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Review

Intracellular pH is a tightly controlled signal in yeast ☆

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ABSTRACT

Background: Nearly all processes in living cells are pH dependent, which is why intracellular pH (pH_i) is a tightly regulated physiological parameter in all cellular systems. However, in microbes such as yeast, pH_i responds to extracellular conditions such as the availability of nutrients. This raises the question of how pH_i dynamics affect cellular function.
Scope of review: We discuss the control of pH_i, and the regulation of processes by pH_i, focusing on the model organism *Saccharomyces cerevisiae*. We aim to dissect the effects of pH_i on various aspects of cell physiology, which are often intertwined. Our goal is to provide a broad overview of how pH_i is controlled in yeast, and how pH_i in turn controls physiology, in the context of both general cellular functioning as well as of cellular decision making upon changes in the cell's environment.
Major conclusions: Besides a better understanding of the regulation of pH_i, evidence for a signaling role of pH_i is accumulating. We conclude that pH_i responds to nutritional cues and relays this information to alter cellular make-up and physiology. The physicochemical properties of pH allow the signal to be fast, and affect multiple regulatory levels simultaneously.
General significance: The mechanisms for regulation of processes by pH_i are tightly linked to the molecules that are part of all living cells, and the biophysical properties of the signal are universal amongst all living organisms, and similar types of regulation are suggested in mammals. Therefore, dynamic control of cellular decision making by pH_i is therefore likely a general trait. This article is part of a Special Issue entitled: Systems Biology of Microorganisms.

1. pH in biological systems

1.1. Definition of biological pH

The pH of a solution is defined as the negative logarithm of the hydrogen ion activity in water. If we assume the inside of a yeast cell to be a watery solution, we can estimate the number of free protons in the cytosol of a single cell (48 μm^3 [1,2] at a pH of 7 [3]) at no more than ~3000. In contrast, global analysis of protein expression in yeast tells us that the number of protein molecules in a cell is in the order of millions [4]. Each of these proteins has multiple protonatable groups which can either donate or take up a proton. In addition, acidic metabolites are also in excess compared to free protons. For instance, the concentration of inorganic phosphate (P_i) in yeast cells is estimated at around 50 mM [5], five orders of magnitude higher than that of protons. This difference in the numbers of free protons and potential buffer molecules is important for our perception of pH_i. The classical view of the pH of a solution as the concentration of free protons in a bulk of water does not seem to apply. Because of this free proton-to-buffer component ratio, cellular pH will be intrinsically buffered by all weakly acidic and basic components of the cell (Table 1). To understand biological function, it is more useful to know the relative protonation of all weakly acidic or basic molecules in the relevant cellular compartment. This requires knowledge of the *in vivo* pK_a values of acidic and basic groups in their actual cellular context, currently unavailable. Estimating the pK_a value of amino acid side chains, for instance, is far from trivial because of the tertiary structure of proteins. Residues on the protein surface, which are largely surrounded by water, have an actual pK_a that will be different from the same residues localized in the protein interior. This can be particularly relevant when these residues are close to, or part of, an active site [6,7].

1.2. Determination of pH_i in yeast cells

To study the effects of pH_i *in vivo* the need for accurate pH_i determinations is paramount. Methods for determination of pH_i in yeast cells usually rely on the determination of the relative protonation state of a particular molecule. They include ^{31}P nuclear magnetic resonance (NMR) [8–12], equilibrium distribution of (radio labeled) weak acids [13–15] or probing cells with pH-sensitive dyes like C.SNARF-1 [16], 9-aminoacridine [17] and fluorescein [16,18,19]. A disadvantage of some of these techniques is that they require

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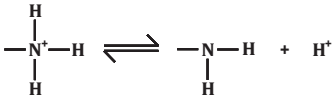
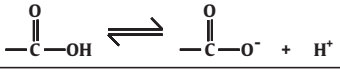
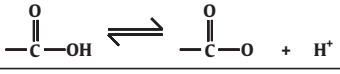
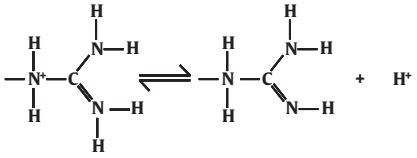
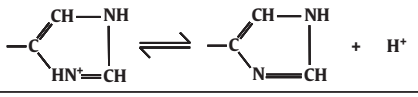
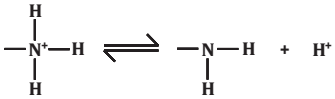
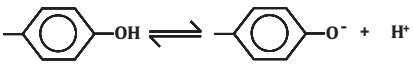
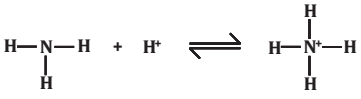
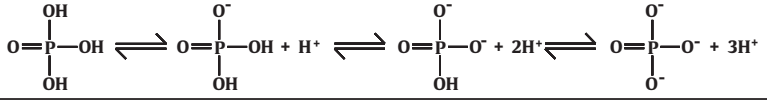
extensive manipulation of cells. This manipulation can have significant effect on the pH_i [20]. In addition, many of these techniques are not able to spatially resolve pH differences within cells. Clearly such spatial resolution is relevant as different organelles within eukaryotic cells have their own specific pH value [21,22]. Recently, the use of pH-sensitive fluorescent proteins has made it possible to determine pH_i in living, unperturbed cells, in a time-resolved and organelle specific manner [23]. One such probe, ratiometric pHluorin [24] was adapted by us and others for *in vivo* pH_i measurements in *Saccharomyces cerevisiae* [3,25–29]. While this method provides a reliable and convenient experimental setup it does have its limitations. The pK_a of the fluorophore is around 7.2 [30] which results in a quick deterioration of measurement resolution at pH values above 8.0 or below 5.5 [3]. In addition, expression of high levels of pHluorin protein could influence a particular luminal pH by buffering, especially if the expected pH of the organelle is near the pK_a of the fluorophore, due to the low number free protons compared to the number of pHluorin molecules.

1.3. pH buffering and homeostasis

As discussed above (Section 1.1), almost all proteins in living cells contain weakly acidic and basic amino acid residues that can act as buffers. In addition to proteins, metabolites can also act as buffers. P_i, for example, has been implicated as an important cellular buffer [31]. In the cell, all weakly acidic and basic molecules together constitute the complex buffer that is the cytosol. The buffer capacity (β) of this buffer is defined as the amount of strong base or strong acid that would have to be added to increase or increase pH by 1 unit. β is dependent on pH and is largest around the pK_a of the buffer molecule. Because the cellular molecules have many different pK_a values, β will change with pH_i. Table 1, adapted from Ref. [32], lists the molecules or groups present in the cell that contribute to β. Because of this complexity, β has not been well defined in yeast. One possible contribution to the complexity of the cellular buffer is the volatile carbon dioxide (CO_2). CO_2 is continuously produced by fermenting yeast cells and, being apolar, can diffuse freely over membranes. In a fully aerated system where CO_2 can escape from the culture the dissolved intracellular CO_2 concentration can be regarded as constant. However, in cells with pH_i ~7.0, it will to some extent react with water forming HCO_3^-. When the cell is faced with a sudden lowering of pH, protons are buffered by this HCO_3^- to form H_2CO_3 (with a $pKa = 3.6$, but a net pK of the total reaction around 6.4) which in turn is converted to CO_2 and H_2O. Mammalian cells are exposed to blood which typically contains about 25 mM of HCO_3^- [21]. This type of buffering accounts for a significant amount of mammalian cellular β (50–66%) [33]. However, in yeast it appears to constitute no more

Table 1
Common pH dependent molecules in biology. Common weak acids and weak-bases in a cell are listed. Note that for amino acid side chains or termini the pK_a values given are those of free amino acids. These values can change drastically when incorporated into a protein depending on the structure of the protein and its surroundings.

protein residues		**pK_a**
amino-terminus		8.0
carboxyl-terminus		3.1
aspartic/glutamic acid		4.4
arginine		12.0
cysteine		8.5
histidine		6.5
lysine		10.0
tyrosine		10.0
metabolites		
ammonium		9.2
phosphate		2.1, 7.2, 12.7