NOTES

Requirement of Adenosine 3′, 5′-Cyclic Phosphate for Flagella Formation in Escherichia coli and Salmonella typhimurium

TAKESHI YOKOTA AND JOSEPH S. GOTS
Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19140

Received for publication 19 March 1970

Adenosine-3′,5′-cyclic phosphate (cyclic AMP) is absolutely required for flagella formation and, hence, motility in cyclic AMP-deficient mutants of Escherichia coli and Salmonella typhimurium.

Adenosine-3′,5′-cyclic phosphate (cyclic AMP) is involved as an intracellular messenger in the mediation of a variety of hormonal effects in higher animals (6). Its role in bacterial physiology has been revealed by its ability to counteract catabolite repression (10), its requirement for the induction of a variety of enzymes (3), and its apparent necessity for the initiation of transcription at the promoter site (2, 9). Further confirmation of some of these effects was obtained with the isolation of an adeny1 cyclase-deficient mutant of Escherichia coli by Perlman and Pastan (11) This mutant showed a pleiotropic deficiency. In the utilization of a variety of carbohydrates which could be phenotypically corrected by cyclic AMP. We have independently isolated similar mutants and find that cyclic AMP is not only necessary for the restoration of sugar fermentation, but is also absolutely required for the formation of flagella and hence motility.

Cyclic AMP-deficient mutants of E. coli were isolated from HfrH and HfrC strains as follows. Overnight cultures in Penassay Broth (Difco) were treated with N-methyl-N′-nitro-N-nitrosoguanidine (150 μg/ml) by the method of Mise and Suzuki (8). Mutants pleiotropic for utilization of sugars as carbon sources and tryptophan as a nitrogen source were selected by penicillin treatment in a minimal salts medium (M-9) containing lactose, arabinose, and maltose as carbon sources and tryptophan as the only nitrogen source. After overnight incubation, the survivors were spread on nutrient agar plates containing triphenyltetrazolium chloride (50 μg/ml) and 0.5% each of lactose, arabinose, and maltose. Red (fermentation-negative) mutants which appeared after 24 hr of incubation were purified on the same media and tested for their sugar fermentation patterns by using EMB agar plates and bromoresol purple semisolid agar tubes (Difco broth base containing 0.3% agar) supplemented with 1% of the various sugars, with and without 10−3 M cyclic AMP (Sigma Chemical Co.). Mutants responding to cyclic AMP and having little adenyl cyclase activity have been assigned the genotype cya. The cya locus was found to map near met E (manuscript in preparation). A cya mutant of Salmonella typhimurium strain DB99 was found by similar fermentation screening of the unclassified pleiotropic sugar-negative mutants in the collection of David Berkowitz. This mutant had been isolated by Berkowitz from strain SL751, a multiauxotrophic, nonmotile mutant carrying a flaA mutation. For subsequent flagellar antigen analyses, it was necessary to convert this to fla+. Strain GP8 (fla+ cya) was obtained by transducing the fla+ allele into strain DB99 using the selection method described by Stocker (12) and P22 transducing phage grown on strain metB23, a fla+ donor. Strain CA7902 is a cya mutant of E. coli, isolated by J. Beckwith and obtained from him after our own isolations and characterizations were completed.

Among the pleiotropic sugar mutants isolated, strain GP1, derived from E. coli HfrH, shows a typical cya phenotype and the most marked deficiency in adenyl cyclase. In the absence of cyclic AMP, it is completely negative for the

1 On leave of absence from Yamanashi Prefectural Institute of Public Health, Kofu, Japan.
utilization of lactose, arabinose, maltose, mannitol, xylose, galactose, and glycerol, but positive for glucose. All sugars are strongly fermented in the presence of 10^{-2} M cyclic AMP. The Salmonella mutant, strain DB99, shows the same pattern except that arabinose utilization does not require cyclic AMP and it is naturally lactose-negative.

The effect on motility was discovered during scoring of the fermentation results in the semisolid stab cultures. It was immediately evident that the growth of the mutant was completely restricted to the line of the stab, whereas that of the wild type, or the mutant grown in cyclic AMP, swarmed through the semisolid medium. This effect was confirmed using Stocker’s semisolid agar-gelatin motility medium (12). Figures 1 and 2 show the effect and Table 1 summarizes the results. The effect was obtained with all of the cya mutants at cyclic AMP concentrations higher than 10^{-4} M. Related compounds, such as adenosine triphosphate, adenosine diphosphate, AMP, adenosine, cyclic guanosine monophos-

![Fig. 1. Motility of E. coli GP1 (fla+; cya) on gelatin-semisolid agar plates. Plate on left does not contain cyclic AMP; plate on right contains 1 mm cyclic AMP.](image)

![Fig. 2. Motility of E. coli GP1 (fla+; cya) and its recombinant GP7 (fla+; cya) by stab culture method in a gelatin-semisolid agar medium. (Strain GP7 is a recombinant between E. coli GP1 and E. coli AB1157, a polyauxotrophic F strain, (pro, his, thi, cya, str)). Tube 1: strain GP1, no cyclic AMP in medium; Tube 2: strain GP1, plus cyclic AMP (1 mm); Tube 3: strain GP7, no cyclic AMP in medium; Tube 4: strain GP7, plus cyclic AMP (1 mm).](image)

Table 1. Cyclic adenosine monophosphate (AMP) requirement for motility of cyclic AMP-deficient mutants (cya) of Escherichia coli and Salmonella typhimurium

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Genotype</th>
<th>Motility at cyclic AMP concentration (m) of</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Hfr-H</td>
<td>flas</td>
<td>10^{-2}</td>
</tr>
<tr>
<td>E. coli</td>
<td>GP1</td>
<td>cya</td>
<td>+</td>
</tr>
<tr>
<td>E. coli</td>
<td>CA7902</td>
<td>flas</td>
<td>+</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>met B23c</td>
<td>cya</td>
<td>+</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>DB99</td>
<td>flas</td>
<td>+</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>GP8</td>
<td>cya</td>
<td>+</td>
</tr>
</tbody>
</table>

* Gene controlling flagella formation flas; gene for cyclic AMP requirement, cya.

* Motility determined by swarming stab culture semisolid agar-gelatin medium. The following chemicals were negative at a concentration of 10^{-2} M: adenosine, AMP, adenosine diphosphate, adenosine triphosphate, cyclic guanosine monophosphate, and dibutyryl cyclic AMP.

* S. typhimurium metB23 is the donor strain for the transduction of flas producing GP8 (flas; cya) from DB99 (flaA; cya)
shown in Table 2. No flagellar antigens could be detected when the mutant was cultured in the absence of cyclic AMP, but after 150 min of incubation under growing conditions with 10^{-7} M cyclic AMP, phase 1 flagella antigen was detected. Phase 2 antigen was not detected. The wild type, cya+, which is also the fla+ donor used to construct strain GP8 showed both antigens, even without cyclic AMP. Susceptibility of the mutant to flagella phage chi could be demonstrated only when it had been both subcultured and plated with the phage in the presence of cyclic AMP. The cya locus maps near metE (manuscript in preparation) and hence cannot correspond to six of the known fla genes (A to F) since their chromosomal locations are known to map far from the fla genes.
from this site (4). The location of flaG is unknown (4) and it remains to be determined whether this gene is similar to cya.

Cyclic AMP is not required for the integrity of another bacterial appendage, the F pilus, as shown by the fact that the Hfr cya strain GP1 can still serve as a normal donor in conjugation and is still sensitive to male-specific phase R-17 in the absence of cyclic AMP.

Exactly how cyclic AMP functions in the formation of bacterial flagella remains to be answered. Its known role in the formation of inducible enzymes would suggest that it is probably involved in the formation of flagellar protein rather than polymerization. In view of the role of cyclic AMP in counteracting catabolite repression (10), it is of special interest to note that Adler and Templeton (1) found that the presence of glucose in a chemically defined growth medium prevented the synthesis of flagella in E. coli.

We are grateful to David Berkowitz for supplying strain DB99, to Jonathan Perlman for strain CA-7902, and to Herbert Blough for assistance with the electron microscopy.

This work was supported by Public Health Service grant CA-02790 from the National Cancer Institute and by grant GB-7453 from the National Science Foundation.

LITERATURE CITED