REVIEW ARTICLE

Attenuation in the control of expression of bacterial operons

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Bacterial operons concerned with the biosynthesis of amino acids are often controlled by a process of attenuation. The translation product of the initial segment of the transcript of each operon is a peptide rich in the amino acid that the particular operon controls. If the amino acid is in short supply translation is stalled at the relevant codons of the transcript long enough for the succeeding segment of the transcript to form secondary structures that allow the transcribing RNA polymerase molecule to proceed through a site that otherwise dictates termination of transcription. This site is the attenuator; the process is attenuation.

IT has long been known that RNA polymerase, with and without accessory factors, recognizes specific nucleotide sequences which signal the initiation and termination of transcription, and that this recognition is exploited in various ways in the regulation of gene expression. But during the past 10 years it has become evident that transcription termination sites can be located within as well as at the end of an operon. These internal termination sites are used in the regulation of transcription and may be used to sense features and components of metabolism that cannot be used to control initiation of transcription. This mechanism of regulation, called attenuation, can be defined as the regulation of gene expression by selective reduction of the transcription of distal portions of an operon. Regulatory studies with bacteriophage λ (refs 1–5) revealed the first case of attenuation. Two operons of this phage contain transcription termination sites which are used to control transcription of the structural genes located immediately beyond. Soon after these studies with λ a different mechanism of attenuation was discovered in the tryptophan[6] (*trp*) and histidine[7] (*his*) operons of bacteria and subsequently in several other operons of the enzymes of amino acid biosynthesis. In this review I will discuss the mechanisms of attenuation, concentrating on the tryptophan operon of *Escherichia coli*.

During the early history of studies on gene expression in amino acid biosynthetic operons, thoughts on possible regulatory mechanisms were dominated by the conceptual contributions of Jacob and Monod. The focus of investigation was therefore on the detection of a repression system and on the relative roles of amino acid and charged transfer RNAs as activators of a presumed repressor protein[8,9]. Nevertheless,

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Definitions of terms

Leader region—The segment of an operon between the transcription start site and the structural gene(s).
Attenuator—a transcription termination site within an operon.
Transcription pause site—a region of DNA at which RNA polymerase pauses in the course of transcription.
Terminated leader transcript—The transcript terminated at the attenuator.
Termination structure—The segment of the leader transcript that is thought to be recognized as a termination signal by RNA polymerase.
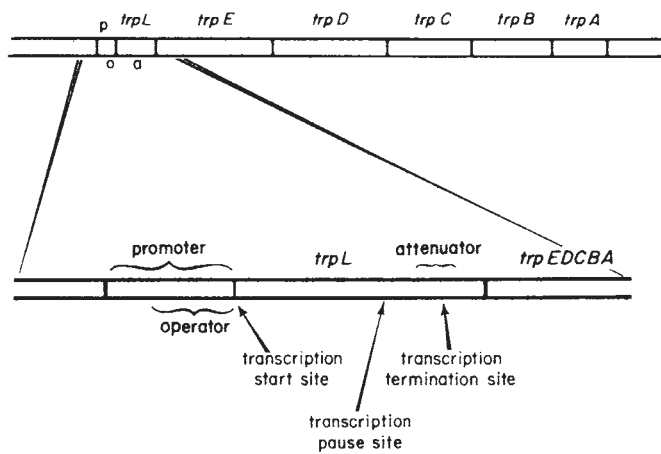*Leader peptide*—The short peptide encoded in the terminated and readthrough transcripts of the leader region.

Ames and his co-workers concluded that transcription of the *his* operon of *Salmonella typhimurium* was probably not governed by a repressor protein and that tRNA^{His} rather than histidine was the signal molecule[10,11]. Martin, Ames and Hartman[12], in some early speculations, proposed that it was translation of the transcript of the initia' ∼ment of the *his* operon that regulated transcription of the remainder of the operon. During the same period investigations on *trp* operon expression in *E. coli* followed a different course because there was convincing evidence that a tryptophan-activated repressor protein regulated transcription of the operon[13–19]. However, several observations were inconsistent with the view that repression was the sole regulatory mechanism of this operon. Tryptophanyl-tRNA synthetase mutants were shown to have regulatory anomalies[20–23]; addition of tryptophan to these strains did not fully depress *trp* operon expression. Moreover, Imamoto observed that the addition of tryptophan to tryptophan-starved cells not only shut down the initiation of transcription but also inhibited transcription in progress on the initial segment of the operon[24,25]. Finally, mutants lacking a functional repressor could still respond to tryptophan starvation by increasing their rate of synthesis of *trp* messenger RNA[26,27].

These findings suggested that repression was not the sole means of regulating the *trp* operon of *E. coli*. Ultimately, two observations forced consideration of alternative regulatory schemes. Among a set of *trp* deletion mutants, in which both deletion termini were within the transcribed region of the operon, some had an unexpected sixfold increase in expression of the remaining genes of the operon[6,28]. Since repressor control was not affected by these deletions, what must have been deleted was a site distinct from the operator but which was also used to regulate transcription of the operon. The second observation was that within the mRNA sequences produced *in vivo* from the 5' end of the *trp* operon, oligoribonucleotides corresponding to the first 140 base pairs of the operon were several times more abundant than those derived from more distal segments[29,30]. These findings suggested that there was a site of transcription discontinuity, possibly a stop site, in the initial segment of the operon[6].

When transcription of the initial segment of the *trp* operon was studied *in vivo* and *in vitro*[28,30–34] it became apparent that a transcription termination site was located before the structural genes of the operon. Moreover, starving bacteria of tryptophan reduced termination of transcription at this site[27,35]. Kasai provided convincing evidence of an equivalent site of regulation of transcription of the *his* operon of *S. typhimurium*[7]. He called regulation at this site 'attenuation', the term now generally used to describe regulation by transcription termination.

Fig. 1 The regulatory and structural gene regions of the *trp* operon of *E. coli*. Transcription initiation is controlled at a promoter–operator. Transcription termination is regulated at an attenuator, in the transcribed 162-base pair leader region, *trpL*. All RNA polymerase molecules transcribing the operon pause at the transcription pause site before proceeding further.

Features of the tryptophan operon and its regulatory region

The *trp* operon of *E. coli* consists of a transcription regulatory region and five structural genes encoding the polypeptides that catalyse the terminal sequence of reactions in tryptophan formation[36,37] (Fig. 1). The operon is approximately 7,000 nucleotide pairs long, and each cistron of the polycistronic transcript of the operon seems to be translated equally. The initiation of transcription is regulated at an operator site located within the promoter region of the operon[15–20,38,39]. A tryptophan-activated repressor protein, the product of the unlinked regulatory gene *trpR*, regulates initiation by controlling the access of RNA polymerase to the promoter[39–41]. The transcription termination site or attenuator (see below for definitions of terms) is located within the 162-nucleotide pair transcribed leader region, *trpL*, preceding the first major structural gene. There is also a transcription pause site within *trpL* (M. Winkler and C. Yanofsky, in preparation). A possible role for this site will be discussed below.

Repression rather than attenuation exerts the greater quantitative effect *in vivo* on transcription of the structural genes of the operon; repression can reduce transcription up to 70-fold[42] while attenuation can only reduce it 8–10-fold[6,35]. Because tryptophan starvation increases expression by relieving both repression and attenuation, transcription of the *trp* operon of *E. coli* can be regulated over a range of about 600-fold. For a more thorough discussion of all aspects of regulation of tryptophan biosynthesis in bacteria the reader is referred to the excellent reviews of Crawford and Stauffer[43] and Platt[37].

Attenuation

In addition to locating the attenuator in the distal segment of *trpL*[6,28], mutants have also provided clues to the mechanism of attenuation. Mutations altering tryptophanyl-tRNA synthetase, tRNA[Trp], or a tRNA[Trp] modifying enzyme and thereby reducing the efficiency of translation of Trp codons, were also found to decrease the termination of transcription at the *trp* attenuator[44–47]. This suggested that attenuation involved the translation of Trp codons rather than simply the recognition of changes in the tryptophan concentration. Attenuation in the *trp* operon is also affected by mutations of functionally important sequences in the leader region. Finally, mutations affecting RNA polymerase and the transcription termination protein rho also affect termination of transcription at the *trp* attenuator[47,48]. The role of rho, if it has one, in transcription termination at the *trp* attenuator is unclear.

The leader transcript

Both *in vivo* and *in vitro* transcription studies established that transcription can be terminated in the leader region of the *trp* operon of *E. coli*[28,32]. Termination occurs at approximately the same position *in vivo* and *in vitro* to give a leader transcript approximately 140 nucleotides in length[31]. The DNA region within which RNA synthesis stops is A + T rich and is immediately preceded by a G + C-rich region that exhibits dyad symmetry. This arrangement is typical of many prokaryotic transcription termination sites[49] and both regions are implicated in the transcription termination event. The question is how, at the *trp* operon attenuator, can tryptophan deficiency be sensed and communicated to the transcribing RNA polymerase molecule, allowing it to transcribe beyond the attenuator into the structural genes of the operon?

Ribosome binding experiments carried out with the 140 nucleotide *trp*-leader transcript yielded the unexpected result that ribosomes protect a 20-base segment of the early portion of the transcript from nuclease attack[50] (Fig. 2). A potential AUG start codon is located in the centre of the protected region. Were translation initiated at this start codon and terminated at the next stop codon (UGA at nucleotides 69–71), a 14-residue peptide would be synthesized with adjacent tryptophan residues near its distal end (Fig. 2). The presence of tandem tryptophan residues in this predicted 14-residue peptide was tantalizing because tryptophan is a rare amino acid in the proteins of *E. coli*, generally being used only once in every 100 amino acid residues. (The amino acid constitution of the predicted leader peptides now known to be specified by other biosynthetic operons (Fig. 3) turned out to be even more striking—see below.) The presence of the tandem tryptophan residues in this small peptide encoded in the leader region suggested a possible role for tRNA[Trp] in the regulation of transcription termination at the *trp* operon attenuator[33]. It was hypothesized that in cells starved of tryptophan a ribosome translating the leader transcript would stall at either of the two Trp codons. Only with sufficient tryptophan would the ribosome complete the entire leader peptide. The final position of the translating ribosome on the transcript could then be the distinctive feature communicated to the transcribing polymerase in the regulation of termination at the attenuator. It was reasonable to consider this hypothesis since in prokaryotes transcription and translation proceed in concert. These considerations raised two crucial questions. Is the potential AUG start codon within the ribosome-protected region (the ribosome binding site) actually used as a site of translation initiation, and, is the existence of adjacent Trp codons in the transcript coincidental?

The *trp* leader ribosome binding site was shown to be an efficient site for the initiation of translation by fusing it to a structural gene or a portion thereof and demonstrating the synthesis of fused polypeptides[51,52]. Since the leader ribosome binding site is used, we must presume that our inability to detect
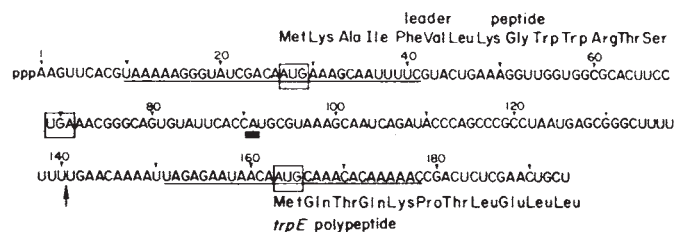

Fig. 2 The nucleotide sequence of the 5′ end of *trp* messenger RNA. The non-terminated transcript is presented. When transcription is terminated at the attenuator, a 140-nucleotide transcript is produced. Its 3′ terminus is marked by an arrow. The 3′ terminus of the pause transcript, at nucleotide 90, is underlined by a bar. The two AUG-centred ribosome binding sites in this transcript segment are underlined. The boxed AUGs are where translation starts and the boxed UGA where it stops. The predicted amino acid sequence of the *trp* leader peptide is shown.

phe, his, leu, thr and *ilv* leader peptides

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pheA:   Met-Lys-His-Ile-Pro-PHE-PHE-PHE-Ala-PHE-PHE-PHE-Thr-PHE-Pro

his:    Met-Thr-Arg-Val-Gln-Phe-Lys-HIS-HIS-HIS-HIS-HIS-HIS-HIS-Pro-Asp

leu:    Met-Ser-His-Ile-Val-Arg-Phe-Thr-Gly-LEU-LEU-LEU-LEU-Asn-Ala-Phe-

        Ile-Val-Arg-Gly-Arg-Pro-Val-Gly-Gly-Ile-Gln-His

thr:    Met-Lys-Arg-ILE-Ser-THR-THR-ILE-THR-THR-THR-ILE-THR-ILE-THR-THR-

        Gly-Asn-Gly-Ala-Gly

ilv:    Met-Thr-Ala-LEU-LEU-Arg-VAL-ILE-Ser-LEU-VAL-VAL-ILE-Ser-VAL-VAL-

        VAL-ILE-ILE-ILE-Pro-Pro-Cys-Gly-Ala-Ala-Leu-Gly-Arg-Gly-Lys-Ala
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Fig. 3   The predicted amino acid sequences of the leader peptides of the *pheA, his, leu, thr* and *ilv* operons of *E. coli* or *S. typhimurium*. Amino acids which regulate the respective operons are in italicized capitals and are underlined. For references see text.

the leader peptide is due to its instability. That the leader peptide as such does not have a regulatory role is indicated by complementation analyses[53].

The significance of the tandem Trp codons in the *trp* leader transcript of *E. coli* is supported by their presence also in the *trp* leader regions of *Shigella dysenteriae, Salmonella typhimurium, Serratia marcescens*[34,54,55] and *Klebsiella aerogenes* (M. Blumenberg and C. Yanofsky, unpublished). The predicted amino acid residues of these *trp* leader peptides are also conserved in the Arg-Thr-Ser sequence that follows the adjacent Trp residues but not in the sequence that precedes it. More importantly, the predicted leader peptides of other amino acid biosynthetic operons that are regulated by attenuation also are rich in the amino acid which is the end product of the appropriate pathway (Fig. 3). Hence, in a bacterial cell starved of any of these amino acids, the ribosome translating the leader transcript of the respective operon would stall over the appropriate codon rather than move to the leader transcript stop codon. Figure 3 reveals that the number as well as the location of the distinctive amino acids varies, presumably reflecting quantitative differences in regulation of the different operons.

If ribosome stalling at the Trp codons of the transcript of the leader region were sufficient to prevent the termination of transcription at the *trp* attenuator, then would stalling anywhere in the coding region of the transcript have the same effect? Starving *E. coli* mutants for each of seven amino acids encoded in the *trp* leader transcript (Met, Val, Leu, Gly, Trp, Arg and Thr) and two that are not (His and Pro)[53] revealed that the starvation response was relatively specific. Only cells depleted of Trp or Arg exhibited reduced termination of transcription at the *trp* attenuator. Comparable starvation experiments with the *trp* operon of *S. marcescens* (I. Stroynowski and C. Yanofsky, unpublished) demonstrated that starvation for His, the second residue before Trp in the leader peptide, as well as for Trp or Arg, reduced the termination of transcription at the *trp* attenuator. These observations suggested that ribosome stalling in the vicinity of the Trp codons is sufficient to exert a regulatory effect.

This conclusion led to the question of how amino acid starvation and presumed ribosome stalling are communicated to the transcribing polymerase. Hoping to answer this question we scrutinized the transcript of the leader region, searching for potential secondary structures that might relate to the transcription termination event. This search was prompted by the suggestion of Marty Rosenberg, on the basis of sequence analyses with a terminated transcript of bacteriophage λ[56], that base-paired segments in RNA may serve as a signal in the transcription termination event. Limited RNase T₁ digestion of the 140-nucleotide long *trp* leader transcript revealed extensive secondary structure in the transcript; many phosphodiester bonds in the latter half of the transcript were relatively resistant to nuclease attack[33]. Also, certain fragments in partial digests of the transcript were observed to migrate together during electrophoresis in non-denaturing gels[57]. Presumably they did so

because they were base-paired. Analyses of the nuclease-resistant RNA fragments, as well as computer matching of complementary sequences, indicated that the transcript contains the two hairpin loops shown in Fig. 4 (left). For simplicity, we designate the four strands participating in these structures 1–4 (Fig. 4). We observed that strands 1 and 2 were paired and that strand 3 remained covalently attached to strand 4. The calculated free energies of these base-paired structures are given in Fig. 4. Note that strand 1 contains the adjacent Trp codons and that structure 3:4 resembles other hydrogen-bonded hairpin structures that immediately precede the 3′ ends of terminated transcripts. Inspection of the sequences of the paired regions revealed an interesting feature—that strand 2 could potentially pair with strand 3 as well as with strand 1[33] (Fig. 4, right). This led to the suggestion that alternative secondary structures in the *trp* leader transcript might provide the termination or readthrough signals that RNA polymerase recognizes.

We could imagine, as shown in Fig. 5, that the position of the ribosome on the transcript determines which one of the alternative RNA secondary structures would be formed[46,57]. For example, a ribosome stalled at the Trp codons or the Arg codon by a shortage of either amino acid would sterically block 1:2 pairing, thereby allowing strand 2 to pair with strand 3 as soon as strand 3 had been synthesized. By the time strand 4 had been synthesized its potential pairing partner, strand 3, would be base-paired and unavailable, preventing the formation of termination structure, 3:4. If 3:4 did not form and if, as we also believe (see next section), the 3:4 structure is the termination signal that RNA polymerase recognizes, then the transcribing polymerase would not stop at the attenuator. Consequently, the structural genes of the operon would be transcribed, leading ultimately to the biosynthetic correction of the tryptophan deficiency. If, however, a ribosome stalled at the Thr codon or reached the stop codon, strand 2 would be sterically prevented from pairing with strand 3 and thus 3:4 would be free to form and signal transcription termination. If the ribosome stalled at the Gly codon strand 1 would pair with strand 2, thereby preventing 2:3 pairing and facilitating pairing of 3 with 4. According to this scheme it is the location of the ribosome on the transcript of the *trp* leader region that determines which alternative RNA secondary structure forms. This, in turn, directs RNA polymerase to terminate transcription or to read through
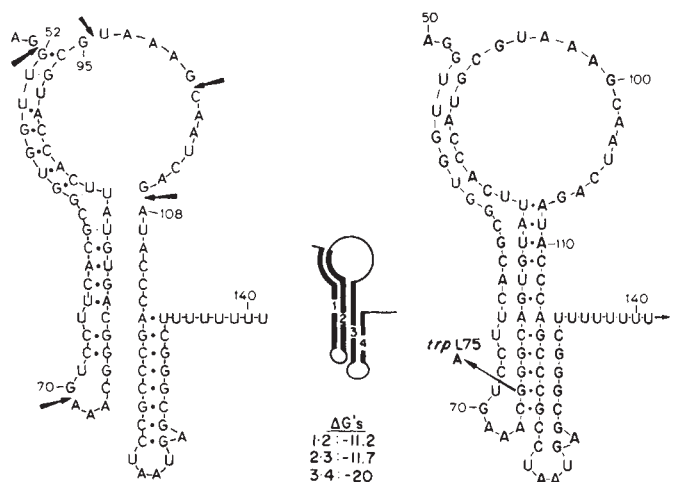

Fig. 4   Secondary structure alternatives in the *trp* leader transcript. In the large structure on the left the two base-paired structures that are detected *in vitro* are presented. The arrows indicate the sites of RNase T1 attack. The G-bonds in the hydrogen-bonded regions are not cleaved, presumably because the Gs are base paired. In the large structure on the right an alternative secondary structure is shown. Formation of this structure is thought to prevent transcription termination at the attenuator. For simplicity we designate the transcript segments that participate in these hydrogen-bonded structures strands 1–4 (see centre inset). The *trp* codons are in strand 1. The *trpL75* mutation is indicated.
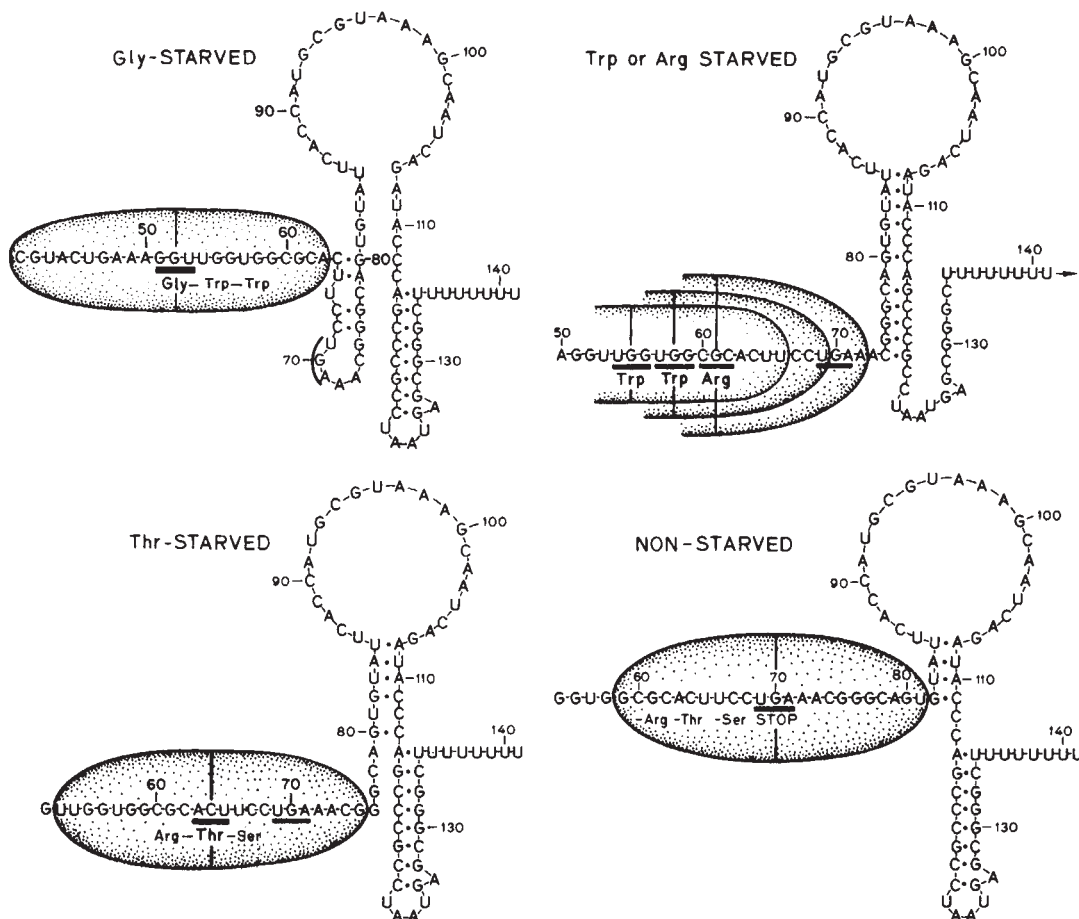
Fig. 5 Schematic representation of ribosome position on the *trp* transcript in cells starved of Gly, Trp, Arg or Thr, or cells that are not starved. It is proposed that a ribosome stalled over one of the codons of the transcript sterically blocks about 10 nucleotides on the downstream segment of the transcript, thereby determining which alternative secondary structure will form during transcription. Only when cells are starved of Trp or Arg is the ribosome positioned so that structure 2:3 will form. The existence of structure 2:3 could prevent pairing of 3 with 4 while 4 is being synthesized. It is assumed that transcription terminates at the attenuator only when structure 3:4 is formed during transcription.

into the structural genes of the operon. With this model for reference we can explain why the Arg-Thr-Ser codon sequence that follows the tandem Trp codons of *trp* transcripts is conserved in the enterobacteria. The Arg-Thr-Ser coding region is within the segment of strand 1 that can base pair with strand 2 (Figs 4, 5). Conservation of this region presumably reflects a requirement for this pairing capability.

Mutations in the leader region of the *trp* operon

Two classes of termination-defective *trp* leader mutants have been isolated. One type terminates transcription at less than the normal frequency and therefore operon expression is increased[58,59]. The base-pair changes in approximately 30 mutants of this type have been determined and, with one exception, all are in the segment of the leader region that corresponds to the 3:4 base-paired RNA structure (Fig. 6). Each of these base-pair changes reduces the predicted stability of the 3:4 structure by about 50%. All of these mutations affect the central G·C base pairs of the structure (Fig. 6), which of course have the greatest effect on stability of the 3:4 structure. *In vivo*, each of these mutations results in a two- to fourfold increase in operon expression. When restriction fragments bearing these mutations are transcribed, 40–70% of RNA polymerase molecules read through the attenuator. With a wild-type template only 5% do so. Clearly, these single mutant changes markedly influence the termination of polymerase activity. Largely on the basis of these findings we believe that the hydrogen-bonded 3:4 structure is the termination signal that the transcribing polymerase recognizes. Consistent with this conclusion is the additional observation[33] that when transcription is carried out *in vitro* with ITP substituting for GTP, transcription is no longer terminated at the attenuator, presumably because I = C base-paired RNA is less stable than G ≡ C base-paired RNA. Similar *in vitro* transcription studies

with other base analogues that affect RNA secondary structure also suggest that a stable 3:4 RNA secondary structure is necessary for transcription termination[60].

A role for the A+T-rich as well as the G+C region of the attenuator in termination of transcription is suggested by our discovery of a termination-defective *trp* leader mutant with a single base-pair change in the A+T-rich region of the attenuator[58]. In addition, a deletion mutant exists in which the distal four A+Ts of the A+T-rich region are replaced by a foreign sequence[31]. This change eliminates transcription termination at the attenuator *in vitro* and reduces it *in vivo*[31,60]. Certain mutants altered in the β subunit of RNA polymerase are also aberrant in termination of transcription at the *trp* attenuator (C. Yanofsky and V. Horn, unpublished). In one class termination is partially relieved, while in a second, termination is more frequent. These mutations influence the termination of transcription only in strains which can form the 3:4 base-paired structure, strengthening the conclusion that this structure is the termination signal that is recognized by RNA polymerase.

There are also mutations in the *trp* leader region which increase termination at the attenuator *in vivo*[53]. Some mutations of this type have a second interesting and important property; they prevent the relief from termination that is normally associated with tryptophan or arginine starvation[53]. This observation provides the strongest evidence for our model of attenuation. In one of these mutants, *trpL29*, which has been isolated on two separate occasions, the AUG start codon for the leader peptide is replaced by AUA, a change which would prevent efficient translation of the leader transcript. Since strains with this mutation do not respond to tryptophan starvation by relieving termination, a prediction of our model—that a ribosome must move to the Trp codon region of the transcript for transcription termination to be relieved—is supported. A second type of mutant, *trpL75* (Fig. 4), of which there have also been two isolates, has a G → A change at position 75 in the leader region. Since this change essentially eliminates the pairing of strand 2 with strand 3, thereby leading to the formation of the 3:4

termination structure, our model explains why termination occurs despite tryptophan starvation. These considerations support two important features of our model: first, that a ribosome must translate the transcript at least to the first Trp codon for termination relief, and second, that pairing of strand 2 with strand 3 is also required for this relief. Thus, these observations strongly suggest that alternative secondary structures in the RNA transcript determine whether or not termination will occur at the *trp* operon attenuator.

Why do these two types of mutants exhibit increased termination of transcription at the attenuator *in vivo*? In the wild-type bacterium we assume that operon expression provides for some transcription readthrough, even in the presence of excess tryptophan[58]. This could be due to rapid dissociation of the translating ribosome from the stop codon of the transcript, allowing the 2 : 3 alternative structure to form. If this happens, formation of the 3 : 4 structure would be prevented and the transcribing RNA polymerase molecule would continue into the structural genes of the operon. This model can explain the increased termination observed *in vivo* in the two types of termination mutants. The AUA translation initiation-defective mutant would, during transcription, form structure 1 : 2. This would prevent pairing of strand 2 with strand 3, thereby promoting the formation of termination structure 3 : 4. In mutant *trpL75*, pairing of strand 2 with strand 3 is presumably defective. Hence, despite ribosome dissociation from the stop codon, strand 2 could not pair with strand 3, and therefore 3 : 4 would form more often. Thus, the increased termination of transcription observed in these mutants can be readily accommodated within our model.

Also consistent with our model is the observation that neither mutant change affects transcription termination at the attenuator *in vitro*[58].

The leader pause site

When restriction fragments containing the *trp* promoter are transcribed we observe the 140-nucleotide terminated leader transcript as the major product along with a small amount of a readthrough transcript. However, at early times during a single round of transcription, a third, major transcript is seen (M. Winkler and C. Yanofsky, unpublished). This species—which we call the pause transcript—is 90 nucleotides long and has at its 3′ terminus the sequence that could form the 1 : 2 base-paired structure (Fig. 4). Enhancement of pausing at this site when BrUTP replaces UTP (P. J. Farnham and T. Platt, unpublished) supports this proposal since numerous U's are present in the 1 : 2 stem. Kinetic studies indicate that every polymerase molecule that transcribes the initial segment of the leader region pauses at the 'leader pause site'. Eventually, these polymerase molecules resume transcription so that the pause transcript grows to become either the terminated transcript or
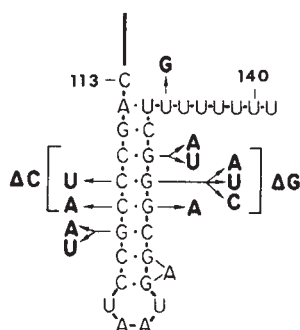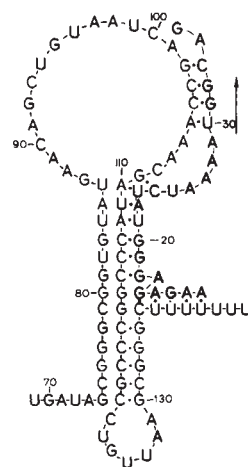

Fig. 7   Pairing of the leader ribosome binding site with the distal portion of the *trp* leader transcript of *S. typhimurium*. Note that both the Shine–Dalgarno region and the start codon region are hydrogen bonded to the distal segment of the transcript. Pairing of the indicated RNA segments has been demonstrated experimentally (K. Brown and C. Yanofsky, unpublished).

the readthrough transcript. The position of the pause site in the leader region suggests that it could be used to synchronize ribosome attachment, movement and positioning on the leader transcript relative to the transcribing RNA polymerase molecule. The polymerase molecule might be held at the pause site until the translating ribosome reached a particular position on the transcript. Thereafter, the polymerase molecule would resume transcription and the translating ribosome would reach the positions on the transcript that favour one or the other of the alternative RNA secondary structures.

The leader ribosome binding site

Perusal of the sequences of the *trp* leader transcripts of various organisms and the results of secondary structure studies with these transcripts suggest an additional feature of the attenuation process. When the secondary structures formed in *trp* leader transcripts from different enterobacteria were analysed, another base-paired species was detected in addition to the 1 : 2 and 3 : 4 structures (K. Brown and C. Yanofsky, in preparation). This species contains an early segment of the transcript—the region containing the leader ribosome binding site—base-paired to a distal segment (Fig. 7). The calculated free energy for this base-paired segment is about $-32 \text{ kcal mol}^{-1}$. If this pairing occurs *in vivo* the distal segment of the transcript could block the leader ribosome binding site, thereby preventing additional rounds of translation. Thus, once the decision was made to terminate transcription or to allow readthrough at the *trp* attenuator, additional translation of the peptide coding region would be prevented by masking the leader ribosome binding site. Sequence arrangements that allow pairing with the leader ribosome binding site may be a common feature of the structure of transcripts of operons regulated by attenuation (ref. 54 and M. Blumenberg and C. Yanofsky, unpublished). This situation is reminiscent of the masking of ribosome binding sites in RNA phages[61,62].

Fig. 6   Base pair changes in the *trp* leader region that relieve transcription termination at the attenuator. The changes in the transcript are shown; all are in the 3 : 4 structure and reduce its stability except the single U → G change at position 135.

Summary of the mechanism of attenuation

Our current view of the events accompanying attenuation in the *trp* operon is as follows: a RNA polymerase molecule that escapes repressor control initiates transcription of the operon.

Soon after the segment of the transcript containing the leader ribosome binding site has been synthesized a ribosome attaches. Synthesis of the leader peptide begins while transcription of the later regions continues. The transcribing RNA polymerase molecule reaches the transcription pause site where it remains briefly, allowing the translating ribosome to approach. When RNA polymerase resumes transcription, the translating ribosome either stalls at one of the Trp codons, if the cell is deficient in tryptophan, or, if tryptophan is plentiful, moves to the translation stop codon. The position of the ribosome on the transcript determines which alternative RNA secondary structure forms. Only if structure 3:4 forms does the polymerase terminate transcription at the attenuator. Whether termination occurs or not, the leader ribosome binding site is then blocked by pairing with a transcript segment.

The interpretation given above presumes that changes in the secondary structure of transcripts mediate the regulatory response. This has not been proved! Structural studies on transfer RNAs have established that interactions other than hydrogen bonding contribute to three-dimensional form. Furthermore, a regulatory role for the DNA template has not been excluded. We should therefore keep open the possibility that other interactions also participate in establishing the informational transcript or template structures that are crucial in attenuation.

Attenuation in other amino acid biosynthetic operons

Extensive regulatory studies have been performed with the *his* operons of *S. typhimurium* and *E. coli*. This operon was the first gene cluster for which there was convincing evidence suggesting that charging of a transfer RNA had a regulatory role[8]. The leader regions of the *his* operons of *S. typhimurium* and *E. coli* have been sequenced[63,64] and appear to have structures resembling that of the *trp* operon. A potential ribosome binding site is situated in the leader transcript sequence, which, if used, would give a 16-residue peptide containing 7 adjacent His residues. A G+C-rich region with dyad symmetry is located somewhat beyond and is followed by an A+T-rich segment within which transcription termination probably occurs. The transcript segment corresponding to the G+C-rich region could form a stable stem and loop that could serve as a termination signal. A potential alternative competing secondary structure can be drawn for the transcript that could prevent formation of the presumed termination structure, depending on the location of the ribosome translating the leader peptide-coding region[63-65]. *In vitro* studies have implicated translation in the regulation of transcription of the *his* operon[66]. In addition, analyses with regulatory mutants altered at key positions in the *his* leader region provide insight into the parts played by different segments of this region or more likely, the corresponding transcript[65]. An ochre nonsense mutation in the leader peptide-coding region decreases operon expression to the extent that the corresponding mutant is a His auxotroph. This observation suggests that translation of at least the initial part of the leader peptide coding region is essential for operon expression. Suppression of the ochre mutation increases *his* enzyme levels and restores the His phenotype. A second mutation conferring His auxotrophy occurs just beyond the translation stop codon for the leader peptide. Oddly enough, although this mutation does not introduce an amber codon, it is suppressed by amber suppressors. Apparently, the mutation prevents formation of the competing RNA structure, resulting in more efficient termination at the attenuator[65]. Presumably, the amber suppressors increase operon expression by allowing the translating ribosome to translate the leader peptide stop codon and disrupt the transcript termination structure[65]. One verified prediction of this interpretation was that amber suppressors would increase expression of the wild-type *his* operon.

Finally, a deletion that removes part of the termination structure increases operon expression. Thus, all aspects of attenuation in the *his* operon are similar to those of the *trp* operon, suggesting that attenuation is used in much the same way.

The *thrA₁A₂BC* and *ilvGEDA* operons of *E. coli* have an additional feature readily accommodated by the attenuation mechanism regulating amino acid biosynthetic operons[67-69]. Expression of these two operons is influenced by two and three amino acids, respectively (multivalent control). Obviously, if a leader transcript contains multiple codons for two or three amino acids in the critical pairing regions, the operon could respond to starvation for any one of these amino acids. The sequences of the leader regions of the *thr* and *ilv* operons suggest that this is exactly what occurs. The *thr* leader region encodes a peptide rich in Thr and Ile residues (Fig. 3); it contains a G+C-rich, A+T-rich region resembling other terminators, and its sequence predicts the formation of alternative, competing paired transcript structures that would be influenced by ribosome position on the transcript[67]. Mutants establish that the G+C-rich region is essential for the termination of transcription. The *ilvGEDA* operon is a more extreme example of multivalent control. It is regulated by changes in the extent of charging of Val, Ile and Leu tRNAs[8]. The leader region codes for a putative 32-residue peptide with multiple Ile, Val and Leu residues and contains adjacent G+C- and A+T-rich regions at which transcription is terminated *in vitro*[68,69]. Mutually exclusive secondary structures have been predicted for the transcript. Their role in the termination of transcription is supported by the finding that substitution of ITP for GTP in the *in vitro* transcription reaction prevents termination. The potential secondary structures in the transcript are complex, perhaps reflecting the need to respond to starvation for any one of the three amino acids. Stalling of more than one ribosome on the transcript may be required for maximum relief of termination[68].

The *leu* operon of *S. typhimurium* has a leader region containing all the features found in the other amino acid operons[70,71]. A 160-nucleotide terminated transcript is produced *in vitro*. The transcript codes for a 28-residue peptide containing 4 contiguous Leu residues. Alternative secondary structures can be predicted for the transcript, including a termination structure, a competing structure and a third structure that could prevent formation of the competing structure if translation of the transcript stopped early in the peptide-coding region. Again, substituting ITP for GTP in the *in vitro* transcription reaction prevents termination.

The Leu codons of the transcript are so positioned that ribosome stalling in the Leu codon region would promote formation of the RNA structure that is the alternative to the termination structure.
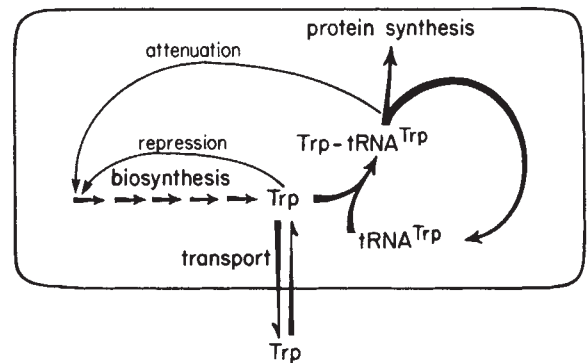

Fig. 8 Metabolic processes involved in tryptophan synthesis and utilization. The existence of the two transcription control mechanisms for the *trp* operon allows the bacterium to sense both the intracellular concentration of tryptophan and the extent of charging of tRNA^Trp.

The *pheA* operon of *E. coli* is a single gene operon containing a leader region with all the features seen in other amino acid operons controlled by attenuation[72]. A terminated transcript is produced *in vitro* and the site of termination is a typical termination region. The predicted *phe* leader peptide is 15 residues in length and contains 7 Phe residues in the last two-thirds of the peptide. Stable RNA secondary structures can be drawn for the *phe* transcript. Ribosome stalling over the Phe codons of the transcript could prevent formation of the transcript termination structure.

Thus, despite considerable differences in the sequences of leader regions of the *trp, his, thr, ilv, leu* and *phe* operons, the predicted structures of each of their transcripts allows the same mechanism of attenuation. When we compare the specific elements of repression systems with those of attenuation as they are used in amino acid biosynthetic operons, it is obvious that from the point of view of information content attenuation is a more economical regulatory mechanism. The unique regulatory information of attenuation is provided by the sequence of 50 or so base pairs of the leader region of each operon. This information is sufficient to be interpreted in terms of the appropriate regulatory response by the transcriptional and translational machinery of bacterial cells.

Conclusions

The existence of two mechanisms for regulating transcription of the *trp* operon leads one to wonder what advantage it is to the organism to have this apparent duplication of regulatory effort. The two mechanisms clearly allow similar responses to the same physiological situations. A possible explanation can be offered by considering the various metabolic reactions involved in the biosynthesis and utilization of tryptophan (Fig. 8). The repression system, by responding to tryptophan exclusively, is tailored to titrate the intracellular concentration of this amino acid. This concentration depends on several factors: first, tryptophan availability in the environment of the bacterium and its entry into the cell via transport systems; second, the rate of tryptophan biosynthesis; and third, the utilization of tryptophan for protein synthesis. By gauging the intracellular concentration of tryptophan, repression is particularly well suited to regulate expression of the operon in response to changes in the availability of tryptophan from the environment. We believe this to be its primary purpose. Attenuation is influenced by the extent of charging of tRNA^{Trp}. The relative concentrations of charged and uncharged tRNA^{Trp} are determined by several factors: the intracellular tryptophan concentration, the amount and activity of tryptophanyl-tRNA synthetase, the amount of tRNA^{Trp} and, perhaps most importantly, the overall rate of protein synthesis. It is this last factor that we believe provides the best explanation for attenuation. If a cell becomes deficient in one of the other amino acids the tRNA^{Trp} pool will become fully charged, resulting in maximal termination at the attenuator. Conversely, if a cell suddenly begins to synthesize proteins rapidly and tryptophan is in short supply, the tRNA^{Trp} pool will be relatively uncharged and there will be little or no termination at the attenuator. Thus, the attenuation mechanism nicely regulates expression of the operon relative to the overall rate of protein synthesis. The combined action of the two regulatory mechanisms enables the bacterium to recognize and respond to the principal external and internal events relevant to expression of the operon.

If it is advantageous to have two transcription control mechanisms for the *trp* operon of *E. coli*, why is attenuation the sole transcription control mechanism used to regulate expression of other amino acid biosynthetic operons such as the *his, leu* and *thr* operons[65,67,70]? The explanation for this apparent contradiction, I believe, is provided by our recent finding that two other operons are regulated by the *trp* repressor. These are the *aroH* operon, specifying one of three enzymes that catalyse the initial reaction in the common pathway of aromatic amino acid biosynthesis[73], and the *trp* repressor operon, encoding the

trp aporepressor[40,74]. In both cases, tryptophan-activated repressor binds to the operator–promoter region of the operon and inhibits transcription initiation. However, the operators in these three operons are at different locations in their respective promoters[40]. This finding is consistent with the hypothesis that these operators evolved independently. Thus, we might speculate that in an organism ancestral to *E. coli* and other enterobacteria, the *trp* repressor might have been used to regulate *aroH* expression while the *trp* operon was regulated by attenuation alone. Subsequently, by very few mutations, an operator may have evolved in the *trp* operon, thereby permitting additional control over transcription of this operon. We suggest, therefore, that two regulatory mechanisms now exist for controlling transcription of the *trp* operon of *E. coli* because it was advantageous to the organism to have evolved a *trp* repressor binding site in this operon so that it could exploit the availability of the *trp* repressor.

Extensions

Attenuation as used in the regulation of amino acid biosynthetic operons is only one of several ways in which gene expression could be controlled through the termination of transcription. Alteration or modification of RNA polymerase, as is presumed to occur when it interacts with the N protein of bacteriophage λ[75-78], or interference with the action of proteins participating in transcription termination[78], are additional attenuation mechanisms. Transcription termination sites could also be used simply to reduce transcription of a gene relative to a leader segment or a preceding gene in the same operon. The *trpR* operon, specifying the *trp* aporepressor, has a termination site in its leader region which may function in this manner[40]. Similarly, the termination site located between *rplL* and *rpoB* in the *rplJL–rpoBC* operon may be used to regulate expression of the two RNA polymerase structural genes relative to the ribosomal protein genes[79]. Inadvertent attenuation also occurs when translation is prematurely terminated within one of the structural genes of a polycistronic operon. Here, in what is termed translational polarity[80], the termination of transcription reduces the transcription of distal genes[81]. In addition to these examples, one could imagine many interactions that would alter the secondary structure of RNA and thereby influence termination. Transcriptional pausing[82-84] should also be considered a mechanism of attenuation. Pause sites could conceivably be used to set the rate of transcription of a gene or operon below that attainable by polymerase–promoter interaction alone. Pause sites may thus fix the maximum rates of transcription. Studies on pausing in phage T7 and in the *trp* operon suggest that pausing can serve different regulatory roles[83,84] (M. Winkler and C. Yanofsky, unpublished). Finally, attenuation may not be limited to bacteria and their viruses but may also occur in animal viruses. In vesicular stomatitis virus (VSV), transcription decreases in an oriented manner at or near the junctions of neighbouring genes, resulting in a cumulative reduction in distal gene expression[85]. In adenovirus and VSV transcription short leader transcripts are accumulated[86,87], an observation suggesting attenuation. It is apparent from the examples cited that transcriptional attenuation is accomplished in diverse ways. We presume that these mechanisms bring into regulatory focus those cellular processes and components that cannot readily participate in the mechanisms that control the initiation of transcription.

1. Taylor, K., Hradecna, Z. & Szybalski, W. *Proc. natn. Acad. Sci. U.S.A.* **57**, 1618–1625 (1967).
2. Luzzati, D. *J. molec. Biol.* **49**, 515–519 (1970).
3. Kourilski, P., Bourguignon, M. F. & Gros, F. in *The Bacteriophage Lambda* (ed. Hershey, A. D.) 647–666 (Cold Spring Harbor, New York, 1971).
4. Heinemann, S. F. & Spiegelman, W. G. *Cold Spring Harb. Symp. quant. Biol.* **35**, 315–318 (1971).
5. Roberts, J. W. *Nature* **224**, 1168–1174 (1969).
6. Jackson, E. & Yanofsky, C. *J. molec. Biol.* **76**, 89–101 (1973).
7. Kasai, T. *Nature* **279**, 523–527 (1974).
8. Brenchley, J. E. & Williams, L. S. *A. Rev. Microbiol.* **29**, 251–274 (1975).
9. Cortese, R. in *Biological Regulation & Development* (ed. Goldberger, R.) 401–432 (Plenum, New York, 1979).
10. Singer, C. E., Smith, G. R., Cortese, R. & Ames, B. N. *Nature new Biol.* **238**, 72–74 (1972).
11. Lewis, J. A. & Ames, B. N. *J. molec. Biol.* **66**, 131–142 (1972).
12. Martin, R. G., Ames, B. N. & Hartman, P. E. in *7th int. Congr. Biochem.* Abstr., 261–262 (Tokyo, 1967).
13. Cohen, G. & Jacob, F. *C.r. hebd. Séanc. Acad. Sci., Paris* **248**, 2490–92 (1959).
14. Shimizu, Y., Shimizu, N. & Hayashi, M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1990–1994 (1973).
15. Zubay, G., Morse, D. E., Schrenk, W. I. & Miller, J. H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1100–1103 (1972).
16. Squires, C. L., Lee, F. & Yanofsky, C. *J. molec. Biol.* **92**, 93–111 (1975).
17. Rose, J. K., Squires, C. L., Yanofsky, C., Yang, H. L. & Zubay, G. *Nature new Biol.* **245**, 133–37 (1973).
18. Squires, C. L., Rose, J. K., Yanofsky, C., Yang, H. L. & Zubay, G. *Nature new Biol.* **245**, 131–33 (1973).
19. McGeoch, D., McGeoch, J. & Morse, D. *Nature new Biol.* **245**, 137–40 (1973).
20. Hiraga, S., Ito, K., Hamada, K. & Yura, T. *Biochem. biophys. Res. Commun.* **26**, 522–527 (1967).
21. Kano, Y., Matsushiro, A. & Shimura, Y. *Molec. gen. Genet.* **102**, 15–26 (1968).
22. Ito, K. *Molec. gen. Genet.* **115**, 349–353.
23. Camakaris, J. & Pittard, J. *J. Bact.* **107**, 406–414 (1971).
24. Imamoto, F. *Nature* **220**, 31–34 (1968).
25. Hiraga, S. & Yanofsky, C. *J. molec. Biol.* **79**, 339–49 (1973).
26. Baker, R. & Yanofsky, C. *J. molec. Biol.* **69**, 89–102 (1972).
27. Morse, D. E. & Morse, A. N. C. *J. molec. Biol.* **103**, 209–26 (1976).
28. Bertrand, K., Squires, C. & Yanofsky, C. *J. molec. Biol.* **103**, 319–37 (1976).
29. Bronson, M. J., Squires, C. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2335–39 (1973).
30. Squires, C., Lee, F., Bertrand, K., Squires, C. L., Bronson, M. J. & Yanofsky, C. *J. molec. Biol.* **103**, 351–81 (1976).
31. Bertrand, K., Korn, L. J., Lee, F. & Yanofsky, C. *J. molec. Biol.* **117**, 227–47 (1977).
32. Lee, F., Squires, C. L., Squires, C. & Yanofsky, C. *J. molec. Biol.* **103**, 383–393 (1976).
33. Lee, F. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4365–4369 (1977).
34. Lee, F., Bertrand, K., Bennett, G. & Yanofsky, C. *J. molec. Biol.* **121**, 193–217 (1978).
35. Bertrand, K. & Yanofsky, C. *J. molec. Biol.* **103**, 339–49 (1976).
36. Yanofsky, C. *J. Am. med. Assoc.* **218**, 1026–1035 (1971).
37. Platt, T. in *The Operon* (eds Miller, J. & Reznikoff, W.) 268–302 (Cold Spring Harbor, New York, 1978).
38. Rose, J. K. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3134–38 (1974).
39. Bennett, G. N. & Yanofsky, C. *J. molec. Biol.* **121**, 179–192 (1978).
40. Gunsalus, R. G. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
41. Oppenheim, D., Bennett, G. & Yanofsky, C. *J. molec. Biol.* **144**, 133–142 (1980).
42. Jackson, E. & Yanofsky, C. *J. molec. Biol.* **69**, 307–313 (1972).
43. Crawford, I. P. & Stauffer, G. V. *A. Rev. Biochem.* **49**, 163–195 (1980).
44. Yanofsky, C. & Soll, L. *J. molec. Biol.* **113**, 663–677 (1977).
45. Vold, B. S., Lazar, J. M. & Gray, A. M. *J. biol. Chem.* **254**, 7362–7367 (1979).

46. Eisenberg, S. P., Yarus, M. & Soll, L. in *Transfer RNA* (eds Soll, D., Schimmel, P. & Abelson, J.) (Cold Spring Harbor, New York, in the press).
47. Korn, L. & Yanofsky, C. *J. molec. Biol.* **103**, 395–409 (1976).
48. Korn, L. & Yanofsky, C. *J. molec. Biol.* **106**, 231–241 (1976).
49. Rosenberg, M. & Court, M. *A. Rev. Genet.* **13**, 319–353 (1979).
50. Platt, T., Squires, C. & Yanofsky, C. *J. molec. Biol.* **103**, 411–420 (1976).
51. Schmeissner, U., Ganem, D. & Miller, J. H. *J. molec. Biol.* **109**, 303–326 (1977).
52. Miozzari, G. F. & Yanofsky, C. *J. Bact.* **133**, 1457–1466 (1978).
53. Zurawski, G., Elseviers, D., Stauffer, G. V. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5988–5992 (1978).
54. Miozzari, G. F. & Yanofsky, C. *Nature* **276**, 684–689 (1978).
55. Miozzari, G. F. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5580–5584 (1978).
56. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. *Nature* **272**, 414–422 (1978).
57. Oxender, D., Zurawski, G. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5524–5528 (1979).
58. Zurawski, G. & Yanofsky, C. *J. molec. Biol.* **142**, 123–129 (1980).
59. Stauffer, G. V., Zurawski, G. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4833–4837 (1978).
60. Farnham, P. J. & Platt, T. *Cell* **20**, 739–748 (1980).
61. Lodish, H. F., in *RNA Phages* (ed. Zinder, N. D.) 301–318 (Cold Spring Harbor, New York, 1975).
62. Fiers, W. in *RNA Phages* (ed. Zinder, N. D.) 353–396 (Cold Spring Harbor, New York, 1975).
63. Di Nocera, P. P., Blasi, F., DiLauro, R., Frunzio, R. & Bruni, C. B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4276–4280 (1978).
64. Barnes, W. M., *Proc. natn. Acad. Sci. U.S.A.* **75**, 4281–4285 (1978).
65. Johnston, H. M., Barnes, W. M., Chumley, F. G., Bossi, L. & Roth, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 508–512 (1980).
66. Artz, S. W. & Broach, J. R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3453–3457 (1975).
67. Gardner, J. F. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1706–1710 (1979).
68. Lawther, R. P. & Hatfield, G. W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1862–1866 (1980).
69. Nargang, F. E., Subrahmanyam, C. S. & Umbarger, H. E. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1823–1827 (1980).
70. Gemmill, R. M., Wessler, S. R., Keller, E. B. & Calvo, J. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4941–4945 (1979).
71. Keller, E. B. & Calvo, J. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6186–6190 (1979).
72. Zurawski, G., Brown, K., Killingly, D. & Yanofsky, C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4271–4275 (1978).
73. Zurawski, G., Gunsalus, R. P., Brown, K. D. & Yanofsky, C. *J. molec. Biol.* (in the press).
74. Singleton, C. K., Roeder, W. D., Bogosian, G., Somerville, R. L. & Weith, H. L. *Nucleic Acids Res.* **8**, 1551–1560 (1980).
75. Adhya, S., Gottesman, M. & deCrombrugghe, B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2534–2538 (1974).
76. Franklin, N. C. *J. molec. Biol.* **89**, 33–48 (1974).
77. Gottesman, M. E., Adhya, S. & Das, A. *J. molec. Biol.* **140**, 57–75 (1980).
78. Greenblatt, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 1991–1994 (1980).
79. Barry, G., Squires, C. L. & Squires, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4922–4926 (1979).
80. Adhya, S. & Gottesman, M. *A. Rev. Biochem.* **47**, 967–996 (1978).
81. Richardson, J. P., Grimley, D. & Lowery, C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1725–1728 (1975).
82. Gilbert, W. in *RNA Polymerase* (eds Losick, R. & Chamberlin M.) 193–205 (Cold Spring Harbor, New York, 1976).
83. Kassavetis, G. A. & Chamberlin, M. J. *J. biol. Chem.* (in the press).
84. Kingston, R. E., Nierman, W. C. & Chamberlin, M. J. *J. biol. Chem.* (in the press).
85. Iverson, L. E. & Rose, J. K. *Cell* (in the press).
86. Fraser, N. W., Sehgal, P. B. & Darnell, J. E. Jr. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2571–2575 (1979).
87. Testa, D., Chanda, P. K. & Banerjee, A. K. *Cell* **21**, 267–275 (1980).

ARTICLES

VLBI structures of the images of the double QSO 0957+561

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Very long baseline interferometry (VLBI) observations of the double QSO 0957+ 561 have revealed radio fine structure in the two 'image' components A and B. The structures are similar, both of the 'core–jet' type typical of many compact extragalactic radio sources. The shapes of the images put strong constraints on the mass distribution responsible for the gravitational imaging.

THE twin QSOs 0957+561A, B are widely believed to be images of a single QSO produced by the gravitational lens effect[1]. The lens has been identified with an 18.5 mag elliptical galaxy in a distant cluster[2-5]. The similarity of the optical spectra of the images has been confirmed[6,7] and the flux ratio of the images has been shown to be the same over a wide range of frequencies covering the radio to UV parts of the spectrum[8-17].

The asymmetry of the geometry of the 'images' A and B with respect to the galaxy indicates that the lens action cannot be modelled by a spherical mass distribution[3]. The gravitational