Determining the intracellular pH of HeLa cells

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Abstract

Changes in the environmental conditions can lead to physiochemical changes inside of cells, which can ultimately affect protein function and assembly (1). Although mammalian cells keep their intracellular pH close to neutral levels, it is known that the intracellular pH can fluctuate under physiological and pathological conditions. For example, the intracellular alkalization and extracellular acidification is a hallmark of cancer cells that contributes to malignancy (2, 3). Here we describe a method based on ratiometric fluorescence intensity microscopy to determine the intracellular pH of mammalian cells. We describe how to prepare a calibration curve and measure the intracellular pH of cells expressing a genetically encoded pH sensor.

Introduction

In order to measure the intracellular pH of HeLa cells, a pH sensor consisting of mCherry-epHluorin was designed and expressed in the cells (adapted from (4)). The emission fluorescence of epHluorin is pH-dependent, decreasing when exposed to acidic environments. In contrast, mCherry emission fluorescence is independent of pH and is used to normalize the expression level of the sensor (Figure 1). By measuring the epHluorin/mCherry fluorescence intensity ratio, we can determine the precise value of the intracellular pH. For this purpose, we first need to prepare a calibration curve with known intracellular pH values.

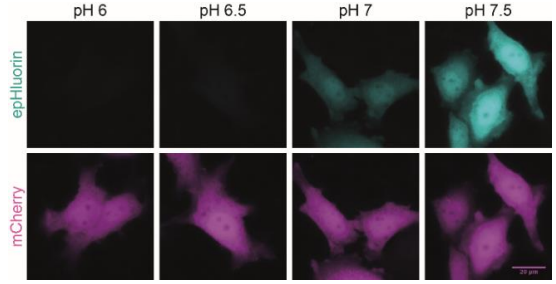

Figure 1: pH sensor expressed in HeLa cells exposed to different pHs. HeLa cells were exposed to Nigericin-containing medium at different pHs. The fluorescence of epHluorin and mCherry is shown.

pH calibration curve

Mammalian cells keep their intracellular pH neutral (pH 7.5). Even when cells are exposed to variable extracellular pH conditions, they can buffer their intracellular pH to some extent. In order to create a pH calibration curve, the intracellular pH needs to be equilibrated with the extracellular pH. This is achieved with the ionophore Nigericin that acts as an antiporter of H+ and K+. Thus, in high K+ medium, the intracellular and extracellular H+ concentrations equilibrate. By exposing HeLa cells to Nigericin-containing medium at different pHs, we generated a pH calibration curve (Figure 2).

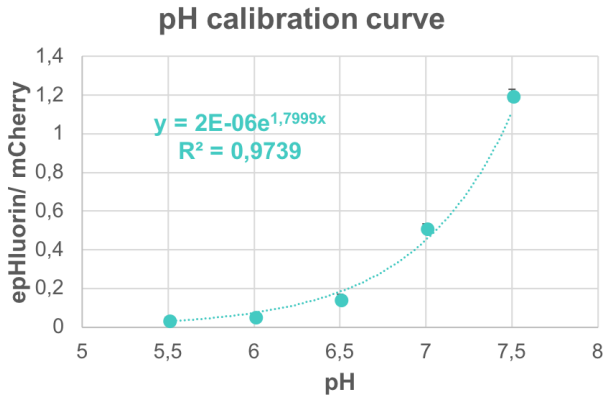

Figure 2: Intracellular pH calibration curve. The ratio epHluorin/mCherry fluorescence from HeLa cells exposed to Nigericin-containing medium at different pHs was calculated. The calibration curve between the epHluorin/mCherry fluorescence and the known pH is shown.

Procedure:

- Grow HeLa cells expressing the pH sensor on imaging plates/dishes, using regular growth conditions.
- Before imaging, expose cells to Nigericin-containing medium adjusted at different pHs (10 mM HEPES, 140 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mg/ml glucose, 10 µM Nigericin). To ensure a proper medium exchange, wash the cells at least one time with the corresponding Nigericin-containing medium (5, 6).

- Image cells at 37 °C (without CO_2 supplementation) after 5-10 minutes of medium exchange (epHluorin is excited at 488 nm, mCherry is excited at 587 nm).
- For every given pH, calculate the average signal ratio epHluorin/mCherry and fit the data to obtain the calibration curve.

Measuring the intracellular pH

Once we have the pH calibration curve, we can use the pH sensor to measure the intracellular pH of cells exposed to different conditions.

We used this system to measure the intracellular pH of HeLa cells exposed to mediums with different pHs (without using any ionophore to equilibrate the intracellular and extracellular pHs). We found that HeLa cells under regular growth conditions (pH 7 - 7.5) keep their intracellular pH slightly higher as compared to the extracellular pH (Figure 3). When cells are exposed to slightly acidic mediums (pH 6 – 6.5), they have some capacity to buffer this pH change, but still their intracellular pH drops (Figure 3).

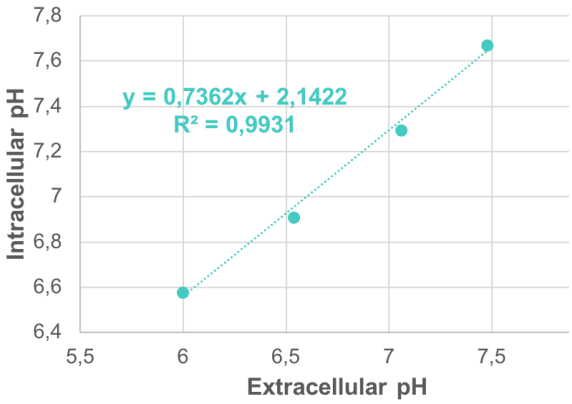

Figure 3: Correlation between intracellular and extracellular pHs. HeLa cells expressing the pH sensor were exposed to mediums at different pHs. The fluorescence ratio epHluorin/mCherry and the pH calibration curve were used to calculate the precise intracellular pH. The correlation between the intracellular and extracellular pHs is shown.

Procedure:

- Grow HeLa cells expressing the pH sensor on imaging plates/dishes, using regular growth conditions.
- Change the cellular medium for DMEM-Hepes medium adjusted at the desired pHs. To ensure a proper medium exchange, wash the cells at least one time with the corresponding DMEM-Hepes medium (DMEM supplemented with 20 mM HEPES, 4 mM $NaHCO_3$, 4.5 gr/L Glucose, 2 mM L-Glutamine).
- Image cells at 37°C (without CO_2 supplementation) after 15 minutes of medium exchange (epHluorin is excited at 488 nm, mCherry is excited at 587 nm). Of note, the change in the intracellular pH upon changing the extracellular pH occurs within 10-15 minutes after medium exchange.
- Calculate the average signal ratio epHluorin/mCherry. Interpolate the corresponding pH by using the previously obtained calibration curve.

Discussion and Conclusions

Using the epHluorin/mCherry pH sensor we can measure the intracellular pH of living mammalian cells. This methodology can be applied to study pH fluctuations upon different conditions, such as changes in the environment or genetic modifications. Alternatively, we can use the described procedure to modulate the intracellular pH and study how changes in the pH affect the functionality of the cell.

References

1. Franzmann TM, Jahnel M, Pozniakovsky A, Mahamid J, Holehouse AS, Nuske E, et al. Phase separation of a yeast prion protein promotes cellular fitness. Science. 2018;359(6371).

2. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. Nat Rev Cancer. 2011;11(9):671-7.

3. Cardone RA, Casavola V, Reshkin SJ. The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis. Nat Rev Cancer. 2005;5(10):786-95.

4. Grillo-Hill BK, Choi C, Jimenez-Vidal M, Barber DL. Increased H(+) efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. Elife. 2015;4.

5. Hudmon A, Lebel E, Roy H, Sik A, Schulman H, Waxham MN, et al. A mechanism for Ca2+/calmodulin-dependent protein kinase II clustering at synaptic and nonsynaptic sites based on self-association. J Neurosci. 2005;25(30):6971-83.

6. Bond J, Varley J. Use of flow cytometry and SNARF to calibrate and measure intracellular pH in NS0 cells. Cytometry A. 2005;64(1):43-50.