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Scaling of *Paramecium*

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Abstract

Scaling analysis was carried out on the unicellular organism, *Paramecium* in terms of the cellular geometry and metabolic activity. Confocal microscopy and computer assisted tomography revealed the conventional assumption of the cell body as a prolate spheroid is enough applicable to represent the cell volume. The tomographic volume could be estimated as 0.75 times of the prolate volume. This linearity between tomographic and prolate volume suggests the isometric scaling of the cytopharynx volume to the cell volume. The prolate assumption of the cell body, which has the short axis scaled to the long by the power 0.56, suggests the allometric relation of surface area to the cell volume by the power 0.71. Allometric scaling of energy expenditure of *Paramecium* was tested based on the result of geometrical scaling using the *SMR* measured by the extrapolation method established by the previous study (Katsu-Kimura *et al*, 2009).

Introduction

Earth is full of various organisms that are different in size and shape. They are results of successive adaptations to the environment on Earth. Needs of changes in size and shape had inspired the new strategy determining the body plan, that is known as "scaling".

Knut Schmidt-Nielsen introduced in his famous book "*Scaling*", a sketch of animal bones drawn by Galileo Galilei who, on the basis of the concept of scaling, was probably the first to point out the non-proportional changes in the geometry of bones in animals of different body sizes (Schmidt-Nielsen, 1984). Scaling is, therefore, the important concept concerning the body plan of the organisms, which is idealized by the subtitle of the book "*Why is animal size so important*" as well as the description that "*scaling deals with the structural and functional consequences of changes in size or scale among otherwise similar organisms*" (Schmidt-Nielsen, 1984).

Galileo's idea is valid under the actual effect of terrestrial gravity, which strongly affects the body plan of the organisms of relatively large scale, such as tetrapod vertebrates. Gravitational force dose not allow the proportional (isometric) increase in geometry with increasing the size of the animals made of the "soft" materials having common mechanical properties. Thus, a question would arise, whether the same rule of scaling would be applied to the small sized organisms such as protists. Gravity governs the world of large size; in the Solar System gravity is the only force that prescribes the motion of the planets. Its effect, on the other hand, decreases with decreasing the size of the object, likely vanishing among the thermal noise in the dimension of protists.

We can observe the protists through microscopy, which gives only a two-dimensional information based on the orthographic projection of the object. This may limit the understanding of the cellular geometry in

three-dimension. The recent advance of confocal microscopy and the computer assisted tomography allows to assess the detailed geometry of microorganisms. This may improve our knowledge about the morphological scaling of unicellular organisms.

In addition to the cellular geometry, scaling analysis could be extended to the physiological function of the organism, especially to the energy metabolism. It is well known that there exists a general rule of thumb for the energy expenditure throughout the Animal (and in some case, Plant) Kingdom. Energy metabolism can be expressed as the allometric function of the body size. For the Standard Metabolic Rate (*SMR*), it could be written as,

$$SMR \propto M^b , \tag{1}$$

where M is the mass of the organism and b is the allometric exponent. By introducing the proportionality constant, a, this formula can be converted to the logarithmic function as,

$$\log SMR = \log a + b \log M \quad . \tag{2}$$

According to the surface rule, where the heat production and dissipation are proportional to the body mass and body surface, respectively, b is referred to as 2/3 for the homeotherms (Rubner, 1883). This value was revised by precise measurements. Kleiber (1932) reported that the exponent was not 2/3 but 3/4 in mammal. On the basis of many other researches, the 3/4 power scaling widely accepted to poikilotherms and even to plants (Hemmingsen, 1960; Schmidt-Nielsen, 1997). For 3/4 power law, 'fractal-like network model' was proposed by West *et al.* (1997) and is widely accepted in the allometry in the wide range of dimensions from cell to ecosystem.

SMR in unicellular organisms has also been suggested to obey 3/4 power law (Hemmingsen, 1960; Fenchel and Finlay, 1983; Schmidt-Nielsen, 1997; Gillooly *et al*., 2001). Researchers who supported 3/4-law were claimed that the fractal model could be applied to allometric scaling of *SMR* in unicellular organisms in a similar fashion to multicellular organisms (West *et al*., 1999).

It should be noted, however, that the majority of the research of the allometry of the unicellular organisms are based on the data found in the literature, which include those from distantly related taxa including bacteria, protozoa, fungi and marine zygotes (Hemmingsen, 1960). In addition, they were obtained in the various conditions, which are not equivalent to those required for the allomertic analysis. Strictly speaking, allometric analysis of the metabolic energy consumption is allowed only on thwhen the analysis is made on the basal metabolic condition. This has not been satisfactorily defined for the allometric analysis of unicellular organisms. Microorganisms live in the viscosity-dominant environment, and thus large amount of energy is dissipated for the movement which must be eliminated for the measurement of the metabolic cost. Another problem is found in the measurement of the body mass. Because simple weighing of a single cell is not necessarily possible, several approximations have been used. Estimation of the cell volume by approximating the cell as a rotating ellipsoid has been commonly used. By this method errors are not avoided that occurs from the incorrect evaluation of the cell geometry; significant depression of the cell surface, cytopharynx of ciliates, for example, may be a major source of such an error.

In the previous study on the measurement of energy cost of locomotion of *Paramecium* (Katsu-Kimura *et al.*, 2009), we first succeeded to evaluate *SMR* of the swimming protist; it was determined for swimming cells without using any agent inhibiting the motile activity. This "physiological method" of evaluation will give *SMR* for the allometric analysis of energy expenditure of unicellular organisms which would be done

on the same basis of strategy adopted in the analysis on the multicellular organisms. In addition to the improvement of the evaluation of the *SMR*, we used the computer tomography for the evaluation of the body size, another parameter for the allometric analysis.

In this study, we focused on the allometric scaling of *Paramecium*. Several representative strains (*P. caudatum* syngen 1, 3, and 4, and *P. aurelia* syngen 8) were used for the estimation of *SMR* and tomographic volume. Allometric scaling of energy expenditure of *Paramecium* will be discussed in combination with the result of geometrical scaling demonstrated by the tomographic measurement.

Materials and Methods

Cells and culture

P. caudatum syngen 1, *3 and* 4, and *P. aurelia* syngen 8 were cultivated at 23 ± 1°C in hay infusion in Dryl's solution (2 mM sodium citrate, 1.2 mM Na_2HPO_4, 1.0 mM NaH_2PO_4, 1.5 mM $CaCl_2$, pH 7.2) (Mogami *et al.*, 2001). Cells at the early stationary phase were collected by low-speed hand-centrifuge (<170 ***g***) and were adapted to the experimental solution (KCM: 1.0 mM KOH, 1.0 mM $CaCl_2$, 0.25 or 1.0 mM MOPS, pH7.2 adjusted by HCl) for longer than 1 h. The K^+ concentration in the experimental solution was controlled by changing the amount of KOH added to the solution in order to achieve slower swimming by membrane depolarization (4mM K^+) (Machemer, 1989).

Analysis of the cell geometry

Volume of the individual cell body was estimated by two separate methods. One is the conventional method with the assumption of the cell body as a prolate spheroid with short rotating axis length d_a and long axial length d_b, and the volume (prolate vlume, V_p) was calculated as,

$$V_p = \frac{1}{6}\pi d_a^2 d_b . \tag{3}$$

Another method is the computer assisted tomographic reconstruction. Cells immobilized by Ni^{2+} (1 to 5 mM) were observed by laser confocal microscope (LSM 700, Zeiss, Germany). From the confocal images generated by autofluorescence of the cell, we obtained cross-sectional images with several micrometers interval (Fig 2A and B). The area of the individual cross section was determined by manually outlining the image and then summing up the pixels within the contour (Fig 2C). The volume (tomographic vlume, V_t) was obtained by summing up the products of cross-sectional area and the interval between the successive cross sections.

Evaluation of SMR

SMR was estimated following the method described in Katsu-Kimura *et al*(2009). 0.74 ml of cell suspension with a density of $3.5 \times 10^3 - 1.9 \times 10^4$ cells ml^{-1}, which is close to the density during the stationary phase, were transferred into the recording chamber. The chamber was placed in the water bath at controlled-temperature (23 ± 1°C). The oxygen concentration in the chamber was measured by an optic fluorescence oxygen sensor (FO-960, ASR, Tokyo, Japan) placed at the top of the chamber. The oxygen consumption rate per cell ($mlO_2\ h^{-1}\ cell^{-1}$) was determined as described in Katsu-Kimura *et al* (2009).

Cell suspension was illuminated through the transparent side wall of the chamber by horizontal slit laser with a known beam thickness (half-maximum intensity width of 0.2 mm; SU-42C- 635-10, Audio Technica,

Tokyo, Japan), and dark-field images of cells that swam in the slit of light were recorded through the transparent bottom with a CCD camera (XC-77RR, SONY, Tokyo, Japan). Swimming speed of the cell was calculated as described in Katsu-Kimura *et al* (2009).

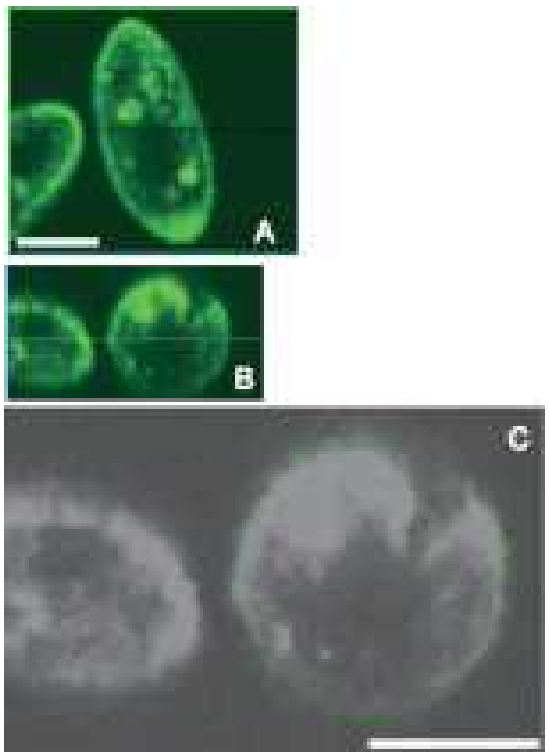

Figure 1 Confocal image of *P. caudatum* syngen 4 generated by autofluorescence of the cell (A) and the reconstructed cross section roughly perpendicular to the long axis (B). In C, the manually outlined contour of cross section is shown that was used for the determination of the cross sectional area. Scale bars, 50 μm.

Results

Geometrical scaling of Paramecium

Paramecium has a slender body. Light microscopy of the cell body shows a ellipsoidal outline of the cell shape. In addition, observation of the swimming of *Paramecium* along the helical trajectory, one can easily imagine the three-dimensional nature of the cell shape, which is confirmed by several images of scanning electron microscopy. The simple assumption of the cell body, therefore, is that as a prolate spheroid. Although fore-aft asymmetry is evident and plays an important role in the swimming behavior (Mogami *et al*, 2001), we started our analysis form this simple assumption.

Fig 2A shows the plots of short axis length (d_a) against the long axis length (d_b) of the cell body. The relation between the two variables was not isometric. From the double logarithmic plot (inset of Fig 2A), the allometric relation was written as,

$$d_a = 2.78\, d_b^{0.56}\,. \qquad (4)$$

Fig 2B shows the plots of tomographic volume (V_t) against the prolate volume (V_p) calculated from the d_a and d_b of the same cell. Interestingly, signals from the double logarithmic plot (inset of Fig 2B) is that the allometric exponent can be considered as unity. It is, therefore, highly likely that the relation between the two variables was nearly isometric. Linear proportional function obtained by the least squares fitting was

$$V_t = 0.75\, V_p\,. \tag{5}$$

Difference between V_t and V_p is the volume of the depression from the surface of the prolate. Since the main part of the depression corresponds to the cytopharyx (Fig 1C), it is highly likely that the volume of the cytopharynx changes in proportion to the total volume of the cell.

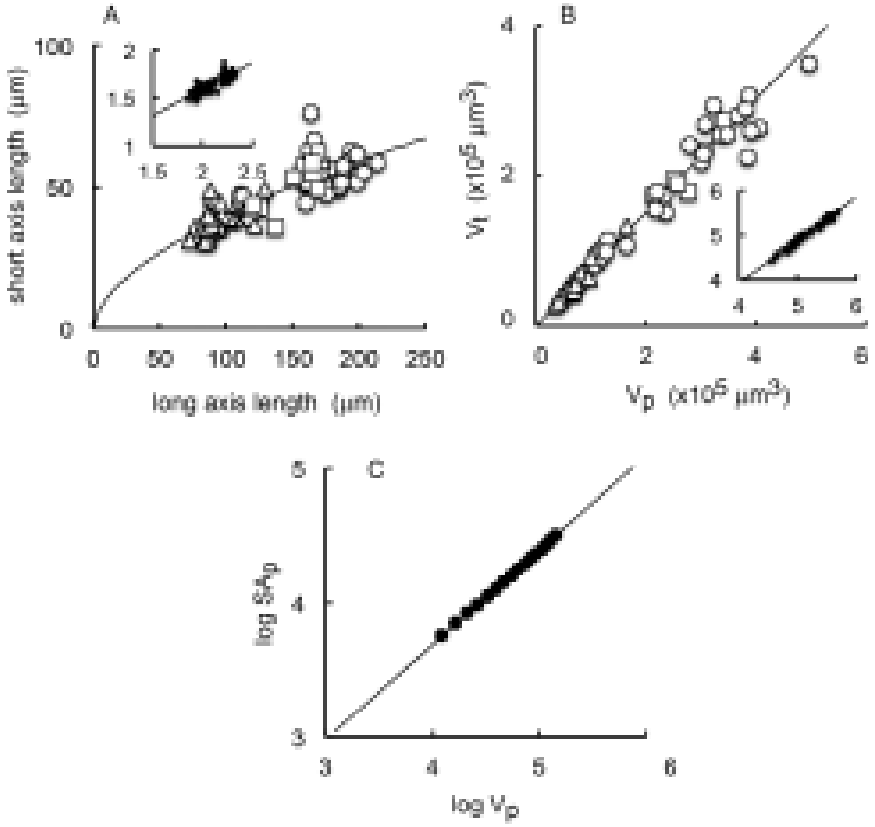

Figure 2 A, plots of d_a as a function of d_b measured from *Paramecium caudatum*, syngen 1 (square), *P. caudatum* syngen 3 (triangle), *P. caudatum* syngen 4 (circle), and *P. aurelia* syngen 8 (diamond). Inset shows the double logarithmic plot of d_a (ordinate) and d_b (abscissa), which signals a linear relation of log d_a = 0.57log d_b + 0.46 (r^2=0.73, N=81). B, plots of V_t as a function of V_p measured from four different strains marked by the same symbols as in A. They show the linear correlation with r^2= 0.96. Inset shows the double logarithmic plot of V_t (ordinate) and V_p (abscissa), which signals a linear relation of log V_t = 0.97log V_p + 0.064 (r^2=0.98, N=53). C, double logarithmic plot of surface area (SA_p, μm^2) and V_p (μm^3) calculated from d_a and d_b with the relation shown in A. Within the range of measured d_a, log SA_p = 0.704log V_p + 0.83 (r^2=0.99).

Linear relation of V_t to V_p suggests that the assumption of the cell body as a prolate spheroid is useful for the evaluation of the cell volume. It may also be suggested that prolate assumption is available for the scaling analysis on the cellular geometry of *Paramecium*. Fig 2C show the double logarithmic plot of surface area (SA_p)

and V_p calculated based on the relation between d_a and d_b. In this figure, SA_p was calculated as

$$SA_p = \frac{\pi d_a^2}{2}\left\{1 + \frac{\sin^{-1} e}{e\left(e^2 - 1\right)}\right\}, \quad (6)$$

where e is the eccentricity of the spheroids defined as

$$e = \sqrt{1 - \frac{d_a}{d_b}}. \quad (7)$$

Signals of the plot indicates that the allometric relation of SA_p and V_p could be written as,

$$SA_p \propto V_p^{0.71}. \quad (8)$$

Metabolic scaling of Paramecium

Figure 3A and B show plots of oxygen consumption as a function of swimming speed measured from *P. aurela* syngen 8 and *P. caudatum* syngen 1, respectively. Both of two variables were determined as the average of the cells confined in the recording chamber. For this measurement K^+ concentration of the experimental solution was changed to slow down the speed at high concentration (4mM). The plots show a positive correlation between increasing swimming speed and increasing oxygen consumption rate. Correlation coefficient (r^2) is 0.38 (N = 32) and 0.35 (N = 42) for *P. aurela* syngen 8 and *P. caudatum* syngen 1, respectively. The linear regression of plots indicates that the intercept is 8.96 ± 5.27 and 17.5 ± 34.3 (10^{-8}mlO_2 h^{-1} $cell^{-1}$, mean ± 95% confidence interval), and can be converted to *SMR* of 8.96 ± 5.27 and 3.52 ± 5.87 (10^{-6}J h^{-1}, mean ± 95% confidence interval) with equating 1 liter of oxygen with 20.1 kJ (Schmidt-Nielsen, 1984).

Figure 3C shows double logarithmic plot of the resultant *SMR* as a function of body mass (W) estimated on the prolate assumption of the cell body with the proportional factor of 0.75, and the mean density of 1.04 g cm^{-3}. Result of the least squares fitting to the data from four strains is log*SMR* = 1.01log$W$ + 4.01 (r^2=0.24). 95 % confidence interval of the exponent was 5.55.

Discussion

It has been conventional for the cell body of *Paramecium* to be assumed as a prolate spheroid. Measurement of the major axes of the cell body from several strains with different size revealed that the aspect ratio changed with body size (Fig 2A). The fact that V_t is proportional to V_p (Fig 2B) suggests that the volume of the cytopharynx, which is a major part of the depression from the prolate surface, increases in proportion to the cell volume. Based on the prolate assumption, on the other hand, surface area of the cell body could be scaled by an allometric function with the exponent of 0.71 (Fig 2C).

For the allometric analysis of metabolic rate of *Paramecium*, we introduced the extrapolation method for the estimation of *SMR*, the metabolic rate at zero swimming speed (Figs 3A and B) (Katsu-Kimura *et al*, 2009). Although several methods are known to make paramecia immotile, none of them are thought to avoid the physiological effects on the cellular metabolism. Ni^{2+}, most commonly used for immobilization, for example, may affect the metabolic activity by inhibiting cytoplasmic motor proteins as well as ciliary motile apparatus. Deciliation may initiate an additional metabolic activity for reciliation. The extrapolation method, however, could only give an estimation with large uncertainty due to the inevitably large scattering of the measurements.

As the result, we could not obtain only a weak correlation (r^2=0.24) in double logarithmic plot of *SMR* and cellar mass calculated on the basis of the prolate assumption (Fig 3C).

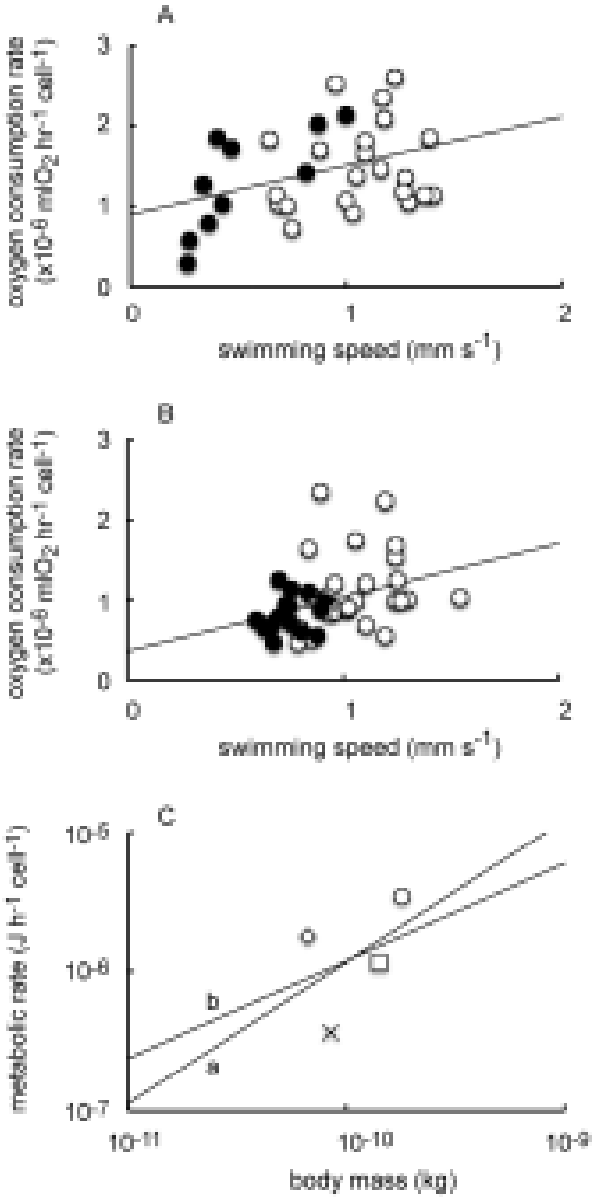

Figure 3 The relationship between the oxygen consumption rate and the swimming speed of *P. aurelia* syngen 8 (A) and *P caudatum* syngen 4 (B). The correlation coefficient (r^2) is 0.38 (N = 32) and 0.35 (N = 42), for A and B, respectively. Open symbols indicate data measured in the standard solution (1mM K^+), closed symbols indicate data in higher concentration solution (4mM K^+). Solid lines show results of the linear regression by least-square fitting. The intercept is 8.96 ± 5.27 and 17.5 ± 34.3 (10^{-8}mlO$_2$ h^{-1} cell^{-1}, mean ± 95% confidence interval), for A and B, respectively. C, double logarithmic plot of *SMR* and cellar mass from four different strains, *Paramecium caudatum*, syngen 1 (square), *P. caudatum* syngen 4 (circle), *P. aurelia* syngen 8 (diamond), and *P. tetraurelia* syngen 4 (cross). *SMR* of *P. caudatum* syngen 1 is from the separate measurement of Katsu-Kimura *et al* (2009). Data of *P. tetraurelia* syngen 4 is from Kato *et al* (2003), and *SMR* was estimated by swimming efficiency described in Katsu-Kimura *et al* (2009). Lines a and b are the results of least squares fitting to the linear function with slope of 1.0 and 0.71, respectively. For details, see text.

It may be interesting to discuss the scaling of metabolism based on the result of the scaling of cell geometry, which indicates the relationship of the cell volume to the cytopharynx volume and the surface area.

Cytopharynx has the role of food intake, and the food taken into the cell is used for generating energy in the course of respiration; oxygen required for respiration is supplied from the environment through cell surface. It is therefore inferred that these two variables, cytopharynx volume and surface area, are included in the

allometric relation of metabolism to the cell size.

Nutrient materials captured by the cytopharynx are then ingested into the food vacuole. The materials are digested within the vacuole and then assimilated into the cytoplasm. If the total volume of the food vacuole formed in a fraction of time is proportional to the volume of the cytopharynx, although there is no clear evidence about the proportionality, the rate of assimilation of energy source is likely to be proportional to the cytopharynx volume. Oxygen uptake occurs through the cell membrane. If the permeability to the oxygen molecule is uniform all over the cell membrane, total flux of oxygen into the cytoplasm is proportional to the surface area. If the assimilation of energy source is rate limiting, metabolic rate may be decided in proportion to the cellular mass. And, it may be decided in relation to the cellular mass by the power 0.71, if the oxygen uptake is rate limiting. The allometric relation with the exponent between 1.0 and 0.71 would be envisioned if the metabolic rate is determined in combination of these two factors. In Figure 3C, lines with slope of 1.0(a) and 0.71 (b) are shown, each of which was obtained by least squares fitting. The root mean squares (*rms*) of these line to the data is 0.31 and 0.32 for a and b, respectively. Although the result of the simple linear regression gave the slope of 1.01 (*rms*=0.30), large confidence interval unfortunately cannot support the possibility that the assimilation of energy source is rate limiting, and also cannot deny another possibility.

Allometry of the metabolic rate of protists is yet controversial. Several researchers claimed 3/4-law as a universal rule proposed for multicellular organisms. Others proposed variable values (0.55 to 0.75) for the allometric exponent (Scholander *et al.*, 1952; Prothero, 1986; Dodds *et al.*, 2001; Phillipson, 1981). However, it should be stressed that the researches so far must be criticized in terms of the measurement of *SMR* and cellular body mass, which had not necessarily been done with the same definition as that applied to the multicellular organisms. Although we only represent a preliminary result, the experimental strategy adopted in this study may pave the way to understand the details of the scaling of unicellular organisms as well as their representative, *Paramecium*.

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