BRIEF COMMUNICATIONS

A POSSIBLE MECHANISM FOR CONCENTRATING SODIUM AND POTASSIUM IN THE CELL NUCLEUS

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ABSTRACT A dynamic, nonequilibrium mechanism is proposed for concentrating both Na^+ and K^+ in the cell nucleus. The model is consistent with experimental observations and with known properties of cell membranes. This model could explain the high nucleoplasm to cytoplasm ratios of Na^+ and/or K^+ reported for liver, kidney, thymus, frog skin, ascites cells, and amphibian oocytes.

The nucleus of a variety of cells appears to contain considerably higher levels of Na^+ than the cytoplasm (1–9). This nuclear Na^+ does not equilibrate freely with that in the cytoplasm and does not seem to be maintained by an energy-requiring process (5). Cells and tissues reported to have a high nuclear Na^+ concentration include amphibian oocytes (1, 2), liver (3–5), kidney (3, 6, 7), thymus (3), frog skin (6), and Ehrlich ascites cells (9). In addition, where studied, these nuclei also contained higher levels of K^+ than the cytoplasm. One recent report (10) confirmed the high gradient between nucleoplasm and cytoplasm in oocytes but found a low nuclear Na^+ concentration. However, since oocyte Na^+ concentrations change markedly during meiotic maturation (11, 12), the two groups may have been using different stages of oocyte maturation.

Nuclear, cytoplasmic, and extracellular Na^+ and K^+ are compared in liver and amphibian oocytes in Table I. The concentration of Na^+ in the nucleus is considerably higher than in the cytoplasm; indeed in the liver it approaches, and in the oocyte exceeds, the extracellular concentration of Na^+. The concentration of K^+ within the nucleus is markedly higher than either cytoplasmic or extracellular levels of K^+ in both systems. Where measured (2, 3, 10), the nucleoplasm was more hydrated than the cytoplasm.

A number of explanations have been offered for the high nuclear Na^+ levels, but the various authors have not attempted to explain *both* Na^+ and K^+ concentrations. The

TABLE I

SODIUM AND POTASSIUM CONCENTRATION GRADIENTS ACROSS THE PLASMA AND NUCLEAR MEMBRANES

Tissue	Ion	Extracellular	Cytoplasm (C)	Nucleoplasm (N)	N/C	Ref.
			millimoles/liter water or cell water			
Liver	Na^+	145	10	131	13.1	5
	K^+	5	163	265	1.63	
Oocyte	Na^+	120	88	281	3.19	2
	K^+	2	106	258	2.43	

explanations for high nucleoplasmic Na^+ concentrations fall into three general classes: (*a*) selective binding of Na^+ to nuclear polyanions such as RNA and DNA (3, 5, 8); (*b*) active transport of Na^+ into the nucleus from the cytoplasm (8); and (*c*) a direct connection between the nucleus and the extracellular compartments (2, 5, 8). A possible contribution from a Donnan-type equilibrium has not been mentioned, but should be considered.

Isotopic Na^+ exchange studies indicate that, at least for liver or oocytes, nuclear ion binding is minimal and would not account for the high Na^+ levels. In the case of liver cells, Langendorf and co-workers (4) and Siebert and co-workers (5) have shown that radioactive Na^+ injected in vivo equilibrates with the nuclear Na^+ within 10 min. More recently, Century and Horowitz (13) reported that in the amphibian oocyte nuclear Na^+ exchanged very rapidly ($t_{1/2}$ = 53 min) when compared with cytoplasm ($t_{1/2} \sim 2$ days). The greater hydration of the nucleoplasm compared with the cytoplasm would also suggest that a significant fraction of the nuclear Na^+ and K^+ is osmotically active and therefore unbound.

Little information is available on the active transport of either Na^+ or K^+ ions across the nuclear membrane. Siebert and co-workers (5) reported that neither Na^+ content nor isotopic Na^+ exchange in in vivo liver nuclei was affected by cooling or by metabolic inhibitors. Although these results do not conclusively rule out an active transport system, they did suggest to these workers (5) that Na^+ exchanged via channels or direct connections between the nucleus and extracellular space.

A connection between the outer nuclear membrane and the membranes of the endoplasmic reticulum has been noted in a variety of cells (8, 14–19). Lowenstein and Kanno (20, 21) have suggested that such a connection might explain the discrepancy between the unusually high capacitance (200 $\mu F/cm^2$) which they observed between nucleoplasm and cytoplasm and the capacitance (1 $\mu F/cm^2$) universally observed in other biological membranes (22). In addition, the occasional observations (8, 14) of continuity between the endoplasmic reticulum membranes and the plasma membrane indicates a possible connection between the perinuclear space and the extracellular space. Several workers have published micrographs demonstrating continuity of the

endoplasmic reticulum from its junction with the plasma membrane to its junction with the outer nuclear membrane (8, 18).

A direct connection could explain the rapid exchangeability of nuclear Na^+ with that in the extracellular space (1, 2, 13) and could also explain the observation that many substances taken up by the amphibian oocyte accumulate in the nucleus (2, 23). These include: leucine, alanine, phosphate, sulfate, as well as ions such as Na^+ and K^+. The existence of a short-circuit connection between nucleoplasm and extracellular fluid would not, however, by itself explain the apparent ability of the *in situ* nucleus to concentrate both Na^+ and K^+ against a concentration gradient. Also, a Donnan effect cannot explain the high nuclear concentrations of both Na^+ and K^+ as is indicated by the lack of agreement between the N/C ratios (Table I, refs. 3, 10, 13) for the two cations.

A possible mechanism for concentrating both Na^+ and K^+ ions in the cell nucleus is illustrated in Fig. 1. The model presented is consistent with the experimental data, with known properties of cell membranes, and is based on known ion regulatory mechanisms. The only assumptions or conditions of the proposed model are:

(*1*) The existence of a direct channel between the extracellular fluid and the perinuclear space (see above).

(*2*) The outer nuclear membrane is selectively permeable to Na^+. This selective permeability is analogous to that of the mucosal membrane of the toad bladder and luminal membrane of mammalian kidney (24).

In this model the primary driving force is the active transport of Na^+ at the plasma

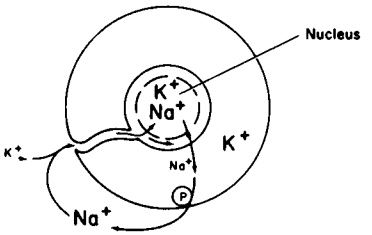

FIGURE 1 A hypothetical mechanism for concentrating both Na^+ and K^+ in the cell nucleus. The relative concentrations of Na^+ and K^+ (see Table I) in the various compartments and the direction of ion flow are indicated by the respective symbols. The conditions of the proposed model are discussed in the text. The active transport of Na^+ outward across the plasma membrane (P) would produce a negative free energy gradient for Na^+ between extracellular fluid and the cell interior resulting in inward Na^+ movement both across the plasma membrane and via direct channels between extracellular fluid and perinuclear space and then from perinuclear space to cytoplasm. Active Na^+ transport across the plasma membrane thus drives a Na^+ current loop via: cytoplasm → extracellular fluid → channel → perinuclear space → cytoplasm. It is assumed that the outer nuclear membrane is selectively permeable to Na^+; K^+ could be carried down the channels by osmotic coupling and, because of low K^+ permeability of the nuclear membrane, would tend to accumulate with Na^+ in the nucleus. In this model it is assumed that the inner nuclear membrane is freely permeable to both Na^+ and K^+ and that these ions are at equilibrium across this particular membrane. As suggested by Lowenstein and Kanno (21), the nuclear pores are assumed not to be freely permeable to ions.

membrane. The Na^+ pump maintains a low intracellular Na^+ concentration and a negative electrical potential difference between the cytoplasm and the extracellular environment. This in turn constitutes a negative free energy gradient between the external fluid and the cell interior. One pathway for the movement of Na^+ down this free energy gradient is through the plasma membrane and a second would be via the channels (assumption 1, above) into the nucleus and across the nuclear membrane (assumption 2, above) into the cytoplasm. Just as the negative free energy gradient produces a flow of Na^+ from the extracellular fluid to the cytoplasm through the plasma membrane, it would also produce a flow of Na^+ through the direct channels into the nucleus and then across the outer nuclear membrane into the cytoplasm. This Na^+ movement through the nucleus would constitute a Na^+ current-loop through the cell interior, driven by the Na^+-pump at the cell surface. A corollary to the Na^+ movement would be the bulk flow (via solvent drag) of water down the channels into the nucleus and out through the cytoplasm to the cell surface.

The free energy gradient (ΔG) between the cell surface and the nucleoplasm has been calculated as a function of varying electrical potential difference across the nuclear

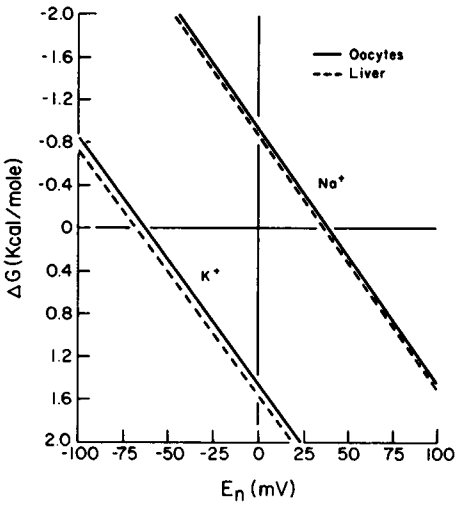

FIGURE 2 The free energy gradient (ΔG) between the extracellular fluid and nucleoplasm as a function of nuclear membrane potential (E_n) in oocytes (———) and liver (--------). The free energy gradient is defined as: $\Delta G = zF(E_m + E_n) + RT \cdot \ln(C_n/C_{ecf})$, where E_m is the plasma membrane potential (sign refers to potential at inside surface relative to outside); E_n, the nuclear membrane potential (sign refers to potential at inside surface relative to outside); E_n, the nuclear membrane potential (sign refers to potential at nucleoplasmic surface relative to cytoplasmic surface); C_n, concentration of cation in the nucleus; and C_{ecf}, concentration of cation in the extracellular fluid. The values for the concentrations of Na^+ and K^+ for oocyte and liver nuclei are taken from Table I. E_m has been reported to be -60 mV for oocyte (11), and -35 mV for liver (26). T is expressed as the absolute temperature for oocyte at 22°C (295°K) and liver at 37°C (310°K). It is assumed that the nuclear cations are unbound. Cation binding would shift the appropriate curve to the right, i.e. increase the negative free energy gradient from extracellular fluid to nucleus for that cation.

membrane. A plot based on the experimental data in Table I is shown in Fig. 2. Under conditions in which the nucleoplasm is negative with respect to the cytoplasm, and over a wide range of positive values, it can be seen that the movement of Na^+ through the channels to the nucleus is down a negative free energy gradient, i.e. spontaneous. In contrast, K^+ inward movement will be spontaneous only if the nucleoplasm is more than 60–70 mV negative relative to the cytoplasm. Thus, K^+ would not be expected to enter the nucleus under its own free energy gradient. However, under physiological conditions the concentration of Na^+ in the extracellular fluid is 25–60 times that of K^+ and the negative free energy gradient for Na^+ movement into the nucleus is likely to be large. As shown in Fig. 2, for nuclear membrane potentials less than about +20 mV, the free energy gradient for K^+ movement outward is only four to five times that of Na^+ movement inward in both liver and oocytes. Since the Na:K ratio in the extracellular fluid entering the channels varies from about 25:1 to 60:1, there is a negative free energy gradient for bulk flow into the nucleus. Thus, under conditions where the nuclear membrane is selectively permeable to Na^+ and the cytoplasmic Na^+ levels are maintained at a low value, net inward flow of K^+ could be driven (through osmotic coupling) by bulk flow and K^+ would tend to accumulate within the nucleus.

An important assumption in this model is that the outer nuclear membrane is selectively permeable to Na^+ ions (assumption 2). Other studies (25) have shown that the oocyte plasma membrane is changed from a K^+ selective system to a Na^+ selective system when extracellular Ca^{++} is removed. Thus, if the nuclear membrane is similar to the plasma membrane in this respect and since cytoplasmic Ca^{++} is largely bound or sequestered (11), one might expect the nuclear membrane to be Na^+ selective.

As described here, the net movement of Na^+ between nucleus and extracellular fluid is a nonequilibrium process. The flow of Na^+ from nucleus to cytoplasm could produce a diffusion potential across the nuclear membrane. The magnitude and direction of this diffusion potential would be a function of the difference between cation (primarily Na^+) conductance and anion (probably Cl^-) conductance of the outer nuclear membrane. The magnitude of the diffusion potential would, in addition, depend upon the driving force for Na^+ movement. The actual potential across both nuclear membranes would be the sum of this diffusion potential and any Donnan potential due to imbalance of fixed charge between nucleoplasm and cytoplasm:

$$E_{nuc} = E_{diff} + E_{Donnan}.$$

In this model, the activity of the Na^+-pump at the plasma membrane would regulate the electrical potential difference between nucleoplasm and cytoplasm. It follows from the model that the magnitude as well as the sign of the nuclear membrane potential would depend upon the difference between the diffusion potential and the Donnan potential. Thus, by altering the magnitude of the diffusion potential, changes in the activity of the Na^+-pump could not only alter the magnitude of the nuclear membrane potential, but also alter its direction.

In summary, a dynamic, nonequilibrium mechanism is proposed for concentrating both Na^+ and K^+ in the cell nucleus. The model is consistent with experimental ob-

servations and with known properties of cell membranes. The cell nucleus should be recognized as a dynamic system undergoing changes during the cell cycle, excitation, etc. Direct channels between the nucleus and extracellular fluid need not be universally present in all cell types and may exist only transiently in certain cells to serve a specific regulatory function. In the proposed model, the driving force is the Na^+-pump at the plasma membrane. As discussed above, changes in the Na^+-pump activity could alter the nuclear membrane potential as well as the nuclear ionic composition and thus play an important role in the regulation of gene activity during the cell cycle or in cellular differentiation.

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