Rotationally Symmetric Structures of the C-ring of Escherichia Coli

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Abstract: The C-ring of a motor contains either 34 or 44 copies of FliM but only about 26 copies of FliG. In addition, the amount of FliMs is influenced both by the direction of rotation and by the number of CheY-P signal molecules in the cytoplasm. The rotationally symmetrical model described here combines all known properties.

Introduction

E. coli is a creature of the simplest construction, whose behavior and inner life are well-known thanks to decades of research. Despite a great deal of detailed knowledge, one hardly understands the overall function. It can be assumed that – due to a lack of brain – any construction detail and reaction of the bacterium is automated and can be described in a mechanistic way. This starts with externalities such as the arrangement of the motors and their synchronous rotation reversals and ends with the technical realization of the analog data memories, which can remember very precisely the concentration of vital substances in the extracellular space for about one second. E. coli bacteria respond to certain changes in the environment and can move to locations with better survival conditions. This is done with the help of tiny rotary drives whose functionality is hardly understood. Thanks to electron microscopy, the size and spatial structure of the motors are well known, and we know the proteins that spontaneously assemble - but not their number[1].

How many proteins form the motor C ring?

The aim of this study is to find rotationally symmetric constructions based on known properties of the proteins involved. Until five years ago, it was largely agreed that the C-ring of the rotor of a flagellar motor is composed of ~26 FliG, ~34 FliM and ~120 FliN proteins. Recent results suggest that one number depends on the direction of rotation[2]: While rotating clockwise, we have about 34 FliMs. In the CCW direction of rotation, there could be 44 molecules or so. Both values, however, are only estimates and it is unclear whether and how the additional molecules affect the diameter of the C-ring. Despite the numerous investigations up to now, it has not been possible to clarify how many proteins the motors of E. coli contain and how these building blocks are arranged.

There are other puzzling measurements: The angular step size for CCW rotation is 13.8°, which corresponds to ~26 steps per rotation. Backwards steps were about ~10.3°, which corresponds to 1/35 of a revolution[3]. This looks as if different "gearwheels" are used.

How does it fit together? Mathematical considerations may help to find possible solutions, based on the known properties of the molecules involved. The top guideline for each solution is the rotational symmetry of the C-ring and a simple explanation of the disproportional module numbers. Finally, the ring is not produced by a complicated pressing tool as in mechanical engineering, it spontaneously assembles out of a disordered liquid. Therefore, we assume that short-range molecular forces are sufficient to assemble and stabilize the ring. We are convinced that no protein can count and there are no magic remote effects. Each protein has a volume and few adhesive sites to connect with immediate neighbors. Because neighboring molecules exert forces on each other, they may deform each other.

This investigation was initiated by the remark "*Moreover, the pattern of suppression suggests that two distinct sites on FliG interact with FliM, perhaps with two FliM molecules in a dimer per molecule of FliG*"[4].

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In the spreadsheet below, the uniformly sized fields are neither a measure of size or shape of the proteins nor are they accurate location data. The non-empty fields indicate approximately the position of the molecule centers and spatial neighborhood relationships. Empty fields may contain parts of particularly large molecules, but they may also symbolize actual gaps between adjacent proteins.

Since the C-ring has no marker, the result *must not* depend on where the count starts. To check rotational symmetry, select any molecule on the circumference as starting point. Then follow the ring and number all other molecules until you return to the starting point. In the table, these numbers are displayed in a vertical column.

CW tumble								CCW run					
FliG (24)	FliM (32)	FliG (25)	FliM (33/34)	FliG (26)	FliM (34/35)	FliG (27)	FliM (36)	FliG (24)	FliM (48)	FliG (27)	FliM (40/41)	FliG (27)	FliM (45)
1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2		2		2		2		2		2		2
2		2		2		2		2	3	2	3	2	3
	3		3		3		3		4				4
3	4	3	4	3	4	3	4	3	5	3	4	3	5
									6		5		
4	5	4	5	4	5	4	5	4	7	4	6	4	6
	6		6		6		6		8				7
5		5		5		5		5	9	5	7	5	8
	7		7		7		7		10		8		9
6	8	6	8	6	8	6	8	6	11	6	9	6	10
									12				
7	9	7	9	7	9	7	9	7	13	7	10	7	11
	10		10		10		10		14		11		12
8		8		8		8		8	15	8	12	8	13
	11		11		11		11		16				14
9	12	9	12	9	12	9	12	9	17	9	13	9	15
									18		14		
10	13	10	13	10	13	10	13	10	19	10	15	10	16
	14		14		14		14		20				17
11		11		11		11		11	21	11	16	11	18
	15		15		15		15		22		17		19
12	16	12	16	12	16	12	16	12	23	12	18	12	20
									24				
13	17	13	17	13	17	13	17	13	25	13	19	13	21
	18		18		18		18		26		20		22
14		14		14		14		14	27	14	21	14	23
	19		19		19		19		28				24
15	20	15	20	15	20	15	20	15	29	15	22	15	25
									30		23		
16	21	16	21	16	21	16	21	16	31	16	24	16	26
	22		22		22		22		32				27
17		17		17		17		17	33	17	25	17	28
	23		23		23		23		34		26		29
18	24	18	24	18	24	18	24	18	35	18	27	18	30
									36				
19	25	19	25	19	25	19	25	19	37	19	28	19	31
	26		26		26		26		38		29		32
20		20		20		20		20	39	20	30	20	33
	27		27		27		27		40				34
21	28	21	28	21	28	21	28	21	41	21	31	21	35
									42		32		
22	29	22	29	22	29	22	29	22	43	22	33	22	36
	30		30		30		30		44				37
23		23		23		23		23	45	23	34	23	38
	31		31		31		31		46		35		39
24	32	24	32	24	32	24	32	24	47	24	36	24	40
									48				
1	1	25	33	25	33	25	33	1	1	25	37	25	41
	2		34		34		34		2		38		42
2		1	1	26		26		2	3	26	39	26	43
	3		2		35		35		4				44
3	4	2		1	1	27	36	3	5	27	40	27	45
			3		2				6		41		
4	5	3	4	2		1	1	4	7	1	1	1	1
	6				3		2		8		2		2
5		4	5	3	4	2		5	9	2	3	2	3

CW rotation (left part of the table)

Rotational symmetry requires that the transition to the next lap occurs smoothly and without tripping. In the table above, the left-most two columns show a possible solution for CW rotation: If the C-ring is built of 24 FliGs and 32 FliMs, the green colored fields at the bottom indicate that we have rotational symmetry.

The same applies to 27/36, but not for 25/34 and 26/35. Here, the beginning and end do not match seamlessly. The red-colored areas indicate arrangements which are not rotationally symmetric. Despite the coarse, digital scheme of the cells of the spreadsheet, the possible molecular bonds may be concluded from the distribution of the non-empty fields. It looks like the FliMs form dimers when the C-ring rotates clockwise. Obviously, there are two types of gaps: some are completed by a

FliG, others are not. The difference will certainly affect the binding of CheY-P. The model provides no indication as to whether the gaps between the dimers are large enough to provide shelter for small "foreign molecules" such as CheY-P.

CCW rotation (right part of the table)

At irregular intervals, the motors of E. coli reverse the direction of rotation to CCW, while at the same time, additional FliMs are installed in the C-ring. Maybe CheY-Ps will be replaced by FliMs. So far it is puzzling where these additional molecules are integrated into the ring structure and whether the diameter changes. The right part of the table above shows three obvious possibilities.

1. The 24/32 solution can be expanded in two ways. If all identifiable gaps are filled with FliMs, you get 24/48. If only every second gap is filled, you get 27/40. In both cases, the number of FliMs deviates noticeably from the experimentally determined value 44.
2. In the 27/36 solution, the remodeling of dimers into trimers is unlikely for several reasons: The solution 27/41 is not rotationally symmetric (red-colored) and shifting molecules costs energy and is error prone, since no molecule can reliably count to three. In contrast to the other two solutions, there is no easy way to recognize how the signal molecules CheY-P could cause the rearrangement of the C-ring.
3. Most likely, certain gaps in the 27/36 solution will be filled by additional FliMs, resulting in 27/45. This requires little energy and no displacement of existing molecules. The rotational symmetry is maintained and the target number of 44 FliMs is reached almost exactly.

The role of CheY-P

Now, we concentrate on the molecular arrangement 27/36 during CW rotation. The C-ring has eighteen gaps that can be divided into two classes. The neighbors of type A are arranged differently than those of type B.

Type A			Type B	
	FliM		FliG	FliM
FliG	A			B
	FliM		FliG	FliM

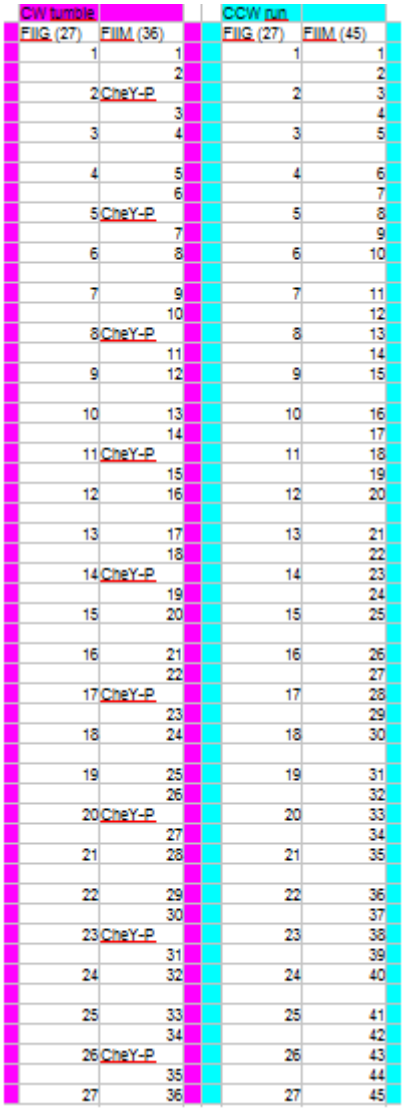
If we confine ourselves to one class, the switching mechanism can be explained on a molecular basis:

Nine gaps of the C-ring are not empty, they may be filled either by FliM or by CheY-P. While the binding force of CheY is not enough to displace a FliM, the signal molecules CheY-P can remove certain FliM proteins from their sites (push them back into the cytoplasm) and cause a configuration change of the C-ring. In the ring, the number of dimers competes with the groups of five. In CW rotation, the number of dimers predominates, but the chain may contain a few groups of five. In CCW direction it is the other way around. The winner takes it all.

Experiments will show if this hypothetical mechanism is realized by type A or type B.

It is known that an activated form of the response regulator CheY destabilizes the parallel arrangement of FliM molecules[5]. If the binding of CheY-P to FliM displaces the carboxy-terminal domain of FliG from FliM, the $FliG_C$-MotA interaction is modulated, causing the motor to reverse the rotational sense[6]. Since most connections between FliG and FliM remain untouched, the diameter of the C-ring will not change, at least not strongly.

The picture shows the two extreme cases (Type A): On the left, all gaps are occupied by CheY-P, on the right, there are only FliMs. However, it is very unlikely that these extreme cases will ever be realized in a living E. coli. At least not with the usual amounts of CheY-P in the cytoplasm. This will be discussed in another paper. Presumably, the allosteric switching of the direction of rotation depends on a threshold value, perhaps five or so. As long as the number of CheY-P falls below the (lower) threshold, the motor turns CCW. Exceeding a certain (upper) threshold initiates an allosteric switching of the entire C-ring and the motor rotates clockwise. A small difference between the two thresholds provides the necessary hysteresis to avoid frequent reversals of the rotational sense.



Binding and unbinding CheY-P

Binding always causes energy dissipation, which mostly escapes into the environment in the form of heat. Part of it is probably used to elastically deform the C-ring. The chemical binding allows the FliMs to identify CheY-P and to repel CheY. As soon as a sufficient number of CheY-Ps are attached (upper threshold), the ring has stored enough energy and can use it to rearrange certain components very quickly. As a result, the inflowing hydrogen ions are diverted, the direction of rotation is reversed and the bacteria starts to tumble.

It is unclear why and how quickly the CheY-P dissolve from the C-ring in order to stop tumbling. In B. subtilis, evolution has found a way out: FliM is replaced by FliY; FliY binds CheY-P and FliY ensures that the phosphate is separated. Since FliY can not bind CheY permanently, because the energy threshold is below the thermal energy of the environment, CheY separates from the C-ring. This elegant path is not possible in E. coli because the CheZ (necessary to dephosphorylate the signal molecules) is hidden far away in the sensor complex.

To separate the bound CheY-P from C-ring of an E. coli motor, either energy must be collected and applied or the energy threshold must be lowered. This can take a while – meanwhile the motor rotates in CW direction. It is unclear whether the C-ring needs to release most or all CheY-P to terminate the tumble. If they stick to the FliMs, the tumble never stops. A fine tuning of the thresholds by hitherto unknown signal molecules may help to adapt the search behavior to changed conditions inside or outside the cell.

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