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The *trp* Promoter of *Escherichia coli* and its Exploitation in the Design of Efficient Protein Production Systems

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Introduction

The *trp* promoter was among the first group of prokaryotic punctuation elements to be exploited for the production of foreign proteins in *Escherichia coli*. The reasons for the early choice of the *trp* promoter had more to do with its widespread availability in cloned form than with a detailed understanding of all the factors that might affect its utilization. During the past decade, our knowledge of the control and modulation of the *trp* promoter have expanded greatly, fully justifying the insight of the early workers who first put it to use in biotechnological applications. As a regulatable promoter of high signal strength, the *trp* promoter has gained widespread adoption in the design of production systems. The aims of this review are twofold: first, I hope to bring together much of the early information that led to the choice of the *trp* promoter in production contexts; second, I have attempted to describe and summarize the vectors, expression systems and successful cases that were built around the *trp* promoter and so provide a ready reference source for individuals wishing to develop new *trp* promoter-driven systems.

No protein production system is ever perfect. Those based on the *trp* promoter are included in this generalization. Continued effort will be needed to obtain the desired degree of control over transcription at all phases of the growth cycle. Plasmid instability has plagued the development of *trp* promoter-based systems since the earliest days of this field. Different *E. coli* strains often show significant variation in the utilization of the *trp* promoter, for reasons that remain obscure. A knowledge of the *trp* promoter literature, reviewed here, should be a useful guide to future workers seeking to modify and improve similar production systems or address fundamental issues in the field of promoter structure–function relationships.

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The *trp* promoter–operator region of *Escherichia coli* and related enteric bacteria

If RNA polymerase (EC 2.7.7.6) is to execute the steps that result in formation of an RNA duplicate of one of the two template strands, precise arrays of functional groups contributed by purine–pyrimidine base pairs must be exposed in the major and minor grooves of duplex DNA. Such punctuation elements, known as promoters, are in essence compactly organized informational cassettes. Their properties include not only the ability to interact with RNA polymerase in a strand-selective fashion but also the ability to govern transcription initiation frequency directly. Many promoters, including the *trp* promoter, contain target sites for regulatory proteins that positively or negatively affect transcription.

Our knowledge of structure–function relationships within the *trp* promoter began with the efforts during the early 1970s of Yanofsky and co-workers at Stanford University. Before the advent of DNA sequencing, which came into general use in 1978, the structural analysis of nucleic acids was accomplished by means of the laborious characterization of overlapping sets of ^{32}P-labelled oligoribonucleotides that had been separated by two-dimensional chromatography and electrophoresis. In most cases, the RNA molecules to be structurally analysed were first isolated by sequential hybridization to DNA molecules that either had an intact *trp* promoter or a deletion that removed part of the promoter-operator-leader region. A *tour de force* for this era of nucleic acid sequence analysis was the determination by Squires *et al.* (1976) of the 5′-terminal sequence of *trp* operon messenger RNA and the demonstration that a leader sequence of 162 nucleotides lay between the startpoint of transcription and the first codon of the *trpE* gene. The assignment of points of overlap between different oligoribonucleotides was made possible by the availability of deletion mutants having 5′ endpoints at various positions within the *trp* operon leader region (Bertrand, Squires and Yanofsky, 1976). The same family of deletions later proved to be valuable sources of DNA fragments harbouring the *trp* promoter but lacking the *trp* attenuator (Miozzari and Yanofsky, 1978a; Russell and Bennett, 1982).

The DNA segment that comprises the RNA polymerase recognition elements of the *trp* promoter is normally never transcribed, so RNA sequencing procedures for determining the structures of upstream regions of promoters would ordinarily not be practical. However, there exist a series of φ80 *trp* and λ-φ80 *trp* specialized transducing phages of the deletion-substitution variety where the *trp* operon had replaced a segment of the viral N operon. In such phages, transcription from the upstream viral P_L promoter generated RNA molecules having *trp* promoter sequences (Zalkin, Yanofsky and Squires, 1974). The structural analysis of such read-through transcripts enabled Bennett *et al.* (1976) to deduce a partial sequence (from −1 to −33) for the *trp* promoter and to locate the *trp* operator (−3 through −20). The advent of chemical sequencing procedures (Maxam and Gilbert, 1977) enabled Bennett *et al.* (1978) to extend the sequenced region of the *trp* promoter upstream to co-ordinate −116. More recently, G. Bogosian (personal communication) has employed dideoxy sequencing techniques (Sanger,

Nicklen and Coulson, 1977) to extend the known sequence upstream of the *trp* promoter to co-ordinate −251. The upstream DNA segment between co-ordinates −175 and −250 has recognition sequences for at least 15 different restriction endonucleases (EC 3.1.21.4). A partial restriction map of the *trp* promoter region of *E. coli* is presented in *Figure 1*, and the DNA sequence of the *trp* promoter-operator is shown in *Figure 2*.

The nucleotide sequences of the *trp* promoters from the related enteric bacteria *Salmonella typhimurium* (Bennett, Brown and Yanofsky, 1978), *Shigella dysenteriae* (Miozzari and Yanofsky, 1978c), *Serratia marcescens* (Miozzari and Yanofsky, 1978b), *Klebsiella aerogenes* (Blumenberg and Yanofsky, 1982a) and *Citrobacter freundii* (Blumenberg and Yanofsky, 1982b; Kuroda and Yanofsky, 1984) have also been determined. In general, there is a high degree of sequence similarity between the enteric *trp* promoter-operator regions. For example, when the *Salmonella* and *E. coli trp* promoters are compared, there are only 10 differences from −1 through −75; five of these differences are clustered in the −25 to −30 region, where conservation of sequence between promoters is rare (Hawley and McClure, 1983). When *Serratia* and *E. coli* are compared, there are 35 differences between co-ordinates −1 and −76. Most of these lie upstream of the −35 region in an area that is not considered important to promoter function (Miozzari and Yanofsky, 1978b).

The boundaries of the *trp* promoter in *E. coli* and *Salmonella* were defined in a series of *in vitro* studies that explored the ability of RNA polymerase holoenzyme to protect *trp* operator DNA from cleavage by pancreatic DNase or restriction endonucleases having target sites near the startpoint of transcription (Bennett, Brown and Yanofsky, 1978; Brown *et al.*, 1978). In addition, DNA segments of varying length generated by cleavage with restriction endonucleases were tested for their ability to function as templates *in vitro* (Brown *et al.*, 1978) and *in vivo* (Oppenheim and Yanofsky, 1980). These studies showed that the *trp* promoter encompassed a DNA segment extending 59 base pairs upstream from the transcriptional startpoint. Although bound RNA polymerase was able to protect blocks of DNA extending to co-ordinate +20 from attack by pancreatic DNase (EC 3.1.21.1), these downstream sequences are non-essential for promoter function (Bennett *et al.*, 1976; Bennett and Yanofsky, 1978). A more detailed examination of the mode of interaction of *trp* promoter DNA with RNA polymerase was carried out by Oppenheim, Bennett and Yanofsky (1980). These workers used a technique pioneered by Johnsrud (1978) that exploits the reactivity toward dimethylsulphate of guanine and adenine residues protruding into the major and minor grooves, respectively, of duplex DNA. When proteins such as RNA polymerase bind to DNA, alkylation reactions may be abolished or enhanced, depending upon the geometry of the complex. For the *trp* promoter, bound RNA polymerase caused decreases in guanine methylation at four points and increases in methylation at three points (*Figure 2*). The model that emerged is one that supposes an asymmetric mode of interaction between polymerase and promoter, mainly involving purines that protrude into the major groove within the antisense strand of *trp* promoter DNA. Close contacts between the *trp*

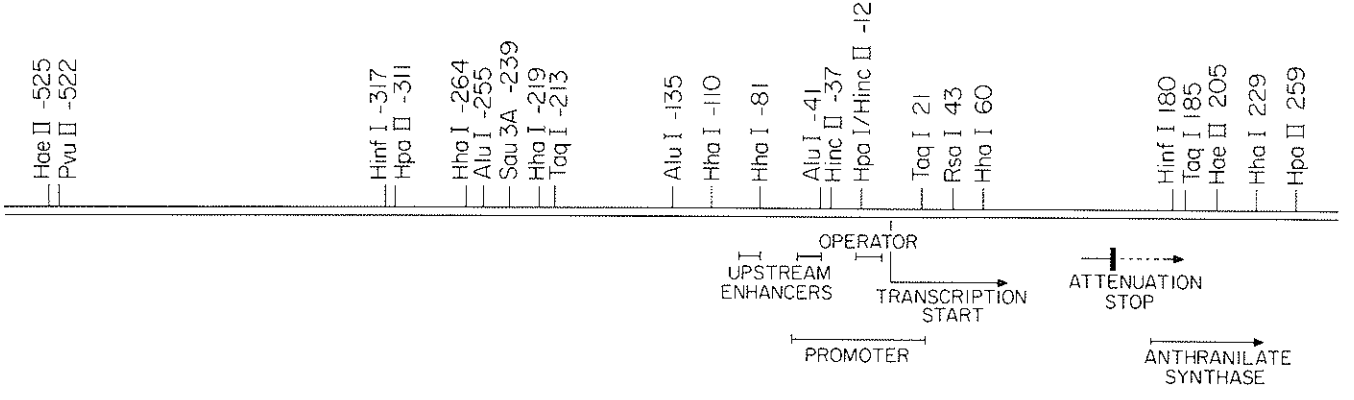

Figure 1. Selected restriction endonuclease cleavage sites in or near the *trp* promoter–operator-attenuation region of *E. coli* K-12. The first nucleotide of *trp* messenger RNA is designated as co-ordinate +1. Taken from the work of Bennett *et al.* (1976, 1978) and G. Bogosian (personal communication). The cleavage sites shown are those that have been most widely used in cloning work or in the functional analysis of the *trp* punctuation elements.

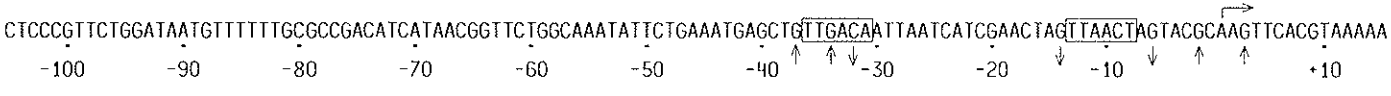

Figure 2. Nucleotide sequence of the *trp* promoter-operator region. The co-ordinate system is identical to that of *Figure 1*. The −35 and −10 recognition elements are enclosed. When RNA polymerase is bound, certain guanine residues tend to be protected against methylation by dimethyl sulphate (↓); other residues show enhanced methylation rates (↑). (Data taken from Bennett *et al.*, 1976; Bennett, Brown and Yanofsky, 1978; and Oppenheim, Bennett and Yanofsky, 1980). Only the *trp* mRNA-equivalent strand is shown. The recognition sequence for Trp repressor is a hyphenated palindrome lying between co-ordinates −20 and −3.

promoter and RNA polymerase evidently occur in the −35 and −10 regions.

Nested within the *trp* promoter is the *trp* operator. This structural element is the major target sequence for Trp holorepressor. The boundaries of *trp0* were defined by experiments that monitored the protection of certain nucleotide residues from chemical or enzymatic attack (Bennett *et al.*, 1976; Bennett and Yanofsky, 1978; Oppenheim, Bennett and Yanofsky, 1980; Kumamoto, Miller and Gunsalus, 1987). These studies were supported by the structural analysis of deletion mutants and point mutants that abolished repressor affinity (Bennett and Yanofsky, 1978; Bass *et al.*, 1987). Taken together, these studies identify the *trp* operator as an 18 bp palindromic sequence that is centred about a recognition sequence for restriction endonuclease *HpaI*, lying between co-ordinate −20 and −3 (*Figure 2*). That the *trp* operator is situated within the *trp* promoter had been foreshadowed by the transcription experiments of Squires, Lee and Yanofsky (1975), who showed that the binding of RNA polymerase and Trp repressor to the *trp* promoter were mutually exclusive. The equilibrium dissociation constant for Trp holorepressor–*trp* operator interaction is 6·7 nM, as measured by protein distribution analysis (Haydock and Somerville, 1984). By filter binding the dissociation complex was found to be 2·6 nM (Klig, Crawford and Yanofsky, 1987). The detailed mode of interaction of Trp holorepressor with its operator target remains to be established. Current models favour the notion that an appropriate constellation of solvent-exposed amino acid side chain residues situated within the E helix of Trp repressor bind to the edges of symmetrically disposed base pairs within the major groove of the *trp* operator, separated by one full turn (10 bp) of duplex DNA (Schevitz *et al.*, 1985; *Figure 3*).

Recently elucidated additional determinants of *trp* promoter strength appear to be two A+T-rich oligonucleotide blocks found upstream of the −35 region, around co-ordinates −50 and −90 (Nishi and Itoh, 1986). When the A+T-rich block at −90 was deleted, the strength of the *trp* promoter was reduced to *c.* one-third of the standard level; when the −90 and −50 A+T-rich blocks were both deleted, the promoter strength was *c.* one-sixth of the standard level. The importance of A+T-rich segments upstream of −35 was supported by the behaviour of two other constructs where the normal −50 and −90 regions were replaced by A+T-rich blocks from the promoter-operator region of phage λ. These so-called *let* promoters were *c.* threefold stronger than the standard *trp* promoter while remaining subject to control by the Trp repressor (Nishi and Itoh, 1986). That powerful *E. coli* promoters tend to have A+T-rich blocks in the region upstream of the −35 area had been noted several years earlier (Nakamura and Inouye, 1979; de Boer, Gilbert and Nomura, 1979).

In a slightly different approach to determining the role of flanking sequences, Russell *et al.* (1984) constructed several *trp* promoter vectors having unique restriction endonuclease cleavage sites either downstream of the normal transcription startpoint or upstream of co-ordinate −39. These vectors were based upon the pKO-1 galactokinase (EC 2.7.1.6) reporter system of McKenney *et al.* (1981). A number of synthetic oligonucleotides were positioned at varying distances from the *trp* promoter. Galactokinase assay values, corrected for plasmid copy number effects, showed that insertions

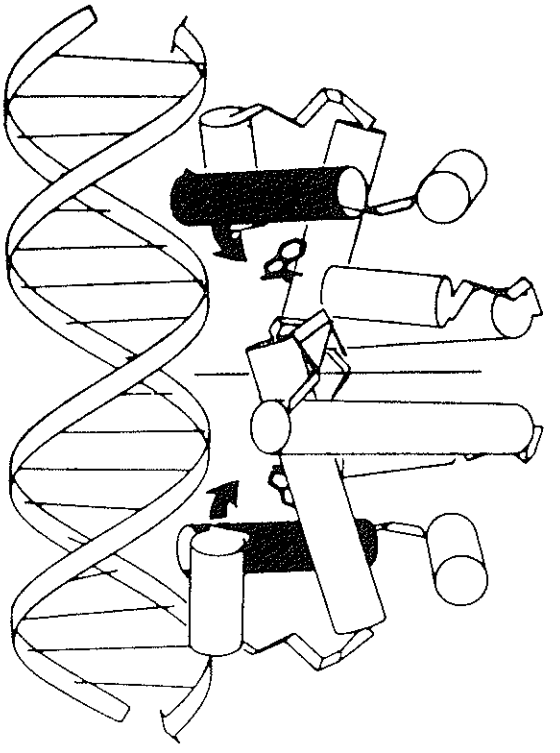

Figure 3. Schematic representation of the interaction of dimeric Trp holorepressor with an operator target in duplex DNA. The recognition helices (black cylinders) contain arrays of amino acid side chains able to bond non-covalently to the edges of purine–pyrimidine base pairs exposed in the major groove. In the absence of ligand, the recognition helices become repositioned (arrows) in locations that disfavour engagement with DNA (Reprinted with permission of the Editors of *Nature* and Dr Paul Sigler; *see* Schevitz *et al.*, 1985).

within the upstream segment had little or no effect on *trp* promoter strength. Insertions at co-ordinate +2 were in some instances stimulatory in terms of the levels of downstream enzyme activity, with short (9–10 bp) GC stretches showing the greatest increases.

The modest effects seen by Russell *et al.* (1984) in no way contradict the findings of Nishi and Itoh (1986) as each group worked with rather different test systems. The possibility that upstream 'enhancer' sequences may improve *trp* promoter strength clearly deserves to be studied in more detail, with emphasis on the nucleotide sequence and location of *cis*-acting segments and the identification of any proteins that might serve to mediate increased promoter function.

Control by Trp repressor of transcription initiation at the *trp* promoter

In quantitative terms, the single most important factor determining *trp* promoter utilization is the availability of functional Trp repressor. Repression lowers the rate of transcription initiation at the *trp* promoter by a factor of 60–80 (Jackson and Yanofsky, 1972; Bogosian and Somerville, 1984). Provided that sufficient amounts of L-tryptophan are present, the liganded complex (holorepressor) becomes firmly but non-covalently bound to an operator target located within the *trp* promoter, thereby excluding RNA polymerase (Squires, Lee and Yanofsky, 1975). Understanding the structure–function relationships and physiology of Trp repressor–operator interaction is thus central to the exploitation of the *trp* promoter in biotechnology.

The structural gene for Trp repressor, *trpR*, was identified and localized within the chromosome of *E. coli* about 30 years ago (Cohen and Jacob, 1959). The phenotypes of *trpR* mutants are resistant to a number of non-metabolizable tryptophan analogues that inhibit the growth of wild-type *E. coli* by acting as false co-repressors (reviewed by Somerville, 1983). The most commonly used tryptophan analogue is 5-methyltryptophan. That Trp repressor is a protein was proved by the characterization of *trpR* amber mutants (Morse and Yanofsky, 1969). Although this might seem to be a trivial matter from the modern perspective, it should be borne in mind that small RNA molecules can function in controlling transcription, as for example in ColE1 replication (Polisky, 1986). The *trpR* gene of *E. coli* was cloned by selecting specialized transducing derivatives of bacteriophage λ carrying the tightly linked *serB* gene (Roeder and Somerville, 1979). The indirect approach was necessary because there are no practical methods for positively selecting *trpR*$^+$ (5-methyltryptophan-sensitive) cells within a large population of *trpR* (5-methyltryptophan-resistant) cells. The primary structure of the *trpR* gene and its control region were determined by DNA sequencing (Gunsalus and Yanofsky, 1980; Singleton *et al.*, 1980).

Trp repressor protein is an α_2 homodimer. Each subunit contains 108 amino acids. The protein has been purified to homogeneity and crystallized (Joachimiak *et al.*, 1983a; Tsapakos *et al.*, 1985). The development of production strains able to yield extracts with elevated levels of Trp repressor was complicated by the fact that the *trpR* promoter is autogenously regulated by its own gene product (Bogosian, Bertrand and Somerville, 1981; Bogosian *et al.*, 1984). Moreover, the translation of *trpR* messenger RNA is relatively inefficient (Kelley and Yanofsky, 1982). Even when the *trpR* gene is present within a multicopy plasmid, autogenous control maintains the level of Trp repressor at a level of approximately 300 dimers per cell (Kelley and Yanofsky, 1982; Gunsalus, Miguel and Gunsalus, 1986). This level of Trp repressor is sufficient to optimize expression from the *trp* promoter under a variety of growth conditions (Bogosian and Somerville, 1984). High-level production of Trp repressor has necessitated the development of schemes for subverting the cellular control mechanisms for this protein by the substitution of strong unregulated promoters and/or the introduction of efficient ribosome-binding sites (Tsapakos *et al.*, 1985; Paluh and Yanofsky, 1986).

A number of physicochemical properties of Trp repressor have been studied in well-defined systems. These properties include measurements of the dissociation constant that governs its interaction with L-tryptophan and several tryptophan analogues. L-tryptophan was shown to bind in a non-cooperative fashion to each of two binding sites on Trp repressor homodimer. The reported K_D values ranged from 14 μM to 48 μM. This variability can probably be attributed to minor differences in experimental protocol; in particular, the affinity of Trp repressor for tryptophan varies over a sevenfold range as a function of temperature (Arvidson, Bruce and Gunsalus, 1986; Lane, 1986; Klig, Crawford and Yanofsky, 1987; Marmorstein *et al.*, 1987). The ability of tryptophan analogues to compete with L-tryptophan for binding to Trp repressor is of particular importance in biotechnological applications. Indoleacrylic acid is frequently employed as a gratuitous inducer of the *trp* promoter owing to the fact that the indoleacrylate–Trp repressor complex is defective in operator binding. Indoleacrylate has a greater affinity for Trp repressor than L-tryptophan; half-saturation of analogue binding occurs at concentrations approximately 30-fold lower than the half-saturating concentration of the normal ligand (Marmorstein *et al.*, 1987).

Trp repressor protein crystallizes readily (Haydock, 1983; Joachimiak *et al.*, 1983b). Efforts over the last several years by Sigler and co-workers have greatly clarified our knowledge of the three-dimensional structure of Trp holorepressor and the unliganded species. A plausible model (*Figure 3*) for the mode of interaction of Trp holorepressor with its operator target has been proposed (Schevitz *et al.*, 1985; Zhang *et al.*, 1987). The recently announced co-crystallization of a simulated Trp repressor–operator complex (Joachimiak *et al.*, 1987) should further expand our understanding of this important regulatory protein, and of others like it that appear to contain a 'helix-turn-helix' motif as an element critical to operator recognition (reviewed by Pabo and Sauer, 1984).

Future studies on Trp repressor should continue to produce interesting and valuable insight into how this protein functions as a controlling element in *trp* promoter function. A number of interesting missense mutants affecting protein–DNA interaction were described by Kelley and Yanofsky (1985) but so far no amino acid switches that affect subunit interaction, ligand binding, or thermostability have been found for Trp repressor. Such mutant repressors could be especially valuable in controlling the *trp* promoter in production systems.

An important aspect of Trp repressor action, the mechanism of which remains to be clarified, is the ability of this protein, when hyperproduced, to reduce the rates of expression from a number of other *E. coli* promoters that are not ordinarily considered to belong to the *trp* regulon (Bogosian and Somerville, 1983).

Effects of guanosine tetraphosphate on transcription from the *trp* promoter

A series of co-ordinated adjustments in cellular activity occur within *E. coli* whenever cells are abruptly starved for an amino acid. Collectively these

transient adjustments are referred to as the stringent response (reviewed by Cashel and Rudd, 1987). The physiological role of the stringent response is to redirect the biosynthetic machinery of the cell away from the formation of ribosomes and tRNA and toward the synthesis or function of enzymes required to overcome the conditions imposed by nutrient depletion. Mutations that abolish the stringent response include a class designated *relA* (for relaxed). The protein product of the *relA* gene (stringent factor) catalyses the transfer of the β-γ-pyrophosphoryl group of ATP to the 3′-hydroxyl of GDP or GTP, yielding ppGpp or pppGpp. This reaction, which occurs on the ribosome, is stimulated by the binding of uncharged tRNA to the aminoacyl (acceptor) site of the ribosome. In *relA* mutants, therefore, the ability of cells to accumulate ppGpp is reduced or eliminated. In wild-type cells, accumulated ppGpp may rise rapidly to levels of *c.* 0·2 mM before hydrolysis sets in, whereupon the level of this molecule declines toward basal levels.

Numerous studies have addressed the ability of ppGpp specifically to stimulate or inhibit the initiation of transcription from a variety of promoters (summarized by Cashel and Rudd, 1987). These studies were conducted either *in vivo*, by comparing mRNA or protein levels in pairs of isogenic *relA*/*relA*$^+$ mutants, or *in vitro* by monitoring the effects of ppGpp on promoter utilization in systems of varying complexity.

When the availability of specialized transducing phages enriched for *trp* operon DNA made it possible to assay *trp* mRNA levels quantitatively, a series of studies were performed to evaluate the role of ppGpp in *trp* operon expression. The experimental strategy was to pulse-label cells with [^{3}H]-uridine, then measure the relative amounts of *trp* mRNA by filter hybridization techniques. Edlin *et al.* (1968) found that *trp* mRNA accumulation during tryptophan starvation was ten times greater in a stringent strain than in an isogenic *relA* control. This result was qualitatively supported by independent studies of Lavallé and de Hauwer (1968) and Morse and Morse (1976). When *trp* mRNA formation was driven by the P_L promoter of phage λ, there were no effects attributable to ppGpp (Kuwano and Imamoto, 1976).

The regulatory effects of ppGpp on transcription initiation *in vitro* have generally supported the idea that this nucleotide can stimulate *trp* promoter utilization. Yang *et al.* (1979) used a cell-free transcription–translation system where the formation of β-galactosidase (EC 3.2.1.23) required transcription initiation at a *trp* promoter within a λd*trp-lac* template. These workers observed a two- to fivefold stimulation of activity in the presence of 0·1 M ppGpp; a slightly lower concentration of pppGpp was also stimulatory. Using a similarly complex *in vitro* system, Pouwels and van Rotterdam (1975) purified a factor that positively stimulated the expression of the *trp* operon. Their protein function, called At (for antitermination) could well have been the protein product of the *relA* gene. Jovanovich and Artz (quoted in Primakoff and Artz, 1979) also found that ppGpp stimulated the *trp* promoter *in vitro*. These conclusions were further supported by the later work of Kajitani and Ishihama (1984) who used a much simpler *in vitro* system where the RNA transcripts were analysed directly by electrophoresis.

Despite the fact that the influence of ppGpp on RNA synthesis *in vitro* is

likely to be very sensitive to assay conditions, the correlation of the *in vitro* studies with the *in vivo* work inspires confidence that the *trp* promoter is subject to specific stimulation by ppGpp. This issue has been addressed in elegant fashion using oligonucleotide-directed *in vitro* mutagenesis for another ppGpp-stimulated promoter, namely that of the *his* operon (Riggs *et al.*, 1986). These workers argue convincingly that the −10 hexamer sequence (especially the fourth and fifth positions) and the adjacent downstream regions are important in the activation of transcription by ppGpp. This nucleotide supposedly interacts directly or indirectly with RNA polymerase to help overcome a rate-limiting step in open complex formation, thereby favouring the initiation of transcription. The work of Riggs *et al.* (1986) also points up the need for caution in drawing conclusions about the mechanistic implications of mutations that affect promoter or operator activity. For example, a mutation scored as 'Promoter-down' might be one where the inherent ability of RNA polymerase to initiate transcription was unaltered, but where a ppGpp requirement had arisen.

The stringent response induces another global regulatory system, namely the heat shock genes (Grossman *et al.*, 1985). A critical element in the heat shock system is σ^{32}, the product of the *rpoH* gene (Grossman, Erickson and Gross, 1984). As discussed below, the *trp* promoter and most other *E. coli* promoters are thought to function in concert with σ^{70}, the product of the *rpoD* gene (Hoopes and McClure, 1987). The possibility that ppGpp could alter the pattern of promoter utilization by interacting with a form of RNA polymerase having σ^{32}, rather than σ^{70} is intriguing but untested.

Influence of the topological state of DNA on transcription from the *trp* promoter

An important property of covalently closed circular DNA molecules is their ability to assume a supercoiled conformation, i.e. they may contain an excess or a deficiency of duplex turns relative to linear DNA. Negative supercoiling (i.e. a deficiency of turns) is the only sort of topological variation found in nature. Supercoiling is central to DNA replication, repair and recombination (reviewed by Drilica, 1984). In a number of cases, the state of supercoiling affects gene expression. Although the reported effects of changes in supercoiling on *trp* promoter utilization are modest, it is important to scrutinize the published data in order to evaluate the status of the *trp* system and to inquire whether the experimental approaches that have been taken produced decisive results.

To a first approximation, the state of supercoiling of DNA in *E. coli* is the result of a balance between the opposing contributions of two enzymes. DNA gyrase (topoisomerase II, EC 5.99.1.3) uses the energy of ATP hydrolysis to inject negative superhelical turns into DNA (Cozzarelli, 1980). A separate enzyme, topoisomerase I (EC 5.99.1.2), catalyses the concerted breakage and reunion of phosphodiester bonds in DNA in a way that leads to the relaxation of negatively supercoiled DNA. DNA gyrase is a tetramer composed of two different subunits, encoded by the *gyrA* and *gyrB* genes. A number of temperature-sensitive mutations have been described for *gyrA* and *gyrB*, and

antibiotics exist that selectively inhibit DNA gyrase. Nalidixic acid inhibits the protein product of *gyrA*; novobiocin and coumermycin A inhibit the product of *gyrB* (Gellert *et al.*, 1976, 1977). Deletion mutants in the gene *topA* are deficient in topoisomerase I (Trucksis and DePew, 1981). Strains with *topA* lesions frequently display higher-than-normal levels of supercoiling, divide slowly, and tend to become overgrown with double mutants having lesions in *gyrA* and *gyrB* (DiNardo *et al.*, 1982).

The experiments that have been done to assess the role of supercoiling in transcription from the *trp* promoter have involved adding an appropriate antibiotic to a whole cell or cell-free system, then measuring *trp* mRNA or an appropriate reporter enzyme in order to evaluate whether the relaxation of template *trp* DNA to a less supercoiled state enhanced or inhibited expression. Controls in such experiments generally involve making the same kinds of measurements using preparations or cells with an antibiotic-resistant mutant form of DNA gyrase.

Smith, Kubo and Imamoto (1978) compared the *trp* promoter and the P_L promoter of a $\phi 80$ *trp* specialized transducing phage where the formation of anthranilate synthase (EC 4.1.3.27) (encoded by *trpE* DNA within the phage) was made to depend on either one or the other of two available promoters. The three antibiotics all strongly inhibited transcription from the P_L promoter but were ineffective against the *trp* promoter. When *gyrA* mutants were infected in the presence of nalidixic acid, anthranilate synthase formation was kinetically indistinguishable from experiments carried out in the absence of the antibiotic. Working with slightly different systems, Kubo *et al.* (1979) measured *trp* mRNA production in order to evaluate the effects of nalidixic acid, oxolinic acid and coumermycin on transcription. Inhibition of *trp* promoter function by oxolinic acid and coumermycin was seen to occur regardless of whether the *trp* promoter was situated in the chromosome, in a multicopy plasmid, or in an infecting λ-*trp* phage. This is the only published report which indicates that loss of supercoiling alters *trp* promoter function.

The effect of novobiocin on β-galactosidase formation in S-30 extracts programmed with *trp-lac* fusion DNA was studied by Yang *et al.* (1979) and Chen *et al.* (1982). In neither case was significant inhibition of the *trp* promoter observed, although other control promoters studied were effectively shut down by coumermycin. Using a temperature-sensitive *gyrB* mutant of *E. coli*, Wahle, Mueller and Orr (1984) concluded from *in vivo* results that the inactivation of thermolabile DNA gyrase was without effect on the *trp* promoter. Finally, Herrin and Bennett (1986) studied the effect of nalidixic acid on a series of plasmid-borne normal and hybrid promoters, including the *trp* promoter. The promoters in question were set up to drive the synthesis of galactokinase, using the system of McKenney *et al.* (1981). A series of hybrid promoters were designed to evaluate whether a given −35 or −10 region was particularly important to the supercoiling response. The *trp* promoter and two others were mildly inhibited by nalidixic acid. In backgrounds with a mutant *topA* gene there was a slight enhancement of *trp* promoter activity, compared with controls. For hybrid promoters that were stimulated by nalidixic acid (*trp-tet*, *trp-lac*), a defect in topoisomerase I lowered galactokinase production.

The experimental approaches that have been taken to evaluate the role of supercoiling in *trp* promoter utilization have been, to say the least, equivocal. Given the range of different — and often interacting — effects that can modify promoter strength, one can accept only order-of-magnitude effects in experiments designed to measure the relative strengths of promoters. To this reviewer's knowledge, nobody has tested whether gyrase-modifying antibiotics such as nalidixic acid or coumermycin could alter the rate of expression of *relA*. If this were to happen, and there were a consequent rise in intracellular ppGpp, certain negative effects attributable to reduced supercoiling might be exactly balanced out by ppGpp stimulation. Nor have detailed studies of the effects of ppGpp on the expression of *gyrA* or *gyrB* been carried out. It is already well established that the stringent response and the heat shock system are interconnected (Grossman *et al.*, 1985). Other examples of mutual interactions between global regulatory networks, of which there may be as many as 20 in *E. coli* (Neidhardt, 1987), have been well documented.

Our fragmentary and incomplete knowledge of the factors that may influence the initiation of transcription at the *trp* promoter calls for a concerted attack employing a variety of *in vivo* and *in vitro* approaches. For example, with present technology one could systematically alter each nucleotide residue within the *trp* promoter, then study how such a series of modified promoters behaved at different levels of supercoiling in a clean *in vitro* system, such as that employed by Borowiec and Gralla (1985). If the same set of promoters were then introduced in single copy form into a suitable series of wild-type and mutant *E. coli* strains, it might be possible to define in a rigorous and definitive way how supercoiling affects *trp* promoter function.

Signal strength of the *trp* promoter in comparison to other prokaryotic promoters

Promoters are punctuation elements of double-stranded DNA that have the potential to form complexes with some form of RNA polymerase holoenzyme. Such interactions, if productive, lead to the synthesis of an RNA copy, or transcript, of one of the two strands of DNA. Of the three forms of RNA polymerase holoenzyme that have so far been established in *E. coli* (Hoopes and McClure, 1987), only the form having σ^{70} as a specificity determinant is considered able to initiate transcription at the *trp* promoter.

The frequency of transcription initiation, a measure of promoter strength, can vary considerably. Some *E. coli* genes are transcribed less than once per generation, whereas others are transcribed as often as once every second, a dynamic range of 10 000-fold (McClure, 1985). Disregarding for the moment any possible influences that accessory proteins or DNA topology might exert on promoter utilization, it is clear that nucleotide sequence is the primary determinant of promoter strength.

Three structural features of the upstream region stand out when large numbers of *E. coli* promoters are compared: these are the consensus sequence elements TTGACA, centred near co-ordinate −35, and TATAAT, centred near co-ordinate −10. The third important feature is the distance separating

these two consensus sequence blocks (17 ± 1 base pairs). Both sequence blocks are important in the binding of the σ^{70} form of RNA polymerase to promoters. The −10 element helps mediate the isomerization of the binary DNA-holoenzyme complex to a form that is appropriate for the catalysis of phosphodiester bond formation (McClure, 1985). Promoter mutations mainly are found within the hexanucleotide blocks at −35 and −10. Depending on whether the mutant promoters approach or diverge from the consensus sequences, such mutations are classified as 'promoter-up' or 'promoter-down'. This is a matter of some importance with respect to the *trp* promoter, because its −10 hexamer (TTAACT) lies within the binding site for a specific regulatory protein, Trp repressor. Thus mutations that strengthen the *trp* promoter might decrease the affinity for repressor; nucleotide switches that altered the operator could be 'promoter-up' or 'promoter-down', depending upon whether the structure of the −10 hexamer had been changed.

Comparisons between the *trp* promoter and other *E. coli* promoters have either been carried out experimentally, using a variety of different *in vivo* or *in vitro* systems, or have been part of computer comparisons employing various algorithms and/or statistical approaches. As we shall see, none of these methods, taken alone, is wholly satisfactory.

One of the earliest comparative studies was that of Davison, Brammar and Brunel (1974), who measured anthranilate synthase levels in cells harbouring constructs where the *trpE*$^+$ gene, in single copy, was either under the control of the P_L promoter of λ or under the control of the *trp* promoter. Under fully constitutive conditions, the P_L promoter was estimated to be 11-fold more efficient than the *trp* promoter. However, at that time the role of the *trp* attenuator was not fully understood. Given the fact that a functioning *trp* attenuator reduces downstream gene expression eight- to tenfold (Bertrand, Squires and Yanofsky, 1976), one could conclude with benefit of hindsight that the *trp* promoter and the P_L promoter of λ are equivalent in strength.

de Boer *et al.* (1982) compared the strengths of the *trp* and *lac*UV5 promoters *in vivo* in plasmid-based *E. coli* systems where the level of resistance to tetracycline or the production of human growth hormone was monitored. Based on tetracycline resistance levels, the partially repressed *trp* promoter was judged to be equal in strength to the constitutively expressed *lac*UV5 promoter. Comparisons of human growth hormone production in the presence and absence of tryptophan led de Boer *et al.* (1982) to conclude that the fully derepressed *trp* promoter was about five times stronger than the *lac*UV5 promoter.

In another study, de Boer and Shepard (1983), used plasmid constructs where galactokinase was the reporter enzyme (McKenney *et al.*, 1981). It was estimated that the *trp* promoter was three times stronger than the *lac*UV5 promoter. The same hierarchy of promoter strengths held true in constructs developed by Windass *et al.* (1982) for the production in *E. coli* of human α-1 interferon; 5–10 times more interferon was synthesized in systems driven by the *trp* promoter, compared with *lac*UV5-driven production. Russell and Bennett (1982) also used galactokinase as a reporter enzyme in a series of comparative studies that included the *trp* promoter. These workers found that the *trp*

promoter was three times stronger than the *lac*UV5 promoter. In a later study, Shirakawa, Tsurimoto and Matsubara (1984) used the β-galactosidase activity of a *cro-lacZ* chimaeric gene as a reporter of promoter strength. The *trp* promoter was found to be 36% stronger than the combined rightward and leftward promoters of λ, but 32% less effective than the *recA* promoter. The former result is somewhat surprising, in that Ward and Murray (1979), studying a system of convergent transcription involving the P_L promoter of λ and the *trp* promoter, had shown that the *trp* promoter was completely blocked by high-efficiency initiation from P_L.

An elegant procedure for inserting a cloned promoter, in single copy, into the chromosome in front of the *malPQ* operon was used by Vidal-Ingigliardi and Raibaud (1985). In such operon fusions the levels of amylomaltase (EC 2.4.1.25), encoded by *malQ*, are proportional to promoter strength. Among seven promoters tested, the *trp* promoter appeared to be the most efficient, exceeding *lac*, λP_R and *tac* in the production of amylomaltase.

In designing a production system for human epidermal growth factor, Oka *et al.* (1987) compared the *trp* promoter with the *phoA* promoter and a tandem P_LP_R promoter derived from λ DNA fragments. The *trp* promoter was 19–45 times stronger than P_LP_R but one- to two-thirds as strong as the *phoA* promoter. In the aforementioned studies, no corrections were made for possible differences in plasmid copy number that might have arisen through the effects on *ori* function of strong waves of transcription. The *in vivo* studies can also be criticized on the grounds that no attempts were made to correct for possible differences in mRNA stability or translation efficiency.

An alternative approach to comparing promoter strength was taken by Kajitani and Ishihama (1983a), who carried out *in vitro* transcription using mixtures of short promoter-bearing fragments of duplex DNA. The promoters initially studied were *lac*UV5, *trp* and *rplJp*. Because each template gave rise to a transcript of unique length, it was possible to compare transcription efficiency quantitatively after the reaction products had been separated electrophoretically. A limitation of the *in vitro* approach is that transcription of the three templates studied was differentially affected by variations in salt concentration and temperature. The order of efficiency established for the three promoters studied was *lacP* > *trpP* > *rplJp*. At least for the *lac* and *trp* promoters, the promoter strength estimations by this method are just the opposite of the *in vivo* results cited above. Kajitani and Ishihama (1983b) extended their studies by analysing the *in vitro* transcription of the *rrnE*, *rpsA* and *recA* promoters. It was confirmed that the *trp* promoter was about half as strong as the *lac*UV5 promoter. This conclusion differs from that of Horowitz and Platt (1982), who carried out *in vitro* transcription using templates carrying both the *trp* and *lac*UV5 promoters within the same fragment of DNA. Horowitz and Platt estimated that the two promoters were equal in strength. It should be noted that transcription proceeded for multiple rounds in the studies of Horowitz and Platt (1982) whereas those of Kajitani and Ishihama (1983a, b) involved single-pass experiments (i.e. the RNA polymerase was prevented from re-initiating by the addition of rifampicin). Thus any features of a system of cyclic transcription that might facilitate re-initiation at a particular promoter

Table 1. The *trp* promoter: computer-assisted comparisons with other selected prokaryotic promoters

Promoter	Homology score*	PHI**	Curvature score†	Helix twist hexamers‡
recA	74·6	−1·1	0	3
pBR322 *tet*	63·9	−1·0	—	—
trp	61·5	−1·7	low	5
λP_L	58·0	−1·4	0·6	—
lac P‡	49·7	−2·0	1·55	0
mal K	32·0	−3·3	1·2	1

* Defined by Mulligan *et al.* (1984). A difference of 10 points corresponds to a tenfold difference in promoter strength.

** Promoter Homology Index, as defined by Harley and Reynolds (1987). As PHI values become more negative, promoter strength decreases.

† Based on phasing of 5′ and 3′ and ends of A_n and T_n tracts; Plaskon and Wartell (1987).

‡ Consensus structures consisting of hexamers having a unique sequence of helix twist angles; may be characteristic of prokaryotic promoters (Tung and Harvey, 1987).

would tend to overestimate the relative strength of that promoter. All of the aforementioned *in vitro* studies employed linear DNA templates. This method of comparing promoter strength may in certain cases be flawed. For example, Borowiec and Gralla (1985) found that *in vitro* transcription from the *lac* p^s promoter was stimulated as much as 40-fold when the template DNA contained negative superhelical turns.

Considerable effort has been devoted to predicting the strength of promoters strictly from a knowledge of nucleotide sequence. This approach began with comparisons between the sequences of known promoters and the emergence of consensus structures for promoter-active DNA (Rosenberg and Court, 1979; Siebenlist, Simpson and Gilbert, 1980; Hawley and McClure, 1983). Mulligan *et al.* (1984) developed an algorithm to compute homology scores for *E. coli* promoters (*Table 1*). In a weighted fashion, based upon a statistical treatment of the occurrence of the four possible sorts of base pairs at each position, a series of promoters were matched against relative strength values determined *in vitro* for 31 promoters. Within a factor of four, the correlation of Mulligan *et al.* (1984) was linear over a range of 10^4 in promoter strength. A similar statistical treatment was employed by Staden (1984), who used somewhat different assumptions about how each base contributes to the homology score. Staden developed a promoter strength prediction algorithm with essentially the same degree of correlation to experimental reality as the algorithm of Mulligan *et al.* (Reznikoff and McClure, 1986). Using approximately the same data base of promoter structure, Harley and Reynolds (1987) reiteratively aligned a set of promoter sequences by a modification of the algorithm of Staden (1984) in order to maximize statistical similarity. These workers compiled and classified 263 promoters. The 'promoter homology index' (PHI) assigned to each entry in their data set was considered to be a numerical reflection of relative promoter strength (*Table 1*). There is general agreement between the conclusions of Harley and Reynolds (1987) regarding promoter strength and the earlier deductions of Mulligan *et al.* (1984).

Other issues that have recently been addressed via the use of computer-

constructed algorithms are the role of DNA curvature in promoter strength (Plaskon and Wartell, 1987) and the possible role of short tertiary structure perturbations (i.e. localized twists and bends dictated by the nucleotide sequence). That bending of DNA in regions upstream from a promoter can affect the efficiency of transcription was experimentaly established by Bossi and Smith (1984), who analysed in detail a 3 bp deletion in a region 70 nucleotides upstream from a $tRNA^{His}$ gene start site in *Salmonella typhimurium*. This deletion led to a twofold decrease in promoter efficiency while altering in a dramatic way the conformation of the corresponding DNA. It is likely that regularly spaced ApA dinucleotides produce additive bending in the DNA axis, and that the deletion of Bossi and Smith (1984) interrupted bending by altering the ApA periodicity. Tung and Harvey (1987) noted that a particular array of helix twist angles was statistically prominent within a series of *E. coli* promoters, compared with structural gene DNA or computer-generated random sequences. Detailed *in vitro* and *in vivo* experimental tests of the correctness of these predictive schemes remain for the future. The ready applicability of oligonucleotide-directed *in vitro* mutagenesis should encourage future workers to define more explicitly those features of the *trp* promoter that affect the rate of transcription initiation, and that distinguish this promoter from others of nominally equal strength.

The most that can be said from a perusal of the experimental and theoretical approaches to estimating promoter strength is that the results give only a broad and general indication of how well the *trp* promoter compares with others. Every study is subject to some form of criticism. The biotechnologist trying to evaluate the merits of *trp*-promoter-driven production systems needs to focus only partly on the issue of 'strength'. The *trp* promoter is certainly powerful enough to satisfy the design criteria of most production systems. A potentially more relevant issue is whether the *trp* promoter ought to be silent during most of the growth of a production culture and, if so, how this may best be accomplished without compromising either cellular physiology or the facile activation of the promoter at the desired moment. The relative merits of the *trp* promoter in comparison with other promoters used in the design of prokaryotic protein production systems have been briefly discussed by Denhardt and Colasanti (1988) and by Brosius (1988).

Complications imposed by *in vivo* instability of plasmids containing the *trp* promoter

In considering what features ought to be optimized in the design of a plasmid-based production system, detailed consideration has been given to copy number and stability, choice of promoter, nature of ribosome binding site, and possible secondary structures in mRNA (Buell and Panayotatos, 1986). Ideally, at the conclusion of a production run, the cell should contain the protein of interest as 20–40% of the total.

For *trp*-promoter-based systems, there are numerous examples of plasmid instability that were encountered during the course of development of practical production schemes. Workers who have studied *trp* promoter plasmids

attributed instability either to some deleterious effect on *ori* function of high-level transcription, or suggested that the continuous overproduction of a particular protein was incompatible with normal cellular physiology. It was proposed that this created a selective condition where cells without plasmids or cells with structurally modified plasmids rapidly overgrew a culture because of their ability to outcompete their enfeebled relatives.

Instability attributable to high rates of transcription initiation from a plasmid-borne *trp* promoter was noted in the first report on the cloning of *trp* operon DNA (Hershfield *et al.*, 1974). Their constructs (pVH5 and pVH15) consisted of two *Eco*RI fragments of DNA (total, 15·3 kb) from φ80 pt190h inserted into the unique *Eco*RI site of ColE1 (6646bp; Chan *et al.*, 1985). After several generations of growth only 5–50% of Trp repressor-deficient host cells retained pVH15. Host cells able to produce a functional Trp repressor were able to maintain pVH15 under a variety of growth conditions. These observations were later confirmed and extended in a detailed study of pVH5 by Kim and Ryu (1984), who showed that physiological derepression of the *trp* promoter using β-3-indoleacrylic acid was also a selective condition that favoured the emergence of plasmid-free segregants. One of the early popular cloning vectors (mini-ColE1, also known as pVH51) arose via a spontaneous deletion of *c.* 19kb in one of the stocks of Hershfield *et al.* (1974) as a Trp^-, colicin-immune segregant (Helinski *et al.*, 1977). One suspects that the presence of highly expressed *trp* DNA in the parental plasmid contributed selective pressure that influenced the emergence of pVH51.

A second generation of plasmids having a cloned *trp* promoter were constructed by Nagahari *et al.* (1977). These workers inserted an *Eco*RI fragment of *c.* 16·4 kb from a specialized transducing phage (λtrp E-$A_{60\text{-}3}$; Fiandt, Szybalski and Imamoto, 1974) into *Eco*RI sites in the vectors pSC101, RSF1010, RSF2124 and RP4. These vectors are superior to ColE1 because they have highly convenient antibiotic resistance phenotypes that may be used as selectable markers. Naghari *et al.* (1977) examined plasmid copy number and *trp* operon enzyme levels in representative constructs. No instability was noted by these workers, although in later studies using RSF2124-*trp* and pSC101-*trp* Imanaka, Tsunekawa and Aiba (1980) and Imanaka and Aiba (1981) reported a marked tendency toward loss of plasmid and/or spontaneous deletion of plasmid-borne *trp* operon DNA. Plasmid instability was exaggerated in host strains having *trpR* mutations and was even greater in strains that were both *trpR* and *tna*. Mutations in *tna* lead to the inability of *E. coli* to produce the degradative enzyme tryptophanase (EC 4.1.99.1). This peculiar effect of a *tna* lesion is quite unexpected and deserves further study. Imanaka, Tsunekawa and Aiba (1980) attributed the instability they observed to stress caused by overproduction of *trp* pathway enzymes and the resultant adverse effects on normal metabolism. However, the observed increases in anthranilate synthase and tryptophan synthase (EC 4.2.1.20) that could be attributed to the presence of plasmid were rather modest (2·5-fold to sixfold), so it is more likely that high-level transcription initiation from the *trp* promoter was interfering with *ori*, the site within plasmid replicons where the events of DNA duplication begin. Despite these drawbacks, Aiba and co-workers (Aiba, Imanaka and

Tsuenkawa, 1980; Aiba, Tsunekawa and Imanaka, 1982) were able to develop production systems for L-tryptophan based on pSC101-*trp*.

Hallewell and Emtage (1980) noted that 96% of the cells in a culture had lost plasmid after growth for seven generations under *trp* promoter-induction conditions, whereas <1% plasmid loss was observed after a comparable period of growth under non-inducing conditions. Rose and Shafferman (1981) developed a *trp* promoter-driven plasmid system for the expression in *E. coli* of the VSV spike glycoprotein. Attempts to introduce such plasmids into *trpR* mutant hosts were unsuccessful. Because deletion derivatives of their plasmid lacking the *trp* control region were viable in *trpR* backgrounds, Rose and Shafferman inferred that overproduction of VSV glycoprotein was lethal to *E. coli*.

An independent series of plasmids bearing the *trp* promoter plus varying amounts of *trp* operon DNA were constructed by Enger-Valk *et al.* (1980). Using *Eco*RI linkers, these workers inserted an 8·7 kb *Sma*I fragment derived from φ80 *trp* EA51 *imm*λ into *Eco*RI-cleaved pBR345 (Bolivar *et al.*, 1977a). The resulting construct (pHP39) had Trp^+ as the sole selectable phenotype. The stability of pHP39 was not addressed by Enger-Valk *et al.* (1980), but this property was later studied in a series of derivatives of pHP39 made by Skogman *et al.* (1983). These workers inserted *trp* DNA into the ColE1 replicon pBR322 (Bolivar *et al.*, 1977b) and the P15 replicon pACYC184 (Chang and Cohen, 1978). Both categories of Trp^+ plasmid were highly unstable, displaying rates of plasmid loss varying between 0·3% and 0·9% per generation. The addition of a partition locus from pSC101 (Meacock and Cohen, 1980) reduced but did not eliminate plasmid instability. A similar approach was taken by Yukawa *et al.* (1985), who achieved excellent stability in a pBR322-*trp* system by the addition of a 9·1 kb *Eco*RI fragment from the F factor. The underlying basis of *trp* plasmid instability was not established by either group, although each speculated that interference with *ori* function by readthrough transcription from the powerful *trp* promoter led to decreases in copy number. The lesson from this study and those cited earlier is that loss of vector from cultures harbouring *trp* plasmids tends to occur in the absence of selective pressure. Elegant solutions to this problem were devised by Rosteck and Hershberger (1983) and by Skogman and Nilsson (1984). Rosteck and Hershberger (1983) investigated plasmid instability found to be associated with the *trp*-promoter-driven production of chimaeric proteins with either the A or B chains of human insulin, as well as another plasmid encoding a TrpE-proinsulin chimaera. Their solution was to add to their production plasmids a segment of DNA encoding a temperature-sensitive allele of λ repressor, then to lysogenize plasmid-bearing strains with a repressor-defective derivative of phage λ. Loss of plasmid from such lysogens led to cell death, because lytic growth of the prophage became induced. Skogman and Nilsson (1984) incorporated the essential gene $valS^+$ into a trp^+ plasmid, which was then stably maintained for over 200 generations in a *recA* host strain having a chromosomal *valS* mutation.

Another (presumably general) solution to the problem of strong promoter interference with plasmid DNA replication via waves of transcription across the *ori* region (*see above*) is to position a strong transcription termination signal

so as to insulate the plasmid replication apparatus from the expression unit. This approach was first devised by Gentz *et al.* (1981) and has been widely applied by others.

Vectors for the *trp*-promoter-driven expression of genes and fusion proteins

A sizeable number of vectors designed to facilitate the controlled high-level production of foreign gene products under the control of the *trp* promoter have been described (*Table 2*). This listing must, of necessity, be incomplete because many of the published reports that allude to *trp*-promoter-driven foreign protein expression in *E. coli* lack detailed information about the vectors employed. Despite this shortcoming, the number of entries that identify *trp* expression vector systems (*Table 2*) is at least double the number of examples cited in two other recent surveys (Pouwels, Enger-valk and Brammar, 1985; Balbás *et al.*, 1986).

The *trp* promoter expression vectors so far described differ markedly in terms of flexibility and degree of sophistication associated with their use. During the past decade there have been numerous technical improvements in the cloning and sequencing of DNA fragments. These advances were paralleled by a steady rise in the commercial availability of restriction endonucleases having a wide range of cleavage specificity and by the perfection of automated machinery for the synthesis of high-purity oligodeoxyribonucleotides. Thus the earliest *trp* promoter vectors contained sizeable amounts of irrelevant DNA derived from the *E. coli* or *S. marcescens* chromosome or from specialized transducing phages generated in the first successful cloning experiments. Important sources of *trp* promoter DNA in the early 1980s were mutationally altered segments from the *E. coli* chromosome where the *trp* attenuator had been deleted (Bertrand, Squires and Yanofsky, 1976; Miozzari and Yanofsky, 1978a). Such *trp*-promoter-bearing fragments were one point of departure for a number of early successful production systems (e.g. de Boer *et al.*, 1982). The successful cloning directly from genomic DNA of fragments harbouring various parts of the *trp* operon of *E. coli* (Hopkins, Murray and Brammar, 1976) and *S. marcescens* (Miozzari and Yanofsky, 1978b), was followed by the determination of the complete nucleotide sequence of the *trp* operon (Yanofsky *et al.*, 1981). These advances paved the way for several useful *trp* promoter vector designs based on smaller and more discrete DNA fragments generated by specific restriction endonucleases (Nishi *et al.*, 1984; Rink *et al.*, 1984; Russell *et al.*, 1984). More recently, two groups have independently carried out the total synthesis of the *trp* promoter of *E. coli*. The syntheses were designed to create expression cassettes that allowed complete freedom in the choice of flanking restriction endonuclease cleavage sites and the positioning of punctuation elements required for translation (Windass *et al.*, 1982; Niwa *et al.*, 1986).

Several *trp* promoter vector designs have featured the use of up to five tandem copies of the same promoter–operator segment of DNA as a way of increasing signal strength (Goeddel *et al.*, 1980b; Fujisawa *et al.*, 1983; Tacon *et al.*, 1983; Nishi *et al.*, 1984; Itoh *et al.*, 1986). When this is done, or when there is

Table 2. Vectors for the expression of heterologous genes in *E. coli* from the *trp* promoter

Plasmid(s)	Parental replicon	Source of *trp* promoter	Useful cloning sites	Comments	Ref.*
p*trp*ED5–1 6·7 kb	pBR322	5·4 kb *Hin*dIII fragment from *E. coli* chromosome	*Hin*dIII, *Bgl*II, *Bss*HII, *Sac*II, *Sna*BI, *Spe*I	IAA-inducible; contains *trp* attenuator	1
pWT111, 121, 131 4·8 kb	pBR322	497 bp *Hin*fI fragment from p*trp*ED5–1	*Hin*dIII	Phase shift plasmids; contain *trp* attenuator	2
pWT500 series 3·8 kb	pAT153	139 bp *Hha*I fragment from pWT121 via pWT 221; 99 bp *Hae*III–*Taq*I fragment from pWT501	*Hin*dIII, *Bal*I, *Eco*RI	Attenuator-deficient; double and triple *trp* promoter vectors	3
pHGH207–1	pBR322	285 bp *Taq*I fragment derived from pGM1*trp*△LE1413	*Xba*I, *Eco*RI	Attenuator-deficient	4
pMBL24 7·2 kb	CloDF13*Cop*–1	*2.6* kb *Eco*RI–*Pvu*I *trp* E^+ fragment from pHP3	*Bgl*III, *Pst*I	Thermoinducible amplification of copy number	5
pDR720 4·0 kb	pKO–1	41 bp *Alu*I fragment derived from *trp*△LC145	*Sal*I, *Bam*HI, *Sma*I	Commercially available (Pharmacia P–L Biochemicals)	6,7,8,
pAS621 4·9 kb	pBR322	497 bp *Hin*fI fragment from wild-type *trp* operon of *E. coli*	*Eco*RI, *Bam*HI, *Sal*I	Contains *trp* attenuator	9
ptrpL1 4·7 kb	pBR322	333 bp *Hin*fI–*Taq*I fragments from wild-type *trp* operon of *E. Coli*	*Cla*I	Attenuator-deficient	10
pTRP771, 801 4·0 kb, 3·25 kb	pBR322	543 bp *Pvu*II–*Taq*I fragment from wild-type *trp* operon of *E. coli*	*Cla*I, *Pst*I	Attenuator-deficient	11
pER103 4·4 kb	pBR322	90 bp *Eco*RI–*Hae*III fragment from *Serratia marcescens trp* operon	*Hin*dIII	May be o^c in *E. coli* owing to T→C change in operator	12
pBN series	pBR322	*S. marcescens trp* promoter–operator	*Eco*RI	May be o^c in *E. coli*	13

pSTP1 3·72 kb	pAT153	82 bp *Eco*RI–*Taq*I fragment chemically synthesized	*Cla*I, *Hin*dIII, *Bam*HI, *Sal*I	Titration of chromosomally encoded Trp repressor leads to partial constitutivity	14
pKYP series 4·5–4·7 kb	pBR322	Fragments of 62 bp, 109 bp and 340 bp derived from wild-type *E. coli* DNA	*Bam*HI, *Hin*dIII, *Cla*I, *Sal*I	Excellent sources of portable *trp* promoter fragments. Double and triple *trp* promoter vectors described	15
pIAT series	pSC101	pDR720	*Bgl*II	High level expression/secretion vector. 5 tandem *trp* promoters. $trpR^+$ cloned in *cis*	16
pTrpEB7 4·15 kb	pBR322	163 bp *Eco*RI–*Bam*HI fragment, chemically synthesized	*Bam*HI, *Sal*I, *Ban*II, *Sph*I, *Xma*III, *Ava*I		17
pKN305 4·7 kb	pBR322	pNS906 (pBR325–*trp*)	*Hin*dIII	Contains attenuator	18
pTrp100 series 6·6 kb	pBR322	2·3 kb *Bgl*II fragment from RSF2124–*trp*	*Eco*RI, *Hin*dIII, *Bam*HI, *Sal*I, *Sph*I, *Pvu*II	Contains attenuator	19
pCT1 4·86 kb	pBR322	0·5 kb *Hin*fI fragment from p*trp*ED5–1	*Cla*I	Contains attenuator; various improved derivatives constructed	20
pTR series 4·86 kb	BR322	pOCT2	*Eco*RI, *Sal*I		21
pHR148 4·6 kb	pBR322	283 bp *Eco*RI–*Taq*I fragment from pGM91	*Eco*RI, *Nco*I, *Kpn*I	Attenuator-deficient	22
pTrS3 3·7 kb	pKYP100	Wild-type *E. coli* DNA (62 bp)	*Cla*I, *Sph*I, *Sal*I	Designed for protein fusion work; 'ATG vector'. Attenuator-deficient	23
slp series	phageλ, pNS906	Wild-type *E. coli* DNA	*Eco*RI	Thermoinducible phage system contains attenuator	24

* References: (1) Hallewell and Emtage, 1980; (2) Tacon, Carey and Emtage, 1980; (3) Tacon *et al.* (1983); (4) de Boer *et al.* 1982; (5) Kos *et al.* 1984; (6) Russell and Bennett, 1982a; (7) Russell and Bennett, 1982b; (8) Russell *et al.* 1984; (9) Rose and Shafferman, 1981; (10) Edman *et al.* 1981; (11) Fujisawa *et al.* 1983; (12) Dworkin-Rastl, Swetly and Dworkin, 1983; (13) Nichols and Yanofsky, 1983; (14) Windass *et al.* 1982; (15) Nishi *et al.* 1984; (16) Itoh *et al.* 1986; (17) Niwa *et al.* 1986; (18) Masuda *et al.* 1986; (19) Nagahari *et al.* 1987; (20) Ikehara *et al.* 1984; (21) Nishimori *et al.* 1984; (22) Rink *et al.* 1984; (23) Nishi *et al.* 1983; (24) Nakano and Masuda, 1982.

Table 3. *trp* promoter-driven production of human proteins in *E. coli*

Protein	Production system	Maximum yield or specific activity	Reference*
Insulin	Not named	High	1
Somatostatin C**	HB101/pLHSdMm *trp*	4×10^5 molecules/cell	2, 3
Somatomedin C	HB101/pCE–SM *trp*	14 mg/litre (crude)	4
Serum albumin	MM294/pHSA1	Modest	5
Tumour necrosis factor	W3110/pTNF *trp*	3×10^5 molecules/cell	6
Tumour necrosis factor	HB101/pHTP 316	20% of total protein	7, 8
Tumour necrosis factor	HB101/pM320	10% of total protein	9
Prorenin**	HB101/pTR501	30% of total protein	10
Epidermal growth factor	YK537/pTA 1732	336 μg/litre of culture	11
β-Endorphin**	N99/pIAT141–βE	1–2 mg/litre	12
β-Endorphin**	RR1/p*trp*βEP	23·5% of total protein	13
Growth hormone**	RR1/p*trp*ED50–chGH800	3% of total protein	14
Growth hormone	D1210/pHGH207–1	42 070 μunits/ml/OD_{550}	15
Growth hormone	HB101/pGH–L9	$2{\cdot}9 \times 10^6$ molecules/cell	16
Tissue plasminogen activator	W3110/pt–PA *trp* 12	$1{\cdot}5$–$2{\cdot}4 \times 10^3$ molecules/cell	17
Transforming growth factor α**	W3110/pTE6	20–30% of total protein	18
β-Urogastrone**	HB101/pWT551–3P3 deriv.	13% of total protein	19
β-Urogastrone	NEM259/pSRW23	High levels	20
IFN-γ/IL2 chimaera	DH1/pIFL9906	$10^6/10^4$ units/litre of culture	21
Basic fibroblast growth factor	MM294/pTB669	23·2 mg/litre of culture	22
α-Atrial natriuretic peptide**	DH1/pClaHtrp3t	32% of total protein	23
Insulin-like growth factor II**	RV308/pCZ21	25% of total protein	24
Pancreatic secretory trypsin inhibitor**	C600/pIFN-γ/PSTI	200 μg/gram of cells	25
Angiogenin	B/pXL694	5–10% of total protein	26

* (1) Burnett, 1983; (2) Niwa *et al.*, 1986; (3) Saito *et al.*, 1987a; (4) Saito *et al.*, 1987b; (5) Lawn *et al.*, 1981; (6) Pennica *et al.*, 1984; (7) Fukui *et al.*, 1985; (8) Yamada *et al.*, 1985 (9) Nobuhara *et al.*, 1986; (10) Imai *et al.*, 1986; (11) Oka *et al.*, 1987; (12) Itoh *et al.*, 1986; (13)Nagahari *et al.*, 1987; (14) Martial *et al.*, 1979; (15) de Boer *et al.*, 1982; (16) Ikehara *et al.*, 1984; (17) Pennica *et al.*, 1983; (18) Derynck *et al.*, 1984; (19) Tacon *et al.*, 1983; (20) Warne *et al.*, 1986; (21) Seno *et al.*, 1986; (22) Iwane *et al.*, 1987; (23) Saito *et al.*, 1987c; (24) Furman *et al.*, 1987; (25) Kikuchi *et al.*, 1987; (26) Denefle *et al.*, 1987.

** Product initially expressed as a fusion protein.

a single copy of the *trp* promoter–operator within a vector of high copy number, the levels of cytoplasmic Trp repressor (encoded by a single chromosomal copy of *trpR*) tend to be insufficient to quench transcription fully from the plasmid-borne *trp* promoter(s). To achieve tight regulation in such systems, several groups have modified their expression vectors through the addition of $trpR^+$ DNA (Osheroff *et al.*, 1982; Itoh *et al.*, 1986; Warne *et al.*, 1986). Expression in such systems is triggered by the addition of indolylacrylic acid.

Examples of systems where protein hypersynthesis in *E. coli* utilized the *trp* promoter

There are almost 80 different proteins for which the structural genes have been cloned in whole or in part and expressed in *E. coli* under the control of the *trp* promoter (*Tables 3–8*). Human proteins other than the interferons and interleukin, the expression of which has been accomplished in *trp* promoter-driven systems, are listed in *Table 3*. The levels of expression ranged up to 32% of total cell protein. In some cases the proteins accumulated in insoluble form as aggregates or inclusion bodies, a property that has proved useful for purification. This aspect of protein production has been reviewed by Marston (1986). In many cases, the desired proteins were produced as chimaeras having some other peptide sequence in addition to the desired product. This strategy has been widely employed as a means of favouring inclusion body formation and/or offering a means for preventing degradation by the intracellular proteases of *E. coli*. In *Tables 4–8* are listed various animal proteins, human interferons, viral proteins and immunoglobulins, interleukin-2 and several miscellaneous proteins from diverse biological sources, the production of which was facilitated via the use of a *trp* promoter expression system. The precise designations of the host cells and plasmid constructs used in these systems are provided to help the interested reader locate the relevant information within papers that invariably contain much additional material that is sometimes focused on issues other than production. It is anticipated that many new examples will continue to be reported in coming years, particularly by workers in Japan, where the adoption of *trp* promoter-driven systems has been widespread.

Conclusions and future prospects

Interest in the use of *E. coli* as a host strain in the hypersynthesis of foreign proteins is predicted to remain high. This is because its genetics and physiology are well understood, because it is readily manipulated using laboratory procedures that are proved and widely practised, and because the final level of accumulated foreign protein can frequently reach 30% of the total, with expression vectors generally available to the scientific community.

The empirical approaches to protein overproduction that characterized the early work with *E. coli* have now given way to more systematic and fundamental studies aimed at identifying the factors that govern the final level of protein that can be recovered. For example, the many instances of cellular

Table 4. *trp* promoter-driven production of animal proteins in *E. coli*

Protein	Production system	Maximum yield or specific activity	Reference*
Calf prochymosin	HB101/pCT70	5% of total protein	1
Calf prochymosin**	C600/CR501	3×10^5 molecules/cell	2
Calf prochymosin	B/r/pCT70	20% of total protein	3
Mouse renin**	HB101/pMR304	10% of total protein	4
Bovine growth hormone**	HB101/pSBBGH	5% of total protein	5
Bovine growth hormone	Am7/runaway replicon	20% of total protein	6
Bovine growth hormone	MM294/pBGH ex-1	1·5 g/litre of culture	7
Porcine growth hormone	MM294/pPGH ex-1	1·5 g/litre of culture	7
Salmon growth hormone	W3110(*strA*)/psGH1B-2	15% of total protein	8
Mouse T-cell receptor (β-chain)	W3110/pMCβK8	High	9

* (1) Emtage *et al.*, 1983; (2) Nishimori *et al.*, 1984; (3) Schoemaker, Brasnett and Marston, 1985; (4) Masuda *et al.*, 1986; (5) Szoka *et al.*, 1986; (6) Langley *et al.*, 1987; (7) Seeburg *et al.*, 1983; (8) Sekine *et al.*, 1985; (9) Kuwana *et al.*, 1987.

** Product initially expressed as a fusion protein

Table 5. *trp* promoter-driven production of interferons in *E. coli*

	Production system	Maximum yield or specific activity	Reference*
IFN αA	MM294/pLeIf A25	12 000 molecules/cell	1
	MM294/pLeIF A9	10^5 molecules/cell	2
	HB101/pER 33	2×10^7 units/litre	3
	JA221/pIFS 201	$2{\cdot}3 \times 10^6$ units/10^{10} cells	4
IFN αB	MM294/pLeIF B7	8×10^7 units/litre	5
	MM294/pIFU16	$1{\cdot}3 \times 10^6$ units/ml	6
Hybrid IFNα	MM294/pLeIF AD (*Bgl*II)	2×10^3–4×10^5 units/mg	7
	MM294/pLeIF DA (*Bgl*II)		
	MM294/pLeIF AD (*Pvu*II)		
	MM294/pLeIF DA (*Pvu*II)		
IFNβ	MM294/pFIF *trp* 69	4 500 molecules/cell	8
	MM294/pFIF *trp*^{3} 69	20 200 molecules/cell	
	MM294/pFIF *trp* 99	2×10^4 molecules/cell	2
	HB101/pM 10	Not stated	9
	HB101/pFJ123	$3{\cdot}9 \times 10^7$ units/litre	10
	9HB101/pMN39–1	6×10^5 molecules/cell	11
	HB101/pMG–1	5×10^9 units/litre	12
	DH1/pVX81F:Trp	2×10^8 units/litre	13
	NEM259/pSRW 25 + pMN45	14% of total protein	14
Modified IFNβ	MM294/pFIF *trp*^{3} 202	Inactive CRM	15
	HB101/GC206	2×10^9 units/litre	16
	HB101/pGC206	$3{\cdot}6 \times 10^8$ units/litre	17
IFNγ	W3110/pIFN-γ *trp* 48	8–80 molecules	18
	HB101/pGKA 2	8×10^6 units/litre	19
	HB101/pGHQ1	$7{\cdot}2 \times 10^7$ units/litre	20
	DH1/pHITrp 1101	3×10^8 units/litre	21
	K-802/pIFN-γ-trp^1	Not stated	22
	K-802/pIFN-γ-trp^2		
BoIFNβ1	MM294/pBoIFN-D1	$2{\cdot}2 \times 10^8$ units/litre	23
BoIFND2	MM294/pBoIFN-β2	$1{\cdot}1 \times 10^8$ units/litre	23
BoIFNβ3	MM194/pBoIFN-β3	$6{\cdot}0 \times 10^8$ units/litre	23
EqIFNα	HB101/pAH52/2	2×10^6 units/litre	24
EqIFNβ	HB101/pAH62	$1{\cdot}1 \times 10^9$ units/litre	24

* (1) Goeddel *et al.*, 1980a; (2) Shepard, Yelverton and Goeddel, 1982; (3) Dworkin-Rastl, Swetly and Dworkin, 1983; (4) Windass *et al.*, 1982; (5) Yelverton *et al.*, 1981; (6) Grundström *et al.*, 1987; (7) Weck *et al.*, 1981; (8) Goeddel *et al.*, 1980b; (9) Nagase *et al.*, 1983; (10) Nishi *et al.*, 1983; (11) Tacon *et al.* 1983; (12) Itoh *et al.*, 1984; (13) Whitehorn, Livak and Petteway, 1985; (14) Warne *et al.*, 1986; (15) Shepard *et al.*, 1981; (16) Porter *et al.*, 1986; (17) Stewart *et al.*, 1987; (18) Gray *et al.*, 1982; (19) Nishi *et al.*, 1985; (20) Nishi and Itoh, 1986; (21) Seno *et al.*, 1986; (22) Sverdlov *et al.*, 1987; (23) Leung, Capon and Goeddel, 1984; (24) Himmler *et al.*, 1986.

Table 6. *trp* promoter-driven production of potential vaccines and immunoglobulins in *E. coli*

Protein	Production system	Yield	Reference*
FPV haemagglutinin	HB101/pWT121/FPV 411 (R)	2–3% of total protein	1
VSV G-protein	C600YS1/pGE4	<1% of total protein	2
HBV core antigen	HB101/pCA 246	10% of toal protein	3
HBV surface antigen: β-lactamase chimaera	HB101/pSA4	8·5% of total protein	3
HBV surface antigen	C600/pTRPSS-50	0·001% of total protein	4
HBV surface antigen	HB101/pTS1038	10^5 molecules/cell	5
HBV surface antigen	MM293/pTRP P31-R	230–250 μg/litre	6
FMDV capsid protein VP3 (as chimaera)	MM294/pFM1	17% of total protein	7
FMDV capsid protein VP1 (as chimaera)	JM101/pMol-72	1–5 mg/litre	8
Rabies surface glycoprotein	W3110/pRabdex 2	2–3% of total protein	9
Exotoxin A (*Pseudomonas*)	MM294/ptrpETA	High levels	10
Murine Ig light chain	HB101/pNP3	High levels	11
Murine Ig light chain	E103S/pNP3	13% of total protein	12
Murine Ig μ heavy chain	*E. coli* B/pNP14	High levels	13
Human Ig chain fragment	WT217/pSC213	18% of total protein	14
Human Ig chain fragment	MM294/pGET302	32 μg/litre	15
Human anti-CEA antibody (heavy and light chains)	W3110/pKCEA trp107–1* △, pγCEAInt 1	0·5–3% of total protein	16
Adenovirus transforming proteins E1A and E1B (as fusion polypeptides)	C/600/pKRS101, pKRS103, pKRS 107, pDR21	1–10 mg/litre	17

* (1) Emtage *et al.*, 1980; (2) Rose and Shafferman, 1981; (3) Edman *et al.*, 1981; (4) Fujisawa *et al.*, 1983; (5) Pumpen *et al.*, 1984; (6) Fujisawa *et al.*, 1985; (7) Kleid *et al.*, 1981; (8) Broekhuisjen *et al.*, 1986; (9) Yelverton *et al.*, 1983; (10) Gray *et al.*, 1984; (11) Boss and Emtage, 1983; (12) Boss *et al.*, 1984; (13) Wood *et al.*, 1984; (14) Kenten *et al.*, 1984; (15) Kurokawa *et al.*, 1983; (16) Cabilly *et al.*, 1984; (17) Spindler, Rosser and Berk, 1984.

Table 7. *trp* promoter-driven production of interleukins in *E. coli*

Protein	Production system	Yield	Reference*
Interleukin 2	K514λ/pTrp Hil 201	10% of total protein	1
	MM294/pLW1	5% of total protein	2
	MM294/pLW21	5% of total protein	3
	MM294/pLW42		
	MM294/pLW44		
	MM294/pLW46		
	C600/pDR720-B	15% of total protein	4
	C600/pWT111-B	10·7% of total protein	
	DH1/pTF1	$1{\cdot}2 \times 10^4$ units/litre	5
	HB101/pT9-11	20% of total protein	6
Interleukin-1β	HB101/ptrpIL-1β	20% of total protein	7

* (1) Devos *et al.*, 1983; (2) Rosenberg *et al.*, 1984; (3) Wang, Lu and Mark, 1984; (4) Marquis, Smolec and Katz, 1986; (5) Seno *et al.*, 1986; (6) Sato *et al.*, 1987; (7) Kikumoto *et al.*, 1987.

Table 8. *trp* promoter-driven production of miscellaneous proteins in *E. coli*

Protein	Production system	Yield or specific activity	Reference*
pp60 src protein of RSV (as fusion protein)	MM294/pCSRex 16	5% of total protein	1
ras p21 protein	MM294/pGa trp	5–10%g of total protein	2
v-H/N-*ras* chimaera	W3110/pCNRA1	5–10% of total protein	3
FBJ murine osteosarcoma virus oncogene	N5115/pTF89	0·1% of total protein	4
Avian erythroblastosis virus *erbB* polypeptide (as fusion protein)	MM294/pHGHerb	40% of protein insoluble in 0·4 M NaCl	5
Avian myeloblastosis virus *myb* polypeptide (as fusion protein)	MM294/pHGHmybML	30–50% of protein insoluble in 0·4M NaCl	6
Isopenicillin N synthetase	RV308/pIT337	20% of total protein	7
Eglin C (leech proteinase inhibitor)	LM1035/pML147	3×10^5 molecules/cell	8
Endonuclease V (phage T4)	HB101/pEndV	Not reported	9
Nicotinic acetylcholine receptor, α-bungarotoxin-binding domain (as fusion protein)	HB101/pR1	Not stated	10
RNA-dependent RNA polymerase (poliovirus) (as fusion protein)	HB101/pPROT-POL	Not reported	11
Peptide C-terminal α-amidating enzyme (*Xenopus laevis*)	W3110/ptrpXAE457 (38–381)	High	12

* (1) McGrath and Levinson, 1982; (2) McGrath *et al.*, 1984; (3) Matsui *et al.*, 1987; (4) MacConnell and Verma, 1983; (5) Privalsky *et al.*, 1983; (6) Klemnauer *et al.*, 1983; (7) Samson *et al.*, 1985; (8) Rink *et al.*, 1984; (9) Inaoka, Miura and Ohtsuka, 1986; (10) Gershoni, 1987; (11) Morrow, Warren and Lentz, 1987; (12) Mizuno *et al.*, 1987.

toxicity and death as a consequence of protein overproduction have focused attention upon controlling the rate of transcription initiation as a critical aspect of practical protein production. In this respect the *trp* promoter continues to find favour. Transcription from this promoter is subject to manipulation through control over Trp repressor–operator interaction. Its signal strength is comparable to the other major promoters now in use, enabling the experimenter to drive the production of abundant amounts of many proteins.

On high-copy-number plasmids the *trp* promoter tends to be incompletely regulated, a complication that has been overcome at least in part by increasing the intracellular level of Trp repressor by DNA manipulations that elevate the *trpR* gene dosage. As our understanding of structure–function relationships in the Trp repressor system continues to unfold, it may be possible to utilize mutationally altered Trp repressors to improve regulation at the *trp* promoter, making this process more amenable to the requirements of biotechnology. For example, if one could modify repressor–operator interaction by heating or cooling a fermentation vessel, thereby activating a *trp* promoter held in check by a thermolabile or cryolabile repressor, the sometimes undesirable use of indolylacrylic acid to activate expression could be abandoned.

Since the previous survey of *trp* promoter-driven systems (Johnson and Somerville, 1985), the number of relevant literature reports has approximately quadrupled. It will be interesting to see whether the number of new production applications for this system continues to grow with an 18-month doubling time.

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References

AIBA, S., IMANAKA, T. AND TSUNEKAWA, H. (1980). Enhancement of tryptophan production by *Escherichia coli* as an application of genetic engineering. *Biotechnology Letters* **2**, 525–530.

AIBA, S., TSUNEKAWA, H. AND IMANAKA, T. (1982). New approach to tryptophan production by *Escherichia coli*: Genetic manipulation of composite plasmids *in vitro*. *Applied and Environmental Microbiology* **43**, 289–297.

ARVIDSON, D.N., BRUCE, C. AND GUNSALUS, R.P. (1986). Interaction of the *Escherichia coli trp* aporepressor with its ligand, L-tryptophan. *Journal of Biological Chemistry* **261**, 238–243.

BALBÁS, P., SOBERON, X., MERINO, E., ZURITA, M., LOMELI, H., VALLE, F., FLORES, N. AND BOLIVAR, F. (1986). Plasmid vector pBR322 and it special-purpose derivatives—a review. *Gene* **50**, 3–40.

BASS, S., SUGIONO, P., ARVIDSON, D.N., GUNSALUS, R.P. AND YOUDERIAN, P. (1987). DNA specificity determinants of *Escherichia coli* tryptophan repressor binding. *Genes & Development* **1**, 565–572.

BENNETT, G.N. AND YANOFSKY, C. (1978). Sequence analysis of operator constitutive mutants of the tryptophan operon of *Escherichia coli*. *Journal of Molecular Biology* **121**, 179–182.

BENNETT, G.N., BROWN, K.D. AND YANOFSKY, C. (1978). Nucleotide sequence of the

promoter-operator region of the tryptophan operon of *Salmonella typhimurium*. *Journal of Molecular Biology* **121**, 139–152.

BENNETT, G.N., SCHWEINGRUBER, M.E., BROWN, K.D., SQUIRES, C. AND YANOFSKY, C. (1976). Nucleotide sequence of region preceding *trp* mRNA initiation site and its role in promoter and operator function. *Proceedings of the National Academy of Sciences, USA* **73**, 2351–2355.

BENNETT, G.N., SCHWEINGRUBER, M.E., BROWN, K.D., SQUIRES, C. AND YANOFSKY, C. (1978). Nucleotide sequence of the promoter–operator region of the tryptophan operon of *Escherichia coli*. *Journal of Molecular Biology* **121**, 113–137.

BERTRAND, K., SQUIRES, C. AND YANOFSKY, C. (1976). Transcription termination *in vivo* in the leader region of the tryptophan operon of *Escherichia coli*. *Journal of Molecular Biology* **103**, 319–337.

BLUMENBERG, M. AND YANOFSKY, C. (1982a). Regulatory region of the *Klebsiella aerogenes* tryptophan operon. *Journal of Bacteriology* **152**, 49–56.

BLUMENBERG, M. AND YANOFSKY, C. (1982b). Evolutionary divergence of the *Citrobacter freundii* tryptophan operon regulatory region: comparison with other enteric bacteria. *Journal of Bacteriology* **152**, 57–62.

BOGOSIAN, G. AND SOMERVILLE, R. (1983). Trp repressor protein is capable of intruding into other amino acid biosynthetic systems. *Molecular and General Genetics* **191**, 51–58.

BOGOSIAN, G. AND SOMERVILLE, R.L. (1984). Analysis *in vivo* of factors affecting the control of transcription at promoters containing target sites for Trp repressor. *Molecular and General Genetics* **193**, 110–118.

BOGOSIAN, G., BERTRAND, K. AND SOMERVILLE, R.L. (1981). Trp repressor protein controls its own structural gene. *Journal of Molecular Biology* **149**, 821–825.

BOGOSIAN, G., SOMERVILLE, R.L., NISHI, K., KANO, Y. AND IMAMOTO, F. (1984). Transcription of the *trpR* gene of *Escherichia coli*: an autogenously regulated system studied by direct measurements of mRNA levels *in vivo*. *Molecular and General Genetics* **193**, 244–250.

BOLIVAR, F., BETLACH, M.C., HEIJNEKER, H.L., SHINE, J., RODRIGUEZ, R. AND BOYER, H.W. (1977a). Origin of replication of pBR345 plasmid DNA. *Proceedings of the National Academy of Sciences, USA* **74**, 5265–5269.

BOLIVAR, F., RODRIGUEZ, R.L., GREENE, P.J., BETLACH, M.C., HEYNECKER, H.L., BOYER, H.W., CROSA, J.H. AND FALKOW, S. (1977b). Construction and characterization of new cloning vehicles, II. A multipurpose cloning system. *Gene* **2**, 95–113.

BOROWIEC, J.A. AND GRALLA, J. (1985). Interaction of RNA polymerase with the *lac* P^s promoter *in vivo* and *in vitro*. In *Sequence Specificity in Transcription and Translation* (R. Calendar and L. Gold, Eds), pp. 3–10. Alan R. Liss, Inc., New York.

BOSS, M.A. AND EMTAGE, S. (1983). Expression of an immunoglobulin light chain gene in *Escherichia coli*. In *Gene Expression* (D.H. Hamer and M.J. Rosenberg, Eds), pp. 513–522. Alan R. Liss, Inc., New York.

BOSS, M.A., KENTEN, J.H., WOOD, C.R. AND EMTAGE, J.S. (1984). Assembly of functional antibodies from immunoglobulin heavy and light chains synthesized in *E. coli*. *Nucleic Acids Research* **12**, 3791–3806.

BOSSI, L. AND SMITH, D.M. (1984). Conformational change in the DNA associated with an unusual promoter mutation in a tRNA operon of *Salmonella*. *Cell* **39**, 643–652.

BROEKHUISJEN, M.P., BLOM, T., VAN RIJN, J., POUWELS, P.H., KLASEN, E.A., FASBENDER, M.J. AND ENGER-VALK, B.E. (1986). Synthesis of fusion proteins with multiple copies of an antigenic determinant of foot-and-mouth disease virus. *Gene* **49**, 189–197.

BROSIUS, J. (1988). Expression vectors employing lambda-, *trp*-, *lac*-, and *lpp*-deprived promoters. In *Vectors. A Survey of Molecular Cloning Vectors and their Uses* (R.L. Rodriguez and D.T. Denhardt, Eds.), pp. 205–225. Butterworths, Stoneham, MA.

BROWN, K.D., BENNETT, G.N., LEE, F., SCHWEINGRUBER, M.E. AND YANOFSKY, C. (1978). RNA polymerase interaction at the promoter–operator region of the tryptophan operon of *Escherichia coli* and *Salmonella typhimurium*. *Journal of Molecular Biology* **121**, 153–177.

BUELL, G. AND PANAYOTATOS, N. (1986). Mechanism and Practice. In *Maximizing Gene Expression* (W. Reznikoff and Gold, L., Eds), pp. 345–363. Butterworths, Stoneham, MA.

BURNETT, J.P. (1983). Commercial production of recombinant DNA-derived products. In *Experimental Manipulation of Gene Expression* (M. Inouye, Ed.), pp. 259–277. Academic Press, New York.

CABILLY, S., RIGGS, A.D., PANDE, H., SHIVELY, J.E., HOLMES, W.E., REY, M., PERRY, L.J., WETZEL, R. AND HEYNECKER, H.L. (1984). Generation of antibody activity from immunoglobulin polypeptide chains produced in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **81**, 3273–3277.

CASHEL, M. AND RUDD, K.E. (1987). The stringent response. In Escherichia coli *and* Salmonella typhimurium. *Cellular and Molecular Biology, Vol. 2* (F.C. Neidhardt, Ed.), pp. 1410–1438. American Society for Microbiology, Washington, DC.

CHAN, P.T., OHMORI, H., TOMIZAWA, J. AND LEBOWITZ, J. (1985). Nucleotide sequence and gene organization of ColE1 DNA. *Journal of Biological Chemistry* **260**, 8925–8935.

CHANG, A.C.Y. AND COHEN, S.A. (1978). Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *Journal of Bacteriology* **134**, 1141–1156.

CHEN, W.-J., YANG, H.-L., ZUBAY, G. AND GELLERT, M. (1982). The effects of supercoiling on promoter function in prokaryotes. In *Promoters: Structure and Function* (R.L. Rodriguez and M.J. Chamberlin, Eds), pp. 242–251. Praeger Publishers, New York.

COHEN, G. AND JACOB, F. (1959). Sur la répression de la synthèse des enzymes intervenant dans la formation du tryptophane chez *Escherichia coli*. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* **248**, 3490–3492.

COZZARELLI, N.R. (1980). DNA gyrase and the supercoiling of DNA. *Science* **207**, 953–960.

DAVISON, J., BRAMMAR, W.J. AND BRUNEL, F. (1974). Quantitative aspects of gene expression in a λ-*trp* fusion operon. *Molecular and General Genetics* **130**, 9–24.

DE BOER, H.A. AND SHEPARD, H.M. (1983). Strategies for optimizing foreign gene expression in *Escherichia coli*. In *Genes: Structure and Expression* (A.M. Kroon, Ed.), pp. 205–248. John Wiley and Sons, New York.

DE BOER, H.A., GILBERT, S.F. AND NOMURA, M. (1979). DNA sequences of promoter regions for rRNA operons *rrnE* and *rrnA* in *E. coli*. *Cell* **17**, 201–209.

DE BOER, H.A., COMSTOCK, L.J., YANSURA, D.G. AND HEYNECKER, H.L. (1982). Construction of a tandem *trp-lac* promoter and a hybrid *trp-lac* promoter for efficient and controlled expression of the human growth hormone gene in *Escherichia coli*. In *Promoters: Structure and Function* (R.L. Rodriguez and M.J. Chamberlain, Eds), pp. 462–481. Praeger, New York.

DENEFLE, P., KOVARIK, S., GUITTON, J.-D., CARTWRIGHT, T. AND MAYAUX, J.-F. (1987). Chemical synthesis of a gene coding for human angiogenin, its expression in *Escherichia coli* and conversion of the product into its active form. *Gene* **56**, 61–70.

DENHARDT, D.T. AND COLASANTI, J. (1988). A survey of vectors for regulating expression of cloned DNA in *E. coli*. In *Vectors. A Survey of Molecular Cloning Vectors and their Uses* (R.L. Rodriguez and D.T. Denhardt, Eds), pp. 179–203. Butterworths, Stoneham, MA.

DERYNCK, R., ROBERTS, A.B., WINKLER, M.E., CHEN, E.Y. AND GOEDDEL, D.V. (1984). Human transforming growth factor-α: Precursor structure and expression in *E. coli*. *Cell* **38**, 287–297.

DEVOS, R., PLAETINCK, G., CHEROUTRE, H., SIMONS, G., DEGRAVE, W., TAVERNIER, J., REMAUT, E. AND FIERS, W. (1983). Molecular cloning of human

interleukin 2 cDNA and its expression in *E. coli*. *Nucleic Acids Research* **11**, 4307–4323.

DINARDO, S., VOELKEL, K.A., STERNGLANZ, R., REYNOLDS, A.E. AND WRIGHT, A. (1982). *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell* **31**, 43–51.

DRILICA, K. (1984). Biology of bacterial deoxyribonucleic acid topoisomerases. *Microbiological Reviews* **48**, 273–289.

DWORKIN-RASTL, E., SWETLY, P. AND DWORKIN, M.B. (1983). Construction of expression plasmids producing high levels of human leukocyte-type interferon in *Escherichia coli*. *Gene* **21**, 237–248.

EDLIN, G., STENT, G.S., BAKER, R.F. AND YANOFSKY, C. (1968). Synthesis of a specific messenger RNA during amino acid starvation of *Escherichia coli*. *Journal of Molecular Biology* **37**, 257–268.

EDMAN, J.C., HALLEWELL, R.A., VALENZUELA, P., GOODMAN, H.M. AND RUTTER, W.J. (1981). Synthesis of hepatitis B surface and core antigens in *E. coli*. *Nature* **291**, 503–506.

EMTAGE, J.S., TACON, W.C.A., CATLIN, G.H., JENKINS, B., PORTER, A.G. AND CAREY, N.H. (1980). Influenza antigenic determinants are expressed from haemagglutinin genes cloned in *Escherichia coli*. *Nature* **283**, 171–174.

EMTAGE, J.S., ANGAL, S., DOEL, M.T., HARRIS, T.J.R., JENKINS, B., LILLEY, G. AND LOWE, P.A. (1983). Synthesis of calf prochymosin (prorennin) in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **80**, 3671–3675.

ENGER-VALK, B.E., HEYNEKER, H.L., OOSTERBAAN, R.A. AND POUWELS, P.H. (1980). Construction of new cloning vehicles with genes of the tryptophan operon of *Escherichia coli* as genetic markers. *Gene* **9**, 69–85.

FIANDT, M., SZYBALSKI, W. AND IMAMOTO, F. (1974). Physical mapping of the *trp* endpoint in the N-t_I segment of λtrpE-A. *Virology* **61**, 312–314.

FUJISAWA, Y., ITO, Y., SASADA, R., ONO, Y., IGARASHI, K., MARUMOTO, R., KIKUCHI, M. AND SUGINO, Y. (1983). Direct expression of hepatitis B surface antigen gene in *E. coli*. *Nucleic Acids Research* **11**, 3581–3591.

FUJISAWA, Y., ITO, Y., IKEYAMA, S. AND KIKUCHI, M. (1985). Expression of hepatitis B virus surface antigen P31 gene in *Escherichia coli*. *Gene* **40**, 23–29.

FUKUI, T., YAMAGISHI, J.-I., FURUTANI, Y., NOTAKE, M., YAMAYOSHI, M., NOMURA, H., YAMADA, M. AND NAKAMURA, S. (1985). Efficient production of a human tumour necrosis factor in *Escherichia coli*. *Nucleic Acids Research Symposium Series* **16**, 273–276.

FURMAN, T.C., EPP, J., HSIUNG, H.M., HOSKINS, J., LONG, G.L., MENDELSOHN, L.G., SCHONER, B., SMITH, D.P. AND SMITH, M.C. (1987). Recombinant human insulin-like growth factor II expressed in *Escherichia coli*. *Bio/Technology* **5**, 1047–1051.

GELLERT, M., O'DEA, M.H., ITOH, T. AND TOMIZAWA, J.-I. (1976). Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proceedings of the National Academy of Sciences, USA* **73**, 4474–4478.

GELLERT, M., MIZUUCHI, K., O'DEA, M.H., ITOH, T. AND TOMIZAWA, J.-I. (1977). Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proceedings of the National Academy of Sciences, USA* **74**, 4774–4776.

GENTZ, R., LANGNER, A., CHANG, A.C.Y., COHEN, S.N. AND BUJARD, H. (1981). Cloning and analysis of strong promoters is made possible by the downstream placement of a RNA termination signal. *Proceedings of the National Academy of Sciences, USA* **78**, 4936–4940.

GERSHONI, J.M. (1987). Expression of the α-bungarotoxin binding site of the nicotinic acetylcholine receptor by *Escherichia coli* transformants. *Proceedings of the National Academy of Sciences, USA* **84**, 4318–4321.

GOEDDEL, D.V., SHEPARD, H.M., YELVERTON, E., LEUNG, D., CREA, R., STOMA, A. AND PESTKA, S. (1980a). Synthesis of human fibroblast interferon by *E. coli*. *Nucleic Acids Research* **8**, 4057–4074.

GOEDDEL, D.V., YELVERTON, E., ULLRICH, A., HEYNECKER, H.L., MIOZZARI, G., HOLMES, W., SEEBURG, P.H., DULL, T., MAY, L., STEBBING, N., CREA, R., MAEDA, S., MCCANDLISS, R., SLOMA, A., TABOR, J.M., GROSS, M., FAMILLETTI, P.C. AND PESTKA, S. (1980b). Human leukocyte interferon produced by *E. coli* is biologically active. *Nature* **287**, 411–416.

GRAY, G.L., SMITH, D.H., BALDRIDGE, J.S., HARKINS, R.N., VASIL, M.L., CHEN, E.Y. AND HEYNECKER, H.L. (1984). Cloning, nucleotide sequence, and expression in *Escherichia coli* of the exotoxin A structural gene of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences, USA* **81**, 2645–2649.

GRAY, P.W., LEUNG, D.W., PENNICA, D., YELVERTON, E., NAJARIAN, R., SIMONSEN, C.C., DERYNCK, R., SHERWOOD, P.J., WALLACE, D.M., BERGER, S.L., LEVINSON, A.D. AND GOEDDEL, D.V. (1982). Expression of human interferon cDNA in *E. coli* and monkey cells. *Nature* **295**, 503–508.

GROSSMANN, A.D., ERICKSON, J.W. AND GROSS, C.A. (1984). The *htpR* gene product of *E. coli* is a sigma factor for heat shock promoters. *Cell* **38**, 383–390.

GROSSMANN, A.D., TAYLOR, W.E., BURTON, Z.F., BURGESS, R.R. AND GROSS, C.A. (1985). Stringent response in *Escherichia coli* induces expression of heat shock proteins. *Journal of Molecular Biology* **186**, 357–365.

GRUNDSTRÖM, T., VON GABAIN, A., NILSSON, G., ANDERSSON, M., LUNDSTRÖM, M., LUND, B. AND LUNDGREN, E. (1987). Expression of an interferon-α gene variant in *E. coli* using tandemly repeated synthetic ribosomal binding sites. *DNA* **6**, 41–46.

GUNSALUS, R.P. AND YANOFSKY, C. (1980). Nucleotide sequence and expression of *Escherichia coli trpR*, the structural gene for *trp* aporepressor. *Proceedings of the National Academy of Sciences, USA* **77**, 7117–7121.

GUNSALUS, R.P., MIGUEL, A.G. AND GUNSALUS, G.L. (1986). Intracellular Trp repressor levels in *Escherichia coli*. *Journal of Bacteriology* **167**, 272–278.

HALLEWELL, R.A. AND EMTAGE, S. (1980). Plasmid vectors containing the tryptophan operon promoter suitable for efficient regulated expression of foreign genes. *Gene* **9**, 27–47.

HARLEY, C.B. AND REYNOLDS, R.P. (1987). Analysis of *E. coli* promoter sequences. *Nucleic Acids Research* **15**, 2343–2361.

HAWLEY, D.K. AND MCCLURE, W.R. (1983). Compilation and analysis of *Escherichia coli* promoter DNA sequences. *Nucleic Acids Research* **11**, 2237–2255.

HAYDOCK, P.V. (1983). *Purification of Trp Repressor Protein of* Escherichia coli. *Physical and Immunochemical Characterization*. PhD thesis, Purdue University. 124 pp.

HAYDOCK, P.V. AND SOMERVILLE, R.L. (1984). Trp holorepressor–*trp* operator interaction studied by protein distribution analysis. *Biochemical and Biophysical Research Communications* **119**, 926–932.

HELINSKI, D.R. HERSHFIELD, V., FIGURSKI, D. AND MEYER, R. (1977). Construction and properties of plasmid cloning vehicles. In *Recombinant Molecules: Impact on Science and Society* (R.F. Beers and E.G. Bassett, Eds), pp. 151–165. Raven Press, New York.

HERRIN, G.L., JR AND BENNETT, G.N. (1986). The effect of nalidixic acid on expression from related *E. coli* promoters. *Biochemical and Biophysical Research Communications* **135**, 411–418.

HERSHFIELD, V., BOYER, H.W., YANOFSKY, C., LOVETT, M.A. AND HELINSKI, D.R. (1974). Plasmid ColE1 as a molecular vehicle for cloning and amplification of DNA. *Proceedings of the National Academy of Sciences, USA* **71**, 3455–3459.

HIMMLER, A., HAUPTMANN, R., ADOLF, G.R. AND SWETLY, P. (1986). Molecular cloning and expression in *Escherichia coli* of equine type I interferons. *DNA* **5**, 345–356.

HOOPES, B.C. AND MCCLURE, W.R. (1987). Strategies in regulation of transcription initiation. In *Escherichia coli* and *Salmonella typhimurium*. *Cellular and Molecular*

Biology, Vol. 2 (F.C. Neidhardt, Ed.), pp. 1231–1240. American Society for Microbiology, Washington, DC.

HOPKINS, A.S., MURRAY, N.E. AND BRAMMAR, W.J. (1976). Characterization of λ-*trp* transducing bacteriophages made *in vitro*. *Journal of Molecular Biology* **107**, 549–569.

HOROWITZ, H. AND PLATT, T. (1982). Regulation of transcription from tandem and convergent promoters. *Nucleic Acids Research* **10**, 5447–5465.

IKEHARA, M., OHTSUKA, E., TOKUNAGA, T., TANIYAMA, Y., IWAI, S., KITANO, K., MIYAMOTO, S., OHGI, T., SAKURAGAWA, Y., FUJIYAMA, K., IKARI, T., KIBAYASHI, M., MIYAKE, T., SHIBAHARA, S., ONO, A., UEDA, T., TANAKA, T., BABA, H., MIKI, T., SAKURAI, A., OISHI, T., CHISAKA, O., AND MATSUBARA, K. (1984). Synthesis of a gene for human growth hormone and its expression in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **81**, 5956–5960.

IMAI, T., CHO, T., TAKAMATSU, H., HORI, H., SAITO, M., MASUDA, T., HIROSE, S. AND MURAKAMI, K. (1986). Synthesis and characterization of human prorenin in *Escherichia coli*. *Journal of Biochemistry* **100**, 425–432.

IMANAKA, T. AND AIBA, S. (1981). A perspective on the application of genetic engineering: stability of recombinant plasmid. *Annals of the New York Academy of Sciences* **369**, 1–14.

IMANAKA, T., TSUNEKAWA, H. AND AIBA, S. (1980). Phenotypic stability of *trp* operon recombinant plasmids in *Escherichia coli*. *Journal of General Microbiology* **118**, 253–261.

INAOKA, T., MIURA, K. AND OHTSUKA, E. (1986). Chemical synthesis of the T4 endonuclease V gene and its expression in *Escherichia coli*. *Nucleic Acids Research Symposium Series* **16**, 105–108.

ITOH, S., MIZUKAMI, T., MATSUMOTO, T., NISHI, T., SAITO, A., OKA, T., FURUYA, A., TAKAOKA, C. AND TANIGUCHI, T. (1984). Efficient expression in *Escherichia coli* of a mature and a modified human interferon-β1. *DNA* **3**, 157–165.

ITOH, M., AIBA, H., INOKUCHI, K., MIZUNO, T., NAGAHARI, K., MUNAKATA, K. AND MIZUSHIMA, S. (1986). A controllable expression–secretion vector constructed from the multiple *trp* promoter–operator, the signal peptide region of the *ompF* gene and the *trpR* gene in *Escherichia coli*. *Agricultural and Biological Chemistry* **50**, 1295–1302.

IWANE, M., KUROKAWA, T., SASADA, R., SONO, M., NAKAGAWA, S. AND IGARASHI, K. (1987). Expression of cDNA encoding basic fibroblast growth factor in *E. coli*. *Biochemical and Biophysical Research Communications* **146**, 470–477.

JACKSON, E.N. AND YANOFSKY, C. (1972). Internal promoter of the tryptophan operon of *Escherichia coli* is located in a structural gene. *Journal of Molecular Biology* **69**, 307–313.

JOACHIMIAK, A., KELLEY, R.L., GUNSALUS, R.P., YANOFSKY, C. AND SIGLER, P.B. (1983a). Purification and characterization of *trp* aporepressor. *Proceedings of the National Academy of Sciences, USA* **80**, 668–672.

JOACHIMIAK, A., SCHEVITZ, R.W., KELLEY, R.L., YANOFSKY, C. AND SIGLER, P.B. (1983b). Functional inferences from crystals of *Escherichia coli trp* repressor. *Journal of Biological Chemistry* **258**, 12641–12643.

JOACHIMIAK, A., MARMORSTEIN, R.Q., SCHEVITZ, R.W., MANDECKI, W., FOX, J.L. AND SIGLER, P.B. (1987). Crystals of the *trp* repressor–operator complex suitable for X-ray diffraction analysis. *Journal of Biological Chemistry* **262**, 4917–4921.

JOHNSON, D.I. AND SOMERVILLE, R.L. (1985). Controlled high-level expression of genes directed by the *trp* promoter-operator of *Escherichia coli*. *Developments in Industrial Microbiology* **26**, 87–94.

JOHNSRUD, L. (1978). Contacts between *Escherichia coli* RNA polymerase and a *lac* operon promoter. *Proceedings of the National Academy of Sciences, USA* **75**, 5314–5318.

KAJITANI, M. AND ISHIHAMA, A. (1983a). Determination of the promoter strength in the mixed transcription system: promoters of lactose, tryptophan and ribosomal protein L10 operons from *Escherichia coli*. *Nucleic Acids Research* **11**, 671–686.

KAJITANI, M. AND ISHIHAMA, A. (1983b). Determination of the promoter strength in the mixed transcription system, II. Promoters of ribosomal RNA ribosomal protein S1 and *recA* protein operons from *Escherichia coli*. *Nucleic Acids Research* **11**, 3873–3888.

KAJITANI, M. AND ISHIHAMA, A. (1984). Promoter selectivity of *Escherichia coli* RNA polymerase. Differential stringent control of the multiple promoters from ribosomal RNA and protein operons. *Journal of Biological Chemistry* **259**, 1951–1957.

KELLEY, R.L. AND YANOFSKY, C. (1982). *trp* repressor production is controlled by autogenous regulation and inefficient translation. *Proceedings of the National Academy of Sciences, USA* **79**, 3120–3124.

KELLEY, R.L. AND YANOFSKY, C. (1985). Mutational studies with the *trp* repressor of *Escherichia coli* support the helix-turn-helix model of repressor recognition of operator DNA. *Proceedings of the National Academy of Sciences, USA* **82**, 483–487.

KENTEN, J., HELM, B., ISHIZAKA, T., CATTINI, P. AND GOULD, H. (1984). Properties of a human immunoglobulin E-chain fragment synthesized in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **81**, 2955–2959.

KIKUCHI, N., NAGATA, K., HORII, T., MIYAZAKI, S., SHIN, M., TAKIMOTO, N., TSURUTA, Y., TAMAKI, M., TERAOKA, H. AND YOSHIDA, N. (1987). Production of recombinant human pancreatic secretory trypsin inhibitor by *Escherichia coli*. *Journal of Biochemistry (Tokyo)* **102**, 607–612.

KIKUMOTO, Y., HONG, Y.-M., NISHIDA, T., NAKAI, S., MASUI, Y. AND HIRAI, Y. (1987). Purification and characterization of human interleukin-1β produced in *Escherichia coli*. *Biochemical and Biophysical Research Communications* **147**, 315–321.

KIM, S.H. AND RYU, D.D.Y. (1984). Instability kinetics of *trp* operon plasmid ColE1-*trp* in recombinant *Escherichia coli* MV12 (pVH5) and MV12 *trpR* (pVH5). *Biotechnology and Bioengineering* **26**, 497–502.

KLEID, D.G., YANSURA, D., SMALL, B., DOWBENKO, D., MOORE, D.M., GRUBMAN, M.J., MCKERCHER, P.D., MORGAN, D.O., ROBERTSON, B.H. AND BACHRACH, H.L. (1981). Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. *Science* **214**, 1125–1129.

KLEMPNAUER, K.-H., RAMSAY, G., BISHOP, J.M., MOSCOVICI, M.G., MOSCOVICI, C., MCGRATH, J.P. AND LEVINSON, A.D. (1983). The product of the retroviral transforming gene *v-myb* oncogene *c-myb*. *Cell* **33**, 345–355.

KLIG, L.S., CRAWFORD, I.P. AND YANOFSKY, C. (1987). Analysis of *trp* repressor–operator interaction by filter binding. *Nucleic Acids Research* **15**, 5339–5351.

KOS, A., BROEKHUISJEN, M.P., VAN GORCOM, R.F.M., VAN ROTTERDAM, J., ENGER-VALK, B.E., VAN PUTTEN, A.J., VELTKAMP, E., VAN DEN HONDEL, C.A.M.J.J. AND POUWELS, P.H. (1984). Construction of vectors suitable for efficient expression of genes in *E. coli*. In *Innovations in Biotechnology* (E.H. Houwink and R.R. van der Meer, Eds), pp. 415–424. Elsevier Science Publishers, Amsterdam.

KUBO, M., KANO, Y., NAKAMURA, H., NAGATA, A. AND IMAMOTO, F. (1979). *In vivo* enhancement of general and specific transcription in *Escherichia coli* by DNA gyrase activity. *Gene* **7**, 153–171.

KUMAMOTO, A.A., MILLER, W.G. AND GUNSALUS, R.P. (1987). *Escherichia coli* tryptophan repressor binds multiple sites within the *aroH* and *trp* operators. *Genes & Development* **1**, 556–564.

KURODA, M.I. AND YANOFSKY, C. (1984). Correction of the nucleotide sequence of the *Citrobacter freundii* tryptophan operon regulatory region. *Journal of Bacteriology* **159**, 1063–1064.

KUROKAWA, T., SENO, M., SASADA, R., ONO, Y., ONDA, H., IGARASHI, K., KIKUCHI, M., SUGINO, Y. AND HONJO, T. (1983). Expression of human immunoglobulin E ϵ chain cDNA in *E. coli*. *Nucleic Acids Research* **11**, 3077–3085.

KUWANA, Y., ITOH, S., NAGASE, F., NAKASHIMA, I. AND KUROSAWA, Y. (1987). Production of the constant domain of murine T-cell receptor β-chain in *Escherichia coli*. *FEBS Letters* **219**, 360–364.

KUWANO, M. AND IMAMOTO, F. (1976). Effect of stringent and relaxed control on transcription of the tryptophan operon from the P_{trp} promoter and the P_L promoter in λtrp phage. *Biochimica et biophysica acta* **454**, 504–513.

LANE, A.N. (1986). The interaction of the trp repressor from *Escherichia coli* with L-tryptophan and indole propanoic acid. *European Journal of Biochemistry* **157**, 405–413.

LANGLEY, K.E., BERG, T.F., STRICKLAND, T.W., FENTON, D.M., BOONE, T.C. AND WYPYCH, J. (1987). Recombinant-DNA-derived bovine growth hormone from *Escherichia coli*. I. Demonstration that the hormone is expressed in reduced form, and isolation of the hormone in oxidized, native form. *European Journal of Biochemistry* **163**, 313–321.

LAVALLÉ, R. AND HAUWER, G. DE (1968). Messenger RNA synthesis during amino acid starvation in *Escherichia coli*. *Journal of Molecular Biology* **37**, 269–288.

LAWN, R.M., ADELMAN, J., BOCK, S.C., FRANKE, A.E., HOUCH, D.M., NAJARIAH, R.C., SEEBURG, P.H. AND WION, K.L. (1981). The sequence of human serum albumin cDNA and its expression in *E. coli*. *Nucleic Acids Research* **9**, 6103–6114.

LEUNG, D.W., CAPON, D.J. AND GOEDDEL, D.V. (1984). The structure and bacterial expression of three distinct bovine interferon-β genes. *Bio/Technology* **2**, 458–464.

MCCLURE, W.R. (1985). Mechanism and control of transcription initiation in prokaryotes. *Annual Review of Biochemistry* **54**, 171–204.

MACCONNELL, W.P. AND VERMA, I.M. (1983). Expression of FBJ-MSV oncogene (*fos*) product in bacteria. *Virology* **131**, 367–374.

MCGRATH, J.P. AND LEVINSON, A.D. (1982). Bacterial expression of an enzymatically active protein encoded by RSV *src* gene. *Nature* **295**, 423–425.

MCGRATH, J.P., CAPON, D.J., GOEDDEL, D.V. AND LEVINSON, A.D. (1984). Comparative biochemical properties of normal and activated human *ras* p21 protein. *Nature* **310**, 644–649.

MCKENNEY, K., SHIMATAKE, H., COURT, D., SCHMEISSNER, U., BRADY, C. AND ROSENBERG, M. (1981). A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase. In *Gene Amplification and Analysis, Vol. II* (J.G. Chirikjian and T.S. Papas, Eds), pp. 383–415. Elsevier/North Holland, New York.

MARMORSTEIN, R.Q., JOACHIMIAK, A., SPRINZL, M. AND SIGLER, P. (1987). The structural basis for the interaction between L-tryptophan and the *Escherichia coli trp* aporepressor. *Journal of Biological Chemistry* **262**, 4922–4927.

MARQUIS, D.M., SMOLEC, J.M. AND KATZ, D.H. (1986). Use of a portable ribosome-binding site for maximizing expression of a eukaryotic gene in *Escherichia coli*. *Gene* **42**, 175–183.

MARSTON, F.A.O. (1986). The purification of eukaryotic polypeptides synthesized in *Escherichia coli*. *Biochemical Journal* **240**, 1–12.

MARTIAL, J.A., HALLEWELL, R.A., BASTER, J.D. AND GOODMAN, H.M. (1979). Human growth hormone: Complementary DNA cloning and expression in bacteria. *Science* **205**, 602–707.

MASUDA, T., NAKANO, E., HIROSE, S. AND MURAKAMI, K. (1986). Synthesis of enzymatically active mouse submandibular gland renin in *Escherichia coli*. *Agricultural and Biological Chemistry* **50**, 271–279.

MATSUI, T., HIRANO, M., NAOE, T., YAMADA, K. AND KUROSAWA, Y. (1987). Production of chimeric protein coded by the fused viral H-*ras* and human N-*ras* genes in *Escherichia coli*. *Gene* **52**, 215–223.

MAXAM, A.M. AND GILBERT, W. (1977). A new method for sequencing DNA. *Proceedings of the National Academy of Sciences, USA* **74**, 560–564.

MEACOCK, P.M. AND COHEN, S.C. (1980). Partitioning of bacterial plasmids during cell division: a *cis*-acting locus that accomplishes stable plasmid inheritance. *Cell* **20**, 529–542.

MIOZZARI, G.F. AND YANOFSKY, C. (1978a). Translation of the leader region of the *Escherichia coli* tryptophan operon. *Journal of Bacteriology* **133**, 1457–1466.

MIOZZARI, G.F. AND YANOFSKY, C. (1978b). The regulatory region of the *trp* operon of *Serratia marcescens*. *Nature* **276**, 684–689.

MIOZZARI, G. AND YANOFSKY, C. (1978c). Naturally occurring promoter down mutation: nucleotide sequence of the *trp* promoter/operator/leader region of *Shigella dysenteriae* 16. *Proceedings of the National Academy of Sciences, USA* **75**, 5580–5584.

MIZUNO, K., OHSUYE, K., WADA, Y., FUCHIMURA, K., TANAKA, S. AND MATSUO, H. (1987). Cloning and sequence of cDNA encoding a peptide *C*-terminal α-amidating enzyme from *Xenopus laevis*. *Biochemical and Biophysical Research Communications* **148**, 546–552.

MORROW, C.D., WARREN, B. AND LENTZ, M.R. (1987). Expression of enzymatically active poliovirus RNA-dependent RNA polymerase in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **84**, 6050–6054.

MORSE, D.E. AND MORSE, A.N.C. (1976). Dual-control of the tryptophan operon is mediated by both tryptophanyl-tRNA synthetase and the repressor. *Journal of Molecular Biology* **103**, 209–226.

MORSE, D.E. AND YANOFSKY, C. (1969). Amber mutants of the *trpR* regulatory gene. *Journal of Molecular Biology* **44**, 185–193.

MULLIGAN, M.E., HAWLEY, D.K., ENTRIKEN, R. AND MCCLURE, R.W. (1984). *Escherichia coli* promoter sequences predict *in vitro* RNA polymerase selectivity. *Nucleic Acids Research* **12**, 789–800.

NAGAHARI, K., TANAKA, T., HISHINUMA, F., KURODA, M. AND SAKAGUCHI, K. (1977). Control of the tryptophan synthetase amplified by varying the numbers of composite plasmids in *Escherichia coli* cells. *Gene* **1**, 141–152.

NAGAHARI, K., HISHIDA, T., NIKI, T., MUNAKATA, K. AND KANEKO, K. (1987). High-level production of human β-endorphin in *Escherichia coli*. *Agricultural and Biological Chemistry* **51**, 845–851.

NAGASE, Y., NAKAMURA, N., TOHYAMA, J., WATANABE, S., OGINO, H., HORIKOSHI, K., NII, A., SOMA, M., NOBUHARA, M., SUZUKI, Y. AND MOCHIDA, E. (1983). Chemical synthesis of a human fibroblast interferon gene and its expression in *Escherichia coli*. *Nucleic Acids Research, Symposium Series* **12**, 83–86.

NAKAMURA, K. AND INOUYE, M. (1979) DNA sequence of the gene for the outer membrane lipoprotein of *E. coli*: an extremely A+T-rich promoter. *Cell* **18**, 1109–1117.

NAKANO, E. AND MASUDA, T. (1982). Construction of 'sleeper' cloning vehicles for enzyme overproduction. *Agricultural and Biological Chemistry* **46**, 313–315.

NEIDHARDT, F.C. (1987). Multigene systems and regulons. In Escherichia coli *and* Salmonella typhimurium. *Cellular and Molecular Biology, Vol. 2* (F.C. Neidhardt, Ed.), pp. 1313–1325. American Society for Microbiology, Washington, DC.

NICHOLS, B.P. AND YANOFSKY, C. (1983). Plasmids containing the *trp* promoters of *Escherichia coli* and *Serratia marcescens* and their use in expressing cloned genes. *Methods in Enzymology* **101**, 155–164.

NISHI, T. AND ITOH, S. (1986). Enhancement of transcriptional activity of the *Escherichia coli trp* promoter by upstream A+T-rich regions. *Gene* **44**, 29–36.

NISHI, T., SATO, M., SAITO, A., ITOH, S., TAKAOKA, C. AND TANIGUCHI, T. (1983). Construction and application of a novel plasmid 'ATG vector' for direct expression of foreign genes in *Escherichia coli*. *DNA* **4**, 265–273.

NISHI, T., SAITO, A., OKA, T., ITOH, S., TAKAOKA, C. AND TANIGUCHI, T. (1984). Construction of plasmid vectors carrying the *Escherichia coli* tryptophan promoter. *Agricultural and Biological Chemistry* **48**, 669–675.

NISHI, T., FUJITA, T., NISHI-TAKAOKA, C., SAITO, A., MATSUMOTO, T., SATO, M., OKA, T., ITOH, S., YIP, Y.K., VILCEK, J. AND TANIGUCHI, T. (1985). Cloning and expression of a novel variant of human interferon-γ cDNA. *Journal of Biochemistry (Tokyo)* **97**, 153–159.

NISHIMORI, K., SHIMIZU, N., KAWAGUCHI, Y., HIDAKA, M., UOZUMI, T. AND BEPPU, T. (1984). Expression of cloned calf prochymosin cDNA under control of the tryptophan promoter. *Gene* **29**, 41–49.

NIWA, M., SATO, S., SAITO, Y., UCHIYAMA, F., ONO, H., YAMASHITA, W., KITAGUCHI, T., SHIGA, Y., NOTANI, J., YAMADA, H., ISHII, Y., UEDA, I. AND TAKAGI, Y. (1986). Chemical synthesis, cloning, and expression of genes for human somatomedin C (insulin-like growth factor I) and ^{59}Val-somatomedin C. *Annals of the New York Academy of Sciences* **469**, 31–52.

NOBUHARA, M., KANAMORI, T., NAGASE, Y., NII, A., MORISHITA, H., TOHYAMA, J., ANDOH, S. AND KURIMOTO, M. (1986). The expression of human tumor necrosis factor in *E. coli*. *Nucleic Acids Research Symposium Series* **17**, 131–134.

OKA, T., SUMI, S., FUWA, T., YODA, K., YAMASAKI, M., TAMURA, G. AND MIYAKE, T. (1987). Efficacies of different secretion vectors for secretion of human epidermal growth factor by *Escherichia coli*. *Agricultural and Biological Chemistry* **51**, 1099–1104.

OPPENHEIM, D.S. AND YANOFSKY, C. (1980). Functional analysis of wild-type and altered tryptophan operon promoters of *Salmonella typhimurium* and *Escherichia coli*. *Journal of Molecular Biology* **144**, 143–161.

OPPENHEIM, D.S., BENNETT, G.N. AND YANOFSKY, C. (1980). *Escherichia coli* RNA polymerase and *trp* repressor interaction with the promoter–operator region of the tryptophan operon of *Salmonella typhimurium*. *Journal of Molecular Biology* **144**, 133–142.

OSHEROFF, N., SHELTON, E.R., OKAYAMA, H., BRUTLAG, D. AND BERG, P. (1982). A cloning vector which permits controlled expression of eukaryotic cDNAs in *E. coli*. *Abstracts of the 12th International Congress of Biochemistry, Perth*, p. 240.

PABO, C.O. AND SAUER, R.T. (1984). Protein–DNA recognition. *Annual Review of Biochemistry* **53**, 293–321.

PALUH, J.L. AND YANOFSKY, C. (1986). High level production and rapid purification of the *E. coli trp* repressor. *Nucleic Acids Research* **14**, 7851–7860.

PENNICA, D., HOLMES, W.E., KOHR, W.J., HARKINS, R.N., VEHAR, G.A., WARD, C.A., BENNETT, W.F., YELVERTON, E., SEEBURG, P.H., HEYNECKER, H.L. AND GOEDDEL, D.V. (1983). Cloning and expression of human tissue-type plasminogen activator cDNA in *E. coli*. *Nature* **301**, 214–221.

PENNICA, D., NEDWIN, G.E., HAYFLICK, J.S., SEEBURG, P.H., DERYNCK, R., PALLADINO, M.A., KOHR, W.J., AGGARWAL, B.B. AND GOEDDEL, D.V. (1984). Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* **312**, 724–729.

PLASKON, R.R. AND WARTELL, R.M. (1987). Sequence distributions associated with DNA curvature are found upstream of strong *E. coli* promoters. *Nucleic Acids Research* **15**, 785–796.

POLISKY, B. (1986). Replication control of the ColE1-type plasmids. In *Maximizing Gene Expression* (W. Reznikoff and L. Gold, Eds), pp. 143–170. Butterworths, Stoneham, MA.

PORTER, A.G., BELL, L.D., ADAIR, J., CATLIN, G.H., CLARKE, J., DAVIES, J.A., DAWSON, K., DERBYSHIRE, R., DOEL, S.M., DUNTHORNE, L., FINLEY, M., HALL, J., HOUGHTON, M., HYNES, C., LINDLEY, I., NUGENT, M., O'NEILL, G.J., SMITH, J.C., STEWART, A., TACON, W., VINEY, J., WARBURTON, N., BOSELY, P.G. AND MCCULLAGH, K.G. (1986). Novel modified β-interferons: Gene cloning, expression, and biological activity in bacterial extracts. *DNA* **5**, 137–148.

POUWELS, P.H. AND VAN ROTTERDAM, J. (1975). *In vitro* synthesis of the enzymes of the tryptophan operon of *Escherichia coli*. Evidence for positive control of transcription. *Molecular and General Genetics* **136**, 215–226.

POUWELS, P.H., ENGER-VALK, B.E. AND BRAMMAR, W.J. (1985). *Cloning Vectors. A Laboratory Manual.* Elsevier Science Publishers, Amsterdam. I-B-ii-1 through I-B-ii-8.

PRIMAKOFF, P. AND ARTZ, S.W. (1979). Positive control of *lac* operon expression *in vitro* by guanosine 5′-diphosphate 3′-diphosphate. *Proceedings of the National Academy of Sciences, USA* **76**, 1726–1730.

PRIVALSKY, M.L., SEALY, L., BISHOP, J.M., MCGRATH, J.P. AND LEVINSON, A.D. (1983). The product of the avian erythroblastosis virus *erbB* locus is a glycoprotein. *Cell* **32**, 1257–1267.

PUMPEN, P., KOZLOVSKAYA, T.M., BORISOVA, G.P., BICHKO, V.V., DISHLER, A., KALIS, J., KUKAINE, R.A. AND GREN, E.J. (1984). Expression of hepatitis B virus surface antigen gene in *Escherichia coli. Gene* **30**, 201–210.

REZNIKOFF, W.S. AND MCCLURE, W.R. (1986). *E. coli* promoters. In *Maximizing Gene Expression* (W. Reznikoff and L. Gold, Eds), pp. 1–33. Butterworths, Stoneham, MA.

RIGGS, D.L., MUELLER, R.D., KWAN, H.-S. AND ARTZ, S.W. (1986). Promoter domain mediates guanosine tetraphosphate activation of the histidine operon. *Proceedings of the National Academy of Sciences, USA* **83**, 9333–9337.

RINK, H., LIERSCH, M., SIEBER, P. AND MEYER, F. (1984). A large fragment approach to DNA synthesis: total synthesis of a gene for the protease inhibitor eglin C from the leech *Hirudo medicinalis* and its expression in *E. coli. Nature New Biology* **12**, 6369–6387.

ROEDER, W. AND SOMERVILLE, R.L. (1979). Cloning the *trpR* gene. *Molecular and General Genetics* **176**, 361–368.

ROSE, J.K. AND SHAFFERMAN, A. (1981). Conditional expression of the vesicular stomatitis virus glycoprotein gene in *Escherichia coli. Proceedings of the National Academy of Sciences, USA* **78**, 6670–6674.

ROSENBERG, M. AND COURT, D. (1979). Regulatory sequences involved in the promotion and termination of transcription. *Annual Review of Genetics* **13**, 319–353.

ROSENBERG, S.A., GRIMM, E.A., MCGROGAN, M., DOYLE, M., KAWASACKI, E., KOTHS, K. AND MARK, D.F. (1984). Biological activity of recombinant human interleukin-2 produced in *Escherichia coli. Science* **223**, 1412–1415.

ROSTECK, P.R. AND HERSHBERGER, C.L. (1983). Selective retention of recombinant plasmids coding for human insulin. *Gene* **25**, 29–38.

RUSSELL, D.R. AND BENNETT, G.N. (1982b). Construction and analysis of *in vivo* activity of *E. coli* promoter hybrids and promoter mutants that alter the −35 to −10 spacing. *Gene* **20**, 231–243.

RUSSELL, D.R. AND BENNETT, G.N. (1982a). Cloning of small DNA fragments containing the *Escherichia coli* tryptophan operon promoter and operator. *Gene* **17**, 9–18.

RUSSELL, D.R., AUGER, E.A., VERMERSCH, P.S. AND BENNETT, G.N. (1984). Role of DNA regions flanking the tryptophan promoter of *Escherichia coli*. I. Insertion of synthetic oligonucleotides. *Gene* **32**, 337–348.

SAITO, Y., YAMADA, H., NIWA, M. AND UEDA, I. (1987a). Production and isolation of recombinant somatomedin C. *Journal of Biochemistry* **101**, 123–134.

SAITO, Y., ISHII, Y., NIWA, M. AND UEDA, I. (1987b). Direct expression of a synthetic somatomedin C gene in *Escherichia coli* by use of a two-cistron system. *Journal of Biochemistry* **101**, 1281–1288.

SAITO, Y., ISHII, Y., KOYAMA, S., TSUJI, K., YAMADA, H., TERAI, T., KOBAYASHI, M., ONO, T., NIWA, M., UEDA, I. AND KIKUCHI, H. (1987c). Bacterial synthesis of recombinant α-human atrial natriuretic polypeptide. *Journal of Biochemistry* **102**, 111–122.

SAMSON, S.M., BELAGAJE, R., BLANKENSHIP, D.T., CHAPMAN, J.L., PERRY, D., SKATRUD, P.O., VAN FRANK, R.M., ABRAHAM, E.P., BALDWIN, J.E., QUEENER, S.W. AND INGOLIA, T.D. (1985). Isolation, sequence determination

and expression in *Escherichia coli* of the isopenicillin N synthetase gene from *Cephalosporium acremonium*. *Nature* **318**, 191–194.

SANGER, F., NICKLEN, S. AND COULSON, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* **74**, 5463–5467.

SATO, T., MATSUI, H., SHIBAHARA, S., KOBAYASHI, T., MORINAGA, Y., KASHIMA, N., YAMASAKI, S., HAMURO, J. AND TANIGUCHI, T. (1987). New approaches for the high-level expression of human interleukin-2 cDNA in *Escherichia coli*. *Journal of Biochemistry (Tokyo)* **101**, 525–534.

SCHEVITZ, R.W., OTWINOWSKI, Z., JOACHIMIAK, A., LAWSON, C.L. AND SIGLER, P.B. (1985). The three-dimensional structure of *trp* repressor. *Nature* **317**, 782–786.

SCHOEMAKER, J.M., BRASNETT, A.H. AND MARSTON, F.A.O. (1985). Examination of calf prochymosin accumulation in *Escherichia coli*: disulphide linkages are a structural component of prochymosin-containing inclusion bodies. *EMBO Journal* **4**, 775–780.

SEEBURG, P.H., SIAS, S., ADELMAN, J., DE BOER, H.A., HAYFLICK, J., JHURANI, P., GOEDDEL, D.V. AND HEYNEKER, H.L. (1983). Efficient expression of bovine and porcine growth hormones. *DNA* **2**, 37–45.

SEKINE, S., MIZUKAMI, T., NISHI, T., KUWANA, Y., SAITO, A., SATO, M. AND KAWAUCHI, H. (1985). Cloning and expression of cDNA for salmon growth hormone in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **82**, 4306–4310.

SENO, M., HINUMA, S., ONDA, H. AND IGARASHI, K. (1986). A hybrid protein between IFN-γ and IL-2. *FEBS Letters* **199**, 187–192.

SHEPARD, H.M., LEUNG, D., STEBBING, N. AND GOEDDEL, D.V. (1981). A single amino acid change in IFN-β_1 abolishes its antiviral activity. *Nature* **294**, 563–565.

SHEPARD, H.M., YELVERTON, E. AND GOEDDEL, D.V. (1982). Increased synthesis in *E. coli* of fibroblast and leukocyte interferons through alterations in ribosome binding sites. *DNA* **1**, 125–131.

SHIRAKAWA, M., TSURIMOTO, T. AND MATSUBARA, K. (1984). Plasmid vectors designed for high-efficiency expression controlled by the portable *recA* promoter-operator of *Escherichia coli*. *Gene* **28**, 127–132.

SIEBENLIST, U., SIMPSON, R.B. AND GILBERT, W. (1980). *E. coli* RNA polymerase interacts homologously with two different promoters. *Cell* **20**, 269–281.

SINGLETON, C.K., ROEDER, W.D., BOGOSIAN, G., SOMERVILLE, R.L. AND WEITH, H.L. (1980). DNA sequence of the *E. coli trpR* gene and prediction of the amino acid sequence of Trp repressor. *Nucleic Acids Research* **8**, 1551–1560.

SKOGMAN, S.G. AND NILSSON, J. (1984). Temperature-dependent retention of a tryptophan-operon-bearing plasmid in *Escherichia coli*. *Gene* **31**, 117–122.

SKOGMAN, G., NILSSON, J. AND GUSTAFSSON, P. (1983). The use of a partition locus to increase stability of tryptophan-operon-bearing plasmids in *Escherichia coli*. *Gene* **23**, 105–115.

SMITH, C.L., KUBO, M. AND IMAMOTO, F. (1978). Promoter-specific inhibition of transcription by antibiotics which act on DNA gyrase. *Nature* **275**, 420–423.

SOMERVILLE, R.L. (1983). Tryptophan: biosynthesis, regulation, and large-scale production. In *Amino Acids: Biosynthesis and Genetic Regulation* (K.M. Herrmann and R.L. Somerville, Eds), pp. 351–378. Addison-Wesley Publishing Co., Reading, MA.

SPINDLER, K.R., ROSSER, D.S.E. AND BERK, A.J. (1984). Analysis of adenovirus transforming proteins from early regions 1A and 1B with antisera to inducible fusion antigens produced in *Escherichia coli*. *Journal of Virology* **49**, 132–141.

SQUIRES, C.L., LEE, F. AND YANOFSKY, C. (1975). Interaction of the *trp* repressor and RNA polymerase with the *trp* operon. *Journal of Molecular Biology* **92**, 93–111.

SQUIRES, C., LEE, F., BERTRAND, K., SQUIRES, C.L., BRONSON, M.J. AND YANOFSKY, C. (1976). Nucleotide sequence of the 5′ end of tryptophan messenger RNA of *Escherichia coli*. *Journal of Molecular Biology* **103**, 351–381.

STADEN, R. (1984). Computer methods to locate signals in nucleic acid sequences. *Nucleic Acids Research* **12**, 505–519.

STEWART, A.G., ADAIR, J.R., CATLIN, G., HYNES, C., HALL, J., DAVIES, J., DAWSON, K. AND PORTER, A.G. (1987). Chemical mutagenesis of human interferon-β: Construction, expression in *E. coli*, and biological activity of sodium bisulfite-induced mutations. *DNA* **6**, 119–128.

SVERDLOV, E.D., TSAREV, S.A., KRYKBAEV, R.A., CHERNOV, I.P. AND ROSTAPSHOV, V.M. (1987). *trp* operon induction during the expression in *E. coli* of two IFN-γ sequences. *FEBS Letters* **212**, 233–236.

SZOKA, P.R., SCHREIBER, A.B., CHAN, H. AND MURTHY, J. (1986). A general method for retrieving the components of a genetically engineered fusion protein. *DNA* **5**, 11–20.

TACON, W., CAREY, N. AND EMTAGE, S. (1980). The construction and characterization of plasmid vectors suitable for the expression of all DNA phases under the control of the *E. coli* tryptophan promoter. *Molecular and General Genetics* **177**, 427–438.

TACON, W.C.A., BONASS, W.A., JENKINS, B. AND EMTAGE, J.S. (1983). Expression plasmid vectors containing *Escherichia coli* tryptophan promoter transcriptional units lacking the attenuator. *Gene* **23**, 255–265.

TRUCKSIS, M. AND DEPEW, R. (1981). Identification and localization of a gene that specifies production of *Escherichia coli* DNA topoisomerase I. *Proceedings of the National Academy of Sciences, USA* **78**, 2164–2168.

TSAPAKOS, M.J., HAYDOCK, P.V., HERMODSON, M. AND SOMERVILLE, R.L. (1985). Ligand-mediated conformational changes in Trp repressor protein of *Escherichia coli* probed through limited proteolysis and the use of specific antibodies. *Journal of Biological Chemistry* **260**, 16383–16394.

TUNG, C.-S. AND HARVEY, S.C. (1987). A common structural feature in promoter sequences of *E. coli*. Nucleic Acids Research **15**, 4973–4985.

VIDAL-INGIGLIARDI, D. AND RAIBAUD, O. (1985). A convenient technique to compare the efficiency of promoters in *Escherichia coli*. *Nucleic Acids Research* **13**, 5919–5926.

WAHLE, E., MUELLER, K. AND ORR, E. (1984). Gene expression in a temperature-sensitive *gyrB* mutant of *Escherichia coli*. *EMBO Journal* **3**, 315–320.

WANG, A., LU, S.-D. AND MARK, D.F. (1984). Site-specific mutagenesis of the human interleukin-2 gene: Structure–function analysis of the cysteine residues. *Science* **224**, 1431–1433.

WARD, D.F. AND MURRAY, N.E. (1979). Convergent transcription in bacteriophage λ: Interference with gene expression. *Journal of Molecular Biology* **133**, 249–266.

WARNE, S.R., THOMAS, C.M., NUGENT, M.E. AND TACON, W.C.A. (1986). Use of a modified *Escherichia coli trpR* gene to obtain tight regulation of high-copy-number expression vectors. *Gene* **46**, 103–112.

WECK, P.K., APPERSON, S., STEBBING, N., GRAY, P.W., LEUNG, D., SHEPARD, H.M. AND GOEDDEL, D.V. (1981). Antiviral activities of hybrids of two major human leukocyte interferons. *Nucleic Acids Research* **9**, 6153–6166.

WHITEHORN, E.A., LIVAK, K.J. AND PETTEWAY, S.R., JR (1985). The effects of hybrid ribosome-binding-site variants on the expression of human interferon-β in *Escherichia coli*. *Gene* **36**, 375–379.

WINDASS, J.D., NEWTON, C.R., DE MAEYER-GUIGNARD, J., MORRE, V.E. AND EDGE, M.D. (1982). The construction of a synthetic *Escherichia coli trp* promoter and its use in the expression of a synthetic interferon gene. *Nucleic Acids Research* **10**, 6639–6657.

WOOD, C.R., BOSS, M.A., PATEL, T.P. AND EMTAGE, J.S. (1984). The influence of messenger RNA secondary structure on expression of an immunoglobulin heavy chain in *Escherichia coli*. *Nucleic Acids Research* **12**, 3937–3950.

YAMADA, M., FURUTANI, Y., NOTAKE, M., YAMAGISHI, J.-I., YAMAYOSHI, M., FUKUI, T., NOMURA, H., KOMIYA, M., JUWASHIMA, J., NAKANO, K., SOHMURA, Y. AND NAKAMURA, S. (1985). Efficient production of human tumour necrosis factor in *Escherichia coli*. *Journal of Biotechnology* **3**, 141–153.

YANG, H.-L., HELLER, K., GELLERT, M. AND ZUBAY, G. (1979). Differential sensitivity of gene expression *in vitro* to inhibitors of DNA gyrase. *Proceedings of the National Academy of Sciences, USA* **76**, 3304–3308.

YANOFSKY, C., PLATT, T., CRAWFORD, I.P., NICHOLS, B.P., CHRISTIE, G.E., HOROWITZ, H., VAN CLEEMPUT, M. AND WU, A.M. (1981). The complete nucleotide sequence of the tryptophan operon of *Escherichia coli*. *Nucleic Acids Research* **9**, 6647–6668.

YELVERTON, E., LEUNG, D., WECK, P., GRAY, P.W. AND GOEDDEL, D.V. (1981). Bacterial synthesis of a novel human leukocyte interferon. *Nucleic Acids Research* **9**, 731–741.

YELVERTON, E., NORTON, S., OBIJESKI, J.F. AND GOEDDEL, D.V. (1983). Rabies virus glycoprotein analogs: Biosynthesis in *Escherichia coli*. *Science* **219**, 614–620.

YUKAWA, H., KURUSU, Y., SHIMAZU, M., TERASAWA, M., OHTA, A. AND SHIBUYA, I. (1985). Stabilization by the mini-F fragment of a pBR322 derivative bearing the tryptophan operon in *Escherichia coli*. *Agricultural and Biological Chemistry* **49**, 3619–3622.

ZALKIN, H., YANOFSKY, C. AND SQUIRES, C. (1974). Regulated *in vitro* synthesis of *Escherichia coli* tryptophan operon messenger ribonucleic acid and enzymes. *Journal of Biological Chemistry* **249**, 465–475.

ZHANG, R.-G., JOACHIMIAK, A., LAWSON, C.L., SCHEVITZ, R.W., OTWINOWSKI, Z. AND SIGLER, P.B. (1987). The crystal structure of *trp* apoprepressor at 1.8Å shows how binding tryptophan enhances DNA affinity. *Nature* **327**, 591–597.