ANNEALING STUDIES OF TRANSCRIPTION IN B. SUBTILIS[1]

Richard A. DiCioccio[2] and Norman Strauss
Department of Biology
State University of New York at Buffalo, Buffalo, New York 14214

Received July 30, 1971

Transcription patterns of B. subtilis have been studied using the technique of annealing RNA to DNA single strands resolved from their complement. It was found that transcription during vegetative growth in broth occurred on at least 5.6% of the L strand and that during sporulation an additional 4.4% of the L strand was "turned on", while little of the original vegetative transcription was "turned off". In contrast, 17.5% of the H strand was rendered during vegetative growth in broth. At the second hour after sporulation onset, the transcription increased to 32%, a figure which is the resultant of a 5-8% "turn off" of vegetative transcription, and a "turn on" of about 20% during sporulation. These studies have accounted for a minimum of 42% of the total B. subtilis transcribing genome. Preliminary comparisons were made of transcription during vegetative growth in minimal medium, with that in broth.

INTRODUCTION

Recent studies of bacterial and phage transcription have revealed that significant regions of the genome may be translated from either strand. This finding is supported by physical and genetic evidence. The origin and evolutionary significance of this "strand-switching" is obscure, and the regulatory implications, if any, are not immediately apparent.

During our studies of regulation of sporulation, we have had occasion to suspect that some transcription occurs on the "non-transcribing" (L) strand. Recently, Yamazawa and Doi (1) have reported that transcription does, indeed, occur on the L strand.

We have reexamined and broadened this problem. Preliminary results of our findings are presented herein.

---

[1] This work was supported by Grant GB23191 from the National Science Foundation.

[2] In partial fulfillment by R.A.D. of the requirements for the degree of Doctor of Philosophy in Biology.

MATERIALS AND METHODS

Vegetative cells of B. subtilis, strain 168 Ura⁻, were obtained by growth either in Schaeffer's nutrient broth (2) or in minimal medium containing uracil (3). Sporulation was induced by a modification of the resuspension method of Mandelstam (4). This method yielded 90% sporulation and good synchrony. At various times during vegetative growth or sporulation, cells were rapidly chilled (within 3 min) to 5C and harvested by centrifugation.

RNA, both labelled and unlabelled, was prepared by the procedure of Dubnau, et al, (5). Extracts were treated 3 times with phenol. Polysaccharide was removed by the method of Ralph and Bellamy (6). Preparations were treated exhaustively with DNAse, extracted with phenol, alcohol-precipitated, resuspended in saline-citrate, and dialysed against 2x standard saline citrate. RNA extracted by this method gave low levels of non-specific binding when annealed to DNA.

RNA was labelled by growing cells continuously in the presence of H^3-uridine to insure that radioisotope counts were a measure of RNA. RNA labelled in steady-state was centrifuged in a sucrose gradient. The specific activity (cpm/O.D.) was shown to be the same in all fractions.

Sporulation RNA was routinely prepared from cells two hours after the onset of sporulation, and was designated "t_2-sporulation RNA". RNA from cells growing exponentially in broth was designated "broth vegetative RNA". Similarly, RNA from cells growing exponentially in minimal medium was designated "minimal vegetative RNA".

The complementary strands of DNA were resolved as previously described (7). Annealing of RNA and DNA was carried out in solution according to the method of Nygaard and Hall (8), as modified by Gillespie and Spiegelman (9), and by McConaughy, et al (10). Labelled RNA was used at saturating levels.

RESULTS

Annealing studies of the L ("non-transcribing") strand of DNA. The hybridization of steady-state labelled RNA extracted from cells in the

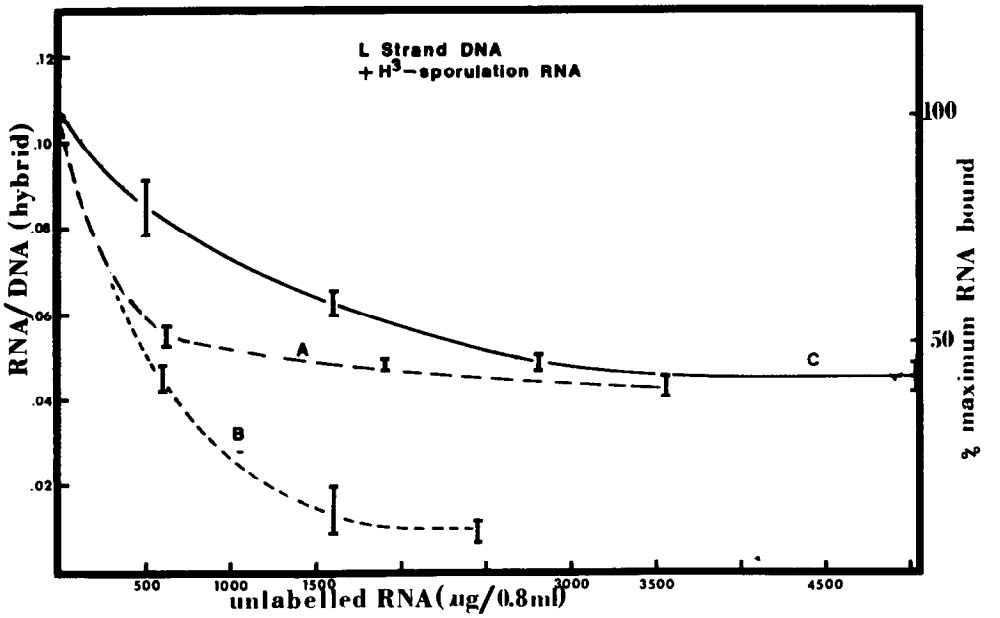

Figure 1. Hybridization Competition. The capacity of various RNA preparations to displace H^3-t_2-sporulation RNA from L strand DNA. Increasing concentrations of unlabelled RNA were mixed with 520 ug of H^3-sporulation RNA and 0.62 ug of L strand DNA in a total volume of 0.8 ml. Curve A, broth vegetative RNA; Curve B, t_2-sporulation RNA; Curve C, minimal vegetative RNA. Each point on the curves is an average of duplicate samples as indicated by the bracketing, short, horizontal lines. Labelled sporulation RNA had a specific radioactivity of 5890 cpm/ug.

---

second hour after onset of sporulation with L strand DNA was shown to reach saturation of the DNA when the hybrid RNA/DNA ratio was 0.10, suggesting that a minimum of 10% of the L strand was transcribed during sporulation. Figure 1 reveals that approximately 56% of this H^3-labelled sporulation RNA can be displaced either by broth vegetative RNA or by minimal vegetative RNA. It would appear, therefore that sporulation-specific RNA is transcribed on at least 4.4% of the L strand. Finally, Figure 2 shows that very little, if any, uniquely vegetative RNA is synthesized from the L strand. This conclusion is based on the finding that virtually all labelled vegetative RNA is displaced from the L strand by unlabelled sporulation RNA. Ribosomal and transfer RNA did not displace any labelled sporulation RNA from the L strand within the limits of sensitivity of the method.

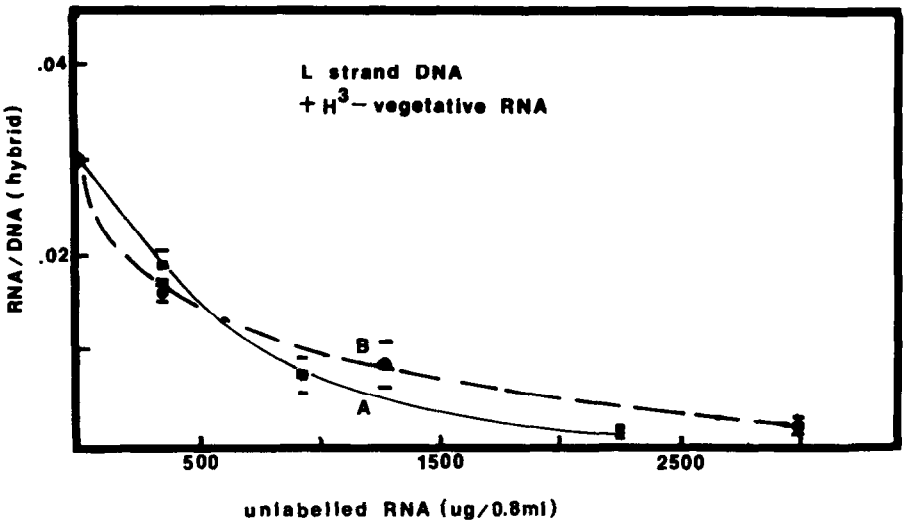

Figure 2. Hybridization Competition. Increasing concentrations of unlabelled RNA were tested for capacity to displace H^3-broth vegetative RNA (345 ug) from L strand DNA (0.62 ug) in a total volume of 0.8 ml. Curve A, unlabelled t_2-sporulation RNA; Curve B, unlabelled broth vegetative RNA. Labelled vegetative RNA had a specific radioactivity of 3437 cpm/ug.

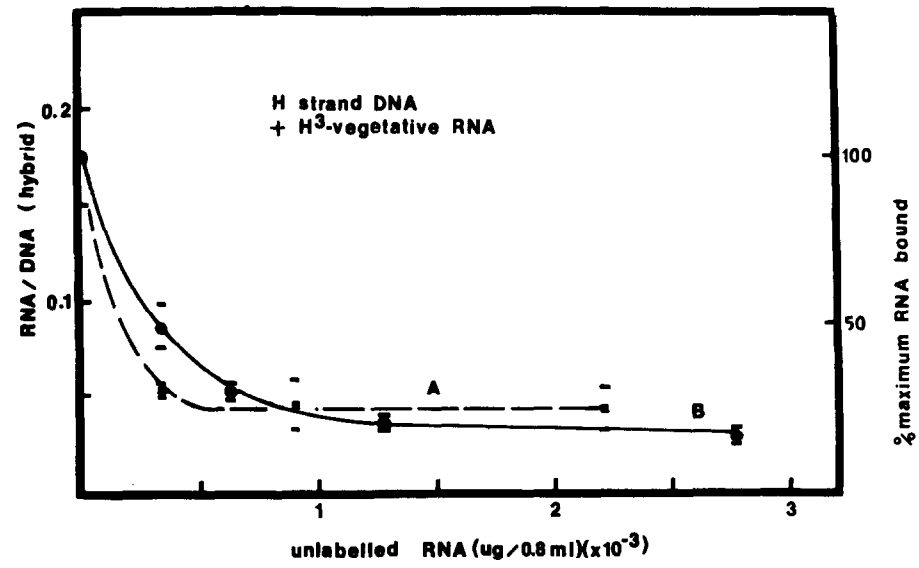

Figure 3. Hybridization Competition. Increasing concentrations of unlabelled RNA were tested for capacity to displace H^3-broth vegetative RNA (345 ug) from H strand DNA (0.45 ug) in a total volume of 0.8 ml. Curve A, unlabelled t_2-sporulation RNA; Curve B, unlabelled vegetative RNA. Labelled vegetative RNA had a specific radioactivity of 3437 cpm/ug.

Annealing studies of the H ("transcribing") strand. Saturation of the

H strand with labelled t_2- sporulation RNA occurs at a hybrid RNA/DNA ratio of 0.32. In contrast, broth vegetative RNA saturates at a hybrid RNA/DNA ratio of 0.175. This finding indicates that more of the H strand is used during sporulation than during vegetative growth in broth. The question of how much of this sporulation RNA which is bound, is unique to sporulation is answered in Figure 3, which reveals that t_2-sporulation RNA displaces all but about 5-8% of the broth vegetative RNA from the H strand. This suggests that only 5-8% of the vegetative transcription in broth is unique when compared to sporulation transcription. The inverse experiment (Figure 4) reveals that broth vegetative RNA can displace no more than 60% of t_2-sporulation RNA, a finding which suggests that 40% of RNA synthesized during sporulation is unique. It also can be seen that minimal-medium vegetative RNA displaces about 80% of the t_2-sporulation RNA, suggesting that minimal

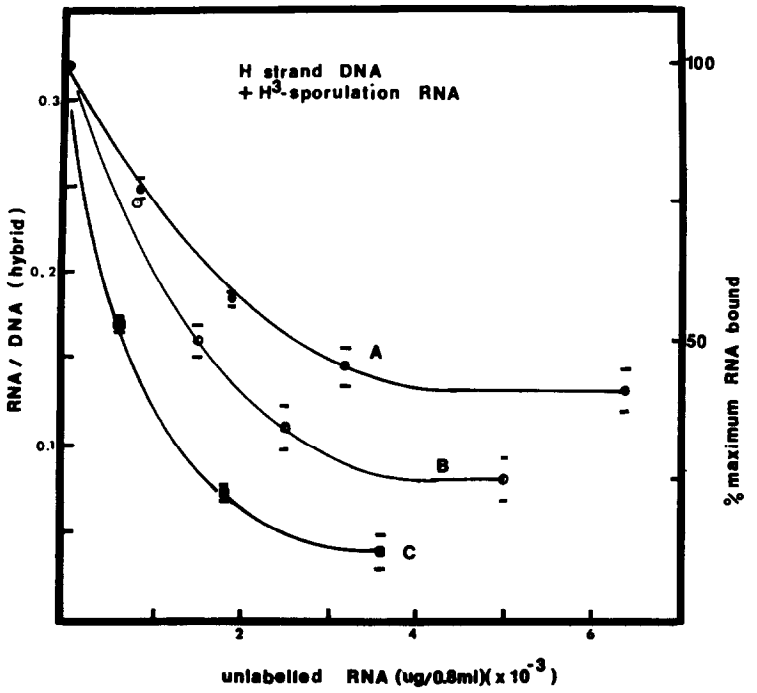

Figure 4. Hybridization Competition. Increasing concentrations of unlabelled RNA were tested for capacity to displace H^3-t_2-sporulation RNA (520 ug) from H strand DNA (0.45 ug) in a total volume of 0.8 ml. Curve A, broth vegetative RNA; Curve B, minimal vegetative RNA; Curve C, t_2-sporulation RNA. Labelled sporulation RNA had a specific radioactivity of 5890 cpm/ug.

RNA is more like sporulation RNA than is broth vegetative RNA.

Ribosomal and transfer RNA did not displace detectable amounts of labelled sporulation RNA from the H strand.

DISCUSSION

The patterns of annealing which we have observed indicate that a surprisingly large fraction (at least 10%) of the L strand is transcribed during sporulation. Of this 10%, about 4.4% is used specifically for sporulation and 5.6% for both sporulation and vegetative growth in broth. These percentages correspond to about 360 and 460 cistrons respectively, assuming a cistron to be 800 nucleotides in length. It is of interest that unlabelled RNA obtained from cells growing vegetatively in minimal medium displaced labelled sporulation RNA from the L strand to the same extent as RNA from broth-grown cells (cf. Figure 1) suggesting that broth vegetative RNA and minimal vegetative RNA contain identical fractions in common with sporulation RNA. This finding does not eliminate the possibility that broth vegetative RNA and minimal vegetative RNA contain unique species of RNA.

Transcription of the H strand during sporulation is characterized by an annealing pattern in which total sporulation RNA corresponds to 32% of the H strand, while broth vegetative RNA corresponds to 17.5%. These values in turn, represent approximately 2600 and 1400 cistrons resp. Broth vegetative RNA can displace only 60% of t_2-sporulation RNA from the H strand, a result which is consistent with the saturation data presented above. In contrast, sporulation RNA can displace all but about 5-8% of the vegetative RNA from the H strand, indicating that the process of sporulation consists primarily in a "turning on" of operons not active during vegetative growth. Very few of the operons functioning during vegetative growth are "turned off".

These findings contradict those of Yamazawa and Doi (1), whose results indicate that barely detectable changes occur in transcription of the H strand in the transition from vegetative growth to sporulation. These discrepancies probably stem,firstly, from the use by Yamazawa and Doi, of t_4- rather than t_2-sporulation RNA; secondly from the use by these workers

of pulse-labelled RNA, a practice which yields heterogeneous labelling of messenger RNA; thirdly, from the use of non-saturating amounts of labelled RNA in their competition experiments, a circumstance which could yield misleading results.

The results described above have accounted for 42% of the transcription of the B. subtilis genome (10% on the L strand, and 32% on the H strand). This figure is probably not smaller than the true value by 20%. We base this conclusion on the assumption that at least 30-40% of the genome is composed of regions (e.g. operators) which do not transcribe at all, and those which are non- or minimally functional under any given set of growth conditions.

The quantitative conclusions presented in this paper must be tempered by the realization that certain assumptions have been made concerning the techniques we have used. Firstly, we have assumed no biased losses to have occurred during the purification of RNA; secondly, we have assumed no biased DNA losses to have occurred during the preparation of resolved complementary strands; thirdly, we have assumed that t_2 sporulation RNA contains insignificant amounts of vegetative RNA which were synthesized during the vegetative growth period, and which persisted during sporulation. Ribosomal RNA, which is not synthesized during sporulation (11), has been shown not to displace detectable amounts of sporulation RNA, probably because it corresponds to a very small percentage of the transcribing region of the genome.

REFERENCES

1. Yamazawa, T., and Doi, R.H. 1971 J. Bacteriol. 106, 305.
2. Schaeffer, P., Millet, J., and Aubert, J.P. 1965 Proc. Natl. Acad. Sci. 54, 704.
3. Anagnostopoulos, C., and Spizizen, J. 1961 J. Bacteriol. 81, 741.
4. Mandelstam, J. 1969 Symp. Soc. Gen. Microbiol. 19, 377.
5. Dubnau, D., Davidoff-Abelson, R., and Smith, I. 1969 J. Molec. Biol. 45, 155.
6. Ralph, R.K., and Bellamy, A.R. 1964 Bioch. Bioph. Acta. 87, 9.
7. Strauss, N. 1970 Genetics 66, 583.
8. Nygaard, A.P., and Hall, B.D. 1964 J. Molec. Biol. 9, 125.
9. Gillespie, D., and Spiegelman, S. 1965 J. Molec. Biol. 12, 829.
10. McConaughy, C.D., Laird, C.D., and McCarthy, B.J. 1969 Biochemistry 8, 3289.
11. Hussey, C., and Losick, R. 1971 J. Molec. Biol. 57, 59.