New Features of the Regulation of the Tryptophan Operon

A new type of regulatory site has been studied.

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In 1961 Jacob and Monod (*1*) presented a detailed description of their model for the regulation of expression of the lactose (lac) operon of *Escherichia coli*, in which a repressor molecule acted at a site on the DNA, the operator, to regulate transcription of the operon by RNA polymerase. Although the fundamental aspects of their model have been substantiated by extensive genetic and biochemical investigations (*2*), more recent studies with the lac operon have revealed that regulation of this gene cluster is more complex than originally thought. There is a second regulatory mechanism, catabolite repression, that also directly influences operon expression (*3*). Furthermore, investigations with other well-characterized systems such as the L-arabinose operon of *E. coli* (*4*) and the histidine degradation operons (*5*) of *Salmonella typhimurium* and *Klebsiella aerogenes* have uncovered regulatory features that cannot be accommodated in a repressor-operator model of regulation. In these cases, it appears that in addition to repressor-operator interactions, and catabolite repression, other mechanisms are used to regulate operon expression. The lesson from these studies is that regulation of expression of any gene or operon may involve mechanisms other than repression which integrate control with other cellular processes.

Studies with the tryptophan (trp) operon of *E. coli* led us to believe that interaction between operator and repressor (*6, 7*) and end product inhibition (*8*) could adequately explain regulation of tryptophan biosynthesis. This view was shaken recently by the unexpected finding (*9*) that mutants with certain internal deletions in the trp operon showed significantly increased expression of the remaining genes of the operon. In these mutants, the deletions terminated in the region preceding the structural genes, yet the trp operator region apparently was unaffected since normal repressor-operator interaction was observed. These findings led to the suggestion that operon expression was limited by a regulatory site early in the operon, distinct from the operator, and that deletion of this site relieved this limitation (*9*). Soon after these observations were made, analyses of the sequence of the 5′ end of trp messenger RNA (mRNA) indicated that there was a long "leader" sequence of about 160 nucleotides preceding the translation initiation codon for the *trpE* polypeptide (*10*). Since this sequence corresponded to the region of the operon altered in the internal deletion strains, it was plausible to assume that this region contained the regulatory site. During the past 3 years we have attempted to identify this site and determine its function. In this article we summarize the results of these investigations which demonstrate that the regulatory site is in a transcribed region of the operon, approximately 30 to 60 nucleotide pairs before *trpE*. The site is used to regulate transcription termination in response to tryptophan deprivation, thereby enabling the cell to control the fraction of initiating RNA polymerase molecules that make structural gene mRNA.

Background information on the trp operon and operator-repressor interactions. The trp operon of *E. coli* and the polypeptides it encodes are illustrated in Fig. 1. There are five structural genes, *trpE* through *trpA* (*11*), preceded by a transcribed leader region, about 160 base pairs in length (*10, 12*). The promoter-operator region is located before *trpE* and precedes the leader region or possibly partially overlaps its initial segment (*13*). Initiation of transcription of the operon is regulated by interaction of the operator with a repressor protein–L-tryptophan complex (*7, 14*). This complex, when added to a minimal transcription system consisting of an appropriate DNA template containing the trp operon, RNA polymerase, and the four nucleoside triphosphates, specifically inhibits transcription of the operon (*7*). Despite suggestive findings in experiments in vivo (*15*), neither tryptophanyl-tRNA (transfer RNA) nor its cognate synthetase could be shown to participate in repression in vitro (*16*). Squires *et al.* (*17*) have shown that the repressor prevents initiation of transcription by excluding RNA polymerase from its trp promoter binding site. The converse situation also appears to hold; that is, RNA polymerase already bound to the operon cannot be inhibited by repressor added subsequently. Therefore it appears that polymerase and repressor compete for binding to the operon, and whichever binds first excludes the other. Thus action of the trp repressor is restricted to inhibition of polymerase binding at the trp promoter. Interestingly, polymerase molecules in the course of transcription, such as those which initiate at a phage promoter and subsequently encounter the trp operon, apparently are not subject to the action of the trp repressor and are capable of transcribing the operon, both in vivo (*18*) and in vitro (*19, 20*).

Internal deletions and operon expression. Selection procedures were developed (*9, 13*) which enabled us to isolate strains with one deletion terminus in the leader region and the other terminus in a structural gene of the operon. The precise extent of each deletion into the leader region was determined by oligonucleotide mapping analysis of the trp mRNA produced by a strain carrying each deletion; that is, the trp mRNA segment extending from the transcription initiation site to the deletion fusion point was isolated, analyzed by oligonucleotide mapping, and identified (*13*). A correlation of gene expression data and the locations of deletion termini in these mutants supports the earlier suggestion that a site in the leader region normally serves to reduce operon expression. The majority of deletions that extend into the leader region result in substantially elevated levels of trp mRNA and polypeptides for the remaining structural genes of the operon (*13*). This increase in operon expression is seen with internal deletions that leave as much as the first 130 nucleotide pairs of the leader region intact. However, deletions which leave the entire leader region (about 160 nucleotide pairs) intact do not elevate operon expression. Two deletions that increase trp mRNA operator-distal to the deletion have no noticeable effect on trp mRNA synthesis in the

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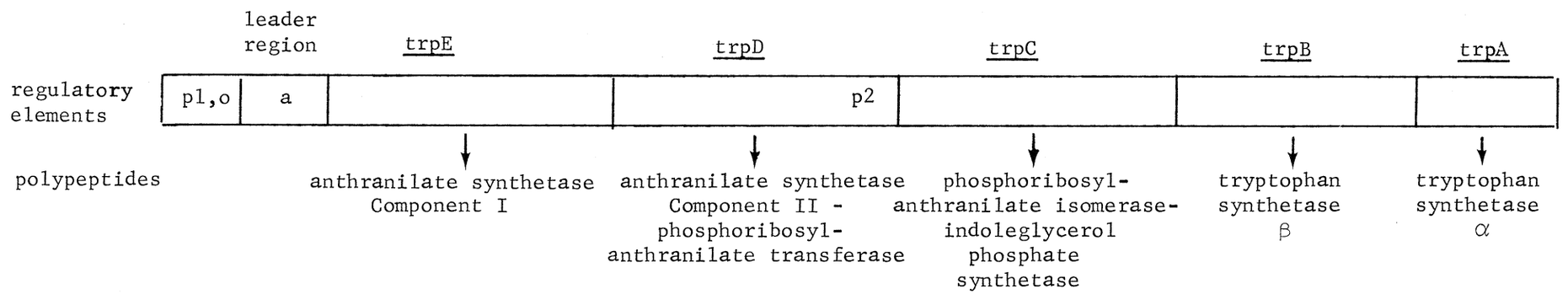

Fig. 1. The organization of the tryptophan operon of *E. coli* and the polypeptide products specified by its structural genes. Abbreviations: p1, the principal promoter; o, the operator; a, the attenuator described in this article; and p2, a low efficiency internal promoter.

Fig. 2. The leader and adjacent transcribed region of the trp operon. Two DNA regions with dyad symmetry are outlined. The sequences of "structural gene" trp mRNA and trp mRNA terminated at the attenuator are indicated, as are ribosome binding sites (underlined sequences) in these trp mRNA's (*12, 32*). The DNA sequence is deduced from the mRNA sequence. The amino acid sequence of the NH_2-terminal region of the *trpE* polypeptide is aligned with the corresponding segment of the messenger. Since the sequenced region is long, there is a possibility that one or more nucleotides are in error or have been omitted. For this reason we are reluctant at this time to predict the amino acid sequence of the polypeptide that would be produced if translation were initiated at the leader ribosome binding site.

leader region preceding the deletion. Furthermore, the increased gene expression observed in these mutants is a *cis*-dominant trait, and therefore not solely the result of inactivation of a diffusable gene product (*13*). These results indicate the presence of a DNA segment, which we will henceforth call the "attenuator" (*21*) or "a" site of the leader region, distal to the trp promoter-operator and within 30 base pairs of the region coding for the amino terminus of the *trpE* polypeptide (*13*).

Analysis of trp mRNA production in various deletion mutants suggests that the coordinate increase in trp mRNA and enzyme levels is the result of an increased rate of distal messenger synthesis rather than an altered rate of trp mRNA degradation. These in vivo properties of mutant trp operons, together with results of studies in vitro that are discussed below, suggest that the attenuator functions at the level of transcription, apparently by serving as a region in which transcription is terminated (*13*).

Sequence of the 5′ segment of trp messenger RNA. The sequence of the first 190 nucleotides of trp mRNA produced in vivo has been determined (*12*) by various experimental approaches and conventional sequencing techniques. This sequence is shown in Fig. 2. There are about 160 nucleotides in trp mRNA preceding the nucleotide sequence corresponding to the NH_2-terminal 11 amino acid residues of the *trpE* polypeptide chain (*10*). Additional evidence that the nucleotide and amino acid sequences correspond has been provided by the demonstration that two *trpE* mutants have base changes in the sequenced region (see Fig. 2) (*22*).

We have been unable to identify the triphosphate-bearing 5′ end of trp mRNA produced in vivo or in vitro. However, the oligonucleotide maps of the mRNA made under both conditions are virtually identical and show only known oligonucleotides.

Transcription of the leader region of the operon in vivo and in vitro. Oligonucleo-

DNA sequence

attenuator region

TTCACGTAAAAAGGGTATCGACAATGAAAGCAATTTTCGTACTGAAAGGTTGGTGGCGCACTTCCTGAAACGGGCAGTGTATTCACCATGCGTAAAGCAATCAGATACCCAGCCTAATGAGCGCCCGCGGGCTTTTTTTTGAACAAAATTAGAGAATAACAATGCAAACACAAAAACCGACTCTCGAACTGCT
AAGTGCATTTTTCCCATAGCTGTTACTTTCGTTAAAAGCATGACTTTCCAACCACCGCGTGAAGGACTTTGCCCGTCACATAAGTGGTACGCATTTCGTTAGTCTATGGGTCGGATTACTCGCGGGCGCCCGAAAAAAAACTTGTTTTAATCTCTTATTGTTACGTTTGTGTTTTTGGCTGAGAGCTTGACGA

leader region

"structural gene" trp mRNA

trpE polypeptide

MetGlnThrGlnLysProThrLeuGluLeuLeu ...

UUCACGUAAAAAGGGUAUCGACAAUGAAAGCAAUUUUCGUACUGAAAGGUUGGUGGCGCACUUCCUGAAACGGGCAGUGUAUUCACCAUGCGUAAAGCAAUCAGAUACCCAGCCUAAUGAGCGCCCGCGGGCUUUUUUUUGAACAAAAUUAGAGAAUAACAAUGCAAACACAAAAACCGACUCUCGAACUGCU. ...

AAAA E9777fs

UAA E9914oc

attenuator-terminated trp mRNA

UUCACGUAAAAAGGGUAUCGACAAUGAAAGCAAUUUUCGUACUGAAAGGUUGGUGGCGCACUUCCUGAAACGGGCAGUGUAUUCACCAUGCGUAAAGCAAUCAGAUACCCAGCCUAAUGAGCGCCCGCGGGCUUUUUUUU$_{OH}$

tide maps of ribonuclease T1 digests of leader trp mRNA labeled in vivo revealed that oligonucleotides derived from positions 1 to 130 in the messenger were generally present in higher molar yield than those derived from more distal regions of the messenger (*13*). This disproportionality suggested the possibility of transcription terminations in the vicinity of position 130 in the leader region. Gel electrophoretic analyses of trp mRNA labeled in vivo support this conclusion since a major, discrete band is observed that corresponds to a fragment containing only the first 130 nucleotides of the leader (*13*).

Disproportional synthesis within the leader region was more dramatically revealed by fingerprint analysis of trp mRNA synthesized in vitro with α-^{32}P-labeled nucleoside triphosphates (*23*). The isolated trp mRNA product contained only the first 130 nucleotides of the leader sequence and no detectable amounts of distal mRNA. Thus under these conditions transcription in vitro terminates in the vicinity of position 130 in the leader region. Two 3′-terminal oligonucleotides released by T1 ribonuclease from the in vitro transcript have the apparent sequences CU_7-OH and CU_8-OH (C, cytidylate; U, uridylate) (Fig. 2); the exact number of U's is difficult to determine (*23*). These sequences are strikingly similar to the 3′ termini of other small RNA molecules, such as 4*S* and 6*S* RNA of bacteriophage λ terminating in U_6A-OH (A, adenylate) (*24, 25*), ϕ80 M_3RNA terminating in CU_5-OH (*26*), and *E. coli* Band IV RNA terminating in U_6A-OH (*27*). Although we do not yet know whether termination in the leader region in vivo is dependent on or facilitated by rho-factor (a transcription termination protein), it appears that accessory components are not needed for termination in vitro. We have not yet determined whether termination in vitro involves dissociation of polymerase from the template as well as cessation of polymerization. Examination of the DNA region in the vicinity of the termination site reveals the existence of two regions of dyad symmetry (Fig. 2). These sequences may be either sites at which multimeric proteins bind or regions of secondary structure which influence polymerase function (*28*).

Termination of transcription within the leader region in a minimal in vitro transcription system may not be absolute. Other results (*7, 29*) suggest that at least a small fraction of polymerase molecules can traverse the entire leader region and continue into the structural genes. Trp operon transcription in a coupled transcription-translation system also yields structural gene mRNA, but the extract employed in such studies may contain cellular components that allow transcription initiated at the trp promoter to proceed beyond the termination site in the leader region (*20*).

The results obtained in in vitro transcription studies with the trp operon must be distinguished from those reported with other systems (*25, 30*), in which pauses or hesitations in transcription were observed. We find that under various experimental conditions most polymerase molecules stop abruptly at the termination site in the leader region. By contrast, transcription in vitro of lac DNA (*30*) and λ DNA (*25*) is characterized by pauses at specific sites in the DNA, but transcription proceeds beyond these sites, resulting in a discontinuous rate of transcription rather than termination.

Additional evidence for the existence of the attenuator. In the course of other studies three new ilv (isoleucine-valine)-linked polarity suppressors have been isolated (*31*). Polarity suppressors reverse the reduction in distal gene expression resulting from nonsense or frameshift mutations in one of the genes of a polycistronic operon, such as the trp or lac operon. They differ from nonsense suppressors in that they restore expression of distal genes without permitting translation of chain termination codons. Under nonstarvation conditions, our polarity suppressors (*31*) are more efficient in relieving polarity than previously studied polarity suppressors (*32*). In addition, two of the new polarity suppressors increase expression of the wild-type trp operon in genetically derepressed (*trpR*$^-$) bacteria—one twofold and the other approximately fivefold (*31*). Since all the trp operon polypeptide levels are increased, we assume that the suppressors affect a site early in the operon which normally limits operon expression. The observed increase in trp enzyme levels was correlated with increased levels and rates of synthesis of trp mRNA corresponding to the structural genes; that is, the suppressors increase synthesis of structural gene trp mRNA without affecting its degradation, By examining the effects of the polarity suppressors on the trp internal deletion mutants mentioned earlier, the site of action of the suppressors was shown to be at or near the attenuator in the leader region (*31*). The existence of mutants which relieve presumed termination events in the leader region implies that some cell component can be altered so as to allow RNA polymerase to proceed beyond the termination site more frequently than in the wild-type cell.

Ribosome-protected regions in the initial segment of trp messenger RNA. When *E. coli* ribosomes are incubated in vitro with the mRNA fragment consisting of the first 190 nucleotides of trp messenger, two distinct regions of the messenger bind ribosomes and are protected from nuclease attack (*33*). One of these two regions contains the AUG codon which serves as the site of initiation of translation for the *trpE* polypeptide. The other protected fragment also contains an AUG codon and is located near the 5′ end of the leader sequence; the distance between these two AUG codons is approximately 140 nucleotides (see Fig. 2). The two sites are bound and protected with approximately equal efficiencies (*33*). Experiments utilizing a smaller RNA fragment lacking the *trpE* initiator demonstrate that ribosome binding to the early region in leader mRNA is independent of the presence of the *trpE* region (*33*).

It is therefore quite possible that ribosomes bind to the leader mRNA in vivo, and that ribosome binding is related to some functional aspect of the leader sequence, although we cannot exclude ribosome protection of the early site as an artifact of the in vitro system. If ribosomes binding in vivo actually initiated translation of the leader mRNA, a small polypeptide would be produced, probably less than 40 amino acid residues in length. Such a polypeptide could be involved in regulation, possibly at the attenuator. Alternatively, ribosomes may bind to leader mRNA without the concomitant initiation of translation, perhaps to protect the messenger from nucleolytic attack, or to preserve some structural feature of leader mRNA that has regulatory significance.

Regulatory role of the attenuator. In vivo, the attenuator may function as an optional termination signal at which the cell governs the fraction of initiated RNA polymerase molecules that will transcribe the entire trp operon. What purpose would such a termination signal serve? It may, for example, respond to some state of cellular metabolic activity related to tryptophan biosynthesis, thereby providing a mode of regulation in addition to repressor-operator control. Accordingly, we have examined the effect of tryptophan deprivation on regulation of termination at the attenuator (*13*). Specific hybridization measurements were made to determine the relative rates of synthesis of trp mRNA corresponding to the leader region, and to a small well-defined region within the structural genes. The results indicate that termination in the leader region is, in fact, less frequent under conditions of tryptophan starvation (*13*). In a genetically derepressed (*trpR*$^-$) culture growing with excess tryptophan, approximately one of every ten polymerase molecules that transcribe the leader region continues through the structural genes to the end of the operon (*13*). (This ratio is in good correlation

with the maximum increase in operon expression observed in mutants in which the attenuator is deleted.) After a shift to conditions of mild tryptophan starvation the fraction of initiating polymerases that reaches the end of the operon increases to approximately one out of four. Thus, when the requirement for tryptophan biosynthesis is great, attenuation allows a greater fraction of initiating polymerases to proceed into the structural genes of the operon.

What regulatory feature does attenuation add to the repression mechanism? We propose, on the basis of our observations to date, that attenuation allows the cell to distinguish between conditions of mild as compared to severe tryptophan limitation. Thus we expect that when *E. coli* is in an environment with abundant tryptophan, expression of the trp operon is minimal because of repression and attenuation. However, as the supply of tryptophan for protein synthesis is reduced, we envision a two-stage response to progressively more acute tryptophan starvation; initially repression would be lifted and, upon greater starvation, attenuation would be relaxed. This would enable the cell to regulate production of structural gene trp mRNA over a greater range than possible by the repression mechanism alone. Relaxation of attenuation coupled with the fact that trp mRNA is degraded more slowly under conditions of tryptophan starvation (*34, 35*) would result in the accumulation of increased levels of structural gene trp mRNA; this could permit increased synthesis of trp operon polypeptides (*36*).

We can imagine different mechanisms by which the attenuator could be used to sense the acuteness of tryptophan starvation. One possibility is that a decrease in the intracellular tryptophan level directly triggers relaxation at the attenuator. Perhaps there is a termination factor which, like the trp repressor, inhibits transcription (in this case at the attenuator) when complexed with tryptophan. If relaxation of attenuation were effected at a lower tryptophan concentration than that which relieves repression, the operon would be regulated over a wider range of tryptophan concentrations than possible by repression alone. Alternatively, tryptophan could directly inhibit a positive acting "readthrough factor" which functions at the attenuator, thereby allowing termination of transcription. A second possibility is that attenuation is governed by the concentration of some cellular product which accumulates during tryptophan or amino acid starvation, rather than by changes in the tryptophan concentration per se. Tryptophan starvation, like starvation for other amino acids, undoubtedly results in the accumulation of guanosine tetraphosphate (ppGpp) (*37*). Thus ppGpp could interact with an attenuator-binding protein and promote continuation of transcription through the trp attenuator region (*38*). We know that when protein synthesis and cell growth are limited by the availability of tryptophan, there is a preferential restriction of ribosomal and tRNA synthesis (*39*). By contrast, the synthesis of structural gene trp mRNA would be amplified through relief of attenuation.

In addition to allowing the cell to distinguish between mild as opposed to severe tryptophan limitation, as we have proposed, the attenuator may serve other regulatory functions. (i) It may be used to regulate the frequency of transcription termination in response to changes in the levels of other key metabolic intermediates, such as compounds involved in carbon or nitrogen metabolism. (ii) Detection of a leader mRNA ribosome binding site in vitro raises the possibility that trp leader mRNA codes for a polypeptide. Attenuation could reflect the need for more leader polypeptide than structural gene polypeptides under certain growth conditions. (iii) Transcription through the attenuator region may be controlled by trp operon gene products directly, providing a mechanism for the cell to gauge the level of trp biosynthetic enzymes. Somerville and coworkers (*40*) have in fact presented evidence suggesting a *trans* regulatory effect of anthranilate synthetase on trp operon expression.

In addition to our findings with the trp operon, studies with two other amino acid biosynthetic operons, the histidine (his) operon and the ilv operon suggest the existence of regulatory attenuator sites. An in vitro protein synthesizing system dependent on DNA has been developed for examining regulation of the his operon of *Salmonella typhimurium* (*41*). Studies with this system support the genetic observations of Kasai (*21*) and provide additional evidence that an attenuator mechanism regulates the his operon. These studies also suggest an independent positive control system for this operon, involving ppGpp (*41*). The properties of the proposed attenuator in the his operon of *Salmonella typhimurium* (*21*) are similar to those of the trp operon in that (i) the site is thought to act as a transcription termination site, (ii) the site is located between the operator and the first structural gene, (iii) deletion of the attenuator enhances expression of the operon, and (iv) a protein factor is thought to interact at the site to regulate transcription termination. It was also proposed (*21*) that the protein factor binds at the his promoter and subsequently allows transcription to proceed beyond the attenuator. A similar mechanism of interference with termination has been considered for the N protein of bacteriophage lambda (*42*). In studies with the ilv operon of *E. coli* it was observed that the polarity suppressor, *suA*, caused a four- to fivefold increase in the repressed levels of the ilv biosynthetic enzymes (*43*). This finding, viewed in the light of our observations with polarity suppressors, suggests that there may be an attenuator in the ilv operon. Further studies with all of these systems should clarify the function of attenuators and indicate how this function is affected by interacting proteins.

In summary, we have detected a new type of regulatory site in the trp operon of *E. coli*. In contrast to other regulatory sites, at which control is exerted over RNA polymerase binding, this new site is used to regulate termination of transcription in a region of the operon preceding the structural genes.

Note added in proof: The 5′ end of trp mRNA synthesized in vitro has been tentatively identified as pppAAG (*44*). This oligonucleotide immediately precedes the sequence in Fig. 2. It has also been found that starvation for amino acids other than tryptophan does not relieve termination of transcription in the leader region (*13, 45*). This suggests that recognition of tryptophan is involved in regulation at the attenuator. Studies in vivo implicate tryptophanyl-tRNA synthetase in this regulation (*45*).

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NEWS AND COMMENT

NSF: House Appropriations Panel Gives Warning Tug on Purse Strings

Through much of the National Science Foundation's formative period in the 1950's and 1960's, the chairman of the House Appropriations subcommittee which handled funds for NSF was Representative Albert Thomas (D–Texas). Thomas was one of the House's old-style, pragmatic philosophers who counseled NSF to stick to slow growth and hard science and avoid controversy that, for example, support of social sciences research might incite. He reinforced his advice by maintaining a firm grip on the purse strings.* Last week the Appropriations Committee followed the lead of the NSF subcommittee, now headed by Representative Edward P. Boland (D–Mass.) and treated NSF in a way reminiscent of the paternalistic, Thomas tradition.

The committee on 19 June approved an appropriations package for NSF which tightened the reins on the agency, most obviously by providing no funds for "implementation" of NSF's school curriculum improvement program. The ban on spending of funds—amounting to $9.2 million—used to inform school authorities about NSF-funded courses and to train teachers to employ them, was directly inspired by criticism within Congress of some behavioral science courses developed with NSF support, particularly one called "Man: A Course of Study" (MACOS) (*Science*, 2 May).

The committee also held the line on the basic research portion of the appropriation, recommending $345 million for the fiscal year compared with an actual appropriation of $340 million for the current year and the President's budget request of $380 million for the coming year.

As the following language in the draft report on the appropriations bill shows, the committee was influenced by recent criticism of the relevance of some research projects:

> In recent months particular activities of the Foundation have been questioned. Members of Congress, representatives of the press, and countless American taxpayers have been openly critical of the uses of tax revenues to finance seemingly frivolous and irrelevant scientific research projects. A number of specific examples have been cited, and responsibility for some has been attributed to the Foundation. Investigation has shown that in *most* cases the responsibility for these grants rests with agencies other than NSF, and some charges of frivolity have stemmed from an inadequate understanding of the scope, purpose and intent of research.
>
> Nevertheless, the Committee is aware of a responsibility to insure that the quality and value of scientific research undertaken is commensurate with the tax dollars provided.

The committee recommended a total of $707 million for NSF in fiscal year 1976, slightly less than the appropriation for the current year, but the carryover of funds deferred last year by the Administration will add $20 million. NSF officials seem to feel that, under the circumstances, the appropriation bill could be a lot worse from the agency's standpoint.

NSF had ample reason to feel it was under the gun. Appropriations Committee chairman Representative George H. Mahon (D–Texas) had written NSF director H. Guyford Stever a letter in May finding fault with NSF in very strong terms. Neither Mahon nor Stever made the letter public, but the contents became fairly widely known. Mahon wrote that if he discovered "damn fool" NSF projects, he would seek to cut millions of dollars out of the agency's budget. He went on to say he was "sick and tired of responding to correspondence from citizens who are blaming Congress for some of the idiotic things done by a few unstable people in the executive branch."

Mahon is said to have commented in the same vein during committee discussion of NSF, but the NSF sections of the pending appropriations bill, which includes funds for NASA and other independent agencies, is said to be essentially the handiwork of Boland and his subcommittee.

NSF officials were apparently braced for the Appropriations action. Under the circumstances, the committee treatment of NSF is being interpreted as reflecting basic sympathy for the agency, but signaling clearly that NSF had better change some of its ways.

NSF has far from finished running the gauntlet of criticism. Representative John B. Conlan (R–Ariz.), who called congressional attention to NSF-supported behavioral sciences courses in general, and to MACOS in particular, continues to press

*A notable exception to Thomas's normal caution and frugality where NSF was concerned was his enthusiasm over the selection of a Houston engineering firm for the ill-starred Mohole ocean-drilling project in the early 1960's. The Houston firm's bid on the Mohole contract was higher than that of two competitors. No impropriety was ever documented, but the fact that officials of the firm were political friends of Thomas, whose district was in the Houston area, and of then Vice President Lyndon B. Johnson, gave rise to speculation that NSF had succumbed to political influence. This was to add embarrassment to what became NSF's most controversial project.