DNA DEPENDENT RNA POLYMERASE FROM VEGETATIVE CELLS AND FROM SPORES OF *B.SUBTILIS:* III. ISOLATION OF A STIMULATING FACTOR

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1. Introduction

The discovery that a protein factor (σ factor) normally associated with *E. coli* RNA polymerase [1] and of an analogous factor isolated from T_4-infected cells [2] determines template specificity *in vitro*, suggested new possibilities for the mode of action of this complex enzyme. It then appeared that this factor (separable from the RNA polymerase by chromatography on phosphocellulose), in conjunction with the "core" RNA polymerase, might promote the transcription, *in vitro*, of a new class of genes otherwise unrecognized. This discovery is of particular interest in explaining the mechanism of regulation related to systems in which genes are activated in a sequential manner, as exemplified by virus infection. Indeed, recent studies on coliphage T_7 [3] have shown that the control of the appearance of phage induced protein is mediated by a protein factor produced by gene 1. This factor replaces or alters the sigma factor of the host RNA polymerase in such a way that the polymerase can then transcribe the rest of the T_7 genome.

The recent and elegant study of Losick and Sonenschein [4] on the change in template specificity of RNA polymerase during sporulation of *B. subtilis* must be considered with the above finding. These authors have shown that RNA polymerase from vegetative cells of *B. subtilis* is able to transcribe, *in vitro*, the phage ϕe DNA, while the enzyme from sporulating cells is inactive with the same template. Two possible explanations were suggested by the authors: either that the RNA polymerase became altered in its structure during sporulation, or that a specific (sigma-like) factor required for the transcription of the ϕe DNA was lost or altered during sporulation.

In the present communication we describe the separation of a sigma-like protein factor, from *B. subtilis* vegetative polymerase by phosphocellulose chromatography. This factor stimulates the transcription of T_4 DNA template by the core polymerase, which is inactive with the same template. We further show that, under identical conditions of isolation, the core RNA polymerase obtained from *B. subtilis* dormant spores still retained the activity to transcribe T_4 DNA as it did with the thymus and *B. subtilis* DNA. Although no stimulating factor was separated from the spore enzyme, the activity of the spore core

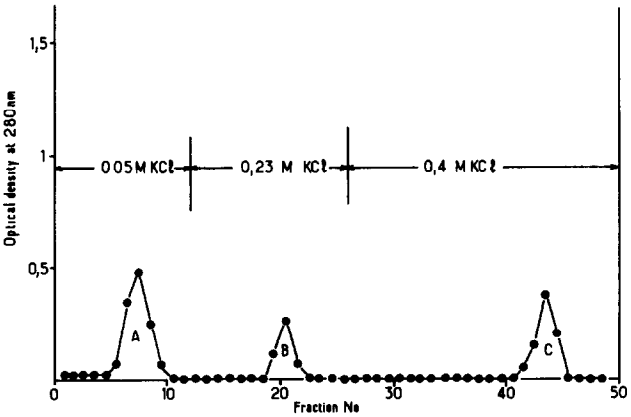

Fig. 1. Stepwise elution profile of vegetative RNA polymerase from a phosphocellulose column according to Burgess et al. [1]. Fractions of 3 ml were collected with a flow rate of 1 ml/3 min.

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Table 1
Stimulation of activity of vegetative core RNA polymerase by vegetative peak A factor. The values in parentheses indicate the amounts in μg (protein or DNA) added to the incubation mixture.

Source of enzyme	DNA template		
	Thymus (50)	T_4 (48)	*B. subtilis* (50)
	(μμmole ^{14}C-AMP incorporated)		
Vegetative polymerase* (56)	194	170	286
Vegetative core polymerase** (57)	245	40	456
Vegetative peak A (11.5)	17	11	16
Vegetative core polymerase (57) + vegetative peak A (11.5)	372	250	414
Vegetative polymerase + boiled peak A (5 min at 100°C)	–	40	–

* RNA polymerase before phosphocellulose chromatography.
** The protein fraction eluted by 0.4 M (peak C) is designed as "core polymerase".

Table 2
Stimulation of activity of spore core polymerase by the vegetative peak A factor.

Source of enzyme	DNA template			
	Thymus (50)	T_4 (48)	*B. subtilis* native (50)	*B. subtilis* denat. (50)
	(μμmole ^{14}C-AMP incorporated)			
Spore RNA polymerase* (60)	123	140	184	–
Spore core polymerase** (51)	107	106	127	
Spore peak A (11)	0	27	22	
Spore core polymerase (51) + spore peak A (11)	145	142	139	160
Spore core polymerase (51) + spore peak A (22)	–	139	148	
Spore core polymerase (51) + vegetative peak A (11.5)	326	245	660	776
Vegetative core polymerase (51) + spore peak A (11)	186	0	234	
Vegetative core polymerase (51) + spore peak A (22)	154	7	223	

* and ** as indicated in table 1.

polymerase can further be stimulated by the *B. subtilis* vegetative factor using calf thymus, T_4 and *B. subtilis* DNA's as templates.

2. Materials and methods

The RNA polymerase isolated from vegetative cells and from spores of *B. subtilis* used in this study were partially purified preparations obtained, after DEAE-cellulose step, by the method previously described [5]. A slight modification was however introduced, viz, the treatment of the crude extract with 2 μg/ml of DNAase. Further purification on a phosphocellulose column was carried out according to Burgess et al. [1]. The assay mixture for RNA polymerase contained per 0.5 ml: 10 μmole tris HCl, pH 7.9; 1 μmole Mg acetate, 0.2 μmole $MnCl_2$; 2 μmole 2-mercaptoethanol; 125 mμmole GTP, CTP and UTP; 125 mμmole ^{14}C-ATP (2500 cpm/μmole) and DNA at the concentrations indicated.

3. Results and discussion

Fig. 1 shows the elution profile, from a phospho-

cellulose column (Whatman P-11) of a *B. subtilis* vegetative RNA polymerase. A similar pattern of elution was obtained with the spore RNA polymerase. By stepwise elution with 0.05 M, 0.23 M and 0.4 M KCl, 3 protein peaks A, B, C were obtained. Each peak was tested for RNA polymerase activity in the presence of calf thymus, T_4 and *B. subtilis* DNA as templates. In both preparations, peak B was shown to retain the same ability to transcribe all three DNA's, as that of the enzyme before treatment by phosphocellulose. This fraction was therefore not further investigated in the present study.

Table 1 shows the activity of the two components, A (factor) and C (core polymerase) of the vegetative enzyme, eluted from a phosphocellulose column, and assayed separately or together in the presence of calf thymus, T_4 and *B. subtilis* DNA's. The results clearly show that core polymerase (peak C) is able to transcribe the thymus or the *B. subtilis* DNA's but is inactive with T_4 DNA. However, the transcription of the latter is stimulated about six fold by the factor present in peak A, which shows no activity by itself. The stimulating activity was completely abolished by boiling the peak A factor. It can also be seen in this table, that the enzyme before chromatography on phosphocellulose, is equally active with thymus or T_4 DNA's as templates.

Table 2 shows the results obtained with the spore RNA polymerase. In this case, the core RNA polymerase shows the same activity with the three DNA's used as templates as that observed with the RNA polymerase before treatment by phosphocellulose. There was no stimulation of activity by the addition of spore peak A (at two different concentrations) to the spore core polymerase. However, when the peak A factor, isolated from the vegetative RNA polymerase was added to the spore core enzyme, a net stimulation of activity was observed with each of the three template DNA's used. It is interesting to note, that the highest stimulation was obtained with *B. subtilis* DNA. On the other hand, addition of the spore peak A to the vegetative core polymerase was without effect.

It can be concluded from the above experiments that the vegetative RNA polymerase contains a sigma-like stimulating factor required to promote transcription of T_4 DNA. As for the spore enzyme, several different explanations can be given for the results obtained at the present stage. It is possible that the spore RNA polymerase factor is irreversibly inactivated by chromatography on phosphocellulose or that such a factor was present but destroyed during the disruption of the spores. A similar observation was made by Bautz and Dunn [6] on the DNA-dependent RNA polymerase, isolated from *E. coli* cells infected with phage T_4 ("modified" T_4 polymerase). The activity of the spore core polymerase might then be due to traces of factor still associated with this core enzyme, and the latter can further be stimulated by the vegetative protein factor. It is, however, more tempting to conclude that the spore polymerase contains little or no dissociable factor at all and that such a factor is only synthesized during germination when it may be required for the transcription of some early functions of the vegetative genome. The fact that, *in vitro*, the spore core polymerase can be stimulated by the addition of the vegetative peak A factor, strongly supports this idea. To promote the transcription of the spore genome during sporulation, there might be a different factor synthesized, as already suggested by Losick and Sonenshein [4].

The purification of all the components of the vegetative and spore polymerase, now in progress in this laboratory, will provide further information on the mechanism of regulation of this enzyme in spore differentiation of *B. subtilis*.

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References

[1] R.R.Burgess, A.A.Travers, J.J.Dunn and E.K.F.Bautz, Nature, 221 (1969) 43.
[2] A.A.Travers, Nature 223 (1969) 1107.
[3] W.C.Summers and R.B.Siegel, Nature 223 (1969) 1111.
[4] R.Losick and A.L.Sonenshein, Nature 224 (1969) 35.
[5] P.Kerjan, J.Marchetti and J.Szulmajster, Bull. Soc. Chim. Biol. 49 (1967) 1139.
[6] E.K.F.Bautz and J.Dunn, Biochem. Biophys. Res. Commun. 34 (1969) 230.