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In Vitro Translation of Messenger Ribonucleic Acid from Sporulating and Nonsporulating Strains of *Bacillus subtilis*

MARYVONNE ARNAUD,[1] INGA MAHLER,[1]* HARLYN O. HALVORSON,[1] HASIA BOSCHWITZ,[2] AND ALEX KEYNAN[2]

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254,[1] and Laboratory of Developmental and Molecular Biology, Department of Biochemistry, Hebrew University of Jerusalem, Jerusalem, Israel[2]

An *Escherichia coli* translation system supplemented with ribonucleic acid from sporulating *Bacillus subtilis* produces unique polypeptides which are missing among translation products of ribonucleic acid from six early sporulation mutants.

Although endospore formation in *Bacillus* is usually considered to involve a process of cell differentiation, the nature of the temporal controls is as yet undetermined. Quantitative isolation of mRNA's during development and their translation into polypeptides with an in vitro translation system provide an insight into the controlling factors between gene transcription and protein synthesis. mRNA from *Bacillus* can be translated in cell-free protein synthesizing systems prepared from *Bacillus subtilis* (5) and *Escherichia coli* (1, 11). Both systems have been employed in efforts to obtain sporulation components from *B. thuringiensis* (10) and *B. subtilis* (8). We used an *E. coli* translation system for a comparative study of RNA isolated from *B. subtilis* 168 during vegetative growth and during early and later stages of sporulation. RNAs isolated from six mutant strains blocked at the initial stage of the morphogenetic event were included in the survey. The bacterial strains used are shown in Table 1.

Cells were grown with shaking to an optical density (600 nm) of 0.5 to 0.6 in the medium of Shaeffer (13) at 37°C. To induce sporulation, cells were suspended to the same optical density at room temperature in Sterlini and Mandelstam resuspension medium (15) and incubated at 37°C for specified time periods. All *spo0* mutants were harvested after 90 min ($T_{1.5}$) in resuspension medium. For the *spo*$^+$ strain, refractile forespores were first visible after 8 h and sporulation was complete by 24 h.

Preparation of total cellular RNA was carried out as described (5) with some minor modifications (G. H. Chambliss, personal communication). NaN_3 at 0.025 was added to the culture before harvesting. After centrifugation, 7 mg of Macaloid (NL Industries, Inc., Hightstown, N.J.) was added per g (wet weight) to the cell concentrate, which was then frozen to −70°C.

Purified RNA was added to an S_{30} extract prepared from *E. coli* MZ9. The preparation of the S_{30} extract and the conditions for mRNA translation were as described previously (3). A 5-μCi amount of [^{35}S]methionine (Amersham) was added per 50 μl of total reaction mixture. Trichloroacetic acid precipitation was carried out as described in a Translation Kit (catalog no. N.E.K. 001; New England Nuclear). The dried filters were counted in a toluene-based scintillator by liquid scintillation spectrophotometry.

The cell-free, protein-synthesizing system was optimized to obtain high-molecular-weight polypeptides from MS_2 RNA (Miles Research Products) by using a magnesium concentration of 10 mM and a 20-min reaction time. Maximal ^{35}S incorporation of every mRNA sample was established by measuring the concentration of mRNA's versus [^{35}S]methionine incorporation. These concentration studies revealed that saturation of the in vitro translation system occurred with 0.2 to 0.24 mg of vegetative mRNA per ml compared with 0.62 to 0.84 mg of sporulation mRNA per ml (data not shown). This suggests that the approximately threefold-lower [^{35}S]methionine incorporation of sporulation mRNA observed by us and in *B. thuringiensis* (10) might be due to the presence of translationally inactive mRNA species. The presence of a possible inhibitor in sporulation RNA was ruled out by translating a mixture of vegetative and sporulation RNAs.

In Fig. 1A and B, lanes c through e display the polypeptides synthesized from mRNA extracted from *B. subtilis* 168 *spo*$^+$ during vegetative growth and during early ($T_{1.5}$) and late (T_5) stages of sporulation. The alteration in polypeptide band pattern from vegetative to sporulating mRNA is evident. Many vegetative bands have disappeared or show considerable diminu-

TABLE 1. *B. subtilis strains used*

Strain	Description			Source
168	*trp*$^+$		*spo*$^+$	J. Marmur
1S11	*trpC2*	*tolB24*	*spo0A12*	BGSC[a]
1S16	*trpC2*	*phe*	*spo0B136*	BGSC
1S19	*trpC2*	*phe*	*spo0F221*	BGSC
1S20	*trpC2*		*spo0H4*	BGSC
1S27	*metC3*	*tal-1*	*spo0J87*	BGSC
1S28	*trpC2*		*spo0K14*	BGSC
646 R15-13	*trpC2*	*phe*	*abrB23*	J. Hoch
646			*spo0A12*	J. Hoch
1S2[b]	*metB5*	*thr5*	*spoA3*	M. Salas

[a] BGSC, Bacillus Genetic Stock Center.

[b] This strain was constructed by transforming *B. subtilis* Mu8U5U5 *leu met thr* Sup$^-$ with DNA from *B. subtilis* Marburg 3NA *spoA* Sup$^-$ (7).

tion in the lanes containing sporulation mRNA products, whereas at least nine new bands (arrows) can be seen in the sporulation lanes. The appearance of new polypeptides and the simultaneous loss of certain vegetative proteins in sporulating cells of *B. subtilis* have been observed in vivo (6). Five new bands appear in the $T_{1.5}$ lane. One of these, a polypeptide of 92,000 daltons, is visible in the top section of Fig. 1B, which represents an autoradiogram of longer exposure. Of the four new bands in the T_5 lane, the smallest, a 10,000-dalton polypeptide, visible in the bottom section of Fig. 1B, is the most prominent. Experiments with 0.1 M phenylmethylsulfonylfluoride (Sigma) and 5 mM EDTA (pH 7) suggest that the 10,000-dalton polypeptide is not derived from protein break-

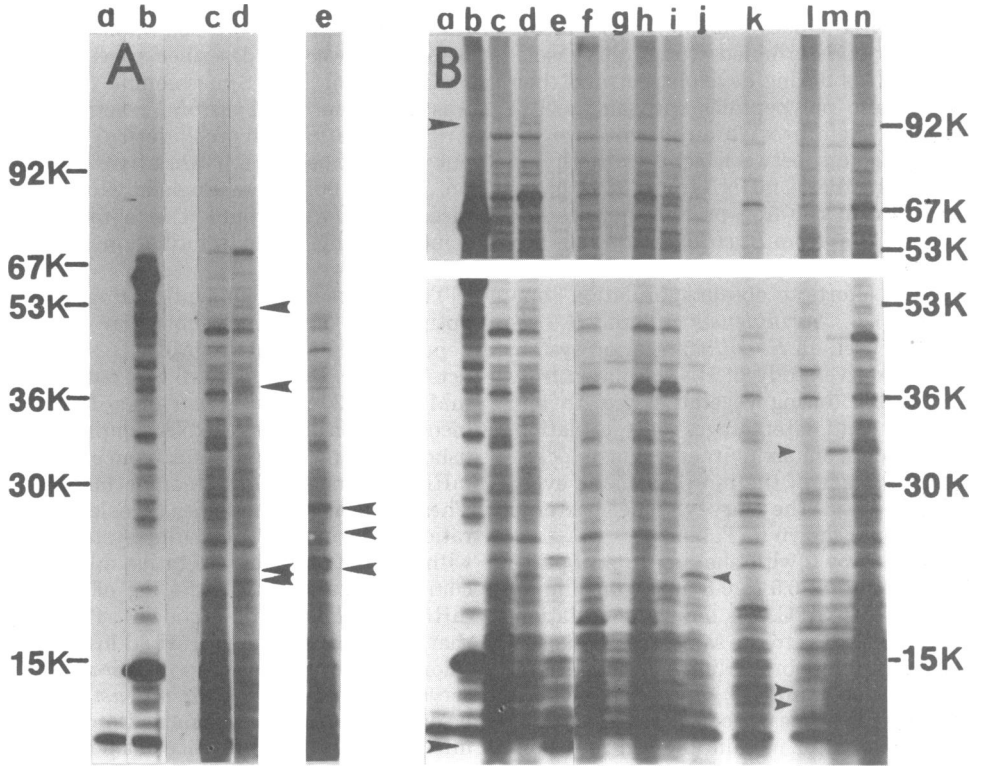

FIG. 1. *Preparation of samples and gel electrophoresis. Aliquots of reaction mixtures, containing equivalent amounts of radioactivity, were loaded on a 10 to 17.5% linear gradient acrylamide gel. To obtain good separation of proteins on acrylamide slab gels, polyethylene glycol was removed from the reaction samples before electrophoresis as described (3). For staining and drying of gels, the procedures of Studier (16) were used. To obtain autoradiograms, the dried gels were exposed to X-ray film (Kodak XR2) for various intervals of time at −70°C. (A) Gel exposure of 48 h. (B) Composite autoradiogram. The upper panel represents a gel exposure of 5 days; the bottom panel represents an exposure of 16 h. Translation products of the following mRNA's are shown: lane (a) no RNA; (b) MS_2; (c) vegetative; (d) $T_{1.5}$; (e) T_5; (f) spo0K; (g) spo0J; (h) spo0H; (i) spo0F; (j) spo0B; (k) spo0A3NA; (l) spo0A12; (m) R15-13; (n) vegetative. Molecular weight markers used were: phosphorylase A (92,500); bovine serum albumin (67,000); glutamate dehydrogenase (53,000); glyceraldehyde-3-P-dehydrogenase (36,000); carbonic anhydrase (30,000); and hemoglobin (15,500).*

down (data not shown).

In Fig. 1B, lanes f through j and lane l show the polypeptides synthesized from six unlinked *spo0* mutants. The two highly pleiotropic loci, *spo0A* and *spo0B*, produce protein products (2). Although functions for *spo0K* and *spo0H* are unknown, it has been suggested that *spo0F* may be defective in high phosphorylated nucleotide synthesis and that *spo0J* may be an RNA polymerase subunit (2).

Control experiments (not shown) of RNA from *spo0* mutants harvested before resuspension, reveal a pattern of polypeptide synthesis very similar to that for exponentially growing wild-type cells with at least one to three extra bands visible per mutant lane. *spo0* mutant RNAs isolated after resuspension additionally show a loss or change in intensity of many vegetative bands. However, with the exception of one 25,000-dalton *spo0B* band (lane J, arrow), which is visible among the $T_{1.5}$ products, none of the other early spore-related bands is present in the *spo0* lanes. Some minor qualitative differences can be detected among the mutant lanes: two bands of 20,000 and 12,000 daltons are missing among the *spo0J* products, whereas *spo0B* is missing a band of 40,000 daltons. Although the overall band patterns are very similar, the ratio of one band to another varies with each mutant. The basic similarity in mutant polypeptide band pattern is a function of the mRNA source and not the translation system as shown by the inclusion of strain *spo0A* (7) in lane K. The mRNA products of this strain differ qualitatively not only from another *A* mutant (*spo0A12*) but also from the other mutants examined.

In Fig. 1, lanes l and m also show translation products from *spo0A12* and a partial revertant, R15-13, which does not sporulate but is capable of reversing many of the pleitropic characteristics of *spo0A* mutants (17). For comparison, lane n contains vegetative mRNA products. In lane m, although several bands of *spo0A12* have disappeared, at least three vegetative bands indicated by arrows have reappeared.

We conclude that a cell-free protein synthesizing system from *E. coli* is capable of synthesizing a number of unique polypeptides from sporulating *B. subtilis* mRNA. These specific polypeptides, with one exception, do not appear among the translation products from cellular RNAs isolated from six *spo0* mutants. The translation system is sufficiently sensitive to detect differences between an *spo0A* mutant and a second site, partial revertant and should be useful in studying unique mRNA species which can hybridize to recombinant DNA molecules (9, 18). Cloned DNA segments which contain *B. subtilis* sporulation markers have already been reported (4, 12, 14; J. Ito, National Science Foundation US/USSR Seminar on the Genetics of Actinomycetes and Bacilli, abstr. no. PB283329-T 1978).

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