duct was isolated in 84% overall yield with greater than 90% incorporation of fluorophore. Addition of the ligand *N*-DNP-glycine to the fluorescein-Fab adduct resulted in a decrease in fluorescence, providing a direct assay of ligand binding (Fig. 4). The association constant of *N*-DNP-glycine to the fluorescein-Fab adduct was determined to be $(3.0 \pm 0.5) \times 10^5 M^{-1}$, almost identical to the $K_A$ of *N*-DNP-glycine to underivatized MOPC 315, $2.0 \times 10^5 M^{-1}$ (*5*). Addition of DNP-glycine to a solution of one equivalent free *N*-fluoresceinthioureido-2-mercaptoethylamine and Fab resulted in no detectable fluorescence change.

These experiments show that it is possible to develop selective catalysts that combine the high binding specificity of the immune system with the diverse and efficient catalytic groups available from synthetic chemistry (*19*). In addition, the chemical strategy described here may find application to the selective modification of other proteins for which the three-dimensional structures are unavailable.


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# Multiple Principal Sigma Factor Homologs in Eubacteria: Identification of the "*rpoD* Box"


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**Genes for the principal sigma factor (*rpoD* genes) of various eubacteria were identified with a synthetic oligonucleotide probe corresponding to a conserved sequence in *rpoD* gene products of *Escherichia coli* and *Bacillus subtilis*. Multiple *rpoD* homologs were found in the strains of *Micrococcus*, *Pseudomonas*, and *Streptomyces*, whereas single genes were detected in *E. coli*, *B. subtilis*, and *Staphylococcus aureus*. The four *rpoD* homologs of *Streptomyces coelicolor* A3(2) were cloned and sequenced. A homologous portion with 13 amino acids was found in the *rpoD* genes of *S. coelicolor* A3(2), *E. coli*, and *B. subtilis* and was named the "*rpoD* box."**


THE RNA POLYMERASE HOLOENzymes of *Escherichia coli* and *Bacillus subtilis* contain the principal sigma factors (*rpoD* gene products) that play a central role in the initiation of transcription (*1, 2*). Several other sigma factors, categorized as minor sigma factors, substitute for the *rpoD* gene products under special cir-


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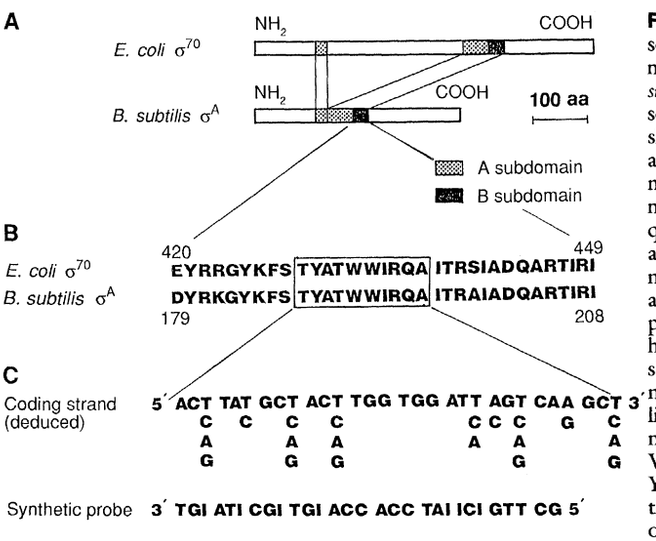



**Fig. 1.** (**A**) Schematic representation of the principal sigma factors of *E. coli* and *B. subtilis*. The two highly conserved regions are shown by shaded boxes (A subdomain) and filled boxes (B subdomain) (*1, 2, 6*). (**B**) Alignment of the B subdomain sequences. The one-letter amino acid notation is used. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. The boxed portion was used to design the oligonucleotide probe. (**C**) The design of the oligonucleotide containing inosines at degenerated codon positions, including the nucleotide sequence of the coding strand deduced from the amino acid sequence boxed in (B) and the oligonucleotide synthesized.

cumstances; for example, the *rpoH* gene product is expressed in *E. coli* at high temperature (*3*), the *spoIIG* gene product is expressed during sporulation in *B. subtilis* (*4*), and the gene 55 product of bacteriophage T4 is expressed during the late period of phage development (*5*).

The comparative study of sigma factors predicts two functional domains, one for core RNA polymerase binding and the other for DNA binding (*6, 7*). The core-binding domain is further divided into A and B subdomains (Fig. 1A). The A subdomain, which is conserved in various sigma factors, appears to be directly involved in core binding. The B subdomain, which is conserved in the principal sigma factors of *E. coli* and *B. subtilis* but not in the minor sigma factors, may modulate core binding by the A subdomain. We synthesized a 29-base oligonucleotide designed from the B subdomain sequence to identify *rpoD* genes from different bacteria (Fig. 1, B and C) (*8–10*).

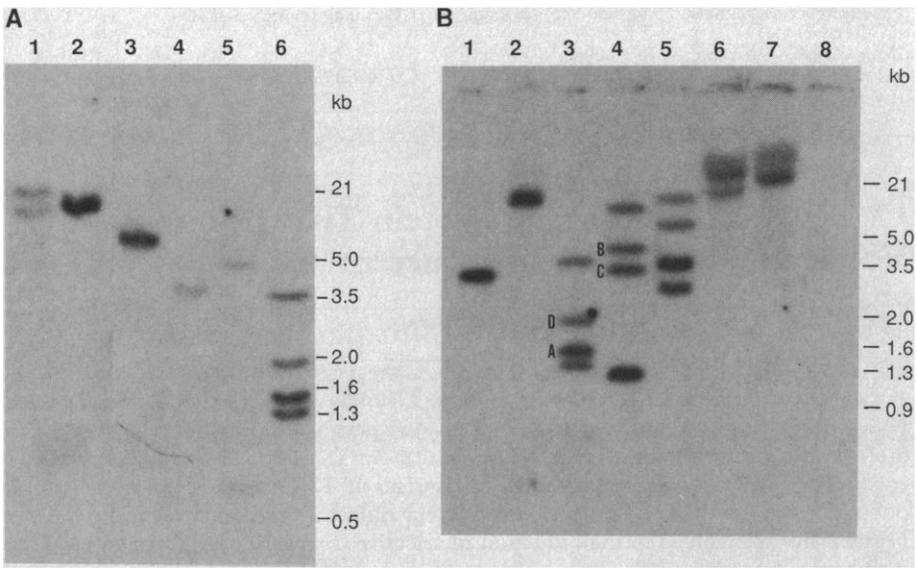
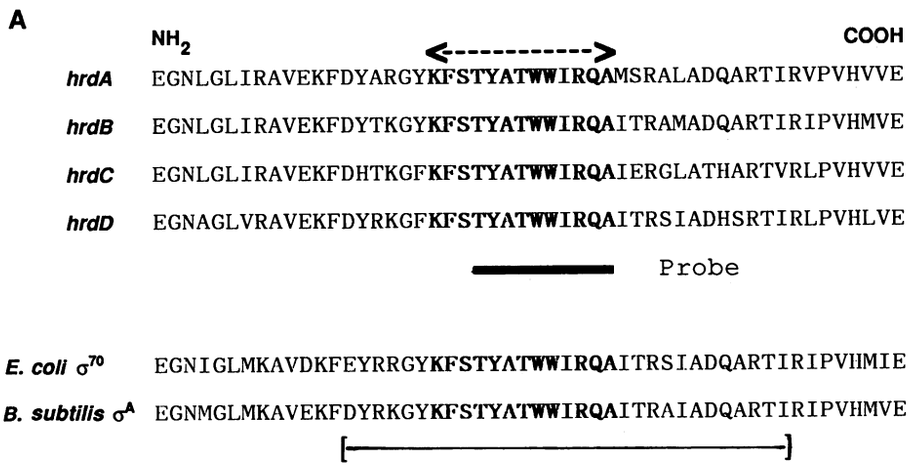



**Fig. 2.** Genomic analysis with the synthetic probe. (**A**) Detection of the *rpoD* signals in bacterial DNAs. Lane 1, *P. aeruginosa* IAM1514 DNA cleaved with Hind III; lane 2, *E. coli* K12 DNA cleaved with Hind III; lane 3, *S. aureus* IAM12544 DNA cleaved with Eco RI; lane 4, *B. subtilis* Marburg 168 DNA cleaved with Eco RI; lane 5, *M. luteus* IAM1056 DNA cleaved with Sma I; and lane 6, *S. coelicolor* A3(2) DNA cleaved with Sma I. (**B**) Identification of the *rpoD* signals in *S. coelicolor* DNA. Lanes 1 and 2, *E. coli* DNA cleaved with Pvu II and Hind III (control); lanes 3 to 8, *S. coelicolor* DNA cleaved with Sma I, Sal I, Pvu II, Bgl II, Pst I, and Bam HI, respectively.



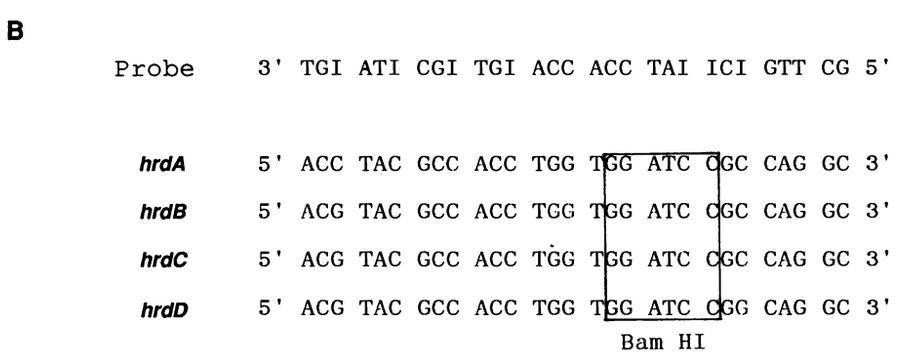




**Fig. 3.** (**A**) Alignment of the six *rpoD* gene products showing the B subdomains and adjacent regions. The four *rpoD* (*hrd*) gene products of *S. coelicolor* and the two *rpoD* gene products of *E. coli* and *B. subtilis* are shown. The B subdomains are shown with a bracket. The *rpoD* box is indicated with a dotted line. The bar shows the amino acid sequence used for the design of the probe. (**B**) The nucleotide sequences of the four *rpoD* genes (*hrd*) corresponding to the probe. The box shows the Bam HI sites.

Fig. 1, B and C) (*8–10*).

DNA hybridization analysis of various bacteria was performed with the synthetic probe (Fig. 2) (*11, 12*). Single signals were detected at the positions expected from the reported *rpoD* sequences of *E. coli* and *B. subtilis*, indicating that the probe can be used to identify the *rpoD* genes of bacteria. A single signal was also detected in *Staphylococcus aureus* DNA. Multiple signals were found in *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Streptomyces coelicolor* DNAs, which gave two, two, and four distinct signals, respectively.

To find out whether the four signals of *S. coelicolor* A3(2) DNA are due to *rpoD* genes, we cloned and sequenced the DNA regions corresponding to the signals (Fig. 2B) (*13*). A common Bam HI site was found in the regions where the probe hybridizes (Fig. 3B) and this may be the reason why the Bam HI–cleaved DNA of *S. coelicolor* did not give any signals (Fig. 2B, lane 8). The amino acid sequences deduced from the nucleotide sequences around the probe (*14–16*) were compared with the corresponding regions of the *rpoD* gene products of *E. coli* ($\sigma^{70}$) and *B. subtilis* ($\sigma^{A}$, also known as $\sigma^{43}$) (Fig. 3A). A 13–amino acid segment was identical in the four regions from *S. coelicolor* and the *rpoD* genes of *E. coli* and *B. subtilis*. Further sequence analysis of the four DNA regions indicated that they contain nearly identical open reading frames throughout the entire regions (*16*). Thus, we designated the four open reading frames (in A, B, C, and D; see Fig. 2B, lanes 3 and 4) as *hrdA*, *hrdB*, *hrdC*, and *hrdD* (the homologs of the *rpoD* gene), respectively. We propose to call the common portion of 13 amino acids the "*rpoD*

box," which presumably bears an intrinsic function in principal sigma factors.

All of the 13 *Streptomyces* strains examined had multiple *rpoD* signals (*16*). Multiple *rpoD* genes may be common in divergent eubacterial strains. The previously identified protein of *S. coelicolor*, which has a functional homology with the principal sigma factor of *B. subtilis* (*17*), may be one of the *rpoD* gene products reported here. More recently, three different RNA polymerase holoenzymes were identified in *S. coelicolor* A3(2) by using the agarase gene (*dagA*) (*18*). Conceivably the biochemical and morphological complexity of differentiating *Streptomyces* cells are guaranteed by the temporal and topological expression of genes through the heterogeneity of the principal sigma factors.

The characterization of *rpoD* homologs from bacteria gives us insights into the gene control mechanisms as well as the transcriptional machinery of divergent bacteria. The study of *rpoD* gene constituents in prokaryotic cells will also give the molecular base for phylogenetic relations.

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# Cloning of a Membrane Protein That Induces a Slow Voltage-Gated Potassium Current


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**A rat kidney messenger RNA that induces a slowly activating, voltage-dependent potassium current on its expression in *Xenopus* oocytes was identified by combining molecular cloning with an electrophysiological assay. The cloned complementary DNA encodes a novel membrane protein that consists of 130 amino acids with a single putative transmembrane domain. This protein differs from the known ion channel proteins but is involved in the induction of selective permeation of potassium ions by membrane depolarization.**


ION CHANNELS EXHIBIT A HIGH DEgree of diversity, varying in their electrophysiological and pharmacological properties (*1*). However, the molecular basis for the different properties of the ion channels remained to be clarified. We previously developed a new strategy to characterize receptors, ion channels, and modulatory proteins by combining molecular cloning in an RNA expression vector with an electrophysiological assay in *Xenopus* oocytes (*2*). Because the kidney is involved in controlling various types of ion permeations to maintain the electrolyte homeostasis (*3*), we began this investigation by characterizing ion currents elicited by membrane polarization after injection of rat kidney polyadenylated [poly(A)$^+$] RNA into *Xenopus* oocytes (Fig. 1A). In the control oocytes injected with distilled water, an outward current appeared as the oocyte membrane potential was stepped from −80 mV to more positive levels. This current probably results from activation of a voltage-dependent $K^+$ channel as reported (*4*). In the oocytes injected with the kidney mRNA, depolarization induced a more markedly activating outward current, and this current was five to ten times as large as that of the control oocytes. To characterize the mRNA responsible for the induction of the outward current, we size-fractionated the kidney mRNA by centrifugation on sucrose gradient. The activity of the mRNA was found in a fraction with an average mRNA size of ~700 nucleotides, which was considerably smaller than those of other ion channel mRNAs (*5*). We therefore initiated the isolation of a functional cDNA clone from a kidney cDNA bank (*6*) and finally obtained a single cDNA clone that was capable of eliciting a markedly elevating outward current by membrane depolarization.

The currents induced by the mRNA synthesized in vitro from the cloned cDNA were characterized in more detail (Fig. 1B). Depolarization from a holding potential of −80 mV elicited large voltage- and time-dependent outward currents. On repolarization to −80 mV, slow outward tail currents were observed. The outward current lasted for at least 20 min, suggesting that the channel responsible for the outward current does not undergo inactivation. Currents with similar amplitude and properties could not be seen in water-injected oocytes or oocytes injected with tRNA.

A series of experiments were conducted to determine ionic mechanisms underlying the outward current. In the external medium containing 2 m*M* $K^+$, reversal of the tail currents occurred at −101 mV (mean ± 2, $n = 4$) (Fig. 1C), similar to the estimated reversal potential of −103 mV for $K^+$ in *Xenopus* oocytes (*7*). At the 20 m*M* $K^+$ concentration, the tail currents reversed at −42 mV (mean ± 3, $n = 3$), in agreement


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