Cell activation

The 'basic' connection

from Walter F. Boron

CHARACTERIZATION of a new biological process almost always includes a description of how it is influenced by changes in pH. In the case of the processes controlled by the enzyme protein kinase C, the tables have been turned; current emphasis is placed on how activation of this kinase affects the pH within the cell (pH_i). Any process that alters pH_i is of potential interest inasmuch as a shift in pH_i could act as a signal to modulate a host of pH-sensitive processes. Activation of protein kinase C is of special interest, because it is believed to mediate the intracellular effects of a wide variety of extracellular signals. On page 371 of this issue Moolenaar *et al.* present evidence that activation of protein kinase C stimulates the plasma membrane Na–H exchanger — which is responsible for the exchange of sodium ions for hydrogen ions across the outer membrane of cells — and thereby leads to an elevation of pH_i in human fibroblasts, HeLa cells and mouse neuroblastoma cells[1]. Grinstein *et al.* have reached similar conclusions in experiments on rat thymic lymphocytes[2]. The implication of these data is that the intracellular alkalinization induced by the activation of protein kinase C is an important step in the process by which certain extracellular signals elicit their physiological effects.

The Na–H exchanger ultimately affected by protein kinase C is normally involved in the active regulation of pH_i, a housekeeping function performed by all studied animal cells except the erythrocyte. The fundamental acid–base problem faced by these cells is their chronic tendency towards intracellular acidosis, usually caused by the passive influx of acids, such as H^+, or efflux of bases, such as HCO_3^-, but sometimes caused by the metabolic generation of acid. Uncontrolled falls of pH_i are prevented by ion-transport systems that have the effect of extruding acid from the cell. The invertebrate system appears to exchange external Na^+ and HCO_3^- for internal Cl^- and H^+, whereas most vertebrate cells use a transporter that exchanges external Na^+ for internal H^+. Both acid-extrusion mechanisms are ideally suited to their role by being virtually inactive at high pH_i values, and progressively stimulated as pH_i falls below a certain 'threshold'[3]. This is at least in part due to an intracellular H^+-binding modifier site, distinct from the site that binds H^+ for transport[4,5]. The first evidence for modulation of a pH_i regulator was that cyclic AMP stimulates the Na^+/HCO_3^- – Cl^-/H^+ exchanger of barnacle muscle[4]. Part of the action of parathyroid hormone on the renal proximal tubule may be due to the inhibition of Na–H exchange by cyclic AMP[5].

A possible role for Na–H exchange in the regulation of cell growth by extracellular signals was suggested by experiments in which exposure of quiescent Swiss 3T3 cells to a combination of platelet-derived growth factor (PDGF), vasopressin and insulin, caused a Na^+-dependent rise in pH_i of about 0.15, as well as a marked stimulation of Na^+ uptake[7]. Subsequently, epidermal growth factor (EGF) was shown to increase pH_i by 0.1 – 0.2 within ten minutes, but not in the presence of amiloride, an inhibitor of Na–H exchange[8,9]. Activation of the quiescent cells culminates in cell division several hours later. The notion that an early rise of pH_i plays a role in triggering the cell into division is supported by the observation that the degree of intracellular alkalinization induced by growth factors closely parallels the subsequent stimulation of DNA synthesis[10]. Thus, the Na–H exchanger, via its effect on pH_i, may be a critical regulator of cell growth.

The cascade of events by which growth factors stimulate Na–H exchange may be as follows: a growth factor, such as EGF[11] or PDGF[12], binds at the plasma membrane to a specific receptor (that also has the activity of phosphorylating proteins at tyrosyl residues). A consequence of the binding is the release of Ca^{2+} and increased breakdown of inositol phospholipids. The latter results in the production of diacylglycerol, a potent activator of protein kinase C[13], which phosphorylates proteins, including the receptor for EGF (see ref. 14), at seryl and threonyl residues. Finally, as indicated by the new data of Moolenaar *et al.*[1], protein kinase C somehow stimulates the Na–H exchanger.

Certain phorbol ester tumour promoters that bear a structural similarity to diacylglycerol are commonly used to stimulate protein kinase C in laboratory investigations. Moolenaar *et al.* show that one such phorbol ester, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), but not a biologically inactive analogue, causes pH_i to increase by about 0.15 within ten minutes, much as it changes after direct stimulation of fibroblasts by polypeptide growth factors. The TPA-induced increase of pH_i is Na^+ dependent and is blocked by a potent amiloride analogue, indicating that it is caused by stimulation of a Na–H exchanger. Furthermore, TPA does not induce a pH_i increase in fibroblasts that have already been stimulated by PDGF. Inasmuch as TPA bypasses the growth-factor receptor/kinase step of the proposed cascade, a simplistic view would be that stimulation of the Na–H exchanger does not require the release of either Ca^{2+} or diacylglycerol *per se*, but only the activation of protein kinase C. Indeed, the observations of Moolenaar *et al.* on Na–H exchange, as well as those of others on secretory processes (see ref. 15), indicate that TPA can produce its pharmacological effect without a measurable rise of intracellular Ca^{2+}.

The simplest explanation for the effects of TPA on pH_i, and the one proposed by Moolenaar *et al.*, is that protein kinase C directly phosphorylates the Na–H exchanger. This remains to be established. The kinetic effect of TPA on the Na–H exchanger could be to increase its pH_i threshold — that is to increase the value to which pH_i must rise before the Na–H exchanger shuts off — and/or to increase its sensitivity to acidosis once pH_i falls below the threshold. Moolenaar *et al.* favour the former. This is an attractive possibility, although it is easy to imagine how phosphorylation near the intracellular modifier site could alter either the pH_i threshold or the sensitivity of the Na–H exchanger.

Finally, it is intriguing to speculate on the possible physiological role of the stimulated Na–H exchanger. Most of the experiments on the effects of growth factors on pH_i have been conducted in HCO_3^--free media. Elsewhere, Moolenaar *et al.* have reported that unstimulated human fibroblasts do not have a detectable HCO_3^- transport system[16]. However, L'Allemain *et al.*[10] report that, whereas a functional Na–H exchanger is necessary for stimulating the mitogenesis of Chinese hamster lung fibroblasts that are incubated in HCO_3^--free media, it is not necessary when the cells are in HCO_3-containing media. Is the Na–H exchanger in these hamster cells functionally replaced by a HCO_3^- transport system? Other, more general questions arise. Is a sudden increase of pH_i really necessary to trigger cell growth? Or do growth factors stimulate Na–H exchange for other reasons, such as to enhance the housekeeping ability of the cell to regulate pH_i in anticipation of the increased requirements of the cell on metabolic activation? □

1. Moolenaar, W.H., Tertoolen, L.G.J. & de Laat, S.W. *Nature* **312**, 371 (1984).
2. Grinstein, S., Cohen, S., Goetz, J.D., Rothstein, A. & Gelfand, E. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
3. Boron, W.F. *J. membrane Biol.* **72**, 1 (1983).
4. Boron, W.F., Russell, J.M., Brodwick, M.S., Keifer, D.W. & Roos, A. *Nature* **276**, 511 (1978).
5. Aronson, P., Nee, J. & Suhm, M. *Nature* **299**, 161 (1982).
6. Kahn, A.M., Dolson, G.M., Bennett, S.C. & Weinman, E.J. *Kidney Internat.* **25**, 289 (1984).
7. Schuldiner, S. & Rozengurt, E. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7778 (1982).
8. Moolenaar, W.H., Tsien, R.Y., van der Saag, P.T. & de Laat, S.W. *Nature* **304**, 645 (1983).
9. Rothenberg, P., Glaser, L., Schlesinger, P. & Cassel, D. *J. biol. Chem.* **258**, 12644 (1983).
10. L'Allemain, G., Paris, S. & Pouyssegur, J. *J. biol. Chem.* **259**, 5809 (1984).
11. Cohen, S., Carpenter, G. & King, L., Jr. *J. biol. Chem.* **255**, 4834 (1980).
12. Heldin, C.-H., Ek, B. & Ronnstrand, L. *J. biol. Chem.* **258**, 10054 (1983).
13. Nishizuka, Y. *Nature* **308**, 693 (1984).
14. Hunter, T. *Nature News and Views*, **311**, 414 (1984).
15. Baker, P.F. *Nature News and Views* **310**, 629 (1984).
16. Moolenaar, W.H., Tertoolen, L.G.J. & de Laat, S.W. *J. biol. Chem.* **259**, 7563 (1984).

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