USE OF λgt 11 AND ANTIBODY PROBES TO ISOLATE GENES ENCODING RNA POLYMERASE SUBUNITS FROM *BACILLUS SUBTILIS*

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A genetic analysis of the complex *Bacillus subtilis* transcriptional apparatus is essential to understand the function, regulation, and interaction of the transcriptase components during growth and sporulation. This approach in *Escherichia coli* has uncovered fundamental mechanisms regulating gene expression Cole and Nomura, 1986; Lindahl and Zengel, 1986) and an analysis of the *B. subtilis* transcriptase will allow comparison of the *E. coli* system to another bacterium that has evolved under different selective pressures. To this end we used antibody probes to isolate the alpha, beta, and beta' core subunit genes from a λgtll expression vector library. To address the question of function and regulation of the minor sigma factors that confer promoter specificity on the polymerase core (Losick et al., 1986), we used the same approach to isolate the gene for the 37,000 dalton sigma factor, sigma-37.

II. GENETIC ORGANIZATION OF THE ALPHA OPERON

We made λgt11 libraries using *B. subtilis* chromosomal DNA as described elsewhere (Suh et al., 1986). Using polyvalent antibody raised against purified alpha subunit as a probe, we isolated phages that carried all or part of the alpha gene, *rpoA* (Suh et al., 1986).

We have now used part of the cloned region as a hybridization probe to isolate from the λ gt11 library a 1.8 kb EcoRI fragment that contains additional upstream DNA (Figure 1). From the DNA sequence of a 3.9 kb fragment from the alpha region we identified open reading frames by homology of their products to *E. coli* proteins. Gene order in this region, given by gene product, is IF1-"X"-S13-S11-α-L17. Immediately following L17 is an open reading frame whose hypothetical product is homologous to *E. coli* HisP and MalK, which are the conserved, inner membrane components of periplasmic transport system (Ames, 1986).

There are striking similarities and differences between the alpha regions of *B. subtilis* and *E. coli*. Gene order for *E. coli* is SecY-"X"-S13-S11-S4-α-L17 (Lindahl and Zengel, 1986). "X" is the last gene in the spc operon; it encodes a 38 residue protein thought to function in protein export. A minor promoter may lie between X and S13, the first gene of the alpha operon (Lindahl and Zengel, 1986).

The *B. subtilis* alpha region differs in the presence of IF1 and in the absence of r-protein S4, which is the translational regulator of the *E. coli* alpha operon (Lindahl and Zengel, 1986). In place of S4, *B. subtilis* has a 180 bp intercistronic region that contains two possible promoter sequences (Suh et al., 1986). However, S1 nuclease mapping using *in vivo* message suggests that the bulk of alpha transcription originates upstream from the cloned region and either terminates or is processed at a site immediately following the gene for L17 (Figure 1).

Two additional lines of evidence support the view that the major promoter of the alpha operon lies upstream. First, an integrative plasmid carry-

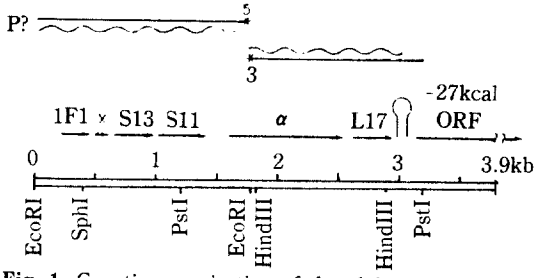

Fig. 1. Genetic organization of the alpha operon. The physical map is derived from the DNA sequence (Suh *et al.*, 1986, and Boylan *et al.*, unpublished); the coding regions above the map are indicated by their products. The results of S1 nuclease mapping are shown by the message protection (wavy lines) of end-labeled DNA probes (solid lines), and the stem-loop following L17 identifies a possible transcription termination sequence (-27 kcal; Zuker and Stiegler, 1981).

ing the 1.8 kb EcoRI fragment cannot transform competent cells to chloramphenicol resistance, suggesting that integration into this transcriptional unit is a lethal event. Second, we moved the 1.8 kb EcoRI fragment to the single-copy

Table 1. Three factor transductional cross to map *rpoA*

Selection	Recipient Class[1] Cm	Linc	Tsc	No	Order Implied
Cm^r	1	1	1	65	*rpo-A-gerD-tscA*
	1	1	0	25	
	1	0	1	1	
	1	0	0	9	
Linc^r	1	1	1	64	*rpoA-gerD-tscA*
	1	1	0	5	
	0	1	1	13	
	0	1	0	15	
TscA$^+$	1	1	1	48	*rpoA-gerD-tscA*
	1	0	1	0	
	0	1	1	13	
	0	0	1	37	

1. 1 and 0 refer to the donor (PB96 *rpoA*: :*cat gerD*: :Tn917 trpC2; strain 5215 from R.L. Sammons with *rpoA*: :*cat* marker transducd) and recipient (PB97 *thyA thyb tscA1*; strain PB321 from A. Galizzi) phenotypes, repectively.

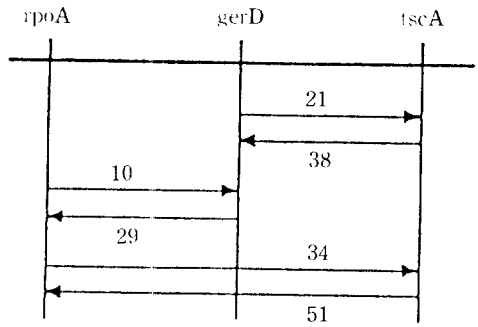

Fig. 2. Genetic map of the alpha region derived from the threefactor transductional cross in Table 1. Genetic distance is given as 100-% cotransduction, with the tail of the arrow indicating the selected marker.

transcriptional fusion vector pDH32 (Dennis Henner, personal communication) and found only low β-galactosidase activity (11-12 Miller units). These results do not preclude the presence of a minor promoter active under special growth conditions but do suggest that the genes in the IF1-L17 interval are largely transcribed from an upstream promoter.

We did additional transductional mapping to determine whether any previously isolated mutations lay within the cloned alpha region (Table 1). As shown in Figure 2, none of the loci clockwise from *rpoA* were closely linked, but the map does orient that alpha region with respect to the recently characterized rRNA operon clones that contain the *gerD* locus (see Bott et al. this volume).

III. ISOLATION OF THE BETA AND BETA' REGION

We used antibody raised against sigma-37 holoenzyme to simultaneously screen for the sigma-37 gene and the beta-beta' region (Duncan et al. 1986). Because the initial screening was done with antiholoenzyme antibody, positive clones might encode any of the four subunits — alpha, beta, beta,' or sigma-37. We used epitope selection (Snyder and Davis, 1985) to quickly identify the gene carried by each of the phages. Two phages encoded part of sigma-37 (Duncan et at., 1987). Eight phages that potentially carried the

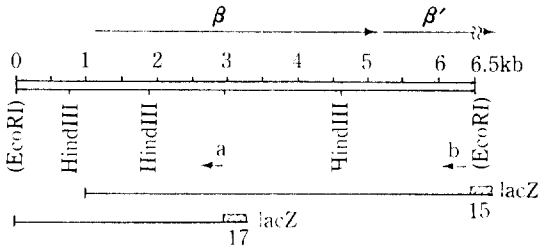

Fig. 3. Physical map of the beta-beta' region, derived from restriction analysis of the λgt11 phage carrying inserts shown beneath the map. Beta and beta' coding regions are estimated from the sizes of the products, aligned by the regions of product homology found by DNA sequence analysis of fragments a and b.

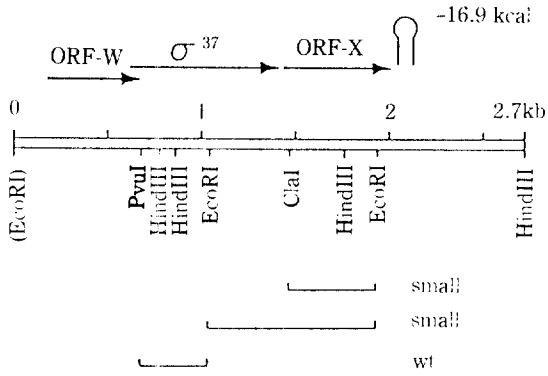

Fig. 4. Genetic organization of the sigma-37 region. The physical map is derived from the DNA sequence (Duncan *et al.*, 1987 and Price *et al.*, unpublished); the coding region for sigma-37 and the flanking open reading frames W (orf-W) and X (orf-X) are indicated above the map. The stem-loop structure identifies a possible transcription termination sequence (−16.9 kcal). The lines beneath the map show the fragments carried by integrative plasmids in the gene disruption experiments (Duncan *et al.*, 1987) with the colony phenotype of each disruption indicated to the right.

beta or beta' gene were characterized by restriction mapping and DNA sequencing and found to carry overlapping inserts that comprise the entire beta coding sequence and part of the bata' gene (Duncan et al., unpublished). A physical map of the beta-beta' region and the inserts carried by two representative phages is shown in Figure 3. Further characterization of these genes is in progress.

IV. GENETIC ORGANIZATION OF THE SIGMA-37 REGION

We are also interested in the *in vivo* function and regulation of the minor sigma factors that associate with the core enzyme to confer different promoter specificities *in vitro*. Our genetic analysis of sigma-37 (Duncan et al., 1987) and the parallel analysis by Losick and his colleagues (Binnie et al., 1986) begin to address these questions.

The DNA sequence of the sigma-37 region suggests that the sigma-37 gene may lie in an operon. Two open reading frames flank the sigma-37 gene: orf-W, which could encode a 18 kdal protein and orf-X, which could encode a 22 kdal protein whose expression may be translationally coupled to sigma-37 expression (Duncan et a., 1987; Figure 4). A structure similar to an *E. Coli* rho-independent terminator closely follows the orf-X termination codon. We have no evidence that orf-W codes for a functional product. However, gene disruptions indicate that orf-X may be cotranscribed with the sigma-37 gene and also suggest that orf-X encodes a product that modulates sigma-37 expression or activity *in vivo* (Duncan et al., 1987).

Additonal analysis *in vivo* and *in vitro* is necessary to test the hypothesis that the orf-X product serves to regulate sigma-37. However, a pattern is emerging that minor sigma factors in *B. subtilis*-and perhaps in *E. coli* as well-lie in operons. Elucidating the functions of these sigmalinked genes may provide the link between cell metabolism and minor sigma control of gene expression.

The lack of an obvious phenotype for a sigma-37 gene disruption was unexpected. To avoid affecting expression of any genes downstream in the same operon, we made an in-frame deletion mutation *in vitro* by removing the sigma-37 fragment between the HincII sites at nt 732 and 1185 (see Figure 3 of Duncan et al., 1987). This deletion was moved to the chromosome by two-step allele replacement procedure (Kalman and Price, unpublished). We found no sporulation-associated functions impaired in the null mutation,

including sporulation frequency, sporulation timing, spore heat resistance, spore germination, production of extracellular serine protease, and transformation competence. Neither was there any marked affect on the vegetative functions tested, including growth in minamal glucose medium, growth at temperatures between 25 and 47°C, carbon catabolite control of histidase expression, chemotactic ability, and motility.

Thus the physiclogical role of sigma-37 remains a mystery. Sigma-37 may function under stress or starvation conditions not yet tested. Alternatively, the function of sigma-37 may overlap the function of another minor sigma factor and only become obvious with the appropriate double mutant. We are now exploring both these possibilities.

V. SUMMARY

We isolated the genes encoding the alpha, beta, beta,' and sigma-37 subunits of *B. subtilis* RNA polymerase by antibody screening of λ gt11 libraries. Characterization of the bata and beta' clones is only beginning, but gene organization of the alpha region is striking in its similarities and differences with the *E. coli* alpha operon. The absence of the gene for r-protein S4, the translational regulator of the *E. coli* operon, is particularly interesting. Does *B. subtilis* lack the translational feedback control that coordinates r-protein gene expression in *E. coli,* or does the *B. subtilis* alpha operon differ only in detail from the *E. coli* theme?

We have used our sigma-37 clone to probe the function and regulation of the sigma-37 gene. Thus far null sigma-37 mutations have revealed no obvious role for sigma-37, but it is clearly not essential for growth or sporulation under standard conditions. We suggest that sigma-37 may lie in an operon with a downstream gene, orf-X, that regulates sigma expression or activity. Regulation of minor sigma function by a linked gene may emerge as a common feature in both the *B. subtilis* and *E. coli* systems.

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