

Biosynthesis of Cellulose by *Acetobacter Xylinum*. III. X-Ray Studies of Preferential Orientation of the Crystallites in a Bacterial Cellulose Membrane

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ABSTRACT: In an undried membrane of bacterial cellulose, it is not clear from X-ray analysis whether well-defined cellulose crystallites with a definite orientation exist or not. There is, however, a tendency toward the formation of a preferential orientation as water is removed from a membrane. Membranes stretched along one direction showed a uniaxial orientation. Membranes dried with a flat surface parallel to a glass plate provided a typical selective uniplanar orientation of the (101) crystallographic plane. It was noticed that a drying membrane adheres to a glass plate, and that shrinkage occurs only normally to a glass plate.

It was recognized that liquids contained in the swollen membranes may influence the preferential orientation of bacterial cellulose upon drying; these liquids can be classified into two broad categories. One is a group of liquids such as acetone and pyridine which give a higher selective uniplanar orientation than water, and the other is a group such as carbon tetrachloride and cyclohexane which disturb the formation of orientation.

KEY WORDS Bacterial Cellulose Membrane / Cellulose Crystallites / Preferential Orientation / Shrinkage / Plastic Flow / H-bonding Ability / External Configuration /

As a partial proof for the preferential orientation of bacterial cellulose membranes, which was mentioned in Part I in the comparison of the X-ray diagrams of ramie and *Valonia* cellulose, the purpose of the present study is to describe the relationship between deformation and orientation during drying processes. Information about the cellulose crystallite orientation is not only of theoretical interest but also of practical importance, especially in the production of regenerated cellulose sheets and fibers. Apart from a study of the characteristics of cellulose fiber diagrams themselves, one of the useful methods of investigating the configuration of the crystallite is to observe with X-rays its plastic behavior. Although bacterial cellulose membranes have a different macroscopic appearance from ordinary natural fibers, they are advantageous for studying certain fundamental problems relating to the fine structure of native cellulose for the following reasons: (1) they

possess a well-formed, native cellulose, crystalline structure; (2) they exist as a uniform membrane in highly swollen or gel conditions; (3) plastic flow can be easily produced in the natural membrane, while with natural fibers it is necessary to use a swelling reagent first. The reagent usually causes the native cellulose to change to the mercerized crystalline form, cellulose II, which is one of polymorphic crystalline structures in cellulose and identical with regenerated cellulose.

The X-ray work on bacterial cellulose was first carried out by H. Mark and G. V. Susich.¹ They confirmed the identity of bacterial cellulose with cotton cellulose by producing well-oriented X-ray diagrams from stretched membranes. Later investigation by W. A. Sisson and G. L. Clark showed that the purified membranes of bacterial cellulose which were well-developed have a preferential orientation with respect to the plane of the membrane. A

similar orientation was observed in regenerated cellulose sheets and fibers and the orientation mechanism was discussed.³⁻⁵ However, in bacterial cellulose membranes, no discussions about a orientation mechanism have been given.

DEFINITION OF TYPES OF ORIENTATION

According to Sisson,³ the preferred orientations can be classified into five types; random orientation, uniplanar orientation, selective uniplanar orientation, uniaxial orientation, and selective uniaxial orientation. The present paper is mainly concerned with the selective uniplanar orientation of bacterial cellulose.

This orientation is similar to a uniplanar orientation in that the *b*-axes (direction of cellulose chains) are parallel to and arranged at random in a sheet, but differs in that the (101) planes of the crystallites have a selective orientation parallel to the sheet.

With the X-ray beam perpendicular to a sheet a random orientation pattern is obtained with line (101) missing and (10 $\bar{1}$) present. With the beam parallel, line (101) is present as a dense arc on the equator, which (10 $\bar{1}$) is present only as a faint arc on the meridian (Figure 1). On the other hand, uniaxial orientation is a general type of orientation, as can be seen in fiber-forming polymers. The only limitation imposed on this orientation is that the *b*-axes of crystallites have a preferred orientation parallel to a fiber axis. With the X-ray beam perpendicular to a fiber axis, lines (101), (10 $\bar{1}$), and (002) exist as intensity maxima at the equator, and their relative densities remain constant with rotation of the sample around the fiber axis as long as the fiber axis is perpendicular to the X-ray beam (Figure 2). If the X-ray beam is parallel to a fiber axis, a random pattern is obtained.

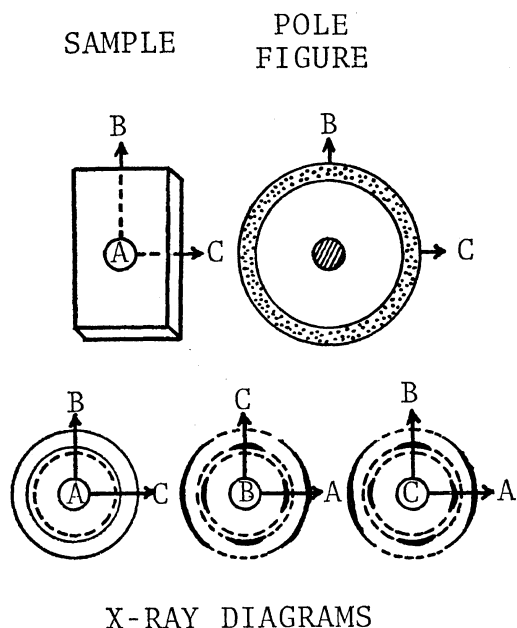


Figure 1. The directional relationships among the sample sheet, the pole figure in reciprocal space, and schematic drawings of X-ray diagrams in selective uniplanar orientation. In the pole figure the dotted areas represent projections of *b* axes (fiber axes) and cross-hatched areas projections of (101) directions. A, B, and C represent arbitrary reference axes within the sample.

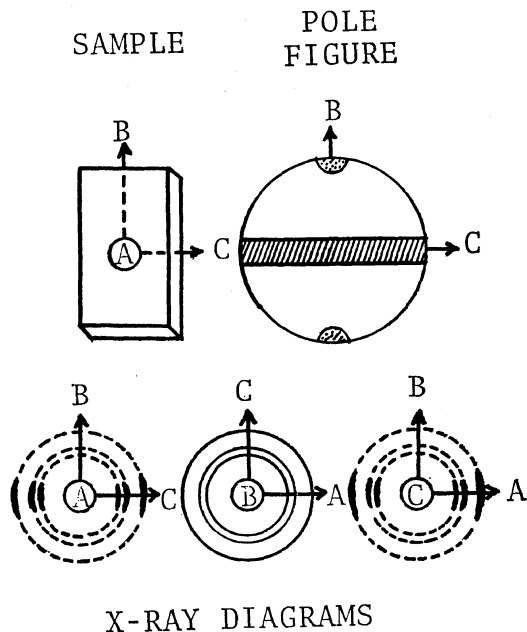


Figure 2. The directional relationships among the sample sheet, the pole figure in reciprocal space, and schematic drawings of X-ray diagrams in uniaxial orientation. In the pole figure the dotted areas represent projections of *b* axes (fiber axes) and the cross-hatched areas projections of (101) directions. A, B, and C represent arbitrary reference axes within the sample.

MATERIALS AND METHODS

Preparation of Membranes for X-Ray Analysis

Membranes were synthesized from glucose by the bacterium *Acetobacter Xylinum*, as described in Part I.⁶ The following four types of specimens of membranes were provided for the X-ray diffraction study: (1) *Undried membranes* pressed into a sheet to remove excess water using filter paper, but containing a large amount of water. They were covered with 0.25-mm Mylar film and subjected to X-ray analysis. (2) *Membranes dried with a flat surface of membrane parallel to a glass plate.* A large section of the membrane was allowed to dry at 105°C in an air oven with the flat surface resting on a horizontal glass plate, then the dried sheet was peeled off and subjected to X-ray analysis. (3) *Stretched membranes.* Stretching was accomplished by suspending in a vertical position a strip of swollen membrane about 4 cm length and applying a weight of 60 g to the lower end. The amount of tension which could be safely applied without breaking was small at first, but became greater as the strip dried. (4) *Specimens with various organic liquids replacing the water and then subjected to drying on a glass plate.* Water in a swollen membrane was replaced with various organic liquids of a different polarity such as acetone, acetic acid, pyridine, cyclohexane, and carbon tetrachloride, and then dried on a glass plate. The membrane is first dried on a glass plate, then immersed in the liquids, and dried under the same stress.

Estimation of Orientation of Cellulose Crystallite

A qualitative estimation of the selective uniplanar orientation of specimens was carried out first by a flat film camera with the X-ray beam perpendicular and parallel to a flat surface of the membranes. X-ray diffractograms by the reflection method, setting the surface of the membranes parallel to the reflecting surface, were also observed with an automatic X-ray diffractometer. The peak intensity ratio (101) to (002) will suggest a pseudoquantitative estimation of the selective uniplanar orientation.

RESULTS AND DISCUSSION

X-Ray Diffractograms of Bacterial Cellulose Membranes

Undried Membrane. The undried membrane

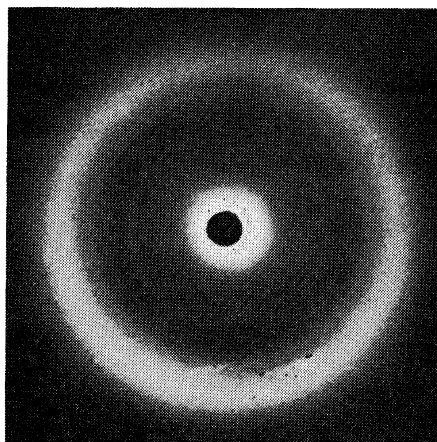
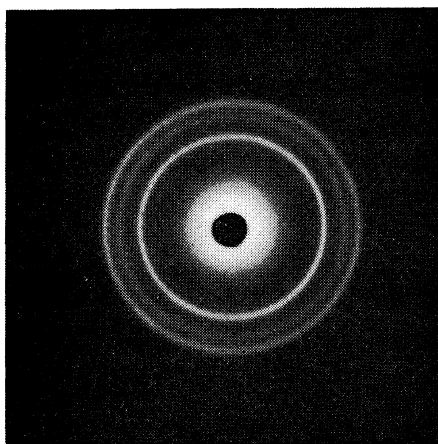


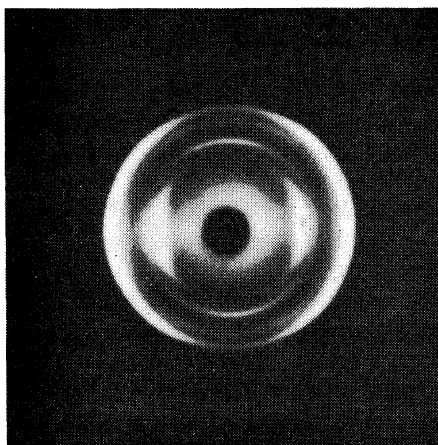
Figure 3. X-ray photograph of undried (never-dried) bacterial cellulose membrane. X-rays perpendicular to a flat membrane surface.

was examined with an X-ray beam perpendicular to the surface (Figure 3). Owing to the large amount of water present, the cellulose diffraction pattern is considerably masked by the characteristic water haloes at 3.3 Å ($2\theta=27.0^\circ$). Although it is not clear whether well-defined cellulose crystallites with a definite orientation exist or not in the swollen membrane, there is a tendency toward the formation of a preferential orientation. As the sample dries, the water pattern is gradually replaced by the cellulose pattern.

Membranes Dried with a Flat Surface of the Membrane Parallel to a Glass Plate. One end of flat strip was clamped, and allowed to contract freely upon drying. It was observed that shrinkage occurred anisotropically normal to the sheet. The strip was also puckered and twisted in the long direction. These puckerings and twistings make it difficult to estimate the orientation. In some cases the specimens may be classified as having a random orientation. However, in the case of a membrane dried with a flat surface of the membrane parallel to a glass plate, it was recognized that the specimen provided a typical selective uniplanar orientation. Representative photographs with X-ray beam perpendicular and parallel to the surface are shown in Figures 4a and 4b, respectively. In the perpendicular pattern, the random orientated pattern is obtained with line (101) missing and



(a)

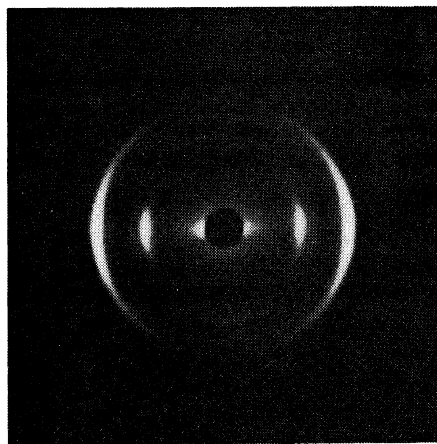


(b)

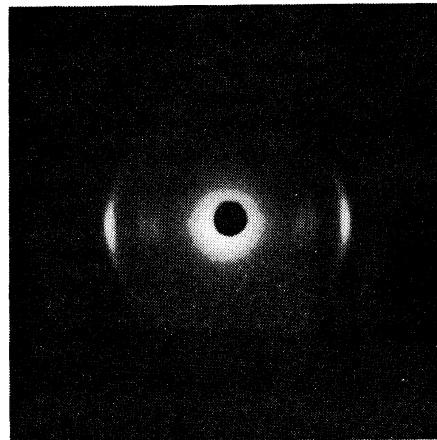
Figure 4. X-ray photographs of bacterial cellulose membrane dried with a flat surface parallel on a glass plate, showing a typical selective uniplanar orientation. X-rays (a) perpendicular and (b) parallel to the membrane surface.

($10\bar{1}$) present. On the other hand, in the parallel pattern, line (101) is present as an intensive arc on the equator, while ($10\bar{1}$) is present only as a weak arc on the meridian. It was noticed that upon drying the membrane adheres to a glass plate, and that shrinkage occurs only normal to the plate. It is assumed that the anisotropical shrinkage causes the orientation.

Stretched Membranes. Typical X-ray photographs of stretched membranes are shown in Figures 5a and 5b. The perpendicular and par-



(a)



(b)

Figure 5. X-ray photographs of bacterial cellulose membrane stretched by applying weight of 60 g attached to the lower end of a strip. X-rays (a) perpendicular and (b) parallel to a flat surface of the membrane.

allel patterns are similar to each other. As the strips were made more sheet-like, their diffraction patterns slowly changed to a selective uniaxial orientation, the so-called "double axial orientation".

These results on drying would seem to indicate that a selective uniplanar orientation of a dried membrane is probably the result of stress produced by a unidirectional shrinkage, which results from a rapid removing of water out of the membrane surface. It is well-known that

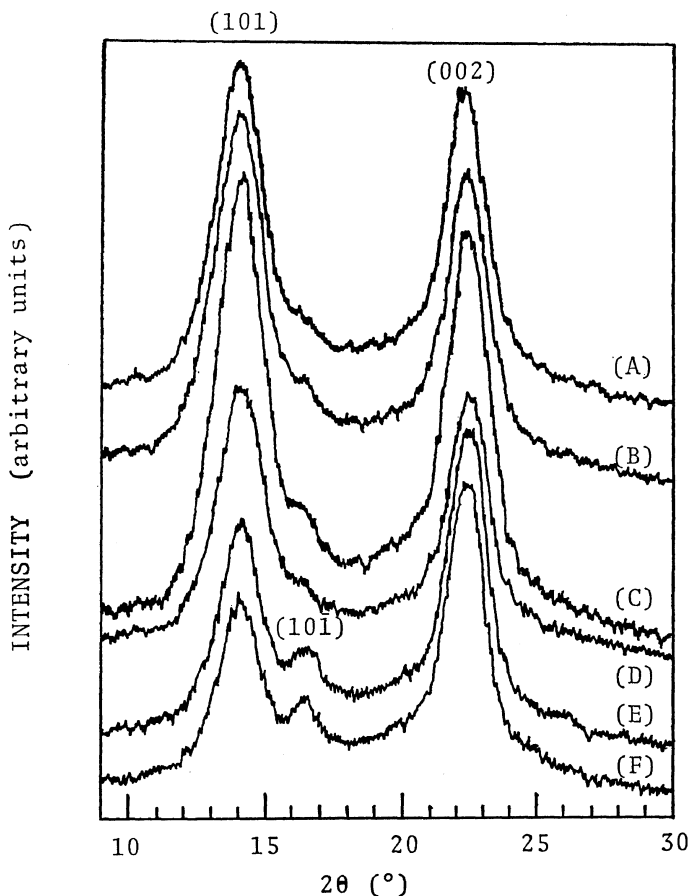


Figure 6. X-ray diffractograms of bacterial cellulose membranes in which water has been replaced by various organic liquids; these were dried on a glass plate. They were measured by the reflection method, setting a flat surface of the membrane parallel to a reflecting surface with an automatic diffractometer. Substituting liquids; (A) acetone, (B) acetic acid, (C) pyridine, (D) water (control), (E) carbon tetrachloride, (F) cyclohexane.



shrinkage is an anisotropic property with a maximum effect perpendicular to the b -axis, but the present data would seem to indicate that the direction of shrinkage is still more specific. It occurs principally normal to the (101) planes. If the bulk of a bacterial cellulose membrane consists of microfibril, it may be assumed that the orientation depends on the shrinkage upon drying. The orientation will now be discussed from a molecular basis. If the molecular mechanism of formation of a bacterial cellulose microfibril is simultaneous polymerization and crystallization by a tip growth of insoluble microfibril from activated glucose residues,^{7,8} it will be the

most reasonable for preferential cellulose deposition. In a ribbon-like microfibril, a selective uniaxial orientation may be already formed.

Therefore, the preferential orientation of a bacterial cellulose membrane appears to be principally affected by the arrangement of microfibril in the membrane.

Membranes with Various Organic Liquids Substituted for Water. It was recognized that liquids contained in the swollen membranes may influence the preferential orientation of bacterial cellulose upon drying. These liquids can be classified into two broad categories. One is a group of liquids which give a higher selective

Table I. Intensity ratios (101) to (002) in X-ray diffractograms^a

Substituted reagents	(A)	(B)
(CH ₃) ₂ C=O	1.256	1.216
CH ₃ COOH	1.251	1.493
	1.196	1.341
H ₂ O	1.050	1.138
CCl ₄	0.669	0.646
	0.602	0.584

^a Column (A) is the ratio for peak intensity and (B) that for integral intensity.

uniplanar orientation than water, and the other is a group of liquids which disturb the formation of the orientation. The X-ray diffractograms by the reflection method are shown in Figure 6. When water in the swollen membranes is replaced with acetone, acetic acid, or pyridine, and then dried on a glass plate, the peak intensity of (101) is more intensive than that of (002). The ratio (101) to (002) is as high as 1.2 (Table I). The selective uniplanar orientation of (101) to the membrane surface was also more conspicuous. On the other hand, for carbon tetrachloride and cyclohexane, the peak of (101) is weaker than that of (002). Furthermore, the diffraction of (10 $\bar{1}$) appeared sharply as a result of the disorientation of (101). The intensity ratio of these is as low as 0.6 (Table I). Such results would seem to indicate that the selective uniplanar orientation is mainly controlled by the H-bonding ability of the substituting liquids. That is, the liquids consisting of molecules which contain electron-donor groups, such as ketones, carboxylic acids, aldehydes, tertiary amines, ethers, and esters, have an attracting ability for the hydrogen atoms of the hydroxyl groups of cellulose. On the contrary, saturated hydrocarbons such as cyclohexane, carbon tetrachloride, and carbon bisulfide have no H-bonding ability. Thus it could be predicted that the more intensive the attracting ability of hydrogen atoms becomes, the higher the degree of orientation which is obtained.

It is interesting to note that the (101) crystallographic plane of cellulose appears to play a

role analogous to a glide plane in metals. The mechanism of obtaining the orientation, however, must be quite different for the two materials. Instead of a glide plane slipping as for metals, all of the evidence points to a movement of the crystallite as a unit for cellulose. The equilibrium position for the cellulose crystallite, therefore, must be governed, not by the symmetry of glide planes, but by the external configuration of the crystallite.

The crystallographic planes, which are distinguished as cleavage surfaces in the crystals, are ordinarily those most densely packed with atoms. In cellulose these are obviously the (101) planes, which are most closely occupied by hydroxyl groups. However, it is impossible to predict to what extent cleavage planes may exist in crystalline cellulose, but from analogy to other crystals one would expect a tendency toward cleavage along (101) planes. These are also the planes whose lattice constant and sharpness of diffraction line are always first affected when cellulose is exposed either to intracrystalline swelling reagents such as sodium hydroxide and potassium acetate, or to chemical substitution reactions in fiber form such as acetylation and nitration. Shift of position, line broadening, or decrease in total intensity of the (101) plane almost invariably precedes changes of the (10 $\bar{1}$) or (102) planes. Therefore, these results suggest that a reasonable interpretation for the elementary structural unit of cellulose was possible by supposing a sheet structure consisting of (101) planes.

As a result of the coherence of these sheets throughout a series of reactions, the products can be best analyzed as an aggregate of such sheets with the possibility of other molecules being held between them. Such aggregations of sheets can give recognizable X-ray diagrams, but such diagrams are not necessarily indications of precise crystal structures. It may well be that more attention should be focused on this sheet structure than on the three dimensional structure within one unit cell. It seems that swelling involves mainly the moving part of sheets of cellulose chains and that the final product formed will depend on the ability of the swelling reagent to facilitate readjustments of chains within the sheets and also the mechanism of

the decomposition of the swelling complex.

The pronounced orientation tendency of cellulose with reference to the *b*-axis, so that for example, a rayon filament can be oriented by stretching is well-known, and this property is usually attributed to rod-like crystallites or fibrous bundles of cellulose chains which are oriented parallel to the direction of stretching. If an elongated shape in the direction of *b*-axis is responsible for the parallel alignment of the crystallites when cellulose is stretched, the question then arises, what configuration of the crystallite is responsible for the preferential orienting tendency with reference to the (101) planes?

Assuming an elongated cellulose crystallite which moves as a unit during plastic flow, there are two possible external configurations which could account for its preferential behavior; (1) it could have a flat or ribbon-like shape, or (2) it could have a greater secondary valence attraction on two sides. In other words, the preferential orienting tendency of the crystallites between them, or perhaps a combination of both factors may be responsible for the behavior.

If the crystallite would have a ribbon-like shape as observed in the electron-microscopic study, it would seem logical to assume that the polar hydroxyl groups of cellulose may be partly responsible for the orienting properties of cellulose. In the crystal structure of cellulose, the (101) planes have the largest number of hydroxyl groups. There are several properties of cellulose which are in harmony with the concept of hydroxyl group influence. For example, since the hydroxyl groups are largely responsible for the sorption of water by cellulose, a crystallite most closely occupied with hydroxyl groups on two sides would be expected to have a major shrinkage normal to (101) planes. Since the secondary valence of cellulose is attributed to hydroxyl groups, the side of the crystallite parallel to (101) planes should have the greatest valence forces, owing to the geometrical arrangement of glucose residues in the crystal lattice, and these hydroxyl groups may assist the preferential orienting tendency of the crystallite. The preferential orienting tendency is not a unique property of bacterial cellulose

membranes. Gelatin,⁹ myosin,¹⁰ and regenerated cellulose films also show a selective uniplanar orientation when they are allowed to dry in sheet form. It is interesting to note that all of these materials which show a preferential orienting tendency have polar groups attached to fibrous molecules which exist as elongated crystallites. Especially in the production of regenerated cellulose sheets, cellophanes, the mechanism of a selective uniplanar orientation of the (101) plane is generally considered to be followed;^{4,5} cellulose zinc xanthate formed on a film surface during a coagulating process plays an important role in regeneration and dehydration. It has been recognized that the skin formation of regenerated cellulose sheets and fibers was based on the effect of dehydration from cellulose xanthates during a coagulation process. On the other hand, we presented the mechanism of the epitaxial growth (or oriented over-growth) of cellulose zinc xanthate molecules on plate crystallites of basic zinc sulfate formed temporarily in a coagulating bath.¹¹

The present results would lead one to conclude that if the specimen is elongated in one direction, the *b*-axes of the crystallites are oriented parallel to that direction; if the sample is compressed in one direction, (101) planes are orientated normal to that direction. Furthermore, a greater secondary valence attraction or hydrogen bonding between polar groups of cellulose crystallites and substituting liquids may influence the preferential orienting properties of cellulose. From the fact that there seems to be little correlation between crystallinity and orientation, it is difficult to predict that crystallite dimensions will have an orienting effect. However, the major point which the present results emphasize in this connection is that, whatever the final concept of the membrane crystallite may be, this concept must take into consideration the fact that the crystallite possesses a major orienting tendency with reference to the *b*-axis, and a major preferential orienting tendency with reference to the (101) plane.

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