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Role of Band 3 in Homeostasis and Cell Shape

Minireview

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Two important functions of membrane proteins are to regulate the internal cellular environment and to determine cell shape. How membrane proteins regulate homeostasis in the cell and throughout the whole organism is a fundamental question of cellular physiology. How membrane proteins anchor the cytoskeleton to regulate cell morphology remains a question of keen interest. The investigation of the erythrocyte anion exchange protein, band 3 (also called AE1) has been at the center of these two questions for the last several decades (reviewed in Jay and Cantley, 1986; Tanner, 1993). Recent studies by Peters et al. (in this issue) and by Inaba et al. (1996) have addressed the importance of band 3 in homeostasis and cell shape. A third study by Sekler et al. (in this issue) implicates the homologous nonerythroid anion exchanger AE2 in a particular role in pH regulation.

Band 3

Band 3, a 95 kDa integral membrane protein, was extensively characterized in early investigations of membrane proteins because it is very abundant (1.2×10^6 copies per cell) and easily purified (Fairbanks et al., 1971). Its biochemical and biophysical characterization (reviewed in Jay and Cantley, 1986) coupled with the early determination of its sequence (Kopito and Lodish, 1985) have made band 3 an excellent model for studying membrane protein structure and function.

There are two putative functions of band 3: anion exchange is carried out by the multi-spanning integral membrane domain and cytoskeletal proteins are bound by the cytoplasmic domain (see Figure 1). Potent inhibitors of anion exchange, including the stilbene disulfonates, allowed assignment of the anion exchange function to band 3 (Cabantchik et al., 1974; Ho and Guidotti, 1975). It carries out passive exchange of HCO_3^- for Cl^-, and is thought to facilitate CO_2 removal and to increase the total CO_2 capacity of blood. One site of Band 3 expression is in the kidney where its anion exchange activity may have a role in pH regulation of blood. Band 3 may also function in the erythrocyte's biconcave shape and resilience to shear force. Band 3 was shown to bind to spectrin, the internal scaffold for erythrocyte shape, via ankyrin, suggesting that band 3 acts in membrane-cytoskeletal interaction to define erythrocyte shape and stability (reviewed in Branton et al., 1981).

The investigation of band 3 has provided much of our basic knowledge of membrane proteins, but its functional relevance to the physiology of the red blood cell and the whole organism has remained unclear. This has now been addressed by studying animals in which band 3 is completely deficient using a gene deletion in mouse (Peters et al., 1996) and a preexisting strain of cattle (Inaba et al., 1996). They used these animals to test the requirement for band 3 in normal physiology (by measuring blood gases and acidosis) and its putative functions in the red blood cell (anion exchange, cell shape, and membrane stability).

Band 3 Deficiency Causes Anemia but Is Nonlethal

The most surprising result of both of these studies is that animals can survive to adulthood in the absence of band 3. They suffer from chronic hemolytic anemia (severe for mice, moderate for cattle) resulting in high rates of neonatal death (higher for mice than cattle). The adult mice examined showed a high rate of hematopoesis which may be a compensatory mechanism for the mice to survive. To address the relative importance of anion exchange and cytoskeletal binding, future work should include experiments using transgenes that encode either the membrane domain or cytoplasmic domain to rescue the observed phenotypes.

CO_2 and pH Regulation

Both studies showed that stilbene disulfonate-inhibitable anion exchange was almost completely absent. Whether there is a stringent requirement for anion exchange in eythrocytes remains an open question. AE2 and AE3 did not increase in band 3^- murine red blood cells, but other anion exchangers were not examined. Neither group measured total CO_2 in erythrocytes which

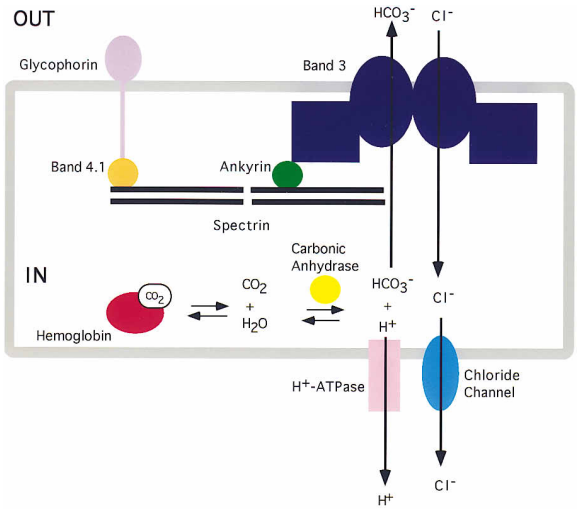

Figure 1. The Interactions of Band 3 in Cell Shape, Anion Exchange, and pH Regulation

Band 3 exists as a homodimer/homotetramer mixture in the membrane, each subunit composed of a multispanning membrane domain that functions in anion exchange and a cytoplasmic domain that binds to ankyrin. Ankyrin in turn binds to the spectrin network which can also attach to the membrane via band 4.1 and glycophorin. The anion exchange function of band 3 is thought to facilitate gas exchange in the erythocyte by allowing bicarbonate out in exchange for chloride. During gas exchange, CO_2 is released from hemoglobin and converted to bicarbonate by carbonic anhydrase-catalyzed hydration. As bicarbonate is much more soluble than gaseous CO_2, this process increases the steady state load of total CO_2 that can be carried by the blood. A function for anion exchange in the kidney is pH regulation of the blood and urine by the asymmetric transport of bicarbonate by band 3 (also called AE-1) and the proton generated during CO_2 hydration by a H^+-ATPase (found in the kidney but not the erythocyte) into the blood and lumen respectively. A kidney chloride channel maintains the charge balance and internal chloride concentration.

would be expected to be higher if anion exchange was absent.

The band 3-deficient cattle had slight acidosis (−0.15 pH units) that was more pronounced upon exercise or acid load by diet. Their blood bicarbonate concentration and total CO_2 were lower than control levels but within a normal range. As CO_2 saturation of blood is rarely reached, it is likely that the additional CO_2 load facilitated by band 3 is probably not critical except under high stress. Thus Inaba's findings are not inconsistent with the proposed roles for band 3 in anion exchange, but do suggest there are multiple strategies for CO_2 and pH homeostasis as the defect was not more severe. A better candidate for pH regulation in nonerythroid cells is the homologous anion exchanger AE2. Sekler et al. (1996) showed that the anion exchange activity of AE2 is steeply sensitive to intracellular pH in contrast to band 3. This sensitivity is due to a cluster of histidine residues found on the cytoplasmic domain that was previously thought to be functionally independent of the membrane domain.

Band 3 and Cell Shape

The most obvious cellular phenotype in both the cattle and mouse studies were erythrocytes that are small, round, and fragile; such cells are typical in hereditary spherocytosis (HS) patients. These cells spontaneously shed membrane vesicles and tubules. That the band 3-deficient red cells exhibited symptoms of HS was not unexpected; even a 20% reduction of band 3 expression results in this phenotype (Lux and Palek, 1995). The comparison of the partial and complete deficiencies of band 3 raises two complementary questions. Why does complete loss of band 3 not result in a more severe phenotype and why is a 20% deficiency of band 3 sufficient to generate the cellular morphology changes even though band 3 is present in 10-fold excess to ankyrin? This latter question is complicated because we do not know what the binding constants for band 3 and ankyrin are in vivo nor how oligomerization affects these interactions. In fact, to my knowledge, we don't know this value for any protein interaction inside cells and generally extrapolate it from in vitro biochemical studies.

The causes of the membrane loss and shape change are unclear. The band 3 deficiency results in the loss of a major structural component representing 10% of the surface area and 25% of the total protein of the membrane. This alone could result in membrane loss and fragility that, in turn, may be sufficient to explain the round shape. A limiting membrane surface area could override membrane–cytoskeletal interactions that are thought to be required for the erythrocyte's biconcave shape (Elgsaeter et al., 1986). Alternatively, the loss of specific cytoskeletal or membrane attachments to band 3 could change morphology or structural stability (Elgsaeter et al., 1986).

The Peters et al. and Inaba et al. studies are at odds concerning the association of cytoskeletal proteins with membranes from band 3-deficient erythrocytes. Peters and coworkers reported relatively normal levels of spectrin and ankyrin (but the absence of band 4.2), normal biosynthesis of spectrin, and normal cytoskeletal structures underlying the membrane. The absence of mouse band 3 does not appear to affect the association of spectrin with the membrane. This may be because cytoskeletal binding via glycophorin and band 4.1 is sufficient to maintain membrane integrity but cannot alone maintain the biconcave shape. In contrast to Peters and coworkers, the Inaba group showed diminished levels of spectrin, actin, and ankyrin, in addition to the absence of band 4.2. Their electron microscopic analysis revealed abnormal underlying cytoskeletal structure.

This difference between the two studies needs to be resolved. Further work is required to investigate the binding properties for mouse and bovine band 3 and glycophorin to their respective cytoskeletal components. Measuring changes in glycophorin–band 4.1–spectrin interactions in response to the loss of band 3 could address the importance of cytoskeletal–membrane contact in determining cell shape and perhaps explain the differences in severity of hemolysis and neonatal mortality between the mouse and cattle studies.

Conclusions

What have these studies told us about the roles of band 3 in cellular physiology? The absence of band 3 leads to predictable physiological and cellular changes based on the functions postulated from biochemical analysis. That these effects are not more severe is a testament to the complex regulation of homeostasis, often by multiple pathways. We are used to thinking about cellular processes as the action of key proteins in single linear pathways. Perhaps a better model is the spider web in which single strands can be broken with minimal effects on fly-catching efficiency. While this complexity is problematic for those of us who study cells by selective loss of function, we should be grateful for the robustness that it provides.

Selected Reading

Branton, D., Cohen, C.M., and Tyler, J. (1981). Cell *24*, 24–32.

Cabantchik, Z.I., and Rothstein, A. (1974). J. Membr. Biol. *15*, 207–226.

Elgsaeter, A., Stokke, B.T., Mikkelsen, A., and Branton, D. (1986). Science *234*, 1217–1223.

Fairbanks, G., Steck, T.L., and Wallach, D.F. H. (1971). Biochemistry *10*, 2606–2617.

Ho., M.K., and Guidotti, G. (1975). J. Biol. Chem., *250*, 675–683.

Inaba, M., Yawata, A., Koshino, I., Sato, K., Takeuchi, M., Takakuwa, Y., Manno, S., Yawata, Y., Kanzaki, A., Sakai, J., Ban, A., Ono, K., and Maede, Y. (1996). J. Clin. Invest. *97*, 1804–1817.

Jay, D.G., and Cantley, L.C. (1986). Ann. Rev. Biochem. *55*, 511–538.

Kopito, R.R., and Lodish, H.F. (1985). Nature *316*, 234–238.

Lux, S.E., and Palek, J. (1995). In Blood: Principles and Practice of Hematology, Handin, R.I., Lux, S.E., Stossel, T.P. eds. (Philadelphia: JB Lippincott Co.), pp. 1701–1818.

Peters, L.L., Shivdasani, R.A., Liu, S.-C., Hanspal, M., John. K.M., Gomzalez, J.M., Brugnara, C., Gwynn, B., Mohandas, N., Alper, S.L., Orkin, S.H., and Lux, S.E. (1996). Cell *86*, this issue.

Sekler, I., Kobayashi, S., and Kopito, R.R. (1996). Cell *86*, this issue.

Tanner, M.J.A. (1993). Semin. Hemat. *30*, 34–57.