TEMPLATE SPECIFICITY CHANGES OF DNA-DEPENDENT RNA POLYMERASE IN B. SUBTILIS DURING SPORULATION[1,2]

Charlyn D. Murray[3], Pattle Pun[4] and N. Strauss
Department of Biology
State University of New York at Buffalo, Buffalo, New York 14214

Received June 25,1974

Previous work has indicated that loss of ability of DNA dependent RNA polymerase, from stationary phase cultures of B. subtilis, to transcribe phage Øe DNA was a sine qua non for sporulation. To ascertain if this change in template specificity was sporulation-specific, we repeated these experiments using a defined sporulation medium. The changes observed previously did not occur in the defined medium although sporulation was normal. The ability of the enzyme to transcribe other DNA templates was also examined. Similar studies were carried out using a polymerase from a rifamycin-resistant, sporulation conditional mutant. The significance of these findings with regard to the regulation of sporulation in B. subtilis is discussed.

One of the earliest biochemical changes observable during the sporulation of Bacillus subtilis is an alteration in the transcribing specificity of the DNA-dependent RNA polymerase (1). It was proposed that this alteration, a loss of the ability of the polymerase to transcribe Øe DNA in vitro at the onset of the stationary phase of growth, reflected sporulation-specific changes in the enzyme. This proposal was supported both by the finding that normal sigma activity was lost during sporulation (2), and by the observation that the enzyme from asporogenous mutants, presumably bearing a lesion in the gene coding for the β subunit, failed to show this decline in ability to transcribe Øe DNA (3). The alteration of core enzyme in the β subunit (2) was shown recently to be an artifact (4,5).

[1]Preliminary results of this work were presented at the Colloque International C.N.R.S. sur la Regulation de la Sporulation Microbienne, at Gif-sur-Yvette, France, September 10, 1973.

[2]This work was supported by Grant GB 23191 from the National Science Foundation

[3]In partial fulfillment by Charlyn D. Murray of the requirements for the degree of Doctor of Philosophy in Biology.

[4]Present address: Department of Biology, Wheaton College, Illinois 60187.

The evidence in support of the hypothesis that the change in template specificity is a sine qua non for sporulation is not conclusive. Absence of the template specificity change in asporogeneous mutants may indicate that the specificity change and the sporulation process are events which have a common metabolic origin but eventually diverge. Such mutants might have a lesion which affects an event on the common pathway prior to that which evokes the specificity change. A crucial test would be to seek other conditions which induce sporulation normally with eliciting a change in template specificity of the polymerase. The results of such a study are presented herein.

METHODS. Organisms. Strain 168 wild-type and a spontaneous derivative, SB2 Arg^-, were used. Strain 168 wild-type was obtained by spontaneous reversion of 168 tryA, and SB2 Arg^- by spontaneous mutation to rifamycin resistance (Rif^R), and arginine independence. This mutant is also a Stage 0 (6, 7) sporulation conditional (Spo^C), cannot sporulate in Sterlini-Mandelstam sporulation medium (SM medium) (8) but sporulates normally in a modified Schaeffer medium (DDS medium) (9, 10). The Spo^C trait presumably stems from a lesion in the gene specifying a subunit of the DNA-dependent RNA polymerase (6).

Media. Organisms were grown in DDS medium, a non-defined medium containing nutrient broth (9, 10), which supports both growth and sporulation of 168 w.t. and of SB2 Arg^-. Cells were also induced to sporulate by transfer from DDS medium, after mid-log stage of growth, to Sterlini-Mandelstam resuspension medium (SM medium) (8), a defined medium which supports sporulation of the wild type but not of SB2 Arg^-. Arginine was used as a supplement in SM medium when the mutant was used.

DNA-dependent, RNA polymerase. Cells were harvested by centrifugation, washed in Buffer 1 (Tris-HCl, 10 mM; pH 7.9; disodium EDTA, 1 mM; $MgCl_2$, 10 mM; KCl, 50 mM; 5% glycerol; β mercaptoethanol, 5 mM; and 0.3 mg/ml

SOLUBILIZATION OF GLUCAGON AND EPINEPHRINE SENSITIVE ADENYLATE CYCLASE FROM RAT LIVER PLASMA MEMBRANES

Judith Ryan and Dan R. Storm

Department of Biochemistry, University of Illinois, Urbana, Illinois

Received July 22, 1974

SUMMARY

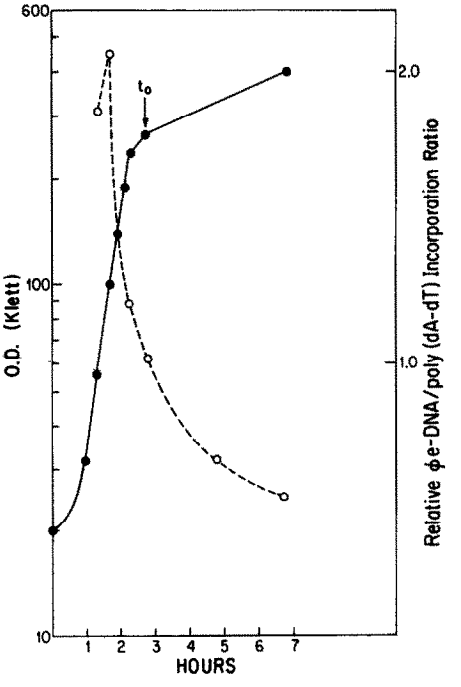
Hormonally sensitive adenylate cyclase has been solubilized from rat liver plasma membranes using Triton X-305 in Tris buffers containing mercaptoethanol and $MgCl_2$. The solubilized enzyme was stimulated 5 fold by NaF, 7 fold by glucagon and 20 fold by epinephrine. Criteria for solubilization included lack of sedimentation at 100,000 x g for one hour, the absence of particulate material in the 100,000 x g supernatant when examined by electron microscopy, and inclusion of hormonally sensitive adenylate cyclase activity in Sephadex G 200 gels. The molecular weight of the solubilized, hormonally sensitive enzyme was approximately 200,000 in the presence of Triton X-305.

INTRODUCTION

Although there has been considerable interest in the mechanism for hormonal stimulation of mammalian adenylate cyclase activities, progress toward the solution of this problem has been severely hindered by one major technical barrier. The enzyme has not been solubilized and purified in a hormonally sensitive form from any mammalian source. However, the bacterial enzyme from Brevibacterium lique-

RESULTS. Effect of sporulation medium on template specificity changes of RNA polymerase. To test the possibility that changes in template specificity of the enzyme are a function of changes in the medium used to induce sporulation, sporulation was induced both in DDS medium and in SM medium. Samples of cells were removed at various times and used to prepare extracts containing enzyme as described above.

To establish that our procedures were consistent with those of previous workers (1, 12), experiments were carried out using sporogenous cells grown in DDS medium. The results, shown in figure 1, are similar to those of Losick and Sonenshein (1). Similar experiments were then carried out using wild-type cells taken from different media and at various times during the growth and sporulation phases. In addition to



The data of this figure were derived from two separate cultures for technical reasons. Both cultures were grown in identical fashion. The first was sampled at $t_{-1.35}$, $t_{-1.0}$, $t_{-0.7}$ and t_0. The second was sampled at t_0, t_2, and t_4. The total change in the Øe DNA/poly(dA-dT) ratio was determined by normalizing the t_0 values of each culture and calculating the corresponding relative values of Øe-DNA/p(dA-dT) ratio at the times indicated. The growth curves were virtually identical for both cultures.

Øe-DNA and poly(dA-dT) as template, B. subtilis DNA and DNA from phage β22 were used. Phage β22 has been found to be virulent throughout the life cycle of B. subtilis (17). The results are shown in table 1.

TABLE I

RNA POLYMERASE ACTIVITY OF WILD TYPE 168 DURING SPORULATION

	Medium	% Sporulation at 24 hours	time	nmoles ATP/10 min/mg protein DNA Template Øe	β22	B.s.	pdA-dT	Øe/pdAT	β22/pdAT	B.s./pdAT	Øe/β22
Exp 1	SM	85.4	t_0 *	15.2**	4.2	3.8	34.6	.44	.12	.11	3.6
			t_3	34.9	13.2	9.1	71.3	.49	.19	.13	2.6
			t_6	26.7	11.2	7.9	60.1	.44	.19	.13	2.4
Exp 2	SM	94.3	t_0	29.2	10.5	8.8	76.0	.38	.14	.12	2.8
			t_2	30.1	10.7	8.0	75.6	.40	.14	.11	2.8
			t_4	29.3	7.9	9.2	69.0	.42	.11	.13	3.7
Exp 3	SM	91.6	t_0	9.7	---	---	21.8	.44	---	---	---
			t_8	3.5	---	---	11.0	.32	---	---	---
Exp 4	DDS	102	t_0 *	22.3	7.8	7.4	56.0	.40	.14	.13	2.9
			t_2	11.1	5.2	4.4	43.3	.26	.12	.10	2.1
			t_4	7.0	2.9	3.2	35.3	.20	.08	.09	2.4

Cells were sampled at the time indicated, disrupted and the resulting extracts brought to 42% ammonium sulfate saturation. The suspension was centrifuged and the supernatant was brought to 62% saturation. The precipitate was resuspended in Buffer 2 and used as enzyme.

* t_0 in DDS medium is taken as the end of exponential growth. t_0 in SM medium is the time of resuspension in SM medium. In DDS medium the value of Øe DNA/pdAT at $t_{-1.0}$ is 0.8 and the total decrease in this ratio between $t_{-1.0}$ and t_4 is four fold. Sporulation in DDS medium was completed by t_{11} and in SM medium by t_{13}.

** This t_0 preparation was derived from a different culture than the t_3 and t_6 preparations.

As mentioned previously, the ratio Øe DNA/poly(dA-dT) also was calculated as an internally consistent means of detecting template specificity changes (1).

It appears that little change occurred in the specific activity of the RNA polymerase with respect to Øe-DNA when wild-type cells sporulated

in SM medium. In those instances in which changes did occur, the Øe-DNA/poly(dA-dT) ratios were constant, indicating that the changes observed were probably a result of variation in efficiency of extraction. Our results, for the case of DDS medium, were in accord with those of previous workers (1, 12, 18). The ratio Øe-DNA/poly(dA-dT) fell some 50% between t_o and t_4. When the decrease was calculated from $t_{-1.0}$, moreover, this drop was four-fold (see figure 1). The ratios β22-DNA/poly(dA-dT) and B.s.-DNA/poly(dA-dT) also fell in parallel with the Øe-DNA/poly(dA-dT) ratio but to a lesser extent. This decline seems to be an indication of loss of sigma activity in stationary phase cells (4). This reduction is not apparent in cells suspended in SM medium.

Mutant SB2 behaves in SM medium with respect to RNA polymerase activity as does the wild type even though the mutant does not sporulate in this medium. In DDS medium, the mutant showed a reduced decline in RNA polymerase specific activity *vis a vis* Øe-DNA over a six hour period. Similarly, the Øe DNA/p(dA-dT) ratio declined only 30% during the period t_o to t_6.

DISCUSSION. The foregoing results suggest that the template specificity changes observed by other workers during sporulation of *B. subtilis* may not be sporulation specific, or may be sporulation specific only in certain media. It is evident from tables 1 and 2 that sporulation of *B. subtilis* strain w.t. 168 and 168 Try^- can occur in the absence of the change in template specificity in SM medium. The obvious interpretation of this finding is that the events leading to the template specificity change and those leading to sporulation have a common origin, and that sporulation in SM medium blocks only those events leading uniquely to the specificity change. Tjian and Losick (personal communication), in contradiction to the results described herein, have reported that RNA polymerase of *B. subtilis* strain SMY underwent a template specificity

TABLE 2

RNA POLYMERASE ACTIVITY OF MUTANT SB2 DURING SPORULATION

	Medium	% Sporulation at 24 hours	time	nmoles ATP/10 min/mg protein DNA Template Øe	β22	B.s.	pdA-dT	Øe/PdAT	β22/pdAT	B.s./pdAT	Øe/β22
Exp 1	SM	7.4	t_o	16.3	1.8	3.8	25.9	.63	.07	.15	9.1
			t_2	15.0	2.5	3.1	21.6	.69	.12	.14	6.0
			t_4	14.9	1.9	3.4	24.2	.62	.08	.14	7.8
Exp 2	DDS	96.2	t_0	5.4	1.3	1.6	11.7	.46	.11	.14	4.2
			t_2	5.3	1.4	1.2	11.9	.45	.12	.10	3.8
			t_4	4.6	1.2	1.1	13.1	.35	.09	.08	3.8
Exp 3	DDS	100	t_0	12.1	---	---	20.8	.58	---	---	---
			t_3	8.3	---	---	20.2	.41	---	---	---
			t_6	7.8	---	---	21.9	.35	---	---	---

Extracts were prepared as described in the legend to Table 1.

change after resuspension in SM medium. This contradiction has not yet been resolved.

It appears that cells which enter stationary phase in DDS medium undergo a loss in RNA polymerase specificity activity (3, 4). Much of this loss is probably non-specific and is reflected as a loss of ability to transcribe all templates. The loss of sigma further decreases the ability of the enzyme to transcribe Øe but increases the ability to transcribe p(dA-dT). The specific activity of the enzyme vis a vis the template, therefore, is a resultant of the non-specific loss in enzyme and the change resulting from the loss of sigma activity. Reports by Szulmajster (20), and by Orrego et al., (4) have indicated that changes in template specificity may result from proteolysis during extraction of sporulating cells even when precautions are taken to prevent such degradation More recently, Tjian and Losick (personal communication) have obtained evidence that sigma is present in extracts of sporulating cells as judged by SDS gel electrophoresis, by antibody precipitation and by

binding to core. They concluded that sigma is unaltered, and that a third factor prevents the binding of sigma to core. The ability of sigma, isolated from sporulating cells, to stimulate the activity of vegetative core was not determined however, leaving the possibility that a minor proteolytic cleavage, not detectable by the methods used, was responsible for the loss of sigma activity.

The original observations of Losick and Sonenshein (1) provided a temporal correlation of the loss of ability of polymerase to transcribe Øe DNA and the loss of ability of the phage to replicate, in sporulating cells. The data of Table 1 indicate that the DNA of virulent phage β22, a phage which can replicate in sporulating cells (17), shows an altered ability to be transcribed by RNA polymerase from sporulating cells. Ito et al., (19), (personal communication) have found that the decrease in the burst size of phages Ø15 and Ø29, occurs somewhat earlier in the growth cycle than that of Øe. These observations suggest that the parallel change in template specificity and the decrease in burst size which was observed for the case of phage Øe (1) were fortuitous and not related to the trapping phenomenon. This problem is far from being resolved.

REFERENCES

1. Losick, R. and Sonenshein, A.L. 1969. Nature 224, 35.
2. Losick, R., Shorenstein, R.G. and Sonenshein, A.L. 1970. Nature 227, 910.
3. Sonenshein, A.L. and Losick, R. 1970. Nature 227, 906.
4. Orrego, C., Kerjan, P. Manca de Nadra, M.C., Szulmajster, J. 1973. J. Bact. 116, 636.
5. Linn, T.G., Greenleaf, A., Shorenstein, R.G. and Losick, R., 1973. Biochemistry 70: 1865.
6. Pun, P., Murray, C.D. and Strauss, N. (In preparation).
7. DiCioccio, R.A. and Strauss, N. 1973. J. Mol. Biol., 77, 325.
8. Sterlini, J.M. and Mandelstam, J. 1969. Biochemical Journal 113, 29.
9. Schaeffer, P., Millet, J. and Aubert, J.P. 1965. Proc. Natl. Acad. Sci. U.S. 54, 571.

10. Leighton, T.J. and Doi, R.H. 1971. J. Biol. Chem. 246, 3189.
11. Burgess, R.A. 1969. J. Biol. Chem. 244, 6160.
12. Brevet, J. and Sonenshein, A.L. 1972. J. Bact. 112, 1270.
13. Whitely, H.R. and Hemphill, H.E. 1970. Biochem. Biophys. Res. Commun. 41, 647.
14. Marmur, J. 1961. J. Mol. Biol. 3, 208.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. J. Biol. Chem. 193, 265.
16. Shorenstein, R.G. and Losick, R. 1973. J. Biol. Chem. 248, 6163.
17. Yehle, C.O. and Doi, R.H. 1967. J. Virol. 1, 935.
18. Brevet, J. Molec. Gen. Genet. 1974. 128, 223.
19. Ito, J., Meinke, W., Hathaway, G., Spizizen, J. 1973. Virology 56, 110.
20. Szulmajster, J. 1973. International C.N.R.S. sur la "Regulation de la Sporulation Microbienne", at Gif-sur-Yvette, France.