3.14: The Transport and Regulation of Ca^{2+} Ions in Higher Organisms

All living organisms need calcium, which must be taken up from the environment. Thus, Ca^{2+} ions have to be distributed throughout the organism and made available where needed. In higher organisms, such as humans, the blood-plasma level of total calcium is kept constant (≈2.45 mM) within narrow limits, and there must be a mechanism for regulating this concentration. On a cellular level we have already seen in the preceding section that the basal cytoplasmic Ca^{2+} concentration, at least in eucaryotic cells, is very low, on the order of 100 nM. At the same time the concentrations of Ca^{2+} in certain organelles, such as endoplasmic (or sarcoplasmic) reticulum or mitochondria, may be considerably higher. If Ca^{2+} ions are to be useful as intracellular "messengers," as all present evidence has it, Ca^{2+} levels in the cytoplasm would have to be raised transitorily as a result of some stimulus. Ca^{2+} ions may enter the cytoplasm either from the extracellular pool or from the Ca^{2+}-rich organelles inside the cell (or both). We could imagine Ca^{2+} channels being regulated by chemical signaling, perhaps by a hormone acting directly on the channel, or by a small molecule released intracellularly when a hormone is attached to a membrane-bound receptor. Some channels may be switched on by voltage gradients, and both these mechanisms may operate concurrently.

Increased intracellular Ca^{2+} levels must eventually be brought back to the basal levels, in some cells very quickly. The ions could be transported out of the cell or back into the Ca^{2+}-rich organelles. This transport will be against an electrochemical potential gradient, and thus requires energy. There are many possibilities for different forms of Ca^{2+} transport and regulation in living systems, and we still know fairly little about the whole picture. Detailed studies are also complicated by the fact that, in higher organisms, cells are differentiated. Nature is multifarious, and what is valid for one type of cell may not be relevant for another. With these words of caution we will start out on a macroscopic level and continue on toward molecular levels.

Ca^{2+} Uptake and Secretion

The uptake of Ca^{2+} from food has mostly been studied in typical laboratory animals, such as rats, hamsters, chickens, and humans. In humans, uptake occurs in the small intestine, and transport is regulated by a metabolite of vitamin D, calcitriol (1,25-dihydroxy vitamin D_3).[34] The uptake process is not without loss; roughly 50 percent of the calcium content in an average diet is not absorbed. To maintain homeostasis and keep the calcium level in blood plasma constant, excess Ca^{2+} is excreted through the kidney. The main factor controlling this phenomenon in vertebrates is the level of the parathyroid hormone that acts on kidney (increases Ca^{2+} resorption), on bone, and, indirectly, via stimulated production of calcitriol, on the intestinal tract (increases Ca^{2+} uptake). Calcium enters the cells from the outside world, i.e., the intestinal lumen, by traveling through the brush-border membrane of the intestinal **epithelial cells**, through the cytosolic interior of these cells, and into the body fluids through the **basal lateral membranes** of the same cells. The molecular events involved need to be studied further. Figure 3.8 outlines the Ca^{2+} transport processes known or thought to occur.

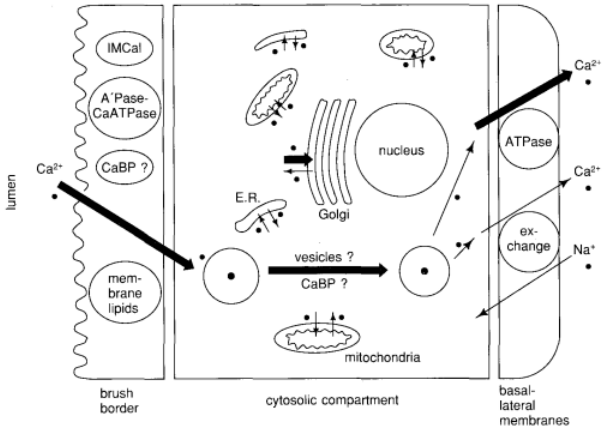

Figure 3.8 - A scheme representing some of the known and hypothetical molecular participants in the transport of Ca^{2+} across intestinal epithelial cells. Transport across the brush-border membrane is generally assumed to be passive or to be facilitated by a carrier (IM Cal), and is also influenced by vitamin D. Transport through the cell may be in vesicles and/or in association with Ca^{2+}-binding proteins (CaBP), notably calbindins D_{9k} (mammals) or D_{28k} (avians). Temporary storage or buffering of Ca^{2+} may be through cytosolic CaBPs, mitochondria, endoplasmic reticula (ER), or other organelles. Transport of Ca^{2+} out of the cell through the basal-lateral membranes is energetically uphill, and appears primarily accomplished by a Ca^{2+}-ATPase and possibly to some extent by a Na^{2+}-Ca^{2+} antiport. Adapted from Reference 35.

Transfer through the brush-border membrane is assumed to be "passive" although indirectly facilitated by calcitriol. The calcitriol effect may be due to synthesis of a carrier protein,[35] but could also be an effect of altered membrane lipid composition. [36] The fate of Ca^{2+} ions, once inside the epithelial cell, is a much-debated subject. What appears clear is that the Ca^{2+} ions entering through the brush-border membrane do not cause an increase of the low cytosolic Ca^{2+} concentration. It is thus quite likely that the Ca^{2+} ions are carried through the cell but the means of transportation is unknown. One plausible carrier is the intracellular low-molecular-weight Ca^{2+}-binding protein calbindin D_{9K} ($M_r \approx 9$ kDa) formerly known as ICaBP (see Section V.C).[35] Its synthesis is induced by vitamin D, and it is mainly found in mammalian intestines. The porcine and bovine calbindin D_{9K} has a Ca^{2+} binding constant of $K_B \approx 3 \times 10^8\ M^{-1}$ in low ionic strength media[37] and $K_B = 2 \times 10^6\ M^{-1}$ in the presence of 1 mM Mg^{2+} and 150 mM K^+.[38] The concentration of calbindin D_{9K} in epithelial cells can reach millimolar levels,[35] which could allow it to facilitate Ca^{2+} diffusion across the cytosol. This was first suggested by Williams, subsequently elaborated by Kretsinger *et aI.* in 1982,[39] and later demonstrated in a model cell by Feher.[40] The basic idea is that, although the diffusion rate of Ca^{2+} ions ($\sim 10^{-5}\ cm^2\ s^{-1}$) is higher than for the $(Ca^{2+})_2$ calbindin complex ($\sim 0.2 \times 10^{-5}\ cm^2\ s^{-1}$), the fact that the concentration of the latter complex may be about 10^3 times higher than that of free Ca^{2+} will result in an increased net calcium transport rate. Calbindin would, in fact, act very much like myoglobin in facilitating oxygen transport through muscle tissue.

Plausible as the above mechanism may seem, it may, however, not be the whole truth. An alternative mechanism is vesicular transport. In chicken intestine it has been shown that the only epithelial organelles that increased in Ca^{2+} content as a result of calcitriol treatment were the lysosomes.[41] The result lends support to a transport mechanism involving Ca^{2+} uptake across the brush-border membrane by **endocytic vesicles**, fusion of these vesicles with lysosomes, and possibly also delivery of Ca^{2+} to the basal lateral membrane of the epithelial cell by **exocytosis**. This process would also explain the vitamin-D-induced alterations in brush-border-membrane lipid compositions as a consequences of preferential incorporation of certain types of lipids into the vesicles. Interestingly, the lysosomes in the chicken studies also contained high levels of calbindin D_{28k}—a type of vitamin-D-induced Ca^{2+}-binding protein found in avian intestines—making it conceivable that this protein acts as a "receptor" for Ca^{2+} at the brush-border membrane and upon Ca^{2+} binding could become internalized in endocytic vesicles.[41]

The basal lateral plasma membrane contains at least two types of Ca^{2+} pumps that also may play a role in Ca^{2+} uptake, one ATP-driven, one driven by a concurrent flow of Na^+ ions into the cytoplasm (i.e., a Na^+-Ca^{2+} **antiport**; see Figure 3.8). We discuss these types of transporting proteins in the next subsection.

There are some apparent analogies between intestinal Ca^{2+} transport and that occurring in the placenta. Transplacental movements of Ca^{2+} increase dramatically during the last trimester of gestation.[42] In mammalian placental **trophoblasts**, high concentrations of calbindin D_{9K} are found.[43,44] The protein synthesis also in this tissue appears to be under calcitriol regulation. Ca^{2+} ions have to be supplied by mammalian females, not only to the fetus during pregnancy, but also to the newborn child through the mother's milk. The molecular details of Ca^{2+} transport in the mammalian glands have not been extensively studied. In milk, Ca^{2+} is bound mainly to micelles of casein, and the average Ca^{2+} content is reported to be 2.5 g/liter (see Table 3.1).
