Nuclear Fusion in *Spirillum* spp.

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SUMMARY: Cellular fusion of entwined fresh-water spirilla, *Spirillum sinuosum* and *S. anulus*, is followed by nuclear fusion. Evidence of nuclear fusion was obtained from impression smears (fixed in acetic-alcohol, hydrolysed in N-HCl and stained with Giemsa) made at regular time intervals during the growth of cultures of these organisms. Following cellular fusion and nuclear fusion of opposing granules into rings of six granules, with the coalescence of the rings into a single, elongate body, the fusion cells enlarge into 'giant' organisms. The chromatinic bodies of these giants divide or separate into numerous rod-like chromatin structures around the vacuoles found in the later stages. The giant organisms either give off or fragment into small rod-like bodies, containing, at first, a triangular-shaped chromatinic structure. This triangular chromatinic structure condenses into a single spherical body as the small rod-like organisms are more clearly formed. The fate of these small rod-like organisms is unknown, but similar rod-like bodies which develop into the normal spirillum have been observed in young cultures of both *S. sinuosum* and *S. anulus*. Nothing resembling classical mitosis or meiosis was observed in any of the stained preparations of either organism.

Cellular fusion has been shown to occur in the marine organism, *Spirillum lunatum* (Williams & Rittenberg, 1956). In this organism, cellular fusion occurs after the spirilla have become entwined and results in the production of microcysts. The phenomenon of entwined spirilla is common to all the spirilla species examined and, by analogy with the process in *S. lunatum*, it might be assumed that cellular fusion also occurs in the other species. To determine whether this hypothesis is correct and whether nuclear fusion follows cellular fusion, two fresh-water species, *S. sinuosum* and *S. anulus*, were selected for study. *S. sinuosum* was chosen because more entwined spirilla occur in cultures of this species than in all others we have studied, and *S. anulus* was selected because of its large size.

Robinow (1956) has objected to calling the chromatin bodies found in bacteria *nuclei* on the basis that the term 'nucleus' is defined as 'something that arises from a set of chromosomes' and that, as yet, chromosomes have not been demonstrated in the chromatin bodies of bacteria. The term 'nucleus' however, is in common usage in bacterial literature to indicate the chromatin bodies of bacteria with the implicit understanding that the use of the term in this connexion does not necessarily imply that these bodies are the exact equivalent of the nucleus of higher organisms; nor that typical mitosis and meiosis such as occur in higher organisms has been established in bacteria. It is in this sense that the term 'nucleus' is used throughout this paper.

METHODS

Nutrient broth of the following composition was used for the cultivation of both experimental organisms: soybean peptone (Case Laboratories, Chicago, Illinois), 0.5 g.; beef extract, 0.3 g.; yeast autolysate (Basamin, Anheuser-Busch Inc., St Louis, Missouri), 0.3 g.; sea water, 100 ml.; distilled water, 900 ml. For solid media, 15 g. agar/l. were added to the liquid medium. Growth occurred very slowly in broth cultures of both *Spirillum sinuosum* and *S. anulus* when the routine bacteriological method of using one loopful of material for transfer was followed. Under these conditions, as a rule, there was no visible turbidity until 48–72 hr. after transfer. For this reason one drop of a broth culture was always used as the inoculum.

Aliquots of broth cultures were examined by dark-phase contrast, in wet mount preparations, during the course of development of the cultures to determine when entwined organisms were most abundant, thus establishing the proper times for making nuclear-stained preparations. For the latter purpose, using 7-day broth cultures as inocula, series of broth cultures of both organisms were prepared. These were incubated at 30° for periods of 12, 16, 20, 24, 30, 48 and 60 hr., at which times the tubes were centrifuged and the sedimented organisms then spread over the surface of nutrient agar plates. These plates were incubated at 30° for 2 hr., after which time small agar squares were removed, aseptically, at 2 hr. intervals and impression smears made on glass slides. The preparations were fixed in a solution of 1 part of glacial acetic acid, 3 parts of absolute alcohol for 5 min., passed through a graded series of ethanol and distilled water dilution to distilled water and hydrolysed in N-HCl solution at 60° for 8 min. After hydrolysis the smears were rinsed in distilled water and stained with Giemsa solution in phosphate buffer (pH 7.0) for 30 min.

The smears were examined with the Leitz Ortholux microscope, equipped with an achromatic condenser, an apochromatic objective ($\times 90$) N.A. 1.25, and compensating oculars ($\times 12$). The photographs were made with the Leitz Aristophot camera using Panatomic X sheet film and a 560 m μ . interference filter.

RESULTS

The number of entwined organisms found in a broth culture of either *Spirillum sinuosum* or *S. anulus* is very small in comparison with the number of those not entwined. As a rule, entwined cells are most numerous in routine broth cultures 24–36 hr. after inoculation, although entwined cells may be found as early as 18 hr. and as late as 5 days after inoculation.

Spirillum sinuosum grows as long slightly curved filaments although shorter more spiral forms are always present in every culture. In the entwining process of this organism, the filamentous spirillum bends near the centre and one end winds around the other from the centre down (Pl. 1, figs. 1A, B), usually leaving the terminal ends free. In *S. anulus*, which usually occurs as an S-shaped organism, entwining takes place in daughter organisms after

division but before separation (Pl. 2, figs. 3A, B). In both organisms fusion follows entwinement (Pl. 1, fig. 1B; Pl. 2, fig. 3B).

Stained preparations show that fusion of the chromatinic granules occurs after fusion in both species. Different aspects of the process are shown in Pl. 1, fig. 1. Fig. 1A shows a spirillum prior to fusion in which the chromatinic granules are aligned opposite each other. Although it is difficult to determine the exact number of these granules at a particular focal depth, there appear to be three granules for each spiral curve of the organism. The other photographs in Pl. 1, fig. 1, are of fused spirilla after fusion of the chromatinic granules. Two appearances predominate: one is the formation of rings from the 'six' opposing granules by fusion of adjacent granules (Pl. 1, figs. 1B, C). The second is the fusion of single granules in the opposing spiral curves (Pl. 1, fig. 1D). It is possible that the two different appearances are merely due to the angle from which they are viewed and that fusion occurs in the same manner in all the organisms.

Beyond this stage a multitude of patterns of the chromatinic material are found and since serial observations cannot be made on a single organism it is difficult, or impossible to construct a sequence of events. As far as can be ascertained from the examination of a large number of preparations, all the granules in opposing spirals of fused spirilla eventually coalesce into a single, intensely staining, elongate body, which can be seen in Pl. 1, figs. 1E, F.

After fusion of the chromatinic granules occurs or just prior to this event, the fused spirilla enlarge to about twice their normal diameter, forming what we have termed 'giant' forms. These are shown in Pl. 1, fig. 2. Giant forms are very difficult to find in the impression smears and several preparations may be examined before finding them. They stain very poorly and are much less resistant to hydrolysis than the ordinary spirillum. Based on the frequency of the normal, the entwined, and the giant forms at various time intervals during the development of a culture, it is our impression that all fused organisms of *Spirillum sinuosum* and *S. anulus* enlarge into giant forms, although the evidence is certainly not conclusive. Unfused ends of the original spirillum are clearly seen at the bottom of the organisms shown in Pl. 1, fig. 2A, and less clearly in the organism in Pl. 1, fig. 2C. Enlargement into giant forms occurs only after fusion, but appears independent of the stage of fusion of the chromatinic granules, as can be observed by an examination of Pl. 1, fig. 2.

Further changes occur in the chromatinic bodies of the giant forms of *Spirillum sinuosum* following which the organisms separate into individual, somewhat angular bodies. Similar changes occur in *S. anulus*. Because of the larger size and better staining characteristics of the latter species, it is used to illustrate the later stages. Pl. 2, fig. 3A, shows an entwined spirillum of *S. anulus* and a normal sized spirillum. Pl. 2, fig. 3B, shows an entwined spirillum after fusion, with the chromatin fusion already initiated. Pl. 2, figs. 3C-3H show the enlargement of the fused spirillum into giant forms 3 to 4 times the size of the normal organism. In the sequence shown the chromatin fuses into single bodies (Pl. 2, figs. 3B-3D). These bodies undergo rearrangement

and reorient themselves into a horizontal bar-like structure which divides longitudinally into smaller bar-like structures (Pl. 2, fig. 3D). These in turn separate into still smaller structures (Pl. 2, fig. 3E), the principal feature of which appears to be a bar with a granule at each end. This type of configuration is more clearly observed in Pl. 2, figs. 3F and 3G (marked with an arrow). It has not been possible to reach a decision as to the mechanism by which this separation of the chromatinic bodies occurs, although separation clearly does occur, as can be observed in Pl. 2, fig. 3H.

In giant forms, occurring during the later stages of growth of the organism in broth cultures (60–72 hr.), the sequence of events occurring in the chromatinic bodies can be interpreted. The giant forms found in the early stages of growth in broth cultures (36–48 hr. after inoculation) continue to enlarge in size until they are approximately 3 to 4 times the size of the ordinary spirillum. At this time, the chromatinic bodies appear in the shape of horizontal rod-like bodies (Pl. 2, fig. 4A) and the vacuoles begin to appear between (or in) these rod-like bodies (Pl. 2, figs. 4B, C); these rod-like bodies then separate into two smaller rods around the edge of the vacuoles (Pl. 2, figs. 4D, E). An examination of Pl. 2, figs. 4D and E, will show that there appears to be a granule at each end of these rod-like structures (marked with an arrow). The giant forms, at this stage, either fragment or give off small rod-like bodies (Pl. 2, fig. 4F). Pl. 2, fig. 4G, shows these rod-like bodies as they are coming off the giant forms. The chromatin in the small rods at first appears to consist of a triangular segment which condenses into a single spherical body as the cells are more clearly formed. The fate of these small rod-like forms is unknown, although the presence of very similar small rods (Pl. 2, fig. 4H) has been noted in young broth cultures of both *Spirillum sinuosum* and *S. anulus*. It is possible that these small rod-like forms represent the growth of the small rod-like bodies observed in Pl. 2, fig. 4G, and that they develop into the small spirilla shown in Pl. 2, fig. 4H-3 and H-4.

Although the data indicate that the chromatinic granules of the paired spirilla fuse into single bodies and these in turn separate into smaller units, the mechanism and significance of both the fusion process and the separation process are still unknown. In none of the photographs presented is there any evidence of configurations resembling those found during meiosis or mitosis in higher organisms.

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EXPLANATION OF PLATES

Spirillum sinuosum and *S. anulus* were both grown in broth and the spirilla were transferred to agar, as cited in text. All photographs are of hydrolysed and stained organisms. Times shown in parentheses include growth from the time of inoculation until preparations were made. Arrows indicate configurations discussed in text. Magnification, $\times 1750$ (Pl. 1); $\times 2333\text{--}33$ (Pl. 2).

PLATE 1. *Spirillum sinuosum*

- Fig. 1A. Entwined spirillum prior to fusion, showing chromatinic granules aligned opposite each other (23 hr.).
Fig. 1B. Fusion form showing formation of 'rings' from the 'six' opposing granules by fusion of adjacent granules (24 hr.).
Fig. 1C. Fusion form in which 'rings' of granules are fusing together (26 hr.).
Fig. 1D. Fusion form in which single chromatin granules, in opposing spiral curves, are fusing (22 hr.).
Figs. 1E, F. Fusion forms in which 'rings' have fused into an intensely staining, elongate body (29 and 30 hr. respectively).
Figs. 2A, B. Fusion forms enlarging into 'giant' forms (34 and 36 hr. respectively).
Fig. 2C. Giant form showing fusion of chromatinic granules (36 hr.).
Fig. 2D. Giant form in which chromatinic granules have completely fused (40 hr.).

PLATE 2. *Spirillum anulus*

- Fig. 3A. Fusion of two daughter forms (18 hr.).
Fig. 3B. Fusion of chromatinic granules in fused forms (24 hr.).
Fig. 3C. Giant forms in which fused nuclei are seen as horizontal bar-like structures (28 hr.).
Figs. 3D, E. Giant forms showing beginning division or separation of the horizontal bar-like structures into two smaller bars (30 and 34 hr., respectively).
Figs. 3F, G. Giant forms showing bar-like chromatinic structures with granules at each end (39 and 42 hr., respectively).
Fig. 3H. Giant form showing small bar-like chromatinic structures with a granule at each end, separating around the vacuoles into still smaller structures (48 hr.).
Fig. 4A. Giant forms, showing bar-like chromatinic structures. (50 hr.).
Fig. 4B. Giant form, showing separation of bar-like chromatinic structures around vacuoles with granules at each end (50 hr.).
Figs. 4C–E. Giant forms, showing separation of bar-like chromatinic structures around vacuoles (52, 52 and 58 hr., respectively).
Fig. 4F. Giant form, in which triangular body has either separated or broken off the end (66 hr.).
Fig. 4G. Triangular and spherical shaped bodies originating from giant forms, showing the intensely staining triangular and spherical chromatinic bodies (92 hr.).
Fig. 4H. 7-day broth culture transferred to broth and incubated for 12 hr. before being plated out on agar. 4H-1. Small rod-like bodies (14 hr.). 4H-2. Small form morphologically recognizable as a spirillum (16 hr.). 4H-3. Small spirilla (18 hr.). 4H-4. Small spirillum (24 hr.).

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