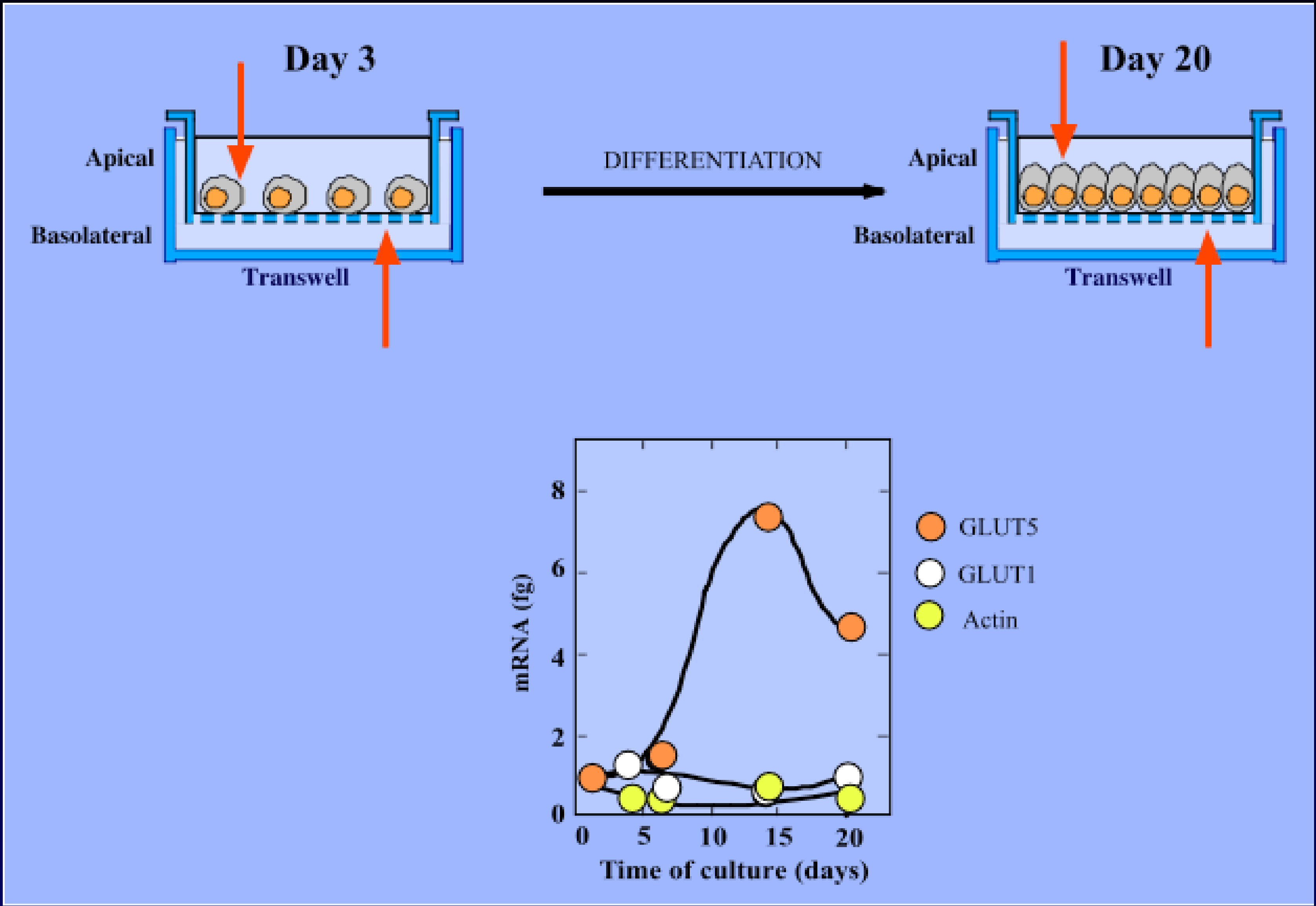


Immunocytochemistry of GLUTs in CaCo-2 cells

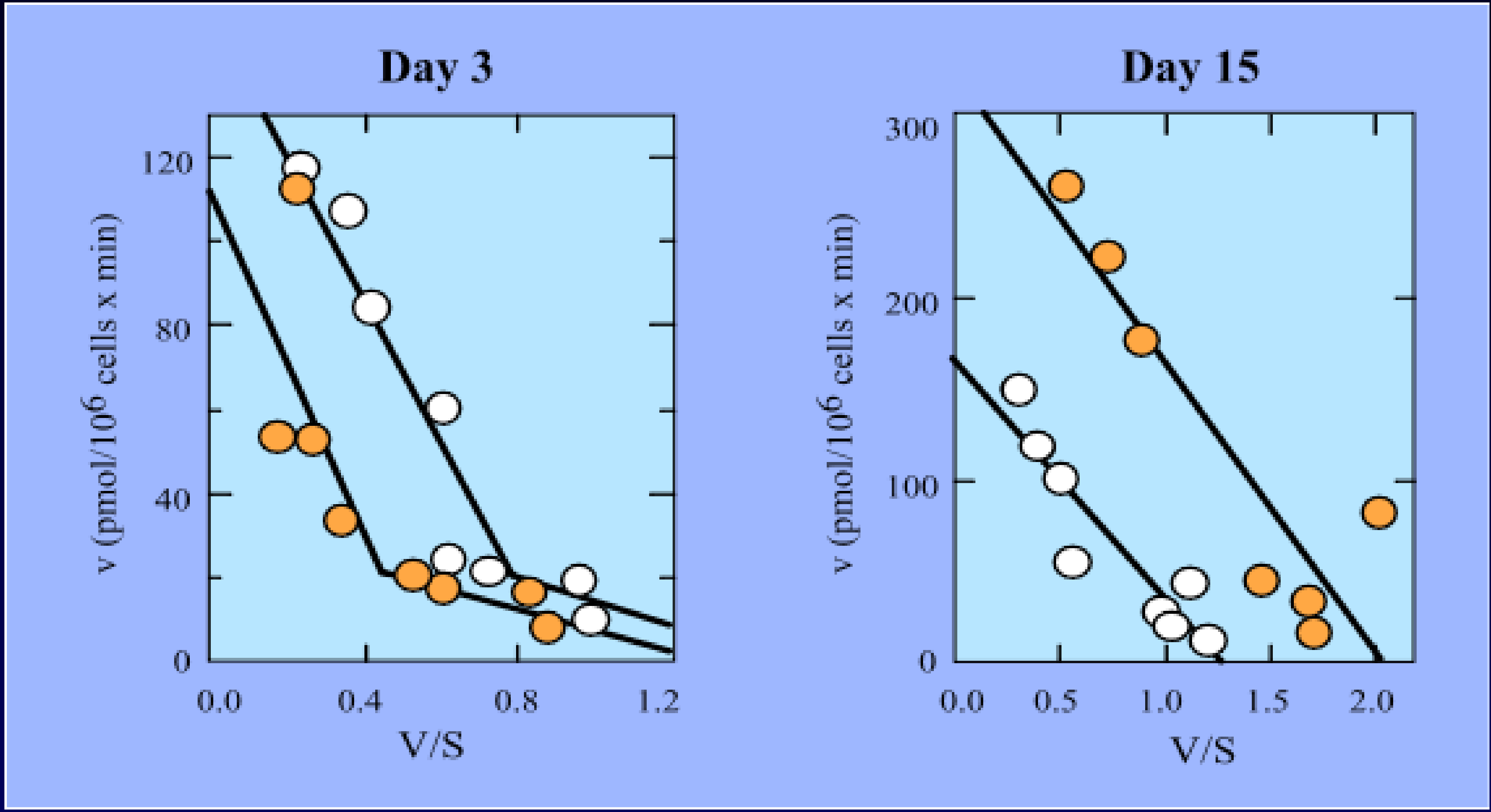


rt-PCR with the LightCycler

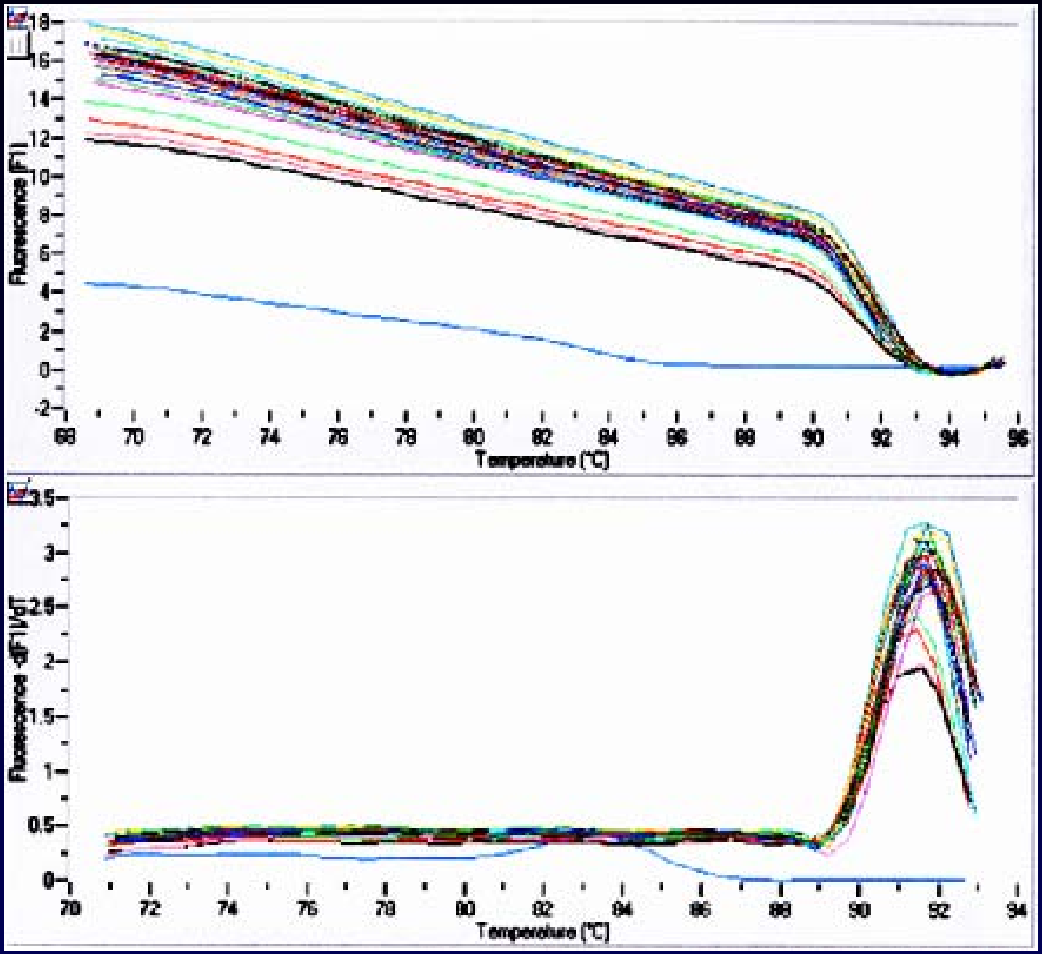
rt-PCR of control genes during CaCo-2 differentiation



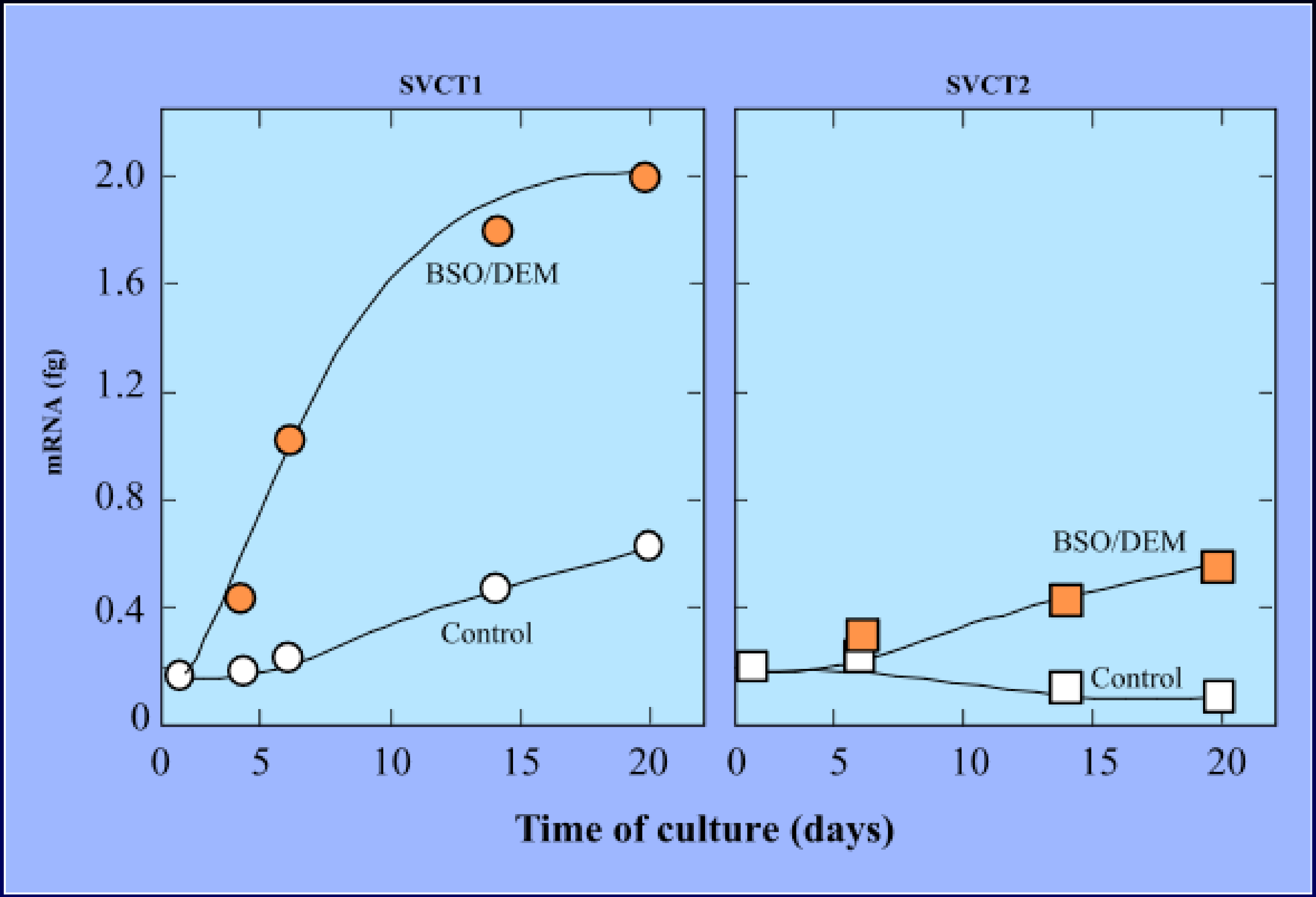
Differentiation of CaCo-2 cells is accompanied by changes In the functional properties of the AA transporters



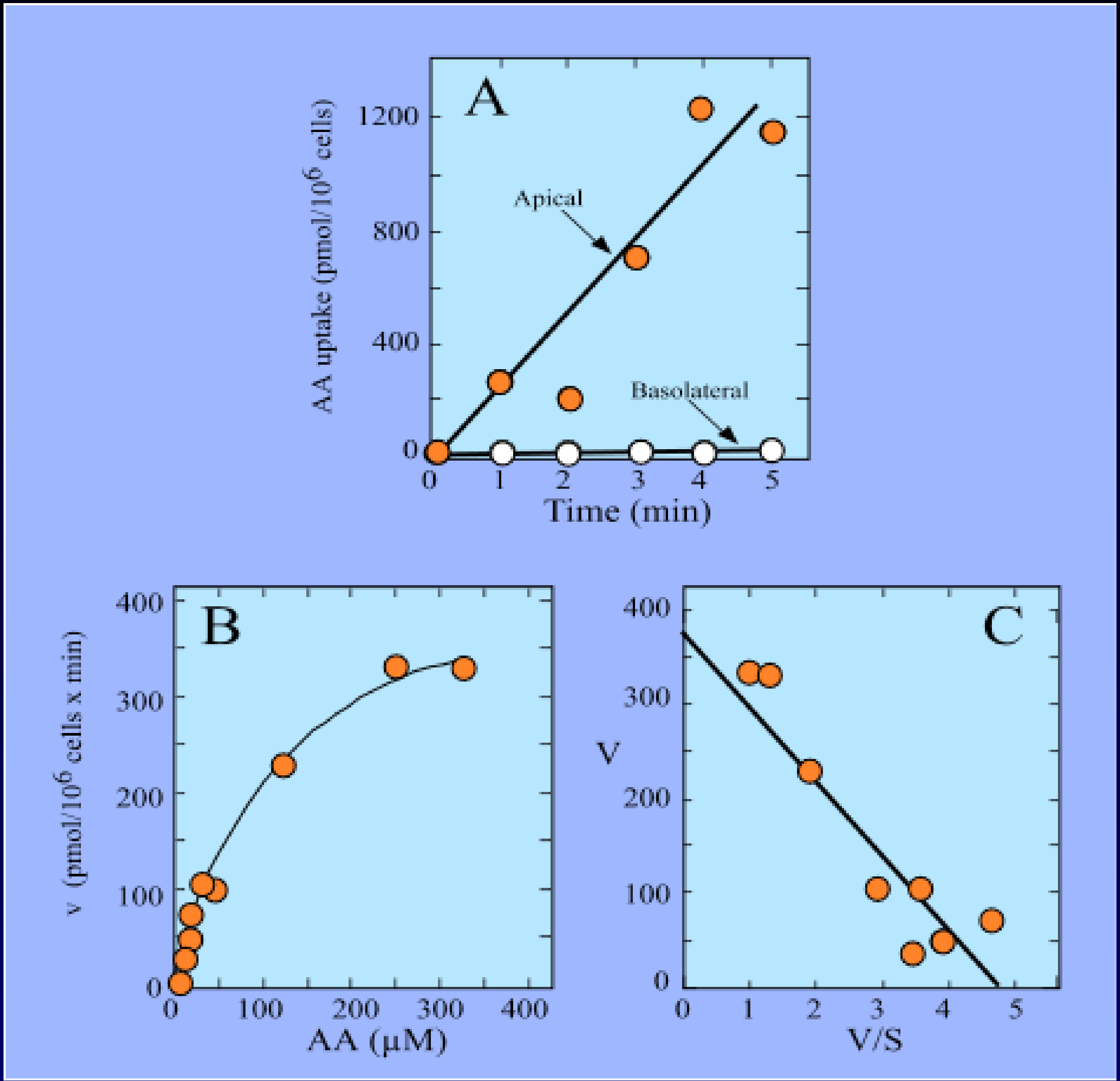
Melting curves for SVCT2



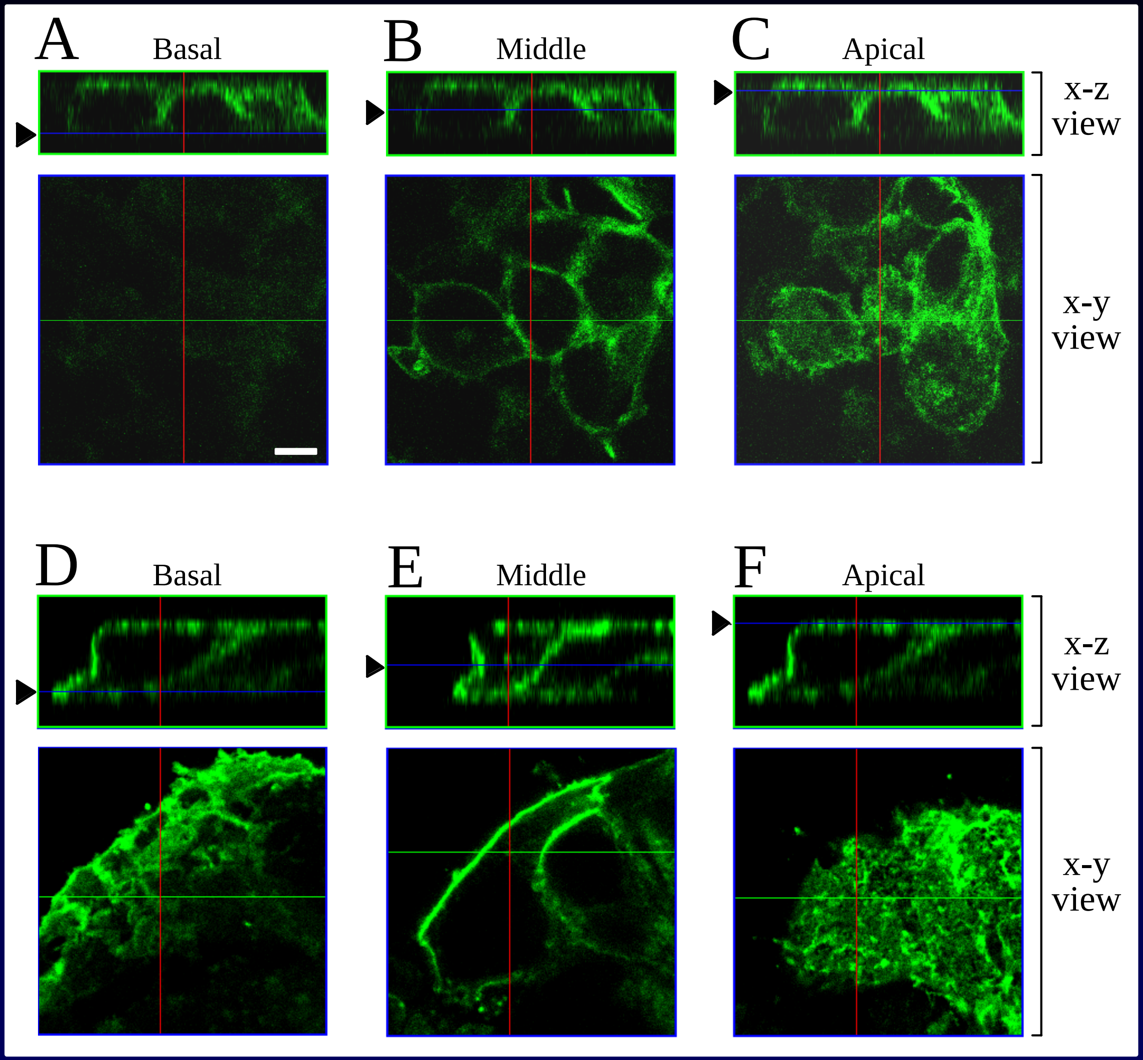
Expression of SVCT1 and SVCT2 in differentiated CaCo-2cells lacking glutathione



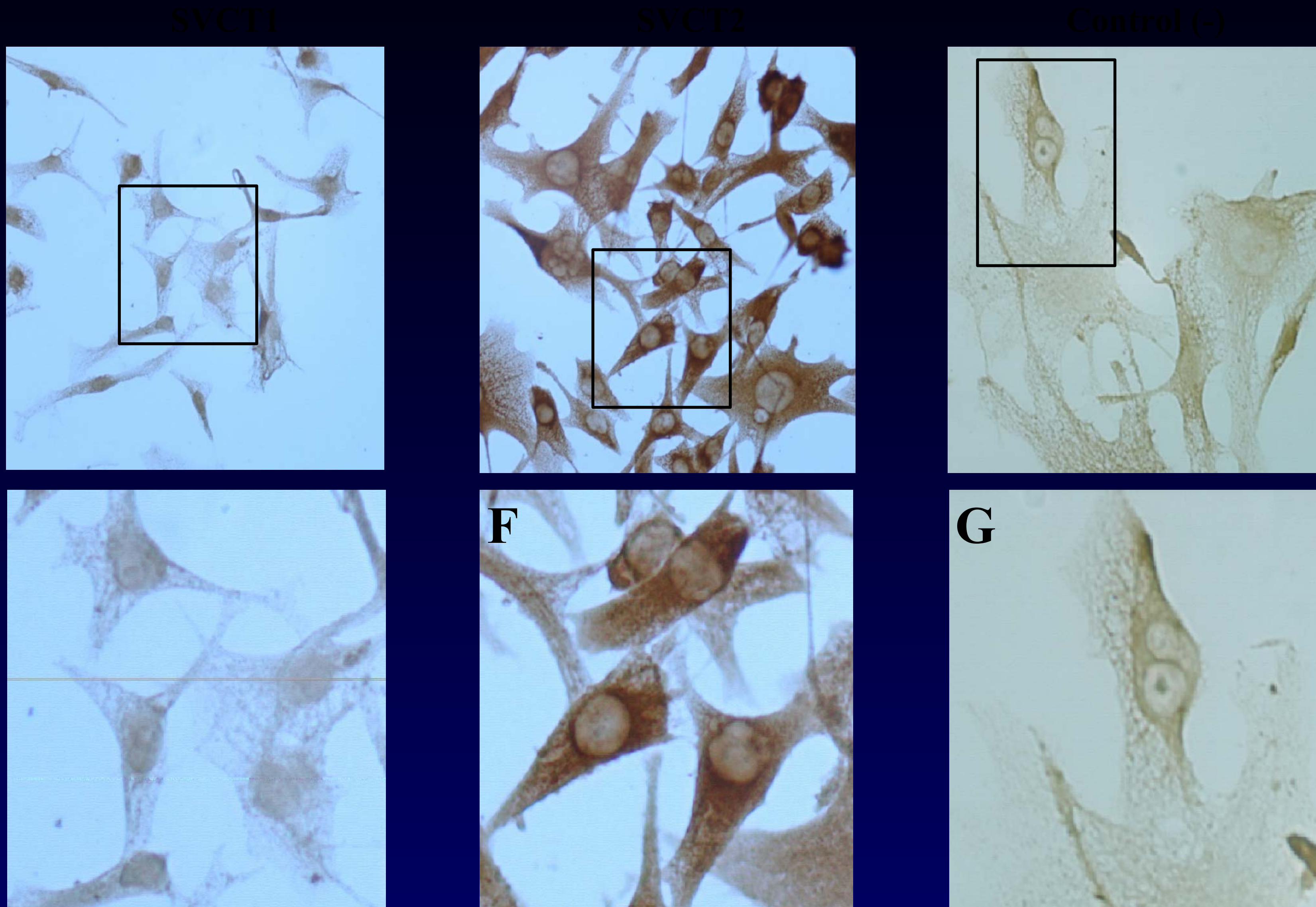
AA transport by differentiated CaCo-2 cells shows evidence of polarization



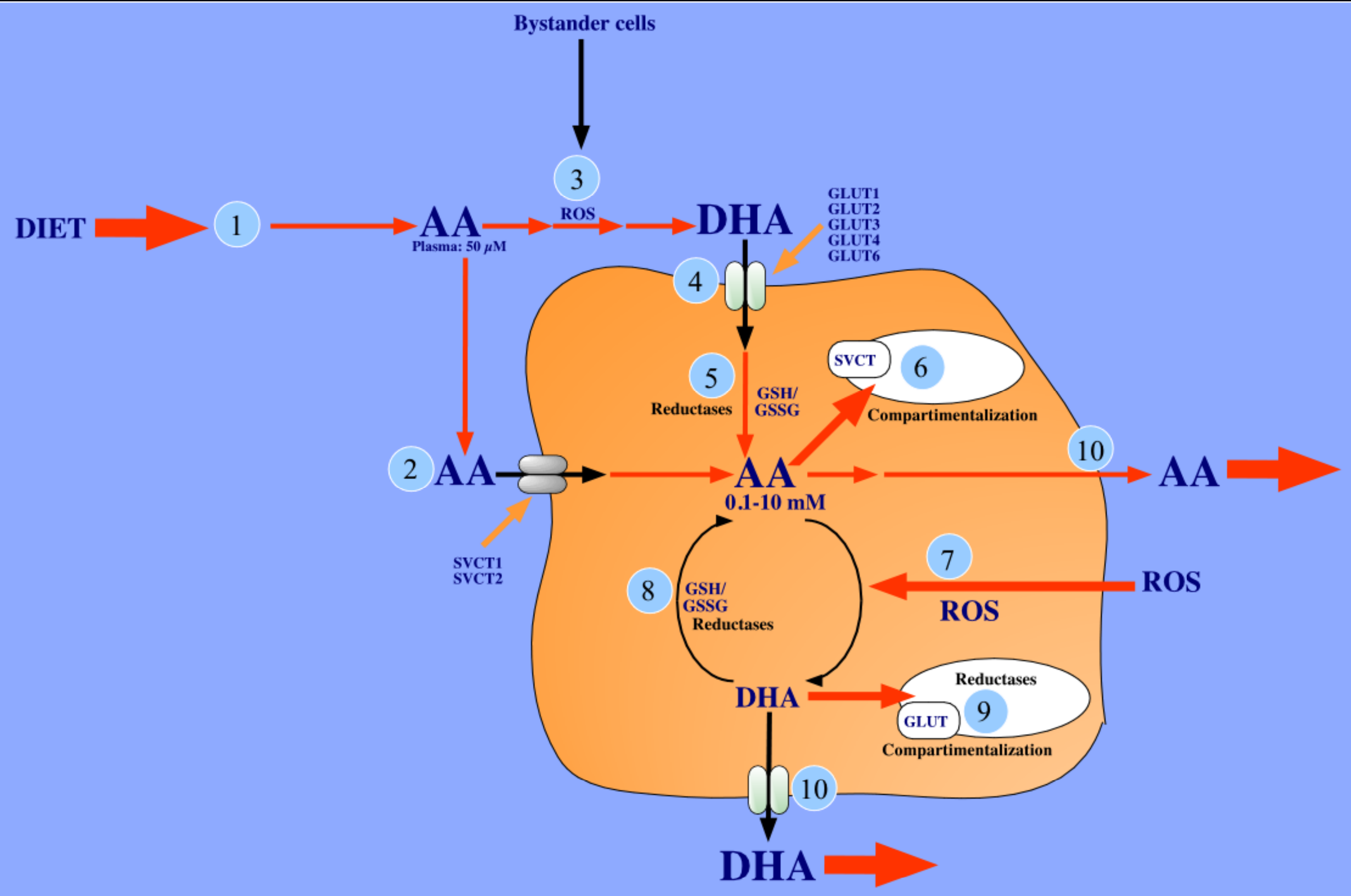
Polarized expression of vitamin C transporters in barrier forming differentiated cells



Intracellular localization of SVCT2



Vitamin C metabolism: The road ahead



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Nualart et al: Recycling of vitamin C by a bystander effect. J. Biol.Chem. 278:10128-10133 (2003)
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