22.37° and a translation of 91.61 Å, −3.69 Å, and −1.75 Å, respectively, in an orthogonal coordinate system.
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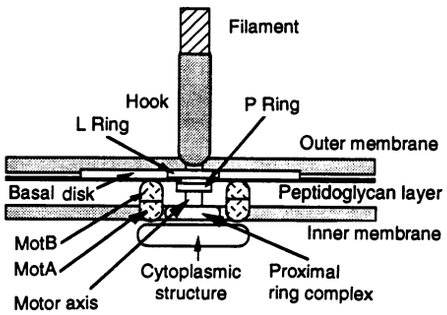

Fig. 1. Schematic illustration of the bacterial flagellar motor of *W. succinogenes*. The flagellar motor is composed of a filament, hook, motor axis, basal disk, L ring, P ring, proximal ring complex, cytoplasmic structure, and the MotA and MotB proteins. The motor penetrates the outer membrane, the peptidoglycan layer, and the inner membrane.

An Archimedian Spiral: The Basal Disk of the *Wolinella* Flagellar Motor

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The motor that powers the rotation of the bacterial flagellum reaches through both membranes into the cytoplasm of Gram-negative bacteria. The flagellum is connected by a flexible link (hook) to the motor axis, which passes through the center of a structure called the basal disk. The basal disk functions with the L-P ring complex as a bushing, enabling the rotation of the motor in the cell wall. The protein subunits of the basal disk of *Wolinella succinogenes* form an Archimedian spiral. The polymerization of subunits from a nucleation point at the motor in the form of a spiral allows constant growth of the basal disk. The disk is thought to provide a reinforcement at the flagellar insertion at the cell pole and to disperse forces that are generated by the momentum of the flagellar rotation.

Flagellar motors (*1–3*) of many bacteria are inserted exclusively at cell poles rather than distributed over the cell body. These motors display a structural element called a basal disk. Basal disks (*4, 5*) are attached to the inner side of the outer membrane and contain the L-P ring complex (*3*), a bushing of the flagellar motor. Both elements form part of the stator, anchoring the motor axis and other rotating parts of the motor to the cell wall (Fig. 1) (*6*).

Earlier reports described basal disk structures associated with the flagellar apparatus in various species of proteobacteria (*7–11*) and more recently one (*12*) in members of the *Comamonadaceae* and disk-like septa occurring between cells of some archaebacteria (*13*). It was assumed that these basal disks were an assembly of concentric rings (*5, 7, 8*). We used electron microscopy to investigate basal disks from *W. succinogenes* (*5, 14*), a Gram-negative uniflagellated bacterium that features a disk of an average diameter of 170 nm. Disks were isolated either with basal bodies or, after disruption of the motor axis, as basal disks with an integrated L-P ring complex in their center (Fig. 2) (*15*). Basal disks from a stationary grown culture display roughly the same diameter, whereas disks isolated from early logarithmic cultures show a large deviation from the average 170-nm diameter toward smaller diameters (Fig. 3) (*16*). This suggests that the disks are assembled by the addition of subunits in a way that does not disturb the preexisting structure. Subunits of the basal disks are assembled in a radial geometry around the central ring structure (L-P ring), as seen by visual inspection. This suggests two possible geometric forms for the underlying construction: a set of concentric rings or a spiral.

We describe here a procedure that allowed us to distinguish concentric rings from spirals by the analysis of their Fourier transforms (*17, 18*). This differentiation is not possible by investigating the optical diffractograms of electron micrographs alone. In the Fourier transforms, concentric rings possess information exclusively in the real part, representing the symmetric structural information. Spirals, however, show symmetric and asymmetric contributions, with the odd-numbered diffraction orders occurring in the imaginary part and the even-numbered diffraction orders in the real part of the Fourier transform. For the disks of *W. succinogenes*, the significant first-order ring is in the imaginary part (Fig. 4D) and consists of fringes that are arcs of a spiral. This proves that the basal disk is a spiral (Fig. 4B). The spiral line can be observed clearly in the filtered image of a small disk (Fig. 4B), and the end of the spiral band is even visible in the original micrograph (Fig. 4A).

The individual subunits along one whorl of the spiral also contribute to the Fourier transform. Their Fourier coefficients are included in the real part as well as in the imaginary part of the transform. Model calculations have shown that their contributions are less prominent than the spiral information. The corresponding diffraction "rings" formed by radially and spatially distributed diffraction spots are weak (Fig. 4C). The filtered image (Fig. 4B) suggests that the spatial distance between the molecules along the spiral line is about equal to the radial distance between neighboring parts of the spiral. Thus, the spatial contributions of subunits along one whorl and between whorls overlap in the Fourier transform (Fig. 4C). The disk has the features of an Archimedian spiral, which is described in polar coordinates by

$$r = \frac{d\varphi}{2\pi}$$

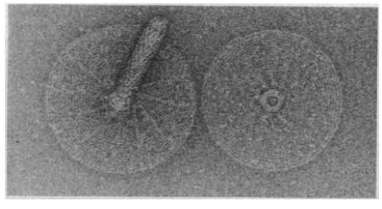

Fig. 2. Basal disks of the flagellar motor from the Gram-negative bacterium *W. succinogenes*. The disk on the left has the hook–basal body complex in place, whereas the disk on the right lost the complex by a short ultrasonic treatment. Disintegration of the motor axis was necessary to obtain free basal disks. The central hole is visible with a diameter of 11 nm, which is close to the diameter of the motor axis. The central ring of the basal disk is part of the L-P ring complex, a bearing of the motor.

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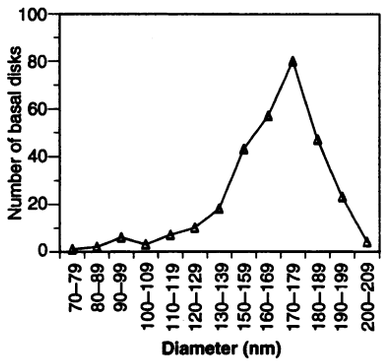

Fig. 3. Size distribution of 297 basal disks from *W. succinogenes*.

where r denotes the radius, φ the angle (in radians), and d the constant radial distance between neighboring whorls obtained by $r_2 - r_1 = d$, if $\varphi_2 = \varphi_1 + 2\pi$. The parameter d was determined by the diffraction ring in the imaginary part of the Fourier transform (Fig. 4D) that appears at the spatial frequency $1/d$. The distance (d) between two whorls is 3.2 nm in disks of *W. succinogenes*. The length (l) of the spiral line is given by

$$l = \frac{d[\varphi\sqrt{\varphi^2 + 1} + \ln(\varphi + \sqrt{\varphi^2 + 1})]}{4\pi} \approx \frac{d\varphi^2}{4\pi}$$

The spiral of a large disk (r_{max} = 100 nm, r_{min} = 12.5 nm) has a length of 9.7 μm (Fig. 2). Assuming a spherical particle size of 3.2 nm in diameter (Fig. 4B), at least 3000 subunits are assembled in a large disk and about 2200 in a disk of the average diameter of 170 nm. Spirillin (*19*), a 20-kD protein, is the predominant molecular constituent of the disk. It is the second most abundant protein of the flagellar apparatus of *W. succinogenes*, with flagellin being the most abundant.

There are two different types of interactions among neighboring spirillin molecules, presumably specific interactions between the molecules along the spiral and unspecific interactions among the molecules of adjacent parts of two whorls. Calculations show that the apparent bonding angles between two spirillin molecules are different at the beginning of the spiral with r_{min} = 12.5 nm (172.7°) and at the end with r_{max} = 100 nm (178.2°). This difference is small enough to allow specific bondings along the entire spiral.

The lateral interactions may be unspecific because systematic contacts appear to be impossible. The number of molecules increases from one whorl to the next by $\approx 2\pi d/a$, where a denotes the repeating distance along the spiral line. There is evidence that carbohydrates are involved in the interaction among the spirillin molecules (*19*), a fact that may explain the flexibility of lateral contact sites. The two types of interactions, specific contact between the molecules along the spiral and unspecific adhesion of lower affinity between adjacent whorls, may establish an efficient and elegant assembly mechanism.

Assembly of the same disk structure by use of concentric rings would present a series of difficulties. After completion of a ring the next ring would have to start at one or multiple nucleation points and would then have to rearrange in order to accommodate the last subunit that performs the ring closure. A further concern is the dimension of the molecular building block (subunit). The ratio of the distances a (monomer to monomer on a ring) and d (whorl to whorl) would have to fulfill the condition $a/d = 2\pi/\Delta n$, where Δn denotes the difference between the numbers of molecules of two consecutive rings, meaning only a whole number of subunits can form a ring. These imposed restrictions can be avoided with the spiral assembly.

The described spiral structure can clearly be distinguished from other macromolecular assemblies such as helices. While the latter are the architectonic principle of filamentous structures, Archimedian spirals make planar disks that grow at one site only (in contrast to two-dimensional crystals). There are a few examples of cylindrical structures with a spiral cross section, for instance ejectisomes from cryptomonads (*20*), R-bodies from bacteria (*21*), and the arrangement of microtubules in the axonemas of heliozoans (*22*), but their basic molecular structures are not spirals.

The basal disk of *W. succinogenes* may function as an anchor for the bushing of the flagellar motor in a way that it bears forces deriving from flagellar rotation of about 100 Hz in both the clockwise and counterclockwise directions (*3*). Isolated disks have a distinguishable structure in their center: a hole surrounded by a ring of high density (thickness). The inner diameter of the ring (18 to 19 nm) is of even higher density in rotationally enhanced averages (*23*), indicating that the inner wall is cylindrical. The size of the structure as well as the relative position of the basal body in hook–basal body–basal disk complexes (Fig. 2) (*4*) identifies this ring as part of the L-P ring complex (*3*), a pair of rings formed from ~24 monomers assembled around the distal motor axis. The formation of this ring is spontaneous (*24*), and the ring may serve as the template for the assembly of the spiral. Functionally the disk may stabilize the position of the L-P ring complex in the outer membrane and may effectively absorb the lateral forces that are created by the rotating flagellum. The surface area of an average disk is about 50 times the area of the L-P ring. The absorbed forces would be distributed over a much larger area and, thus, be reduced by one to two orders of magnitude with respect to a certain site. This may be of particular significance for

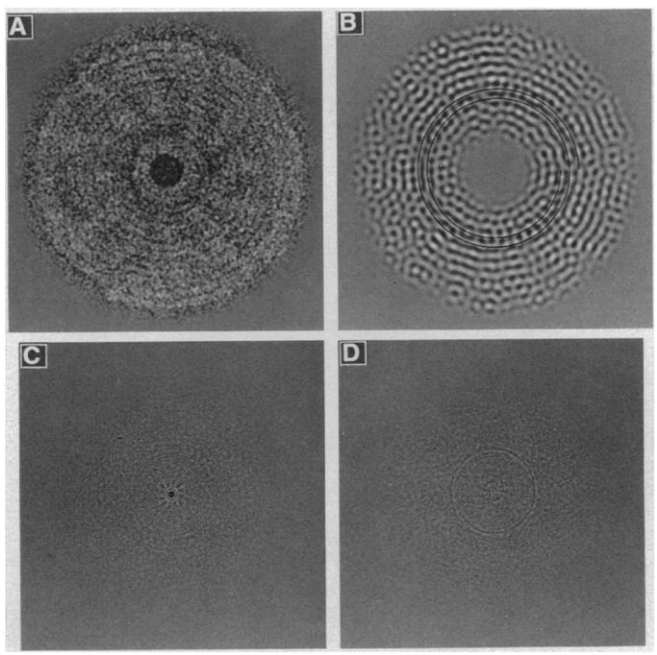

Fig. 4. Small basal disk of 94 nm in diameter. (**A**) The end of the spiral is visible at the bottom left of the disk. (**B**) Filtered image of the disk comprising the information between 1/3.5 to 1/2.9 nm^{-1} spatial frequency that represents the spiral structure of the disk. Two whorls of the spiral are indicated (black line). The substructure of the whorls indicates the position of the spirillin molecules. Real (**C**) and imaginary (**D**) part of the Fourier transform of the disk shown in (A). The imaginary part contains the complete information of the spiral line, whereas both the real as well as the imaginary part contain the (weak) Fourier coefficients defining the substructure of the spiral. The diffraction ring in the imaginary part consists of two intercalating (dark and bright) arcs that represent the negative and positive Fourier coefficients. This pattern is a characteristic feature for the first-order diffraction ring of an Archimedian spiral. The image of the disk was centered such that the antisymmetry center of the spiral coincides with the center of the image.

bacteria possessing only one polar flagellum.

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15. The basal disks were isolated from a filamentless mutant that has an active motor. Spheroplasts of this mutant were prepared as described for the wild type (*4*) and sonicated for 3 min. They were lysed by 2% $C_{12}E_8$ in 10 mM tris-HCl (pH 8), and DNA was digested as described (*4*). We added EDTA to a concentration of 27.5 mM and KOH to a pH of 11. After 2 hours of incubation the basal disks, of which about 50 to 70% were without a hook–basal body complex, could be isolated and purified by repeated low- and high-spin centrifugations in the detergent buffer used above. This preparation was negatively stained with uranyl acetate (2%). Stained areas appear black in the micrographs, whereas biological material is bright. Micrographs were taken in a Philips CM12 electron microscope at minimal-dose conditions at a primary magnification of ×35,000. The micrographs were digitized by means of an Eikonix 1412 camera at an aperture that gave a pixel size of 0.43 nm on the specimen level. Image analysis as well as model calculations were done with the Semper 6.2 image processing system.
16. The disks, isolated from an exponentially growing culture, were photographed and analyzed for their diameters. While the largest diameter might reflect the natural maximum of size, the smallest disks may be a result of the isolation procedure. It cannot be excluded that disks having an even smaller diameter may exist but were not observed because of their low number.
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25. H.E. and S.C.S. contributed equally to this study. Supported by a research grant to E.B. from the Deutsche Forschungsgemeinschaft.

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Replication-Specific Inactivation of the pT181 Plasmid Initiator Protein

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Replication of the *Staphylococcus aureus* plasmid pT181, which occurs by the rolling circle mechanism, is accompanied by the covalent attachment of a ≈12-residue oligodeoxynucleotide to one subunit of the dimeric plasmid-coded initiator protein, RepC. This oligonucleotide represents the plasmid sequence immediately 3′ to the initiating nick site. The resulting heterodimeric protein lacks the topoisomerase and replication activities of unmodified RepC, suggesting that the regulation of plasmid DNA replication requires postreplicational inactivation of the initiator protein as well as control of its synthesis.

Although the initiation of DNA replication is closely regulated in all organisms, the control of replication is not fully understood. We have used the *S. aureus* pT181 plasmids to study the regulation of DNA synthesis because these plasmids are maintained by precise control systems (at about 22 copies per cell). The pT181 plasmids replicate by the rolling circle mechanism, and initiation of their replication is regulated primarily by antisense-mediated control of synthesis of the 38-kD initiator protein, RepC (*1*). Leading strand replication begins with a site-specific nick introduced by RepC at the origin of replication (*ori*) (*2*). The protein is attached to the 5′ nick terminus by a phosphotyrosine bond (*3*). Although the synthesis of RepC is regulated precisely, we consider this regulation to be insufficient to ensure stable maintenance, as reutilization of the protein would probably lead to uncontrolled replication. Therefore, the Rep protein is likely to be degraded or inactivated after use.

To examine the fate of RepC, we incubated *S. aureus* containing a pT181 copy number mutant, *cop-608*, in the presence of chloramphenicol (Cm). Chloramphenicol blocks pT181 replication by inhibiting the synthesis of RepC (*4*). After addition of the drug, new plasmid initiation continues at a very low rate, for 5 to 10 min, during which the presumably small intracellular pool of active RepC is used up (*4*). In the presence of Cm, we did not find degradation of RepC; rather, we found two proteins (38 and 42 kD) that reacted with antibody to RepC (anti-RepC) and that remained present at constant amounts (Fig. 1) over a 30-min incubation. Both protein bands were detected also in the absence of Cm, but only the previously identified (*5*) 38-kD protein was seen in a preparation of RepC from *Escherichia coli*, in which pT181 does not replicate. Neither protein was seen in preparations from *S. aureus* strains that lacked plasmids. This result indicates that RepC is not degraded after its use and suggests that the 38-kD RepC molecule may be converted during replication to a different, slower migrating form. Residual replication in the presence of Cm is completed in less than 10 min, despite the continuing presence of a substantial amount of 38-kD material, suggesting that the 38-kD form is incapable of participating in replication under these conditions. There is no major change in the relative amounts of the 38- and 42-kD forms in the presence of Cm.

To confirm that the 42-kD material repre-

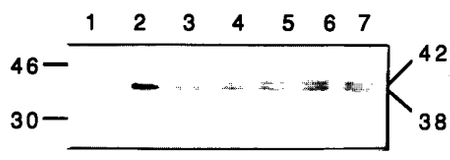

Fig. 1. Effect of chloramphenicol on RepC. A culture of *S. aureus* RN27 containing the high copy number mutant pT181*cop-608* was grown to a cell density of 7 × 10^8 bacteria per milliliter. Chloramphenicol was then added to a concentration of 150 μg/ml, and the culture was serially sampled. Whole-cell lysostaphin lysates from these samples were analyzed by SDS-PAGE followed by protein immunoblotting with rabbit anti-RepC. Blots were developed by treatment with goat antiserum to rabbit alkaline phosphatase conjugate and then stained for alkaline phosphatase. Lane 1, RN27 (plasmid$^-$); lane 2, RepC purified from MB2(pSK184) (*5*), an *E. coli* strain containing a cloned *repC* gene; lane 3, RN27(pT181*cop-608*) before Cm treatment; lanes 4 to 7, samples taken after 5, 10, 20, and 30 min of incubation in the presence of Cm. Numbers 46 and 30 represent molecular sizes (in kilodaltons) of protein standards; numbers 42 and 38 represent apparent molecular sizes of the two forms of RepC.

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