Active Force Generated by the Motility of the Outer Hair Cell*

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Although the force production of the outer hair cell (OHC) leads to the fine tuning of the mammalian cochlea, the value of the force generated by the motility of the OHC *in vivo* has not been clarified yet. In this study, first, the active force generated by the motility of the load-free OHC in response to an electrical stimulation was measured from the deflection of the force probe. Next, to establish cell conditions similar to those of the intact cochlea, a compressive load was applied to the OHC using a force probe driven by a bimorph actuator, and then the active force generated by the motility of the loaded OHC was determined. Comparing the active force generated by the motility of the load-free OHC with that by the motility of the loaded OHC, it was found that the active force decreased due to application of the compressive load.

Key Words: Acoustic, Biomechanics, Measurement, Outer Hair Cell, Force, Motility, Patch Clamp Technique

1. Introduction

The outer hair cell (OHC) is able to elongate and contract in response to an electrical stimulation, which is known as electromotility⁽¹⁾⁻⁽⁵⁾. *In vivo*, the OHC is located between the reticular lamina and Deiters' cell on the basilar membrane (Fig. 1), and the force produced by the OHC, which accompanies its electromotility, is thought to affect the motion of the basilar membrane. As a result, the force production of the OHC is presumed to lead both to the fine tuning and the wide dynamic range of the mammalian cochlea.

The electromotility of the OHC has been reported to be associated with the nonlinear gating charge movement or the nonlinear capacitance based on whole-cell patch-pipette recording⁽⁶⁾. In addition, simulation has indicated that a similar nonlinear charge movement is closely associated with the conformational change of the membrane particles of the OHC⁽⁷⁾. Based on these results, the motility of the

OHC is thought to be associated with voltage-dependent conformational changes of membrane particles which are called motor proteins.

To determine the OHC's ability to influence the basilar membrane motion and tuning, it is important to evaluate the active force generated by the motility of the OHC. In earlier reports, this active force was measured in isolated OHCs *in vitro*^{(8),(9)}. However, *in vivo*, it seems that the matrices surrounding the OHCs impose axial restrictions on the motility. Therefore,

Fig. 1 Schematic of the cochlea and a cross section of the organ of Corti

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to evaluate the force generated by the motility of the OHC, it is necessary to take the axial restrictions into account. However, the force generated by the motility of the loaded OHC has not yet been clarified.

In this study, first, a force probe was attached to the apical part of the OHC, and the active force generated by the motility of the load-free cell was measured based on the deflection of the force probe in response to the electrical stimulation. Next, a compressive load was applied to the OHC using the force probe which was driven by a bimorph actuator, and the active force generated by the motility of the loaded OHC was obtained. Finally, the force generated by the motility of the loaded OHC was compared with that generated by the motility of the load-free OHC.

2. Materials and Methods

2.1 Cell preparation

Guinea pigs weighing between 200 and 300 g were decapitated and the temporal bones were removed. After the bulla was opened, the cochlea was detached and transferred to an experimental bath composed of 140 mM NaCl, 5 mM KCl, 1.5 mM CaCl_2 , 1.5 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 mM HEPES and 5 mM glucose and adjusted to pH 7.2 and 300 mOsm. The bony shell covering the cochlea was removed and two basal turns of the organ of Corti were gently scraped off from the basilar membrane. The organ of Corti was enzymatically incubated with trypsin (1 mg/ml) for 4 minutes and then with dispase (500 PU/ml) for 6 minutes. The OHCs were isolated by gently pipetting the organ of Corti, and then transferred to a chamber. To obtain the local deformation of the OHC, cells were perfused with the medium which contained microspheres 0.75 μm in diameter; these microspheres became randomly attached to each cell. All experiments were performed under an inverted microscope (TE300, Nikon) on a vibration isolation table at room temperature. The care and use of the animals in this study were approved by the Institutional Animal Care and Use Committee of Tohoku University, Sendai, Japan.

2.2 Experimental apparatus

A block diagram of the measurement system is shown in Fig. 2. A patch pipette was used both to hold the OHC at its basal part and to apply an electrical stimulation to the cell. The patch pipette had an initial resistance of 2–5 M Ω , corresponding to tip sizes of 1–2 μm ⁽¹⁰⁾, and it was filled with a pipette solution composed of 150 mM KCl, 2 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 10 mM EGTA and 10 mM HEPES, adjusted to pH 7.2.

To obtain the active force generated by the

Fig. 2 Block diagram of the measurement system

motility of the OHC, it is necessary to know the axial stiffness of the cell in advance. The basal part of the cell was held by the patch pipette and a compressive load was applied to the apical part of the cell using a force probe driven by a bimorph actuator. The displacement of the probe was controlled by the drive voltage induced by a function generator (NF-1944, NF Electronic Instruments) and an amplifier (HSA-4001, NF Electronic Instruments). The axial stiffness of the OHC was obtained from the relationship between the compressive load applied to the cell and the bending displacement of the force probe, i.e., the displacement of the cell. As the force probe had a diameter of a few micrometers and the bending stiffness of the force probe was small, it was difficult to measure the bending stiffness directly. Therefore, the cross calibration method⁽¹¹⁾ was used and the bending stiffness of the force probe was found to be a few mN/m.

To measure the active force generated by the motility of the load-free OHC, electrical stimulation was applied to the cell using the whole-cell patch clamp technique. The whole-cell patch clamp technique is an electrophysiological method which experimentally allows control and manipulation of the membrane potential via a pipette electrode within the patch pipette. Electrical stimulation originally induced by the function generator (NF-1944, NF Electronic Instruments) was applied to the OHC through a patch amplifier (Axon 200B, Axon Instruments). The OHC elongated in response to the hyperpolarizing electrical stimulation and the force probe was deflected. The active force was obtained from this deflection. Next, a compressive load was applied to the OHC using the force probe driven by the bimorph actuator, and the active force was obtained in the loaded cell in the same way as in the load-free cell.

The images of the cell movements were captured

Fig. 3 Relationship between the load applied to the OHC and the change in axial length of the cell (cell length is $33.5\ \mu\text{m}$). The data is fitted by a linear regression line with a slope of 4.22 and a correlation coefficient r of 0.967.

by a high-speed camera (HSV-500DM, nac) and analyzed off-line using a personal computer.

2.3 Mechanical compression for obtaining the axial stiffness of the OHC

To obtain the axial stiffness of the OHC, the length change of the cell was determined from images before and after compression of the cell by the force probe driven by the bimorph actuator, and the load applied to the cell was calculated from the deflection of the force probe. An example of the relationship between the length change of the cell and the load applied to the cell is shown in Fig. 3. The data were fitted by a linear regression line with a slope of 4.22 and a correlation coefficient r of 0.967. From the slope of this regression line, the axial stiffness of the OHC K_c obtained in this example was $4.22\ \text{mN/m}$.

2.4 Active force generated by the motility of the load-free and loaded OHC

To determine the active force generated by the motility of the load-free OHC, electrical stimulation was applied to the cell from the patch electrode. In the measurements, first, the OHCs were voltage clamped at $-80\ \text{mV}$ as a holding potential. Then, the membrane potential was changed from $-80\ \text{mV}$ to $-180\ \text{mV}$ with sinusoidal voltage of $1\ \text{Hz}$, $100\ \text{mV}_{\text{p-p}}$, and the deflection of the force probe accompanied by the elongation of the load-free cell was determined by image analysis. Then, a compressive load was applied to the OHC in the same manner as for obtaining the axial stiffness of the OHC, and the active force generated by the motility of the loaded cell was measured in the same way as with the load-free cell.

2.5 Data analysis

Images of the cell movements were captured by a high-speed camera and analyzed off-line using a

personal computer. One pixel in the recorded digital picture from the high-speed camera system corresponded to $120\ \text{nm}$. Each pixel was quantified using a gray scale of 256. To analyze displacement of the objects, such as the force probe or microspheres smaller than 1 pixel, a high-resolution motion detection method was employed⁽¹²⁾. In this analysis, the analytical error corresponded to $19\ \text{nm}$. This analytical error resulted from noise in the recorded digital picture.

3. Results

3.1 Active force generated by the motility of the load-free OHC

First, to determine the local deformation of the OHC in response to the hyperpolarizing electrical stimulation, the movements of the force probe and microspheres were measured. The measurement points on one of the cells are shown in Fig. 4(a) and time histories of the displacement of each measurement point are depicted in Fig. 4(b). The direction of the arrow in Fig. 4(a) represents the positive displacement. From Fig. 4(b), it is found that measurement points on the apical part of the cell move in the positive direction. However, although the basal part of the cell was attached to the fixed patch pipette, due to the slip between the patch pipette and the cell membrane, measurement points on the basal part move in the negative direction. This means that there is an immobile point in the middle region of the cell which does not move in either the positive or the negative direction. Therefore, it was estimated that the positive displacement of the force probe was evoked by the conformational change of the motor protein in the region of the cell between the immobile point and the apical end of the cell. To determine the force generated by the motility of the whole cell, the immobile point was obtained (see Appendix A). The deflection of the force probe evoked by the motility of the whole cell was calculated from the data shown in Fig. 4(b) and is displayed in Fig. 4(c). From the deflection of the force probe when the cell elongates, which corresponds to two regions, from 0 to 0.5 s and from 1.0 to 1.5 s in Fig. 4(c), the active force F_{iso} generated by the motility of the OHC was calculated with the equation $F_{iso} = (K_p + K_c)X_p$, where K_p , K_c and X_p represent the bending stiffness of the force probe, axial stiffness of the OHC and deflection of the force probe, respectively (See Appendix B). The mean values calculated for the two cycles are defined as the force generated by the motility of this cell. An example of the active force generated by the motility of another OHC is shown in Fig. 5. The abscissa represents the membrane potential. The data are fitted by a logarithmic regression line which has a correlation

(a)

(b)

(c)

Fig. 4 Local deformation of the OHC. (a) Measurement points on the cell, (b) Time histories of the displacements of the force probe and microspheres when electrical stimulation (sinusoidal, 1 Hz, 100 mV_{p-p}) was applied to the cell, (c) Compensated values of the deflection of the force probe

coefficient r of 0.975. The slope of this regression line decreases with hyperpolarization, and the force sensitivity, which is defined as the values of the slope of the regression line divided by the cell length, changes from 0.85 pN/μm/mV at -80 mV to 0.07 pN/μm/mV at -180 mV.

In the same way as shown in Fig. 5, the data from six different cells were analyzed. Figure 6 shows the regression lines obtained from these six cells. The cell

Fig. 5 Example of the averaged values of the active force generated by the motility of an OHC (cell length is 33.5 μm and axial stiffness is 4.22 mN/m). The data are fitted by a logarithmic regression line which has a correlation coefficient r of 0.975.

Fig. 6 Active force generated by the motility of load-free OHCs in six cells. The correlation coefficients r of the 6 curves range from 0.903 to 0.992. The force sensitivity ranges from 0.07 pN/μm/mV to 3.77 pN/μm/mV.

length used in this analysis ranged from 31.2 μm to 41.8 μm. The correlation coefficients r of the six lines are in the range between 0.903 and 0.992. The force generated by the motility of the load-free OHCs has a nonlinear function with the membrane potential, and the force sensitivity ranges from 0.07 pN/μm/mV to 3.77 pN/μm/mV.

3.2 Active force generated by the motility of the loaded OHC

After measurement of the active force generated by the motility of the load-free OHC, a compressive load was applied to the cell via the force probe, and then the active force generated by the motility of the loaded OHC was measured in the same way as that in the load-free condition. An example of the active force generated by the motility of the OHC under

Fig. 7 Active forces generated by the motility of the OHC (cell length is $31.8 \mu\text{m}$). Filled circles represent the value of the force generated by the motility of the OHC when compressive load was not applied, and open squares and triangles represent the value of the force when compressive loads of 1.32 nN and 1.50 nN were applied to the cell, respectively.

load-free and loaded conditions is shown in Fig. 7. In the load-free OHC, the force sensitivity ranged from $0.15 \text{ pN}/\mu\text{m}/\text{mV}$ to $3.49 \text{ pN}/\mu\text{m}/\text{mV}$, and the maximum force measured at -180 mV was 1.58 nN . When compressive loads of 1.32 nN and 1.50 nN were applied to the cell, the force sensitivities ranged from $0.23 \text{ pN}/\mu\text{m}/\text{mV}$ to $0.67 \text{ pN}/\mu\text{m}/\text{mV}$ and from $0.30 \text{ pN}/\mu\text{m}/\text{mV}$ to $0.51 \text{ pN}/\mu\text{m}/\text{mV}$, respectively. Comparing the maximum force sensitivity under the load-free condition with that under the loaded condition, the latter ($0.67 \text{ pN}/\mu\text{m}/\text{mV}$ and $0.51 \text{ pN}/\mu\text{m}/\text{mV}$) was smaller than the former ($3.49 \text{ pN}/\mu\text{m}/\text{mV}$). In addition, the maximum forces measured at -180 mV were 1.18 nN and 1.22 nN when the compressive load of 1.32 nN and that of 1.50 nN were applied, respectively. These values were also smaller than the value of 1.58 nN under the load-free condition.

The active forces generated by the motility of two other OHCs were measured, and the value of the force sensitivity and that of the maximum force are summarized in Table 1. The value of the force sensitivity and that of the maximum force under the loaded condition were smaller than those under the load-free condition, except those of the cell to which the compressive load of 3.54 nN was applied.

4. Discussion

4.1 Force generation of the load-free OHC and comparison with previous studies

To obtain the active force generated by the motility of the OHC, hyperpolarizing electrical stimu-

Table 1 Force sensitivity and maximum force in three cells

	Load-free	Loaded	Loaded
Load applied to the OHC (nN)	0.00	1.32	1.50
Range of force sensitivity ($\text{pN}/\mu\text{m}/\text{mV}$)	0.15 - 3.49	0.23 - 0.67	0.30 - 0.51
Maximum force (nN)	1.58	1.18	1.22
Load applied to the OHC (nN)	0.00	0.46	
Range of force sensitivity ($\text{pN}/\mu\text{m}/\text{mV}$)	0.20 - 8.93	0.27 - 1.10	
Maximum force (nN)	2.54	1.68	
Load applied to the OHC (nN)	0.00	3.54	3.85
Range of force sensitivity ($\text{pN}/\mu\text{m}/\text{mV}$)	0.06 - 0.71	0.06 - 0.82	0.03 - 0.47
Maximum force (nN)	0.67	0.65	0.38

lation was applied to the cell. As a result, as shown in Fig. 4, the cell was found to show complex deformation in response to the electrical stimulation, i.e., the basal part of the cell moved in the negative direction and the apical part moved in the positive direction. Mammano⁽¹³⁾ and Frolenkov⁽¹⁴⁾ also reported such a complex deformation of the OHC when the cell was depolarized within the intact organ of Corti. One of the reasons for this complex deformation of the cell might be the boundary condition of the cell. *In vivo*, the basal part of the OHC is cupped by Deiters' cell and the apical part of the cell is firmly held within the reticular lamina. In this experiment, the basal part of the OHC was held by the patch pipette and the apical part of the cell was attached to the force probe, which is similar to the condition *in vivo*. Therefore, it is reasonable that complex deformation was found in the experiment. The complex deformation of the cell may lead to the dispersion in the values of the active force generated by the motility of the OHCs as shown in Figs. 5, 6 and 7.

It was reported that the active force generated by the motility of a load-free OHC had a linear relationship with the membrane potential when the cell was hyperpolarized⁽⁶⁾. In that previous report, the electrical stimulation was applied across the cell membrane and the change in the membrane potential was estimated to be less than 20 mV . In this study, however, the membrane potential was changed from -80 mV up to -180 mV , a wider range than that in the previous study. As a result, as shown in Fig. 5, the values were fitted better by a logarithmic regression line which had a correlation coefficient r of 0.975 than by

a linear regression line which had a correlation coefficient r of 0.912. A logarithmic regression line was also used in other cells for the optimum fitting of data (Fig. 6). These results indicate that the force generated by the motility of the OHC has a nonlinear function with the membrane potential in a wide range. In addition, as shown in Fig. 5, the slope of the force generated by the motility of the OHC decreased with a decrease in the membrane potential. As the cell length change in response to the electrical stimulation shows a nonlinear function with the membrane potential⁽⁶⁾ in this wide range, it seems to be reasonable that the active force generated by the motility of the OHC has a nonlinear function.

In this study, from Fig. 6 and Table 1, the force sensitivity was determined to be $0.06 - 8.93 \text{ pN}/\mu\text{m}/\text{mV}$ from the load-free OHCs. On the other hand, in previous studies, Hallworth⁽⁸⁾ and Iwasa⁽⁹⁾ reported that the force sensitivity was $0.15 - 5.59 \text{ pN}/\mu\text{m}/\text{mV}$ and $0.78 - 2.9 \text{ pN}/\mu\text{m}/\text{mV}$, respectively. Comparing our value with the previous ones, the maximum value of the force sensitivity in this study is consistent with those in the previous reports. However, the minimum value is smaller than the previous ones. This might be because, in this study, the force generated by the motility of OHCs has a nonlinear function and the slope of the force decreases with a decrease in the membrane potential.

4.2 Effect of the compressive load on the force generation of the OHC

It is still unknown what kinds of loads are applied to the OHC *in vivo*. However, it can be assumed from the study done by LePage⁽¹⁵⁾ on the motion of the basilar membrane in response to the acoustical stimulation. He reported that the motion of the basilar membrane included not only the AC component but also DC offset. The displacement of the DC offset was toward the scala vestibuli at a characteristic frequency and its value was about 0.2 nm. This result indicates that the OHC is compressed by the DC offset of the basilar membrane *in vivo*; the amount of the load applied to the OHC by the DC offset is calculated to be less than 0.1 nN. However, in the present experiment, it was difficult to apply a compressive load of 0.1 nN to the OHC because the deflection of the force probe corresponded to a load of 0.1 nN which is in the range of analysis error. As a result, compressive loads of more than 0.4 nN were applied to the OHC.

As shown in Table 1, both the maximum force and the force sensitivity of the loaded OHC were smaller than those of the load-free OHC. This means that the active force generated by the motility of the loaded OHC is smaller than that by the motility of the load-free OHC. As shown in Eq. (2) of Appendix B,

the active force generated by the motility of the OHC is dependent on both the axial stiffness of the cell and the displacement of the motor. Therefore, the decrease in the force resulting from application of a compressive load to the OHC is due to a decrease in the axial stiffness of the cell or a decrease in the displacement of the motor. In this study, as shown in Fig. 3, the axial stiffness of the OHC was obtained from the relationship between the cell length change and the load applied to the cell, and the slope of the relationship was linear, i.e., the axial stiffness of the cell was constant in the range of the compression up to $0.8 \mu\text{m}$. Therefore, it is supposed that the axial stiffness of the OHC is not related to the decrease in the force generated by the motility of the loaded OHC.

Iwasa⁽⁷⁾ has reported that an increase in the membrane tension led to a positive shift in the voltage at peak membrane capacitance in OHCs. This result implies that the increase in the membrane tension elevates the fraction of the extended state of the motor protein for a given membrane potential. Kakehata et al.⁽¹⁶⁾ confirmed that the increase in the membrane tension decreased the amount of cell elongation in response to electrical stimulation because the contracted state of the motor protein decreased as a result of the increase in membrane tension at a given membrane potential. In the present study, when a compressive load was applied, the membrane tension increased, which resulted in an increase in the fraction of the extended state of the motor protein. As a result, it is considered that the active force generated by the motility of the cell may be decreased by applying a compressive load.

5. Conclusions

In response to the hyperpolarizing electrical stimulation, the active force generated by the motility of the load-free OHC was measured from the deflection of the force probe attached to the apical part of the cell. Then, the OHC was loaded by the force probe driven by a bimorph actuator and the active force generated by the motility of the loaded OHC was obtained. The following conclusions can be drawn:

(1) The active force generated by the motility of the load-free OHC has a nonlinear function with the membrane potential, and the force sensitivity ranges from $0.06 \text{ pN}/\mu\text{m}/\text{mV}$ to $8.93 \text{ pN}/\mu\text{m}/\text{mV}$.

(2) The active force generated by the motility of the loaded OHC is smaller than that of the load-free OHC.

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Fig. 8 Compensated displacements of all measurement points shown in Fig. 4(a) at 0.5 s. Data are fitted by a linear regression line with a slope of 0.568 and a correlation coefficient r of 0.971. From this regression line, the immobile point is determined to be 0.597 from the basal end.

Fig. 9 Motor distribution. The immobile point is in the middle region of the cell, and l represents the normalized distance from the basal end to the immobile point. In this analysis, the force generated by the motility of the cell is assumed to be caused by motors distributed in the hatched area, which is represented by $(1.0-l)L$.

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Appendix A: Compensation to Obtain the Active Force Generated by the Motility of the Whole Cell

To calculate the active force generated by the motility of the whole cell, the immobile point was obtained. The displacements of all measurement points shown in Fig. 4(a) at 0.5 s, i.e., the membrane potential of -180 mV, were obtained from Fig. 4(b) and are shown in Fig. 8. The abscissa represents the distance of each measurement point from the basal end of the cell, and the positions of the basal end and apical end along the cell axis are converted to 0.0 and 1.0, respectively. The values are fitted by a linear regression line which has a slope of 0.568 and a correlation coefficient r of 0.971. From this regression line, the position of the immobile points is determined to be

Fig. 10 Equivalent mechanical models. (a) Equivalent mechanical circuit of the OHC under the isometric condition, (b) Equivalent mechanical circuit that represents the experimental configuration. The upward direction represents positive displacement.

0.597 from the basal end. When the immobile point was in the middle region of the cell, as shown in Fig. 9, force generated by the motility of the cell was caused by the motors in the hatched area which is represented by $(1.0-l)L$, where L and l are the length of the cell and the normalized distance from the basal end to the immobile point, respectively. Since the motors could be assumed to be distributed equally along the axial direction of the cell, the deflection of the force probe X_p which would be evoked by the whole cell was estimated as follows:

$$X_p = \frac{L}{(1.0-l)L} X'_p \quad (1)$$

where X'_p represents the deflection of the force probe measured in the experiment (Fig. 4(c)), which is evoked by the cell in the region of $(1.0-l)L$.

Appendix B: Isometric Force

Hallworth⁽⁸⁾ reported that the active force generated by the motility of the OHC was dependent on the boundary condition of the cell and increased with an increase in the boundary stiffness. To remove the effect of the boundary stiffness on the active force generated by the motility of the OHC, the active force under the isometric condition, i.e., isometric force, was calculated in this study. An equivalent mechanical circuit of the OHC under the isometric condition is shown in Fig. 10(a)⁽⁹⁾. In this figure, an upward direction represents positive displacement. As shown in this figure, the isometric condition means that the cell length is kept constant. In the model, the protein motor was assumed to be an ideal extension generator and to be in a series with the stiffness of the OHC. When the electrical stimulation was applied to the OHC, the motor produced the displacement X_0 . Then isometric force F_{iso} was given by

$$F_{iso} = K_c \cdot X_0, \quad (2)$$

where K_c represents the axial stiffness of the cell. As the displacement of the motor X_0 could not be obtained directly, it was calculated from the deflection of the force probe X_p using the equivalent mechanical circuit which represents the experimental configuration (Fig. 10(b)). When the electrical stimulation is applied to the OHC, the deflection of the force probe X_p is evoked by the generation of the motor displacement X_0 . Assuming that the stiffness of the cell does not vary during its deformation, the force F measured from the bending displacement of the force probe is given by

$$F = K_p \cdot X_p, \quad (3)$$

where K_p represents the stiffness of the force probe. At the same time, the OHC is compressed by the force F , and the force applied to the OHC F is given by

$$F = K_c(X_0 - X_p). \quad (4)$$

Equations (3) and (4) lead to

$$K_p \cdot X_p = K_c(X_0 - X_p), \quad (5)$$

From Eq. (5), X_0 is given by

$$X_0 = \frac{K_p + K_c}{K_c} X_p. \quad (6)$$

Substituting Eq. (6) into Eq. (2), F_{iso} is expressed as

$$F_{iso} = (K_p + K_c) X_p. \quad (7)$$

From Eq. (7), it is clear that the isometric force can be calculated from the stiffness of the force probe K_p , the axial stiffness of the OHC K_c and the deflection of the force probe X_p .

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