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Insights & Perspectives

How potassium came to be the dominant biological cation: of metabolism, chemiosmosis, and cation selectivity since the beginnings of life

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Abstract

In the cytoplasm of practically all living cells, potassium is the major cation while sodium dominates in the media (seawater, extracellular fluids). Both prokaryotes and eukaryotes have elaborate mechanisms and spend significant energy to maintain this asymmetric K^+/Na^+ distribution. This essay proposes an original line of evidence to explain how bacteria selected potassium at the very beginning of the evolutionary process and why it remains essential for eukaryotes.

KEYWORDS
chromatin, histone chaperones, ion-selective channels, ionic homeostasis, ionic selectivity, membrane potential, peptide bond, replication, transcription

THE ORIGIN OF SELECTIVITY OF POTASSIUM CHANNELS

Potassium-selective cation channels are essential components both in eukaryotes and prokaryotes. Molecular dynamics (MD) studies show that the discrimination between K^+ and Na^+ in the selectivity filter of the KcsA channel does not originate from its rigid geometry but is a cumulative effect of its dynamic structure and the intrinsic properties of the peptide carbonyl groups. Here, I present experimental data that confirms this modeling result. A higher affinity of the peptide carbonyl for K^+ relative to Na^+ was detected by comparing the K^+/Na^+ selectivity of an acidic polypeptide, poly(L-glutamic acid), to that of a simpler polyanion, polyacrylic acid. The gain in free energy of K^+ versus Na^+ binding by the peptide carbonyl is small ($\delta g^K{}_{Na} = -100 \div -250$ cal/mol) but can result in large-scale K^+/Na^+ discrimination when taken collectively, like in the KcsA channel where the passage of an ion includes 32 coordination/dissociation events with the oxygen atoms of the peptide carbonyl.

K^+ channels have high permittivity as well as high selectivity

All living cells exploit membrane proteins to separate (actively and passively) potassium from sodium, which leads to an "asymmetric" distribution of these ions between the cytoplasm (rich in K^+) and the extracellular medium (dominated by Na^+). Over 20 years ago, a decisive breakthrough was made in determining the structures of the ion-selective channels,[1–3] which brought us an understanding of the mechanisms of ion selection and permeability in the channels. In the KcsA potassium channel from the soil bacterium *Streptomyces lividans*, the separation between Na^+ and K^+ is achieved at a selectivity filter; a pore lined with five layers of carbonyl groups of a peptide backbone with four carbonyls in each layer.[4,5] In the original crystallographic work[4,5] and in the following discussion (for a summary see ref. [6]), it has been postulated that the discrimination between K^+ and Na^+ ($K^K{}_{Na} > 10^3$) of the KcsA selectivity filter is the result of the precise geometry of the pore, which allows octahedral coordination to peptide carbonyls (perfect for K^+ but not for Na^+) at each of the binding sites.

This explanation, however, does not take into account the necessity for the pore to have significant flexibility to achieve high ion permeability. Mutations of the aromatic amino acids, which were considered crucial for the structure of the filter, did not show much change in the K^+ selectivity.[7] Large values of crystallographic B-factors and MD simulations of the KcsA channel confirmed that the structure of the selectivity filter is indeed variable and dynamic.[3,8]

The major challenges in solving the problem of how high potassium selectivity can combine with the dynamic structure of the selectivity filter and its high permittivity are that estimations of the cation selectivity of the pore are based on the calculation of differences in the four

free energy terms.[8,9]

$$\delta G(Na^+ \rightarrow K^+) = [G_{pore}(Na^+) - G_{pore}(K^+)] - -[G_{bulk}(Na^+) - G_{bulk}(K^+)] \quad (1.1)$$

where $G_{pore}(Na^+)$, $G_{pore}(K^+)$ and $G_{bulk}(Na^+)$, $G_{bulk}(K^+)$ are free energies of the K^+ and Na^+ interaction, respectively, with carbonyl oxygen atoms in the selectivity pore and with the bulk water. In Equation 1.1, all four values are so large that the uncertainties in their determination by most experimental and theoretical methods exceed the precision required to calculate a reliable value for the $\delta G(Na^+ \rightarrow K^+)$.

Applying *ab initio* calculations and MD simulations with a finely tuned force field, Noskov et al.[6,10,11] showed that K^+/Na^+ selectivity in the filter originates not from its static construction but from a cumulative effect of the pore structure and intrinsic dynamic and electrostatic properties of the peptide carbonyl groups forming the selectivity filter. Still, to support these theoretical results, it would be useful to get a reliable experimental estimation for the cation preferences of this structural element of the pore.

Here, I present an experimental result that demonstrates the natural higher affinity of the peptide carbonyl for K^+ relative to Na^+. Unlike the significant uncertainties in the determination of the free energy terms in Equation 1.1, counting the numbers of the bound K^+ and Na^+ ions by the atomic absorption spectroscopy method allows direct and precise determination of the cation selectivity.

Why polyglutamic and polyacrylic acids have different K^+/Na^+ selectivity?

In an earlier study,[12] we reviewed and quantified the influence of the nature of alkali metal cations on reactions and structural transitions of biological molecules. As part of that work, we carried out a number of additional experiments that gave us data that was lacking in the existing literature. Particularly, we compared K^+/Na^+ selectivity of a model anionic polypeptide, poly(L-glutamic acid) (PGA) and a simpler carboxylate polyanion, polyacrylic acid (PAA). In Figure 1a, the selectivity of PGA and PAA in dependence of ethanol concentration in H_2O/EtOH mixtures is shown (the data is from ref. [12]). The Na^+/K^+ selectivity of a polyanion, PA (PGA or PAA), of a cation exchange reaction:

$$PA^{N^-} \cdot K^+ + Na^+ \Leftrightarrow PA^{N^-} \cdot Na^+ + K^+ \quad (1.2)$$

is described by a selectivity constant, $D^{Na}{}_K$

$$D_K^{Na} = (C_{Na}/C_K)_{PA} \cdot (C_K/C_{Na})_{solvent} \quad (1.3)$$

where C_{Na} and C_K in each of the brackets are Na^+ and K^+ concentrations, respectively, bound to the polyanion and in the bulk solution. An ultrafiltration technique described in refs [12,14] was used to analyze the amounts of Na^+ and K^+ bound to the polyanion equilibrated against a 1:1 molar mixture of NaCl and KCl in the H_2O/EtOH solvent.

From 40 to 85% EtOH, the PAA shows preferable binding of Na^+, $D^{Na}{}_K \approx 1.4 - 1.6$ (Figure 1a). The preference of Na^+ over K^+ is a long-known feature of the carboxylate anion (see, e.g., [15] and references cited in[12]). In contrast, the PGA does not select between Na^+ and K^+, $D^{Na}{}_K \approx 1$ in the range 0-60% EtOH. The affinity of the PGA for Na^+ appears at EtOH > 60% and reaches $D^{Na}{}_K \approx 1.8 - 1.9$ for 75-85% EtOH. Remarkably, Na^+ selectivity of the PGA increases in parallel with the progression of a coil to α-helix structural transition that has earlier been discovered for its Na- but not K-salt[13,16] (Figure 1b; this observation has been recently confirmed[17]).

Both the PGA and PAA have negatively charged carboxylate groups that must be neutralized by a cation. The PGA has one more cation-binding site, an electronegative oxygen atom in the carbonyl group of the peptide backbone ($C^{\delta+} = O^{\delta-}$). The logical explanation for the absence of the Na^+/K^+ selectivity of the PGA at EtOH < 60% is that the Na^+ preference of the COO^- group is balanced by preferable interaction of K^+ with the carbonyl oxygen atom resulting in $D^{Na}{}_K \approx 1.0$.[12] At EtOH > 60%, the coil to α-helix transition leads to the formation of a hydrogen bond between the peptide α-amino and carbonyl groups and to the exclusion of the oxygen atom from interaction with cations (illustrated in Figure 1c). The high charge density of the PGA α-helix leads to its Na^+ selectivity being even higher than that of the less densely charged PAA.

The net selectivity of the PGA at 40-60% EtOH ($D^{Na}{}_K = 1.0$), combined with that of the PAA ($D^{Na}{}_K = 1.3\text{-}1.5$), provides an estimation for the K^+/Na^+ selectivity of the peptide carbonyl: $D^{Na}{}_K \approx 0.67–0.83$ or $D^K{}_{Na} = 1/D^{Na}{}_K \approx 1.2 - 1.5$ in the range 0 – 60% EtOH (Figure 1a). The variation of free energy in the reaction (1.2), $\delta g^K{}_{Na}$, is calculated from the experimental values of the ion-exchange equilibrium constant and plotted in Figure 1d ($\delta g^K{}_{Na} = -RT \cdot \ln D^K{}_{Na}$; T is the temperature in Kelvin, R is the gas constant). Estimated dependence of $\delta g^K{}_{Na}$ on EtOH concentration is shown in Figure 1d as red squares and line. At T = – 298 K, $\delta g^K{}_{Na} \approx -100$ cal/mol for $D^K{}_{Na} = 1.18$; and $\delta g^K{}_{Na} = -250$ cal/mol for $D^K{}_{Na} = 1.52$. These two $D^K{}_{Na}$ values correspond to the selectivity of the peptide carbonyl oxygen atoms, respectively, in water and 60-70% EtOH (Figure 1d). The conclusion is that interaction of the oxygen atom of the peptide carbonyl group with K^+ is slightly more favorable than that with Na^+, $\delta g^K{}_{Na} \approx -100 \div -250$ cal/mol. The reported $\delta g^K{}_{Na}$ range is not an error margin but an indication of the variation of $\delta g^K{}_{Na}$ in dependence of the EtOH concentration. The weakest preference for K^+ is observed in water; the peptide carbonyl shows an increase of the K^+ selectivity at the higher EtOH concentrations.

Notably, the absence or delay in the formation of the PGA α-helix in the presence of K^+, Rb^+, and Cs^+ (but not Na^+) was obtained not only in the EtOH/H_2O mixtures but several other organic solvent/water systems.[13] This observation allows for the conclusion that K^+ preference of the peptide carbonyl is rooted in the properties of this chemical entity and most certainly persists inside the selectivity pore buried in the lipid bilayers with a low dielectric constant.

In an additional note, unrelated to the main topic of this essay, I would like to indicate that specific K^+ - peptide carbonyl interaction might influence protein secondary structures *in vivo* (Box 1).

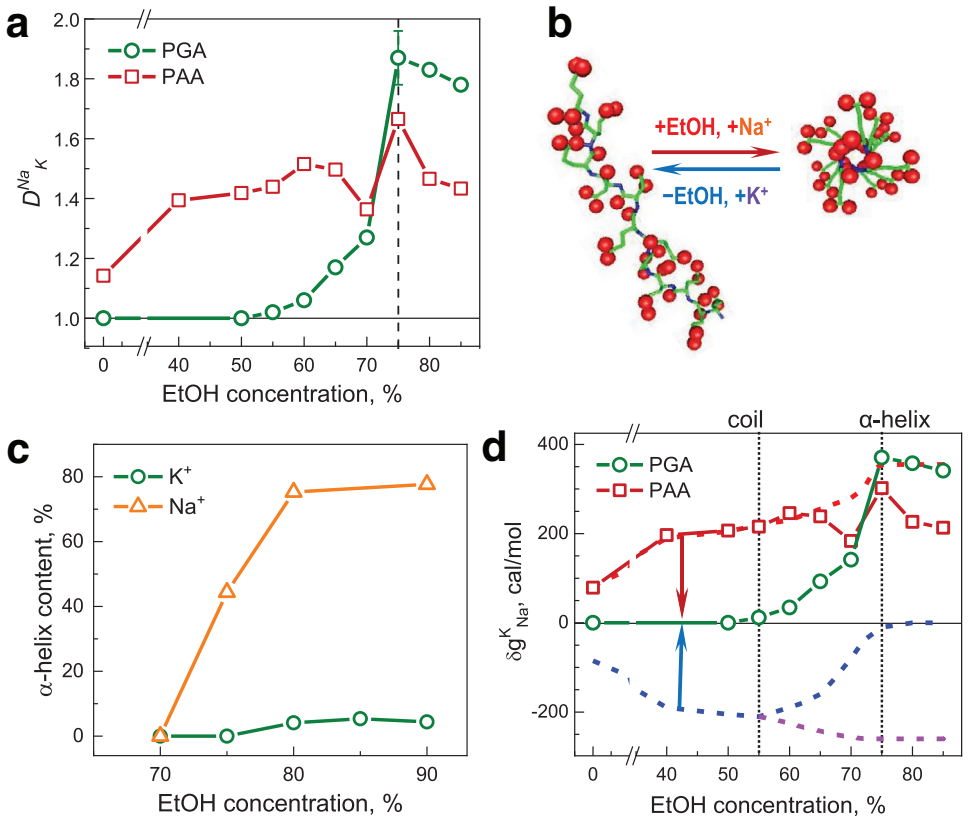

FIGURE 1 (a) The K^+/Na^+ selectivity of ion exchange (D^{Na}_{K}) depends on ethanol concentration for poly(L-glutamic) (PGA) and polyacrylic (PAA) acids. The PGA or PAA were equilibrated with a solution of KCl + NaCl (25 mM each salt), 1 mM EDTA, pH 7.6 and quantities of K^+ and Na^+ neutralising the polyanion charge that was measured by atomic absorption spectroscopy. Data from ref. [12]. (**b**) Structural presentation of the coil – α-helix transition of the PGA in ethanol/water mixtures. Electronegative oxygen atoms of the carboxylic group and peptide carbonyl are highlighted as red spheres. (**c**) Dependence of α-helix content of the PGA Na^+- and K^+-salts on EtOH concentration. Data from ref. [13] adapted with permission © John Wiley & Sons. (**d**) Interpretation of the data shown in (**a**) and (**b**) in terms of the free energy of Na^+ for K^+ ion exchange, δg^{K}_{Na}. The solid curves with points are experimental data for the PGA (green) and PAA (dark red). For the PAA, substitution of Na^+ for K^+ is unfavourable at all EtOH concentrations ($\delta g^{K}_{Na} > 0$). The free energy of PGA interaction with Na^+ and K^+(δg^{K}_{Na}) is close to 0 at EtOH concentration from 0 to 55%; K^+ binding becomes unfavourable at EtOH > 55% and coincides with the PGA coil to α-helix transition marked by vertical bars. It is suggested that two contributions, one from the side-chain carboxylate, the other from peptide carbonyl, add to the total δg^{K}_{Na} value of the PGA. The dashed blue and red lines are estimations of δg^{K}_{Na}, respectively for the carbonyl and carboxylate groups of the PGA. At EtOH < 55% the two δg^{K}_{Na} terms compensate each other as indicated by blue and red arrows. At EtOH > 55%, the PGA carbonyl group is eliminated from interaction with cations being involved in the formation of the α-helix. The dashed magenta line shows estimation for the δg^{K}_{Na} term of the free peptide carbonyl group at EtOH > 55%

Peptide carbonyl preference for K^+ is the basis of potassium channels' selectivity

The weak K^+ selectivity of the peptide carbonyl oxygen atom can, however, result in large $K/^+Na^+$ discrimination when taken collectively. Cation transfer through the selectivity pore of the KcsA channel is accompanied by coordination/dissociation with the 32 carbonyl groups. Even with some caution about the correctness of simple summation, the accumulated difference in free energy between Na^+ and K^+ upon passage through the "chromatography column" of the selectivity filter can be quite significant, δG^{Na}_{K} = 3.2 – 8.0 kcal/mol. Since the incremental accumulation of free energy leads to an exponential increase of the cation selectivity, it results in a substantial separation between K^+ and Na^+ in the pore, $K^{K}_{Na} = (D^{K}_{Na})^{32} = 3.5{\cdot}10^2 - 4{\cdot}10^5$. It is in general agreement with the experimental value $K^{K}_{Na} > 10^3$ found for the KcsA channel.[8] For the selectivity filter inside the lipid membrane, one might expect that the carbonyl selectivity corresponds to the values observed at low dielectric permittivity.

It is interesting to note that selectivity for the K^+ ions is observed not only in specialized potassium-selective channels but also in other channels that are not necessarily selective for K^+[9,18] where this is termed "equilibrium selectivity."[18] Therefore, the intrinsic minor K^+ selectivity of the peptide carbonyl group makes it a useful building block to construct a selectivity pore where the flexible spatial arrangement of several layers of the $C^{\delta+} = O^{\delta-}$ groups creates conditions for highly selective but fast potassium transport through the channel.

BOX 1: Affinity of the Peptide Carbonyl to Potassium might influence Secondary Protein Structures *In Vivo*

An interesting and important observation, although not related to the main point of this essay, is that potassium cations can block a coil — α-helix transition of the PGA. This effect was observed for pure K^+ salt of the PGA[13,16,17] (Figure 1c) . An explanation of this strong effect might be that specific K^+ association with the $C^{\delta+} = O^{\delta-}$ group blocks formation of its hydrogen bonding with the α-amino group. In water, the mutual repulsion of the negative carboxylate groups prevents PGA folding into an α-helix. The decrease of dielectric constant with increasing EtOH concentration leads to an increase of $Na^+ - COO^-$ binding, that reduces monomer-monomer repulsion and makes the formation of the α-helix favorable (Figure 1c). For the uncharged peptides where repulsion between amino acids' side-chains is absent, internal hydrogen bonding is expected to be favorable in a water solution even in the absence of EtOH (if, of course, K^+ ions are not present). The carbonyl oxygen - α-amino group hydrogen bond is responsible for the formation of the protein secondary structures, like α-helices and β-sheets. Potassium is the major cation in the cytoplasm of all living cells, which means that under the *in vivo* conditions, the degree of protein folding might be significantly different from that observed in a test tube where sodium salt is commonly used. This observation urges to use potassium, not sodium salts, to model "physiological" salt conditions *in vitro*.

The computational work[6,10,11] underlines the importance of both the coordination number and the mutual repulsion between carbonyl oxygen atoms for the selectivity of the pore. Our data allows for only a crude estimation of the selectivity of peptide carbonyl; hence, other methods need to be applied to disclose the details of this phenomenon. Nevertheless, the theoretical work[6,10,11] shows that the K^+ affinity of the carbonyl group persists even when the coordination number is below its optimal value of eight. MD and ab initio modeling[6,10,11] also predicts an inversion of the $K/^+Na^+$ selectivity ($D^{Na}{}_K > 1$) upon an increase of the $C^{\delta+} = O^{\delta-}$ polarity that is in agreement with a Na^+ affinity of the more highly charged carboxylate group. To some extent, our experimental setup with the PGA as polyanion replicates the "toy models" described by Noskov et al.[6,10,11] These authors analyzed multiple structural arrangements that are possible for the K^+ and Na^+ ions coordinating oxygen atoms. In the PGA/PAA/K^+/Na^+ system studied in our work,[12] various combinations of peptide carbonyl and carboxylate groups interacting with K^+ and Na^+ under different conditions were experimentally tested.

An essential flaw of the presented experiment is that the K^+ affinity of the peptide carbonyl was suggested based on comparisons of the two polyelectrolytes, with, and without, the peptide bond. This begs the question of the possibility to measure ionic selectivity of the polar groups in the neutral polypeptide. In our experiment, the COO^- anion is required to get the polypeptide negatively charged, so a cation must neutralize this charge. This method of direct analysis of the alkali cations cannot be used for the uncharged peptides since after equilibration with the mixture of salts followed rinsing with water will wash all the ions. Therefore, one needs to invent some other technique for counting the ions in the vicinity of the partially charged groups. To my knowledge, no reliable method for this kind of measurements currently exists. Probably, an NMR relaxation method that has been applied for DNA using ^{23}Na relaxation[19,20] can be suitable. However, the sensitivity of this method might be insufficient to detect the minuscule differences expected in the neutral peptides. An interesting endeavor would be to study the ionic selectivity by combining negatively charged (Asp and Glu) amino acids with neutral ones in the protein sequence, thus keeping some negative charge of the polypeptide, while having an increased number of peptide carbonyl groups.

In conclusion, condensing the previously discussed features into a conceptual whole, potassium channels and pumps are the principal components of all living cells; the peptide carbonyl group with intrinsic K^+ versus Na^+ preference provided the Nature with a readily available building block for constructing cation channels that are highly selective for potassium.

THE MECHANISM OF POTASSIUM SELECTION BY BACTERIA

"*E. coli*, like other bacteria and cells in general, accumulate K^+ ions and exclude Na^+. Just why they do this has never been quite resolved, perhaps because there is no single simple answer that would satisfy our penchant for linear thinking."[21] In this section of the essay, I demonstrate that cation-conductive channels are an absolute necessity for all bacteria, which use proton motive forces to produce ATP and for other cellular functions. It is shown that even a single cation channel can protect the cell membrane from dangerous jumps of the electric potential caused by minor misbalances in the H^+ influx and efflux. I also show that cation channels, which exploit oxygen atoms of the peptide carbonyl to dehydrate cations and to prevent protons from unproductive leaking, have an intrinsic ability to separate K^+ against Na^+ in addition to their protective function. The potassium selectivity of these channels results in an instant K^+ enrichment of the cytoplasm in all chemiosmosis-driven bacteria.

Protecting the cell from membrane potential fluctuations produces cation specificity

Chemiosmosis[22,23] (Figure 2) is a universal mechanism used by all living cells for the transformation of energy from biochemical reactions or from the Sun to the common energy carrier, ATP. This drives essentially all cellular processes (synthesis and degradation of the biomolecules, transmembrane transport, motility, etc.). Like the central dogma of biol-

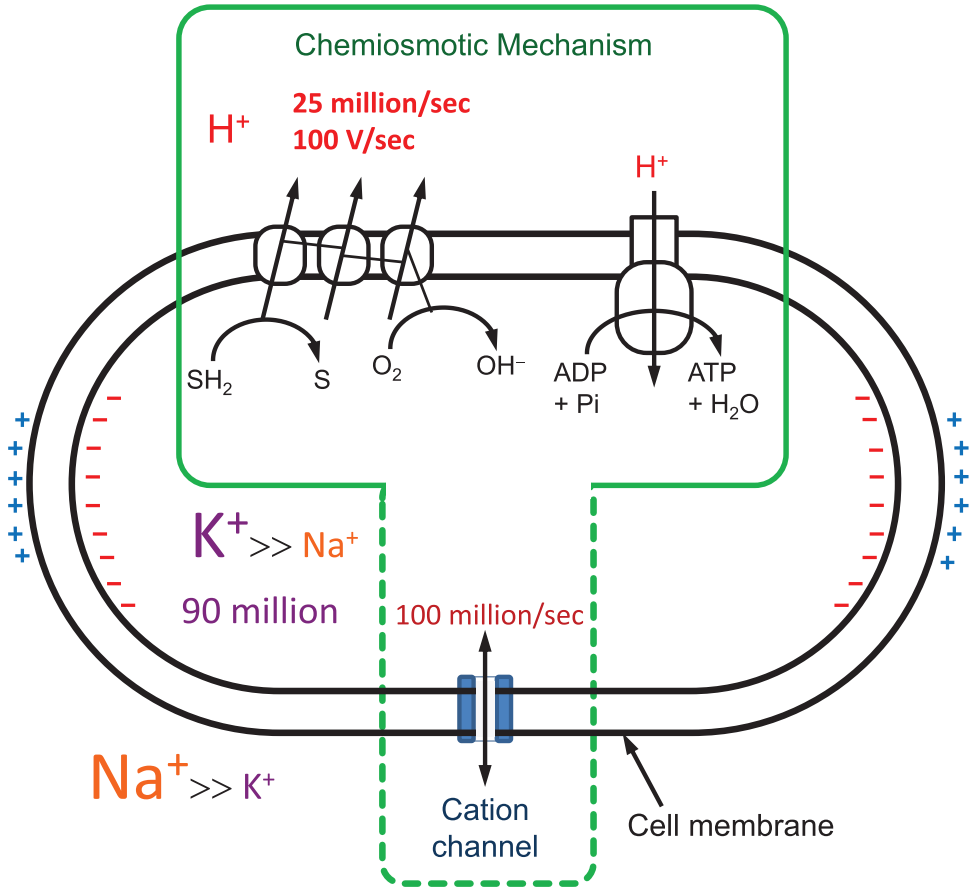

FIGURE 2 Principal components of the bacterial energy conversion cycle. The green frame at the top of the figure displays canonical elements of the chemiosmotic mechanism, cytochrome oxidases (left) and ATPase (right) that create and consume the electrochemical proton gradient. The lower part of the illustration shows cation conducting channel and the asymmetric distribution of Na^+ and K^+ ions observed in most living cells. This work demonstrates that cation channels must be considered as an integral part of the chemiosmotic machinery, and that enrichment of the cytoplasm in K^+ is a natural consequence of the cation's channel operation (indicated by a dashed green frame)

ogy (DNA → RNA → protein), the chemiosmotic coupling of the proton transfer across the cell membrane to the generation of the ATP by the ATP synthase is considered as one of the universal principles inherent to every living cell. It is even possible that chemiosmosis was the true origin of life on this (and other) planet(s).[24,25]

However, most of the living organisms have one more universal feature that is rarely acknowledged - the selection of potassium as a major monovalent cation in the cytoplasm. Living cells have developed numerous K^+-selective channels, porters, and sodium-potassium pumps.[26,27] They spend significant energy on creation and maintenance of high K^+ concentration inside, against the continuous invasion of very similar sodium ions that are overwhelmingly dominant in typical extracellular media like seawater or blood serum. There are several justifications for such an asymmetric distribution between K^+ and Na^+, for example, its importance for osmotic regulation, maintenance of membrane potential and other functions (see insightful analysis in refs[28–30]). Still, the reason(s) for a universal enrichment of cell cytoplasm in potassium remains enigmatic.[21,31] It challenges life scientists to search for a single explanation for an origin or reason for this phenomenon, and few such hypotheses have been proposed. One of the hypotheses claims that K^+ was abundant in underwater thermal vents that were the cradle for life[32] and now-living cells keep maintaining this ancient ionic medium. The other heretic theory is more extreme: it claims that the dominant presence of K^+ is a consequence of the magical, "living" state of water inside the cell.[33]

In this part of the essay, I show that in a typical bacterium, which uses chemiosmotic coupling for energy conversion, even a minute imbalance between proton efflux and influx can be hazardous for the cell membrane. I prove that the high-voltage jumps of the membrane potential can destroy the fragile lipid bilayer in a fraction of second. However, there exists a straightforward protective mechanism, namely the cation conducting channels, which saves the bacterium from this threat. These channels should be selective to cation species with a minimal requirement to be impermeable for the protons while allowing other cations to pass in response to fluctuations of the potential. I show that the simplest cation conducting channels are able to not only to separate H^+ from all other cations but that they are also naturally selective for K^+ against Na^+. That way, the dominance of K^+ in the cytoplasm of bacteria is a natural consequence of the necessity to protect the cell from dangerous fluctuations of the membrane potential.

Escherichia coli is used as a model bacterium. Numerical values are taken from either original papers or databases BioNumbers[34] (https://bionumbers.hms.harvard.edu/search.aspx) and *CyberCell* Database, CCDB[35] (http://ccdb.wishartlab.com/). For the parameters taken from BioNumbers database, identification numbers (BNID) are given.

Estimation of H^+ efflux rate in *E. coli*

First, let us estimate the intensity of proton fluxes in an *E. coli* cell with a division time of 30 min (1800 sec). Each *E. coli* requires 64 mmol ATP/(g dry weight) to divide in 30 min[36] that includes 59.81 mmol ATP/(g dry weight) (BNID 110421) for the creation of a new cell, and 4.19 mmol ATP/(g dry weight) is a maintenance cost (BNID 110422). An ATP synthase uses about 4 protons to generate one ATP molecule,[37] which means that approximately 250 mmol H^+/(g dry weight) passes through the ATP synthase during one division cycle. Assuming *E. coli* dry cell weight $0.3{\cdot}10^{-12}$ g (0.3 pg),[38] at least $75{\cdot}10^{-15}$ moles (75 femtomoles) of H^+ should be pumped out through the cell membrane to feed ATP synthases and to support other cellular functions. It means that $45{\cdot}10^9$ protons are evicted in 1800 s by the *E. coli* respiratory system with efflux rate $25{\cdot}10^6$ H^+ per second (25 million protons per second).

Challenges related to the high-intensity of proton fluxes

To the best of my knowledge, no evidence has been found showing a direct connection or some feedback mechanism that warrants an exact balance between hydrogen ions efflux and intake. Therefore, one must assume that proton intake by the ATP synthases and other complexes using a proton motive force should proceed with the same rate, $25{\cdot}10^{+6}$ ion/s. If the rates of the proton efflux and intake do not match, then two situations are possible: (a) ATP synthase (plus flagella and proton-dependent porters) can consume more protons than are pumped; (b). Cytochrome complexes extrude H^+ ions with a higher rate than all proton-consuming machinery can handle (including cases when some external or internal factors abruptly block proton intake). When the cytochrome complexes are pumping fewer protons than the bacterium can reabsorb (case "a") if the proton motive force is reduced or reverted, the ATP synthases can operate not at the maximum speed or even work in the opposite direction.

If proton efflux exceeds H^+ intake, then a bacterium cell has two major problems:

(1) An alkaline shift of pH in the cell cytoplasm. Several mechanisms exist that can cope with the pH shift ensuring a stable pH inside the bacterium. Box 2 explains that the increase in the cytoplasmic pH is a much lesser problem than the membrane electric polarization.

2. A dangerous jump of potential on the cell membrane.

Let us calculate a potential (V) created by H^+ efflux on the lipid membrane. The number of ions transferred across the membrane, and the membrane potential is strictly connected: $V = q/C$ (q is the charge of the ions; C is the membrane electric capacitance).[40] For the rate $25{\cdot}10^6$ H^+/s, $q \approx 40{\cdot}10^{-13}$ Coulomb/s is the charge of the ejected protons ($25{\cdot}10^6$ H^+ per second multiplied by the unit charge, $1.602{\cdot}10^{-19}$ Coulombs), $C = A{\cdot}c_s$, where $A = 6{\cdot}10^{-12}$ m^2 is a surface area of the E. coli inner membrane (BNID 101792) and $c_s = 6\text{-}7{\cdot}10^{-3}$ Coulomb/($V{\cdot}m^2$) is a specific capacitance of the lipid bilayer.[41] So C is about $40{\cdot}10^{-15}$ Coulomb/V. Plugging these numbers into a simple equation $V = q/C$ results in an incredibly high value of $V \approx 100$ V/s! Yes, cytochrome complexes of a rapidly dividing *E. coli* bacterium generate 100 volt membrane potential every second! This outcome is in qualitative agreement with an even higher recent estimate[42] (p. 198-199). [Authors of the cited book calculated that the extrusion of only 10^4 H^+ (10,000 protons) generates a potential of 100 mV meaning that in a rapidly growing *E. coli* 25 million H^+ ions create 250 V/s membrane potential.]

Overall, the fact that proton pumping persistently generates a significant and dangerous membrane potential in bacteria has been known for a number of years (see, e.g.[21]). A biological lipid membrane, which maintains its structure by noncovalent hydrophobic forces, can be destroyed if its potential exceeds 200 mV.[43] Therefore, in fast-growing bacteria, the proton-pumping section of the chemiosmotic machinery can blow the cell in just 2-4 ms if the proton current in the

BOX 2: Ability of the Bacterium to keep Fixed pH

Buffering capacity of *E. coli* is about 85 nmol H^+/(pH unit × mg protein).[39] The estimated protein mass of single cell is 0.3 pg = $0.3{\cdot}10^{-9}$ mg = > capacity of one cell is $17{\cdot}10^{-18}$ mol H^+/(pH unit) or about 10^{+7} H^+ ions/(pH unit); ten million protons per pH unit. A dangerous jump in pH from typical 7.4 – 7.8 value is not one unit but about 0.4 – 0.5 units, so a misbalance in 3-5 million H^+ is already hazardous. This jump in pH could occur in just a fraction of a second (200 ms) if the proton influx is somehow completely blocked. In a more realistic situation, when say there is only 0.1% discrepancy between proton efflux and uptake, dangerous alterations of intracellular pH can happen in 3-4 min. It is clear that on a time scale, pH fluctuations caused by misbalances in the proton currents, are about two-order of magnitude less dangerous than the jumps of the membrane potential caused by the same reason. It is reasonable to suggest that minute-scale pH variations give sufficient time to mount a biochemical response to conquer undesirable pH shifts. Indeed, bacteria maintain their pH homeostasis using multiple mechanisms:[39]

1. metabolic switching to generate acidic or neutral end-products;
2. acid-induced amino acid decarboxylases, and base-induced amino acid deaminases;
3. use of urease activity, sometimes working together with carbonic anhydrase activity.

opposite direction were abruptly blocked! A complete blockade of the proton uptake is a highly unlikely situation. Still, even a minor misbalance, say 0.1%, in the in-and-out H^+ currents can destroy the membrane in just 2-3 s.

A simple solution to save the bacterium from dangerous membrane polarization

From the argument above, it is evident that every bacterium must have a mechanism capable of reliably preventing its membrane from disastrous millisecond-scale voltage fluctuations. An obvious candidate for this function is found in the cation-conducting channels that allow ubiquitous Na^+ or K^+ ions to shuttle between the cytoplasm and extracellular medium in response to the potential jumps. At present, it is considered that the chemiosmotic mechanism consists of the two components—the membrane complexes extruding protons and the ATP synthase consuming them. Since the voltage fluctuations can be very fast, frequent and destructive, I propose that the cation-conducting channels must be added as an integral and essential element of the chemiosmotic machinery (Figure 2).

Next, let us estimate how many channels are required to cope with the voltage fluctuations. Unitary conductance of known channels varies in a broad range. For example, for the K^+-selective channels, the conductance varies from 5 to 270 picoSiemens (pS).[44] Then, in a channel with a reasonable conductance of, say, 160 pS and membrane potential of 100 mV, the electric current would be 16 pA = $16 \cdot 10^{-12}$ Coulomb/s. Dividing this number by the unit charge gives 10^8 monovalent ions per second (100 million cations per second). That means that in a bacterial cell, a single cation channel of rather average permeability can easily cope with any misbalance between the proton currents. As shown above, the rate of H^+ efflux is 25 million H^+ per second.

Cation-conducting channels are naturally selective for K^+

In addition to a reasonable cation permeability, the channels safeguarding the operation of the chemiosmotic complex must reliably prevent unproductive proton leakage through themselves. This means that the channels must be cation-selective with a minimal requirement to separate H^+ ions from Na^+ or K^+. In order to avoid the "short circuit" of the protons leakage through the channel, it is essential to strip the hydration shell from the cations. Substitutive coordination to oxygen atoms of the peptide carbonyl is a natural choice for the working element of the cation filter. (Note that Na^+/K^+ selectivity has yet nothing to do with the major safeguarding function of the channel.)

In potassium channels, K^+ selectivity is achieved through a cascade of peptide carbonyl groups in the selectivity filter. Therefore, as shown in the preceding section, it is plausible to suggest that first cation channels used peptide carbonyls for cation dehydration and that these channels appeared to be naturally selective for K^+ against Na^+. The higher preference for potassium in the pores lined by peptide carbonyl groups was also noted for a number of channels not necessarily selective for K^+.[18]

K^+/Na^+ selectivity of the peptide carbonyl is equal to $D^K_{Na} = 1.20 - 1.5$ or in terms of free energy $\delta g^K_{Na} = -100 \div -250$ cal/mol (see Figure 1). However, this weak K^+ selectivity can result in large $K/^+Na^+$ discrimination when taken collectively. The cation transfer through the channel is accompanied by the coordination of 8 carbonyl groups in each of the four sites of the selectivity filter. It means that the enrichment in K^+ at each site will be from 4 to 25 times. Subsequently, four to five layers of carbonyl oxygen atoms would effectively block Na^+ passage through the pore even if the outside medium is Na^+-rich seawater (Na^+ concentration is equal to 450 mM; K^+ is 10 mM).

Genomic analysis shows that K^+ channels are probably the most ancient (see, e.g.[45]). Therefore, it is reasonable to suggest that these channels have been developed as early as the chemiosmotic mechanism. Also, the molecular structure of the channels is much simpler than the structures of cytochrome complexes and ATP synthase. Moreover, an observation that K^+ channels are exploited not only in the cellular membranes but are also essential for the regulation of potential and ionic homeostasis in the other cellular compartments[26] further substantiates the claim that potassium channels are the simplest and earliest invented.

Since the cation channels are required for the protection of the membrane (see above), it is plausible to suggest that their K^+ selectivity is simply a side effect or a "bonus." It is just a consequence of the small natural selectivity of the peptide carbonyl oxygen for K^+ versus Na^+. And this affinity has been multiplied in the selectivity filter that consists of several layers of the carbonyl groups.

How quickly K^+ replaces Na^+ in the bacterial cytoplasm?

How many monovalent cations are inside a bacterium cell? Well, there are only 90 million K^+ ions in the *E. coli* cell (and 2 million of Na^+).[35] (Another estimate gives 42 million K^+).[46] Thus, a single K^+ channel can supply all potassium ions to the cell in less than a second! It also means that conditions for different cations to migrate in and out of the bacterium are asymmetric: while it is reasonable to assume that passive cation leakage from the cell has low cation selectivity; K^+-selective channels greatly enhance the flux of these ions into the cell. Even a small excess of H^+ efflux over influx allows for quick substitution of virtually all monovalent cations for K^+. Therefore, it is not a mystery why K^+ is so abundant in all bacteria. High conductivity of the K^+-selective channels makes potassium the dominant cation almost instantly after sealing the cell membrane.

Other consequences of the K^+ selection

The chemiosmotic mechanism requires the presence of the cation conducting channels that can buffer dangerous jumps of membrane potential. Potassium channels became a significant player in defining the

ionic composition of the cytoplasm. As a result, K^+ concentration in cytoplasm becomes much higher than in the medium that (in combination with free K^+ movement through the channels) results in a negative electrochemical potential (defined by Nernst equation for K^+). This negative potential allows for the maintenance of the proton electromotive force, while free movement of K^+ prevents dangerous membrane overcharging.

At the same time, the negative membrane potential resolves the major osmotic problem for all living cells. The excess of negatively charged polymers (RNA, DNA, actins, and some other proteins) dictates the exclusion of low-molecular-weight anions from the cytoplasm (Donnan salt-exclusion effect). As a result, the equilibrium between Cl^- inside and outside the cell is impossible unless a negative potential on the cell surface equilibrates the in-and-out electrochemical activities of chloride ions.[29,30] The data on the activities of K^+ and Na^+ ions in the cytoplasm shows that most of these cations remain hydrated and mobile with their activities reduced by localization in the vicinity of densely charged RNA and DNA abundant in the bacterial cytoplasm.[47,48]

Also, a mechanism for the protection from dangerous fluctuations of the membrane potential, which explains the potassium enrichment of the cytoplasm, resolves the mystery of a high (roughly 50% of total) cost of maintaining the bacterial membrane in the "energised state"[49] (see also https://openwetware.org/wiki/Ecoli_ATP_requirement). It is plausible that the protection of the membrane performed by cation conducting channels consumes a substantial portion of the energy generated by proton efflux working as a "Maxwell demon" selecting K^+ over H^+ and Na^+.

The chemiosmotic mechanism of ATP production creates an absolute necessity for cation-conducting channels. Selectivity for potassium is a natural property of the carbonyl oxygen atoms of the peptide bond that are the major construction elements of all cation channels. Substitution of Na^+ for K^+ in the bacterial cytoplasm was instant due to natural potassium selectivity of the cation channels.

However, eukaryotic organisms do not use their outer cell membrane for ATP production. Therefore, the direct threat of membrane disruption as a consequence of misbalances in proton currents disappears. Still, potassium remains a major cation in eukaryotic cytoplasm, and cells spend a lot of energy to keep K^+ in and Na^+ out. Why? See next section of the essay.

THE POTASSIUM INFLUENCE ON DNA PROCESSING

Potassium is a major cation in the cytoplasm of practically all cells, whereas sodium dominates in the medium (seawater, blood serum), Table 1. The cells employ numerous ionic channels and pumps to maintain an asymmetric K^+/Na^+ distribution. Some theories, like osmotic regulation, seem to explain the necessity for high K^+ both in eukaryotes and prokaryotes. However, it is puzzling that a universal phenomenon, the $K/^+Na^+$ asymmetry, is justified by different motives not applicable to all types of cells. In the previous section, an explanation has been suggested, which explicates the reason and the mechanism of K^+ selection by chemiosmosis-driven bacteria. However, eukaryotes do not use their outer membrane for chemiosmosis but still maintain high K^+ - low Na^+ concentrations in the cytoplasm. In this final part of the essay, using the methodology of physical chemistry of polyelectrolytes, I analyze a universal life process, chromatin disassembly and assembly. I show that a substitution Na^+ for K^+ allows DNA-processing machinery to operate more efficiently on the chromatin template. The ability to select potassium gives the cells a small but crucial evolutionary advantage that has resulted in the elimination of species incapable of replacing Na^+ for K^+. This novel hypothesis does not contradict other existing explanations while justifying their nonuniversal applicability.

TABLE 1 Major inorganic ions in the typical extracellular medium (seawater, blood serum, laboratory tube) and inside the eukaryotic cell cytoplasm[50]

Ion	Extracellular concentration, mM	Intracellular concentration, mM		Separation factor[a]
		total	"free"	
Na^+	140–150	5–15	5–10	$D^K_{Na} > 300$
K^+	4–5	140–160	100–120	
Ca^{2+}	1–2	1–2	0.0001	$D^{Mg}_{Ca} \sim 10–40$
Mg^{2+}	1–2	10–40	0.5	
Cl^-	110	5–15	5–10	

aSeparation factor, D, is used to describe relative affinities of polyelectrolytes and ion exchange resins. The cell is considered as an ion exchanger. $D^{M2}_{M1} = (C_{M2,inside}/C_{M2,outside})\cdot(C_{M1,outside}/C_{M1,inside})$ where C_{M1} and C_{M2} are concentrations of the cations M2 and M1 inside and outside the cytoplasm.

Eukaryotes have strong K^+ gradients in the absence of a threat of membrane hyperpolarization

In the two preceding parts of this essay, I have shown that cation-selective channels with a selectivity pore lined with oxygen atoms of the main-chain peptide carbonyl group are intrinsically selective to potassium. Furthermore, that dominance of K^+ ions in bacterial cytoplasm is an immediate and natural consequence of the operation of the chemiosmotic machinery. However, in eukaryotic cells, ATP production is localized in separate organelles, mitochondria, or plastids. Since there are no intense proton currents through their outer cell membrane, the mechanism of enrichment of the cell cytoplasm in potassium that is a direct consequence of the protective function of the cation conducting channels does not apply to eukaryotes. Still, practically all eukaryotic organisms maintain a high K^+ concentration in the cell cytoplasm and have sophisticated energy-consuming mechanisms for K^+ intake and Na^+ exclusion against sodium abundance in media like seawater or blood serum (Table 1).[50]

The mechanisms of the channels' and pumps' operation get much attention, that has resulted in substantial progress. However, the

problem of *why* cells spend up to 40% of their energy on keeping K^+ in and Na^+ out remains less examined. Undoubtedly, cation transport by K^+, Na^+-ATPase and controlled $K/^+Na^+$ permittivity of the K^+- and Na^+-channels are crucial for the existence of animals since nerve signaling is based on playing with cell membrane potential. Other theories (e.g., osmotic regulation) seem to explain the necessity for $K/^+Na^+$ asymmetry in simple organisms, including prokaryotes.

A few insightful papers[28-30,51] give an analysis of the main challenges that living cells should address to maintain their ionic homeostasis. The stoichiometry of charged species in the cytoplasm of all cells (RNA, DNA, proteins, NTPs, polyphosphates) is a net negative. As a result, Cl^- anions are excluded from the cytoplasm (Donnan salt-exclusion effect) that creates conditions for indefinite cell swelling driven by the difference in Cl^- activities in the cytoplasm and the media (seawater, blood serum). Therefore, to equilibrate Cl^- electrochemical activities in and out of the cell, the membrane potential should be negative.[28,29,51] Also, constant concentration of not only K^+ but a *sum* of K^+ and Na^+ ions is sufficient to maintain both the charge balance and the cell volume.[30] From the above arguments, it follows that eukaryotic cells could equilibrate cell volume to handle turgor pressure by steering membrane potential through Cl^--selective channels and pumps. Instead, sophisticated energy-expensive machinery is used to mount and to maintain high K^+/low Na^+ in the cytoplasm. Free K^+ diffusion through K^+-selective channels creates negative potential, thus keeping the electrochemical equilibrium of both K^+ and Cl^- between the cell and the medium.

It has also been known for many years that K^+ depletion generally inhibits macromolecular synthesis in both prokaryotic and eukaryotic cells[52–54] (and references cited therein). However, the ability of halotolerant and halophilic bacteria to thrive when Na^+ dominates in their cytoplasm, as well as experimental data showing that the proliferation of some types of eukaryotic cells is not affected by K^+ levels,[55] indicate that cells can accommodate the presence of Na^+. Therefore, it is plausible to suggest that sensitivity to K^+ is a consequence of proteins' adaptation to potassium abundance rather than a requirement for effective macromolecular synthesis. Despite decades of studies, the reason for the universal preference for potassium of all living cells remains mysterious.[21,31]

Below, I describe how the nature of a monovalent cation, Na^+ or K^+, can affect the fundamental life processes, DNA replication, transcription, repair, and recombination. I show that the replacement of Na^+ for K^+ facilitates access to the compacted DNA by acidic components of transcription, repair and replication machinery. I explain how eukaryotic cells that preserved mechanisms of K^+ selection from their prokaryotic ancestors have gained a small but decisive evolutionary advantage over the cells unable to keep K^+ in and Na^+ out.

DNA packing and unpacking are an electrostatic problem

Concentrations of RNA and DNA in bacteria[56] and dsDNA in the eukaryotic nucleus[57] are very high. The most important molecule of life, the carrier of genetic information, double-stranded DNA (dsDNA), is a very long polymer. The limited volume of the eukaryotic nucleus requires dense DNA compaction into an ordered but dynamic phase, chromatin, by specialized proteins, histones. Chromatin composition and structure are highly conserved (Box 3). The dsDNA carries one negative charge per each 1.7 Å of its length, making it one of the most densely charged polyelectrolytes. Consequently, electrostatic DNA-DNA or RNA-RNA repulsion is a major force that resists DNA packing or RNA folding. An elementary building block of chromatin, the nucleosome core particle (NCP), is a polyanion – polycation complex of the 146 bp DNA with a −292e charge and octamer of the histone protein with a net charge of +146e (Box 3, Table 2).

TABLE 2 Charged amino acids in the histone octamer $(H3/H4)_2$ tetramer, H2A/H2B dimer, and in the histone tails[a]

Histones	Net charge (Lys, Arg, Asp, Glu)	Unit charge per amino acid
Octamer	+146 (118, 100; −24, −48)	0.15
Tetramer $(H3/H4)_2$	+76 (48, 64; −14, −22)	0.16
Dimer (H2A/H2B)	+35 (35, 18, −5, −13)	0.14
Histone tails[b]		
H2A (1-24)	+9 (4, 4; −0, −0)	0.33
H2B (1–31)	+12 (12, 1; −1, −1)	0.39
H3 (1–39)	+13 (8, 4; −0, −0)	0.33
H4 (1-31)	+10 (6, 4; −1, −0)	0.30

[a]Amino acid sequences, according to Sperling and Wachtel.[64]
[b]Amino acids of the histone tails (numbers in parentheses) according to Hansen et al.[65]

DNA transcription and replication must include multiple stages of DNA separation from the basic proteins (histones in eukaryotes, histone-like proteins of prokaryotes). Chromatin disassembly must include the relocation of the histone proteins to other negatively charged domains in order to avoid the considerable penalty associated with separation DNA from the histones. Therefore, acidic domains of the proteins involved in DNA processing must "take over" the basic histones and as such should be integral constituents of the DNA replication and transcription. One can argue that DNA helicases and RNA polymerases are mighty machines and that the energy of the NTP hydrolysis is more than enough to, not only unwind the DNA double helix but also to strip any molecules attached to the DNA. Still, uncontrolled sticking and clogging of the released histones should be avoided to maintain genomic stability and the epigenetic landscape. Indeed, the molecular machines breaking chromatin have negatively charged surfaces to deal with positively charged species involved in DNA folding (Figure 3). After displacement from the DNA by helicases or polymerases, the histones are relocated to specialized acidic proteins, chaperones. The controlled treatment of charges ensures an orchestrated reassembly of the chromatin structures after transcription or replication. There is abundant evidence that histone chaperones are integral

BOX 3: Eukaryotic Chromatin is a Conserved Polyanion – Polycation Complex of the DNA and Histone Proteins

On its primary level, eukaryotic chromatin is remarkably uniform and consists of linear arrays of nucleosomes; complexes of the dsDNA with highly conserved octamer of four histone proteins, H2A, H2B, H3, and H4. An elementary building block of the chromatin, the nucleosome core particle (NCP) is formed by 145-147 bp double-helical DNA wrapped as a 1.8-turn superhelix around one $(H3/H4)_2$ tetramer and two H2A/H2B dimers. The 10-90 bp linker dsDNA connects NCPs, the length of which varies depending on the eukaryote's species, the type of the cell within the species, the cell cycle, the location in the cell nucleus, or the specificity of the chromatin domain within each chromosome. Linear arrays of nucleosomes (NCP + linker DNA) are folded and compacted into multiple higher-order structures. Various other proteins contribute to higher-level chromatin compaction with several types of so-called linker histones being the most abundant and essential. Since the primary chromatin structure was settled, [58,59] higher levels of chromatin organization continue to be the subject of intensive studies, debates and controversies (see, e.g.[60–63]).

To compact DNA inside the limited volume of a cell nucleus, the negative charge of the DNA must be substantively reduced by binding to the positively charged histones. To describe the electrostatic aspects related to DNA compaction, accessibility and dynamics in chromatin. Here, the nucleosome core particle is used as a model object. From the electrostatic point of view, an NCP is a polyanion-polycation complex of the dsDNA (total charge −292e from 146 bp) and the histone octamer (HO) with a net charge of +146e with a significant portion of the positive charge on unstructured flexible N-termini "tails" (see Table 2).

The interaction of cationic species with DNA, RNA, or other polyanions can be described as a counterion exchange reaction or as a competition between a multicharged ligand (L^{Z+}) and monovalent cations (M^+) for the association to the polyanion (e.g., DNA^{N-}):[12,66,67]

$$DNA^{-N} \cdot B_1 M^+ + L^{+Z} \Leftrightarrow [DNA \cdot L]^{Z^{-N}} \cdot B_2 M^+ + (B_1 - B_2) M^+ \quad (1)$$

where $-N$ and $+Z$ are the charges on the DNA and the ligand (in general $N \neq Z$); B_1 and B_2 are the numbers of monovalent cations, which are bound to respectively the DNA polyanion and the polyanion-oligocation complex, [DNA·L]. Here, a similar but smaller contribution related to the binding and release of anionic counterions (e.g. Cl^-) to the oligocation L^{Z+} is not taken into account. The binding of both M^+ and L^{Z+} includes all types of interactions: site-specific non-ionic binding, the formation of cation-anion pairs, solvent-separated cation-anion interactions, and diffuse localization of fully hydrated cations by the polyanion electric field.

Experimental and theoretical biophysical studies have shown that a major driving force for the formation of the oligocation-DNA complex is the entropy increase related to the release of monovalent counterions that are confined near the double helix by the negative electric potential. The strong dependence of the DNA·L complex dissociation constant (K_d) on both the salt concentration (C_{M+}) and the ligand charge is an indication of the decisive entropy contribution. The linear dependence of $\log K_d$ versus $\log C_{M+}$ with a slope $b_1 \cdot Z$:[12,68]

$$\log K_d = \log K_d\,(1M) + b_1 Z \cdot \log C_{M+} \quad (2)$$

is universally observed for a wide variety of cationic species[67,68] and proteins.[68,69] Here, $K_d(1M)$ is the value of K_d extrapolated to $C_{M+} = 1$ M and includes all non-electrostatic contributions to the ligand-DNA interaction; $b_1 = B_1/N \approx 0.9$[66] is close to the unity that reflects a significant degree of counterions M^+ binding to the dsDNA. Using experimental data on oligocation – DNA interaction, we concluded that *in the eukaryotic cytoplasm, oligocations (histones, basic domains of proteins, protamines, polyamines) with charge $Z > +3$ are almost completely bound to the oligo- and polyanions (RNA, DNA, acidic/phosphorylated domains in proteins).*[70,71] It follows from the ionic composition of the eukaryotic cytoplasm that histone proteins can never be present in free form; they are *always* bound either to the DNA, histone chaperones or to the other polyanions (e.g., RNA, polyphosphates, actin, etc.).[71]

Also, the polyelectrolyte approach proves that the electrostatic component of the free energy of negative DNA – positive HO interaction is very favorable and greatly exceeds contributions from the factors opposing the formation of the NCP (DNA bending, DNA-DNA and histone-histone electrostatic repulsion, loss of entropy due to macromolecular ordering). Detailed presentation of evidence proving the absence of free oligocations in the cell cytoplasm and the very high stability of chromatin is given in our earlier work.[70-72] In general, delocalized, dynamic, and structurally undefined electrostatic interactions can be very strong (see, e.g.[73]). The nonspecific electrostatic histone – DNA interactions make a major contribution to chromatin formation.

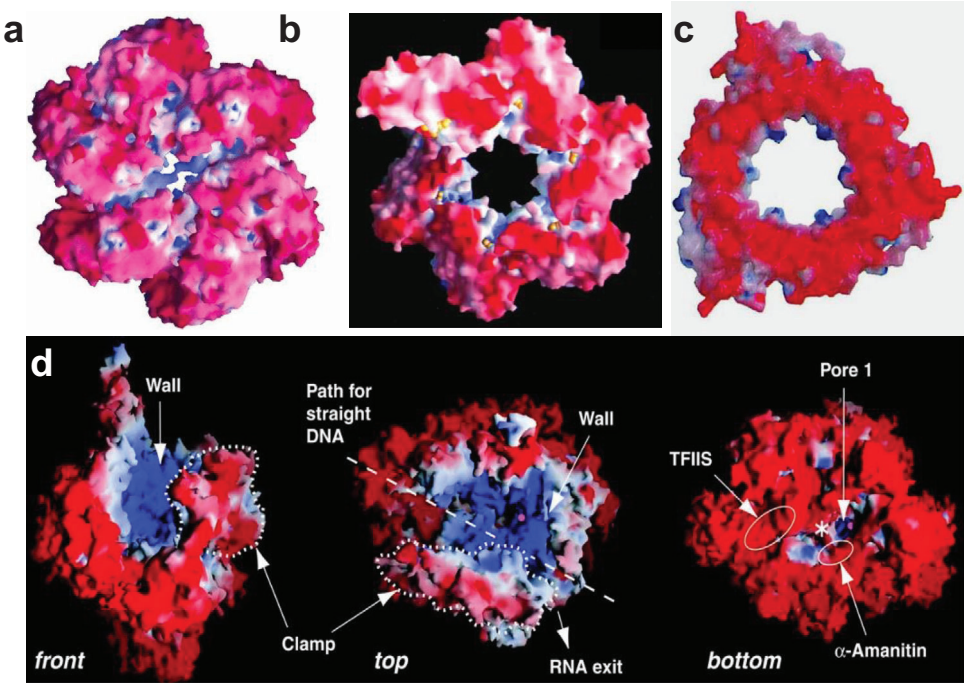

FIGURE 3 Examples of the proteins involved in DNA processing, which show their negatively charged surfaces at locations where they face chromatin. Negative (red) and positive (blue) surfaces of the helicases (**a**)[81] and (**b**),[82] (**c**) polymerase processivity factor of T4 bacteriophage[83] and (**d**) RNA polymerase II.[84] The processivity factor T4 operates in bacterial chromatin where the charge of DNA condensing proteins is less positive. Still, acidic residues are required to handle the positive charge of the proteins. Reprinted with permission from Cell Press (**a**) and (**b**); Academic Press (**c**); American Association for the Advancement of Science (AAAS) (**d**)

and obligatory participants of the DNA replication, transcription, and repair.[74–80]

Acidic chaperones are compulsory for chromatin disassembly

The histone chaperones (nucleoplasmin, NAP-1, N1/N2 proteins, etc.) are indispensable not only for chromatin assembly but also for chromatin disassembly. The chaperones are acidic proteins (AcProt) containing domains rich in glutamate (Glu) and aspartate (Asp) amino acids. Formally, chromatin disassembly can be described as a polycation exchange reaction or as a competition of two polyanions (DNA and acidic proteins) for a polycation (histones):

$$[\text{DNA} \cdot \text{Histones}] + \text{AcProt} \cdot B_2M^+ \Leftrightarrow [\text{AcProt} \cdot \text{Histones}]$$

$$+\text{DNA} \cdot B_1M^+ + (B_2 - B_1)M^+ \quad (3.1)$$

where B_1 and B_2 are the numbers of monovalent cations, which are bound to, respectively, the DNA polyanion and the acidic protein (also see Box 3).

Chromatin disassembly (Equation 3.1) does not only include the transfer of the histones from the DNA to the acidic protein. It is also accompanied by the cations' relocation from the carboxylate groups of the Asp/Glu amino acids to the phosphate groups of the DNA. The polycation exchange reaction (3.1) is the combination (difference) of two reactions of the charged ligand-polyanion complex formation (Equation 1 in Box 3). As a result, entropic contributions related to the release of the counterions (which is the major driving formation of the DNA·L complex) cancel each other out (term $B_1 - B_2$ is small). Anion selectivity of a polycation does not influence reaction (3.1) since it is always bound to the DNA or acidic protein. Correspondingly, other contributions that have had a lesser influence on the equilibrium of the DNA - oligocation interaction (Box 3) can exert a more significant effect on the outcome of reaction (3.1). One of these factors is *the nature* of the monovalent cation neutralizing the charge of the DNA and the acidic proteins.

K^+ and Na^+ can influence an outcome of chromatin disassembly: Data of a model study

The influence of the nature of the monovalent cation on the transformations and reactions of biomolecules has been analyzed and quantified in our earlier work.[12] The sensitivity to the cation nature originates from the chemical identity of electronegative and charged groups of the biomolecules. Change in free energy associated with Na^+-to-K^+ substitution in a system of DNA—histones—acidic proteins, is:

$$\delta g^K{}_{Na} = \left(\delta g^K{}_{Na}\right)_{PO4-} - \left(\delta g^K{}_{Na}\right)_{COO-} \quad (3.2)$$

where $(\delta g^{K}_{Na})_{PO4-}$ and $(\delta g^{K}_{Na})_{COO-}$ are changes in free energy of interaction with the cation of, respectively, a DNA phosphate and an Asp or Glu carboxylate upon substitution of Na^+ for K^+. This change in free energy is applied to each negatively charged group of the DNA and the acidic protein that in the course of the reaction (3.1) exchanges it is counterion from the positively charged groups of the histone (Arg or Lys) to a monovalent cation.

To the best of my knowledge, there is no published experimental data on the influence of monovalent cation nature (Na^+ or K^+) on the assembly/disassembly of chromatin. Hitherto, a relevant system was studied comprising two polyanions, DNA and poly(methacrylic acid), PMA, (labelled with a fluorescent tag). There, two polyanions compete for binding to a polycation, poly(N-ethylvinylpyridinium) (PEVP), in solutions of NaCl or KCl:[85]

$$[\text{DNA} \cdot \text{PEVP}] + \text{PMA} \cdot B_2M^+ \Leftrightarrow [\text{PMA} \cdot \text{PEVP}]$$

$$+\text{DNA} \cdot B_1M^+ + (B_2 - B_1)M^+ \quad (3.3)$$

In this system, the negatively charged carboxylate group of the PMA was the same as in the acidic domains of the histone chaperones. The charge ratio between the polycation and the DNA was equal to 0.4, which is close to the histone – DNA charge balance of about 0.5 in the nucleosome core particle (Table 2). The PEVP polycations ($Z = +30e$ or $+130e$) were completely bound to the PMA or the DNA under the conditions applied in the cited work. Figure 4 shows distributions of the PEVP between DNA·PEVP and PMA·PEVP complexes in dependence of the NaCl or KCl concentration. Three combinations of the PMA and PC were studied: large ($N = -2100e$) PMA with $Z = +30e$ on the PC; comparable PMA and PC ($N = -50e$, $Z = +30e$); and large charge on PC – smaller charged PMA ($N = -50e$, $Z = +130e$).

Figure 4 shows that the nature of alkali cations has a significant influence on the equilibrium of the reaction (3.3). The data in Figure 4 can be explained by connecting the equilibrium of the polycation exchange to counterion preferences of the DNA and PMA (Equation 3.2). It is well-established that in water, a carboxylate anion has a higher affinity for Na^+ relative to K^+ (see, e.g. [15] or data for the PAA in Figure 1a). A phosphate group of the DNA either does not select between Na^+ and K^+[12,86,87] or has a slight preference for K^+.[88] Free energy terms of the reaction (3.2) associated with $Na^+ \rightarrow K^+$ substitution (Equation 3.2) estimated from the literature data cited above are $(\delta g^{K}_{Na})_{COO-} \approx 100 - 250$ cal/mol and $(\delta g^{K}_{Na})_{PO4-} \leq 0$ cal/mol. Substituting Na^+ for K^+ shifts the equilibrium of the reactions (3.1) and (3.3) to the right, favouring polycation transfer from DNA to the carboxylate polyanion with the change in free energy $\delta g^{K}_{Na} \approx -100 \div -250$ cal/mol per monovalent cation.

Figure 4 shows that an increase of salt concentration causes the redistribution of the PEVP polycation between the PMA·PEVP and DNA·PEVP complexes with an increase of the PEVP binding to DNA. Remarkably, in the physiological range of salt, 100–250 mM, the amount of the polycation bound to DNA in NaCl is two-three times larger than that in KCl for the all PMA – PEVP combinations. This result is in agreement with cation preferences of the DNA phosphate and PMA carboxylates. Na^+ selectivity of the PMA carboxylate groups in combination with the lack of DNA selectivity for Na^+ or K^+ results in stabilization of the DNA·PEVP complex in the presence of NaCl.

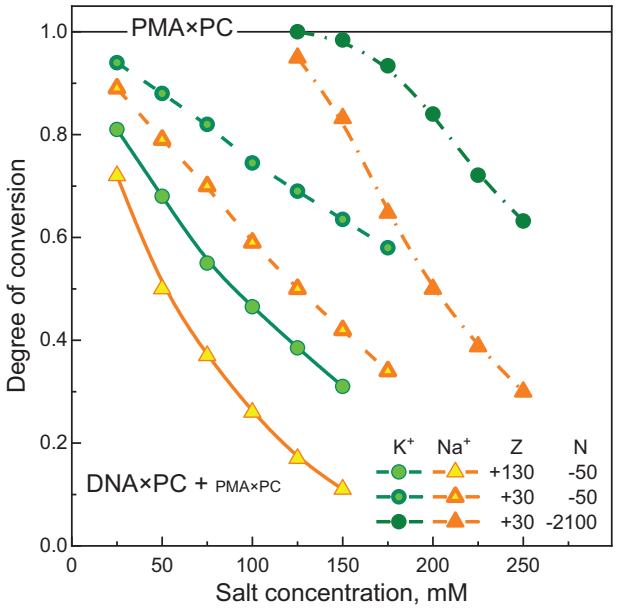

FIGURE 4 Competition between two polyanions, high molecular weight dsDNA and poly(methacrylate), PMA, for binding to a polycation (PC) in solutions of KCl (green) or NaCl (orange). Complete binding of the PC to PMA corresponds to 1. Polycation is poly(N-ethyl-4-vinyl-pyridinium), PEVP. The PMA is tagged with fluorescent pyrenylic groups. Three combinations of the PC and PMA were studied; charge Z of the PC and charge N of the PMA are indicated in the graph. In all experiments, DNA and PMA concentrations were the same and equal to 40 μM in charged groups; charge ratio PC/DNA = PC/PMA = 0.4; 10 mM Tris buffer, pH 9.0. Data from ref. [85] was used to build the graph with permission from John Wiley & Sons

A significant difference in the PEVP distribution between DNA and PMA is caused by a relatively small variance in free energies of Na^+ and K^+ binding to the polyanions' charged groups. For the range of δg^{K}_{Na} $-100 \div -250$ cal/mol, one would expect a modest increase of polycation binding from 1.2 to 1.6 times per charge. However, this small effect of a single Na^+ to K^+ substitution is amplified by the PEVP multivalency ($Z = +30e$). In the system studies by Izumrudov et al.,[85] equal concentrations of DNA and PMA in charged groups were used. Thus, an excess of the carboxylate polyanions is required to release the DNA from the polycation in the KCl solution, and even more COO^- groups should be supplied in the sodium salt which inhibits the chaperone activity.

Is the model data relevant for chromatin *in vivo*?

Other notable observations can be drawn from the experimental results reported by Izumrudov et al.[85] A PMA with a high charge

(−2100e) is more competitive than the lower molecular weight polyanions. A polycation with a high charge (+130e) has a preference for binding to the DNA rather than to the shorter PMA (case Z = +130e and N = −50e, Figure 4). An experimental system studied by Izumrudov et al.[85] might favor preferential binding of the PEVP to the fluorescently labeled PMA rather than to the DNA. It was shown,[89] that labelling of the PEVP facilitates the formation of the PMA·PEVP complex due to the additional favorable interaction between aromatic pyridinium of the PEVP and the label, pyrenylmethane. Therefore, in the absence of the aromatic fluorophore, the reaction (3.3) might be more sensitive to the nature of the cation, and its equilibrium can be shifted more to the left, in favor of the DNA·PEVP complex.

Histone chaperones (e.g., nucleoplasmins) have acidic domains with the net charge lower than PMA with N = −50e. For example, acidic domains of the nucleoplasmin of *H. sapiens* (aa 161-188) and *X. laevis* (a.a. 121-148) have a net charge −26e and −20e respectively (a.a. sequence according to UniProt database[90] entries P06748 and P05221). *In vivo*, nucleoplasmin forms a homopentamer in the shape of a flat cylinder with five unstructured acidic domains merged at the distal part of the cylinder.[91] Integration of the five acidic domains (−130e in *H. sapiens* and −100e in *X. laevis*) allows the matching of the positive charge of histones H2A/H2B dimer (Z = +35e) or $(H3/H4)_2$ tetramer (Z = +76e, see Table 2) and dramatically increases the chaperone competitiveness with the DNA. Again, the stronger association of Na^+ (than K^+) with carboxylates of the acidic domains can diminish the histone-binding potential of the chaperones. Comparison of the chaperones' histone-binding affinities in the presence of Na^+ or K^+ could be important for the verification of the hypothesis suggested in the present work.

Another negatively charged group, phosphate ($-OPO_3^{2-}$) of the phosphorylated serine or threonine amino acids, can also take part in the histone removal during DNA replication and transcription. Phosphorylation of the histone chaperones[92] and the RNA polymerase II C-terminal domain[93,94] correlates with active DNA processing. Thus, the cation preference of the phosphate group is also essential for chromatin disassembly. An unprotonated doubly-charged phosphate group is the primary form of the phosphorylated amino acids at physiological pH (7.3 -7.8).[95,96] The selectivity of the $-OPO_3^{2-}$ anion is similar to the one of the carboxylate: it is slightly selective for Na^+ relative to K^+.[97] Therefore, similar to the COO^- anion, K^+-rich environment might facilitate the activity of the phosphorylated proteins.

A small variance in the interaction of Na^+ and K^+ with chromatin components can nevertheless result in noticeable differences in chromatin folding and compactions. The presence of potassium stimulates open chromatin structures, while Na^+ facilitates chromatin packing. For example, increasing K^+ concentration in the solution of the nucleosome arrays compacted into a 30-nm fiber by addition of Mg^{2+} leads to the array unfolding. In contrast, the addition of Na^+ to the same Mg^{2+}-folded array results in further array compaction, aggregation and precipitation.[98] Even in the absence of Mg^{2+}, the folding of the nucleosome arrays in KCl and NaCl solutions is different. In K^+ salt, an unfolding effect is observed similar to that produced by acetylation of the functionally important H4 histone Lys 16. The arrays with the unmodified H4 Lys 16 are always more compacted in Na^+ solution than in the presence of K^+.[99,100]

Potassium is the "Grease"; sodium is the "Sand" inside the cell motor

Overcoming the repressive influence of chromatin is a rate-determining stage of DNA replication and transcription.[101] *In vivo*, unlike the model reaction (3.3) DNA – PEVP – PMA, DNA replication and transcription as well as the preceding stage, chromatin disassembly, can only proceed as a continuous chain of events, from the first to the last nucleotide. Every aspect of DNA processing is important. As I have shown above, minute differences in K^+/Na^+ affinity, $\cdot(\delta g_{COO-})^K{}_{Na}$, are multiplied by the number of the histone positive charges (Z_{hist}) transferred from the DNA to the acidic proteins:

$$\delta G^K{}_{Na} \approx -Z_{hist} \times (\delta g_{COO-})^K{}_{Na} \tag{3.4}$$

For one nucleosome with a net histone charge Z_{hist} = +150e, the substitution of Na^+ for K^+ gives a huge advantage in free energy for the acidic protein in competition with DNA for binding to the histone octamer, $\delta G^K{}_{Na} \approx -15 \div -37$ kcal/mol. Importantly, for the sequence of mutually dependent steps, the ultimate effect of counterion specificity does not depend on the mechanism of the histone and histone-like protein displacement. Figure 5 shows that in the presence of Na^+, the nucleosome disassembly by histone chaperones is energetically more costly and requires an increase of the histone chaperone concentration.

The total charge of the histones removed from DNA during replication (e.g., of 2 m of the human genome packed by 30 million nucleosomes) is immense. I claim that Na^+ (like sand in a motor) creates "friction" for the anionic groups in proteins (carboxylates of the Asp and Glu, phosphates of the modified Ser and Thr) involved in the removal, the temporary storage and the reassembly of histones. Meanwhile, the speed and smoothness of the cell replication are crucial in the struggle for survival. Faster spreading species have a clear advantage compared to their competitors (see Box 4), even if the cell must pay the high energetic cost for the advantage of having "oil" (K^+) instead of "sand" (Na^+) inside its "motor."

This new hypothesis might explain a few observations reported in the literature. For example, mature red blood cells of carnivores simultaneously lose the nucleus and the ability to maintain $K/^+Na^+$ asymmetry.[104] It is plausible to imply that the maintenance of high K^+ in the cytoplasm becomes redundant since DNA processing is ceased in these cells. Another example is halotolerant and halophilic bacteria, which have proteins with a higher content of the acidic and lower one of the basic amino acids compared to the similar bacteria living at lower salinity.[105] The halotolerant and halophilic bacteria cannot exclude Na^+ from the cytoplasm, so this change in amino acid composition

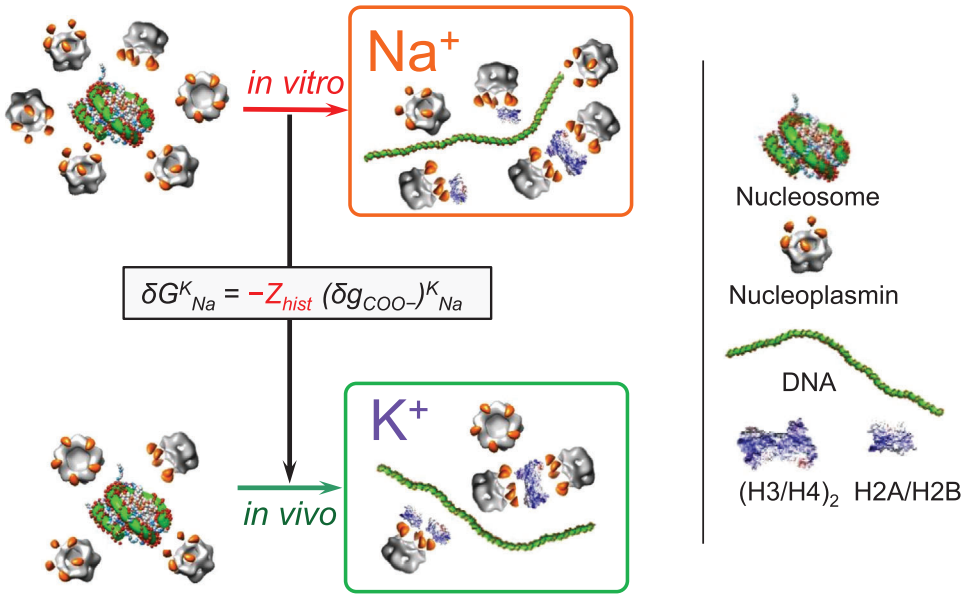

FIGURE 5 Schematic presentation of the nucleosome disassembly as a competitive reaction between DNA and acidic chaperones for binding to the histone octamer composed of $(H3/H4)_2$tetramer and two H2A/H2B dimers. The free-energy difference between the decomposed nucleosome states in the presence of $Na^+$and K^+ions,δG^K_{Na}, is proportional to the charge of the histone octamer(Z_{hist}), the difference in the interaction of free energy of the protein COO^-group with $Na^+$and K^+,$(\delta g_{COO-})^K_{Na}$. In the figure, nucleoplasmin pentamer is used as an example of histone chaperone using recent cryo-EM structure.[91]Acidic domains of the pentamer are in orange

BOX 4: Division Rate is a Driver of Evolutionary Selection

Consider a situation when in a population of one billion bacteria with a division time of 31 min, one cell emerges that divides in 30 min. A simple calculation shows that it would take less than 40 days to eliminate the slower-dividing cells. After the indicated time, there will be a billion cells with 30 min division time to only the one dividing in 31 min. Forty days is just a blink of an eye in the context of 3.7 billion years of evolution.[102,103] This small advantage in DNA processing might have been an additional bonus in bacteria that gained dominance of the potassium in their cytoplasm as a natural consequence of the emergence of the chemiosmotic mechanism as described above. Since any change that shortens a cell's division time is a superior evolutionary advantage, the beneficial K^+ presence in the cytoplasm has been preserved in eukaryotes.

might be an adaptation against the inhibitive influence of Na^+ that ensures the effective and fast chromatin disassembly. It is similar to the effect observed in the model reaction (3.3): a reduction of the polycation charge and an increase of the charge of the polyanion facilitate the polycation transfer from the DNA (see Figure 4). One more observation of how Nature exploits the small differences in K^+/Na^+ affinities: many polyanionic biopolymers designated for extracellular functions are heavily sulfonated (e.g., heparan sulfonate[106]). It is well-known that the sulfonate group, $-SO_3^-$, is slightly selective to K^+.[107,108] This means that in the extracellular liquids, rich in sodium, negative charges of these polyelectrolytes are less shielded by Na^+ and therefore are more active than if they were performed in the K^+-rich cytoplasm.

From physiology to experimental implications

Mimicking chromatin's "preferred" ionic environment

I have shown above how the nature of the monovalent cation can modulate the major cell processes, DNA replication and transcription. Small differences in Na^+ or K^+ interaction with the acidic/phosphorylated domains of the proteins accumulate over all positive charges removed from the replicated or transcribed DNA. I put forward the hypothesis that a positive influence of K^+ on the ability of the acidic domains to strip the histones from the DNA is the reason for eukaryotic organisms to retain and to maintain a K^+-rich environment inherited from bacteria.

From my analysis, an important conclusion follows that the data of chromatin studies *in vitro* routinely carried in NaCl might give incorrect information about chromatin properties *in vivo* (that is in the presence of K^+). The arguments presented in this essay imply that it is essential to use K^+ in the *in vitro* studies modeling intracellular processes. I hope that my analysis will inspire comparative studies of chromatin transformations in the presence of K^+ and Na^+ to either refine or to refute this hypothesis.

CONCLUSIONS AND OUTLOOK

Many significant advances in science have originated from seemingly minor findings. For example, an observation of small differences in the X-ray diffraction patterns of hemoglobin crystals with silver or mercury ions solved the X-ray crystallography phase problem and gave rise to enormous progress in structural biology. An unexpected finding that the quick cooling of solutions does not lead to water crystallization; thereby keeping biological structures intact, has made possible the recent exponential growth of cryo-EM research. Even beautiful maps of the expanded Universe were drawn because a curious person had noted a small red shift in the galaxies' spectra.

In this essay, a hypothesis is proposed that minor differences in cation affinities of the electronegative sites of biomolecules might explain the universal features of living cells. Namely, that the origin of a ubiquitous dominance of the potassium cations in the cytoplasm can be traced to a small affinity for K^+ over Na^+ of the peptide carbonyl oxygen atom. The peptide bond is the most common part of all proteins, so its oxygen atoms become a natural choice for constructing the selectivity pore of the cation-conducting channels. Next, it is shown that the cation channels should be considered an essential part of the chemiosmosis machinery because they can reliably protect the bacterial membrane from wild fluctuations of the electric potential. The intrinsic K^+ selectivity of these channels results in an instant replacement Na^+ for K^+ in the cytoplasm. However, this hypothetical mechanism of potassium selection by bacteria seems to be inapplicable to eukaryotes, which do not use their outer-membrane for chemiosmosis. In the final part of the essay, an approach based on the physical chemistry of polyelectrolytes is used to show that the DNA transcription and replication should include a transfer of the histones from the DNA to the acidic proteins. It is demonstrated that small differences in the Na^+ and K^+ affinities of the DNA and acidic proteins can modulate the activity and the rate of the histone transfer. In K^+-rich environments, the histone chaperones might perform with higher activity and rate, and it might result in the small but essential evolutionary advantage. This hypothesis has a few weak points, some of which are mentioned throughout the essay, and some that may not yet be apparent to the author. In the spirit of progress, the author invites further scrutiny and testing to help challenge and develop the hypothesis.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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