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"Perspectives on Protein-Nucleic Acid Interactions on the Regulation of Biosynthetic Pathways in Bacteria and Fungi"

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ABSTRACT: *Bacteria and fungi have developed stringent and sophisticated mechanisms for the control or regulation of both anabolic and catabolic pathways for survival of the species. The control of aromatic amino acid biosynthesis has been extensively studied in fungi and bacteria, and it is clear that nature has evolved many solutions to the problem of control in branched pathways. It is pertinent to understand that these control or regulatory mechanisms have been detected or depicted in only a few selected bacteria and fungi, and they have in vast cases been untested and unproven to occur in several other bacteria or fungi; however, when ardent research is extant, they are obtainable or discovered. Certain normal mechanisms by which bacterial and fungal cells regulate or control their metabolic activities are stated in this paper. This study of protein-nucleic acid interactions in the regulation of amino acid biosynthetic pathways in bacteria and fungi reveals the unique regulatory mechanisms in these organisms.*

KEY WORDS: proteins, nucleic acids, regulation biosynthetic, pathways, bacteria, fungi

INTRODUCTION

Bacteria and fungi have developed stringent and sophisticated mechanisms for the control or regulation of both anabolic and catabolic pathways for survival of the species. Certain normal mechanisms by which bacterial and fungal cells regulate or control their metabolic activities are stated in this paper. There are invariably three conditions in which certain proteins function as both enzymes and repressors in a regulatory pattern and capacity. These are (i) the rate at which gene expression is conducted by the protein specified by the same gene; (ii) synthesis of pathway-specific enzymes by the first enzyme in the pathway; and (iii) synthesis of pathway-specific enzymes by aminoacyl-tRNA synthetases. In these systems, mutations affecting the structure of an enzyme culminate in alteration of regulatory trajectories. Whereas certain enzymes undoubtedly play a role in regulation, it is difficult to assign that function to a repressor role instead of some

other influence (Calvo & Fink 1971). The control of aromatic amino acid biosynthesis has been extensively studied in fungi and bacteria, and it is clear that nature has evolved many solutions to the problem of control in branched pathways. It is pertinent to understand that these control or regulatory mechanisms have been detected or depicted in only a few selected bacteria and fungi, and they have in vast cases been untested and unproven to occur in several other bacteria or fungi; however, when ardent research is extant, they are obtainable or discovered.

Dihydrofolate reductase:

As enzymes do function as repressors, it is remarkable that the product of a gene represses its own synthesis as exemplified for the locus specifying dihydrofolate reductase in Diplococcus pneumonia (Sirotnak et al 1969). The uncommon attributes of specific *ame* mutants are suggestive that dihydrofolate reductase represses the rate of its own synthesis, with the plausibility of several other pertaining models (Calvo & Fink 1971).

Thus, certain alternative explications need to be considered before the determination of a repressor function is allocated tosignifcant consideration such as (i) that dihydrofolate reductase makes rapid turnover, and the mutations resulting in elevated enzyme concentrations decrease the enzyme destruction rate. (ii) Derepression is dependent upon certain small molecule and dihydrofolate reductase is associated with its harnessing or production. For instance, dihydrofolate reductase catalyses the reactions: folate ------- dihydrofolate -------- tetrahydrofolate. The induction of dihydrofolate reduction by folate leads to certain mutations in the structural gene which may reduce the enzyme activity for the first reaction, culminating in folate accumulation as well as simultaneous and consequent induction. (iii) The role of dihydrofolate reductase synthesis is regulated remarkably at the translation level. Augmented enzyme synthesis might emanate from mutations in which certain condons become replaced by those with a less limitation impact upon translation. It is suggested that any significant difference depicts that the original codon must have undergone inadequate translation that it effectively restricted the translation rate; thus mutations at other loci cause slight effect on the translation rate (Calvo & Fink 1971).

Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) are the two folate-dependent enzymes which are critical for the provision of the requisite nucleotide precursors for the maintenance of DNA synthesis and repair. Also, with these essential functions in enzyme catalysis, the two enzymes have been demonstrated to act as RNA binding proteins. By means of in vitro and in vivo experimental model systems, it was shown that the fundamental resultant impact of binding of TS protein to its cognate mRNA including DHFR binding to its own DHFR mRNA is translational repression. Restriction of these usual translational autoregulatory feedback

mechanisms portends the molecular basis for the enhanced development of cellular drug resistance (Tai et al 2004).

E. coli D3-157 is a streptomycin-resistant bacterial strain that has undergone mutation as to relinquish its DHFR activity and necessitates the augmentation of thymidine to the medium for growth. It was observed that recombinant DHFRL1 contains adequate DHFR enzyme activity for the complementation of DHFR-negative phenotype in a bacterial system (McEntee et al 2011). It became perspicuous that DHFRL1 has the potential of the same translational autoregulation as DHFR by binding to its own mRNA, with every single enzyme being able to replace the other. Data on the dihydrofolate reductase gene family suggest a single functional gene within multiple intronless pseudogenes. The active DHFR gene is located on chromosome 5, and encodes an enzyme that catalyzes the dihydrofolate reduction to the biologically functional state, tetrahydrofol;ate.

Anthranilate synthetase:

At the translation level, anthranilate synthetase plays a role in the regulatory mechanism of tryptophan-producing enzymes (Lavalle & de Hauwer 1970). The mechanism of the translational regulation is unclear. It could involve the two enzymes, anthranilate synthetase or tryptophanyl-tRNA synthetase which have interactions with tryptophan. As the two enzymes are encoded by genes in disparate operons, it is suggestive that a diffusible agent is associated in the inhibition of enzyme synthesis (Lavalle 1970).

AcDNA and compatible promoter region for a naturally occurring, insensitive anthranilate synthasae (AS) subunit gene, ASA2 was isolated from a random, but 5-methyl-tryptophan-resistant (5MTr), tobacco, Nicotiana tabacum cell line, AB15-12-1 (Song et al 2001). The ASA2 cDNA has a putative transit peptide sequence, and Southern hybridization revealed that more than a single intextricably linked sequence is available in the tobacco genome. It was suggested that expression of the altered AS is controlled in a tissue-specific pattern, and that 5MTr selection generates lines with elevated concentrations of the feedback insensitive AS form that is expressed in culture cells but not in plants.

Several aromatic compounds can be synthesized via the shikimate pathway in higher plants (Haslam 1993). Chorismate resides at a branch point of the pathway and is ultimate prevalent precursor of a lot of these compounds. AS catalyzes the first committed reaction in the Trp-biosynthesis branch by the conversion of chorismate to anthranilate, and it is feedback-inhibited by the endproduct, Trp. It is perspicuous that AS is the control point in the Trp branch in plant

cells due to (i) pathway-intermediate feeding studies (Widholm 1974); (ii) enzyme activity concentrations; (iii) feedback inhibition of the specific enzyme activities (Singh & Widholm 1974); and (iv) 5MT resistance selection that resulted in lines with altered feedback-inhibited AS and increased free Trp (Widholm 1972).

Phosphoribosyltransferase (PR-ATP synthetase):

This enzyme plays a role in repression. It is suggested that when histidine and triazole alanine (or attached to $tRNA^{His}$) are bound to the wild type PR-ATP synthetase at the feedback site causes conversion of the enzyme into a repressor or a unit of a repressor. With regard to strains with feedback insensitive PR-ATP synthetases, it is possible that histidine rather than triazole alanine (and His-$tRNA^{His}$ and TRA-$tRNA^{His}$) for the wild-type and feedback-insensitive enzymes. If the first enzyme in a pathway is involved in repression, certain mutants within the structural gene for that enzyme must culminate in constitutive and depressed states of pathway-specific enzymes (Kovach et al 1969). PR-ATP synthetase possesses a high affinity for charged $tRNA^{His}$, and also has interactions with uncharged $tRNA^{His}$ (Kovach et al 1970).

An investigation of the specificity of the interaction between phosphoribosyltransferase and partially purified preparations of several species of tRNA using a filter binding assay exhibited a higher affinity of the enzyme for histidyl-tRNA than for arginyl- or glutamyl-tRNA. Competition experiments demonstrated that the enzyme did not discriminate between the aminoacylated and deacylated states of arginine tRNA or glutamic acid tRNA as every binding of the aminoacylated tRNA is liable to be inhibited by deacylated tRNA. The enzymes discriminated between the the aminoacylated and deacylated states of histidine tRNA, though Vogel et al 1972). There existed about 70% specificity of the binding of aminoacylated histidine tRNA as merely 30% of the binding was inhibited by deacylated tRNA. It is suggested that the regulatory role of phosphoribosyltransferase is conducted as a complex with histidyl-tRNA (Vogel et al 1972).

Contrary to the classic-operator model of negative control, an activator-attenuator model of positive control was presented for the principal operon-specific mechanism influencing histidine gene cluster expression of S. typhimurium using coupled in vitro transcription-translation system and a minimal in vitro transcription system (Artz & Broach 1975). The product, G enzyme or N-1-[5’ phosphoribosyladenosine triphosphate: pyrophosphate phosphoribosyltransferase of the primary structural gene, hisG of the histidine operon is unconnected in the positive control mechanism. A suggestive role for G enzyme as an accessory negative control component is preferential in this model. The operon-specific mechanism functions together with guanosine 5’-

diphosphate 3'-diphosphate [ppGpp] that could be a positive effector connected with the regulation of amino acid-forming systems.

Threonine deaminase:

Threonine deaminase of S. typhimurium comprises four identical subunits but contains just two moles of pyridoxal phosphate per mole of tetramer (Burns et al 1966; Zarlungo et al 1968; Sanderson 1970). Research conducted on the mechanism of the isoleucine-mediated inhibition of threonine deaminase from Bacillus subtilis and S. typhimurium (Hatfield & Burns 1970a) demonstrates its immature state as implicated in repression (Hatfield & Burns 1970b). Conversion to the active form is dependent upon pH. The presence of any of the enzyme effectors, being isoleucine, threonine or valine decreased the maturation half-time to circa 50 sec (Hartfield & Burns 1970c). Combinations of isoleucine and valine or isoleucine and threonine, but not that of valine and threonine caused the inhibition of the maturation process. The immature state of threonine deaminase specifically binds leucyl-$tRNA^{leu}$ (Hatfield & Burns 1970b) but is not bound to other charged tRNAs, and it does not bind to uncharged $tRNA^{leu}$. Moreover, leucyl-$tRNA^{leu}$ was not shown to prevent subsequent enzyme maturation elicited by one of its effectors. It is suggestive that the immature threonine deaminase form that is bound to leucyl-tRNA functions as a repressor to control expression of the *ilvADE* operon. Restriction of valine or isoleucine results in derepression due to depleted repressor via enzyme repression. L-threonine deaminase, the *ilv*gene product is necessary for repression of its own synthesis as an autoregulatory model, as also the enzyme is pertinent for induction of the ilvC gene product, and it is also a pivotal component for ilvADE and ilvB operon repression, as well as for the induction of the ilvC operon (Calhoun & Hatfield 1973). Threonine deaminase was partially purified from a nascent extreme thermophilic bacterium, Thymus X-1 that is identical to T. aquaticus YT-1 (Higa & Ramaley 1973). The threonine deaminase of strain X-1 exhibited a minimal reaction rate at 85-90^{o}C with greater thermostability than the mesophilic threonine deaminases. The molecular weight of the enzyme is ca 100,000-105,000, a K_m of 8.0, and as other threonine deaminases also catalyzes serine deamination. The Thermus X-1, however, did not exhibit a portent feedback isoleucine inhibition. It is probable that the isoleucine biosynthesis regulation in this extreme thermophile resembles that documented in Rodospirillum rubrum. Furthermore, the main characteristic of multivalent repression in which leucine is necessary to repress isoleucine and valine-producing enzymes is explicated that a restriction of leucine culminates in restriction of leucyl-tRNA, a required component of the presenting holorepressor (Freundlich et al 1962). The leucine analogue, fluoroleucine that replaces leucine for repression of the isoleucine-valine biosynthetic enzymes in S. typhimurium exhibits differential effects due to its ability to bind the different species of leucine

tRNA as shown by its ability to attach to leucine transfer ribonucleic acid tRNA, and its ability to protect approximately 70% of leucine tRNA from periodate oxidation (Freundlich & Trela 1969). It was revealed that the tertiary structure of an isolated polypeptide chain differed from its determined stable conformational state as a unit of an oligomer (Alpers & Paulus 1971). The concomitant assemblage of free subunits into an oligomeric form may be associated with an ostensibly unstable oligomeric intermediate form where the conformation of the components is compatible to that of the free polypeptide chains. This sort of metastable intermediate structure is separable from the stable oligomeric state by a significant energy barrier, and the pronounced temperature dependence of subunit corresponding reactions is attributable to an extant conformational state (Alpers & Paulus 1971).

Aminoacyl-tRNA synthetases:

In certain cases, aminoacyl-tRNA synthetases function in the repression of pathway-specific enzymes. It is imperative to show if amino acid-activating enzymes are invariably consistently involved in repression regulation; and if when involved, whether they act directly as aporepressors, or whether they act indirectly, catalyzing corepressor, that is, charged tRNA production. Experimental designs of these actions have either employed analogues of amino acids or mutants presenting altered aminoacyl-tRNA synthetases. Mutants of E.coli or typhimurium with altered aminoacyl-tRNA synthetases have been isolated for a vast majority of the 20 amino acids (Alexander et al 1971; Hoffman et al 1970) with depressed levels of pathway-specific enzymes (Hoffman et al 1970). In certain cases, mutants having altered synthetases have been demonstrated to present normally repressible pathway-specific enzymes (Calvo & Fink 1971). This does not preclude a role for these synthetases in regulation as there is no expectation that every mutation altering synthetase might influence repression.

The function of aminoacyl-tRNA synthetases in repression has been investigated via the mode of action and physiological impacts of amino acid analogues (Nass et al 1969). The problem is the interpretation of the studies using amino acid analogues entails the difficulty to investigate or predict all the potential impacts of the analogue. For instance, it is probable that coupled to inhibitory histidyl-tRNA synthetase that alpha-methylhistidine presents a higher affinity than histidine as an aporepressor, and that the complex has the attribute of an inactive repressor.

Aminoacyl-tRNA synthetases (RS) constitute pertinent elements of the cellular translation machinery exploitable for antibiotic development and utilization. It may be well-nigh impossible to identify specific enzyme targeted by a nascent natural or synthetic inhibitor since cells present numerous varied RS, normally for a specific amino acid. Using the primer extension technique

with specifically synthetic genes for the identification of the RS targeted by an inhibitor, it was demonstrated that synthetase activity of suppression decreased the level of the cognate aminoacyl-tRNA in a cell-free translation system culminating in the abrogation of translation as the corresponding codon penetrates the ribosome decoding centre (Orelle et al 2013). The importance of the technique is defined due to revelation of a switch in target specificity of certain synthetic inhibitors of threonyl-tRNA synthetase. The main function of an aminoacyl-tRNA synthetase (RS) is to charge tRNA with the cognate amino acid (Ibba & Soll 2000).

There are also those cases whereby analogues ostensibly do not influence aminoacyl-tRNA synthetases and still induce repression, as in analogues of tyrosine (Ravel et al 1965) and tryptophan (Mosteller & Yanofsky 1970). E. coli K-12 mutants were isolated from a nascent strain having only a single DAHP synthetase isoenzyme via the selection for resistance to the tyrosine analogue 4-aminophenylalanine where various enzymes associated with tyrosine biosynthesis were derepressed (Wallace & Pittard 1969). The mutation concerned with the derepression was mapped and the gene was demonstrated as not inextricably linked to arof and tyrA was designated as tyrR.

Ornithine, citrulline and hormoarginine exhibit a regulatory role on arginine biosynthetic enzymes in wild-type, but not so in argR mutants, as well as non-interference in the aminoacylation of $tRNA^{Arg}$ in vitro and in vivo. Similar aminoacylation reaction is apparently not modified in argR mutations, thus undergirding the mutation hypothesis associated with aporepressor or aporepression.

These could present significant evidence excluding the participation of synthetases or tRNA in repression; if there exists ardent demonstration that the analogue was not found to bind to the synthetases, and that there was specific inhibition of the synthesis of pathway-specific enzymes instead of being the etiologic agent for the synthesis of ‘false protein’ or the wholesome inhibition of protein synthesis. The former position is verifiable by demonstrating if the analogue is activated by or inhibits synthetase action. These demonstrations could not have been amenable to detect activation (Martin 1969). It is suggested that the synthetases having amino acid or analogue that is bound at an allosteric site functions as a repressor. It is, therefore, pertinent that binding in lieu of activation or inhibition be evaluated to unravel the polemics.

It has been variously determined that charged tRNA acts as a corepressor, for instance, in the valine and histidine pathways, whereas there are divergent suggestions for arginine and tryptophan pathways. All presenting data are compatible that the activating enzyme in association with an amino acid or aminoacyl-tRNA is a repressor of pathway-specific enzyme. Arguments exist

regarding the rate of synthesis of an aminoacyl-tRNA synthetase and its possible control by the intracellular content of cognate amino acid. Findings present arginyl- and histidyl-tRNA synthetases as not constitutively synthesized, rather there are disparities within a ten- to fifty-fold range in response to amino acid restriction. In addition, a not so preponderant data are suggestive that rates of synthesis of isoleucyl-, leucyl-, methionyl-, and prolyl-tRNA synthetases are dependent on growth conditions. Another observation was that the inclusion of excess amino acid to a culture previously restricted for that amino acid abruptly culminated in the cessation of synthetase formation, with the resumption of synthesis merely following the mass doubling of the cells (Lavalle & Hauwer 1970; Lavalle 1970).

Identification of genetic loci in either or both E. coli and S. typhimurium for synthetases specific for certain amino acids depicted from the map positions that the loci influencing aminoacyl-tRNA synthetases are affected by the energy charge intracellularly, viz, energy charge = [(ATP) + 0.5 (ADP)]/[(ATP) = (ADP) + (AMP)], and that purified histidyl-tRNA synthetase was noncompetitively inhibited by ADP and AMP (Brenner & Ames 1972; Brenner et al 1972).

The topography of the histidyl-tRNA synthetase active site was investigated by the determination of K_I values for certain structural analogues of histidine by means of ATP-PP_i exchange with tRNA aminoacylation reactions. It was revealed that (i) the enzyme is stereospecific in aminoacyl-tRNA complex formation, as the D-isomer of histidine has no effect on the two reactions; (ii) the carboxyl group is not necessary for binding; (iii) bulky carboxyl group derivatives preclude the molecules from being bound to the enzyme; (iv) the amino group allows for a convenient binding affinity; (v) the length of the ring side chain serves as a vital attachment point to the enzyme; and (vi) the inhibitory attributes of the analogues were determined by the types of ring heteroatoms (Lepore et al 1975).

Secondary metabolites

Fungi produce several low molecular weight compounds named secondary metabolites which function in remarkable cellular processes like transcription, development and intracellular communication. Genome mining demonstrates that the potential of fungi to form secondary metabolites has been grossly underestimated because a vast majority of the gene clusters of the fungal secondary metabolite biosynthesis are silent under conventional cultivation states (Brakhage 2013). With respect to fungi, there is an extant problem because they usually present with an excess of fifty biosynthetic gene clusters, and also the pertinent synthases and synthetases are iterative, depicting complex and cryptic programming. Gene clusters devoid of linkage to the biosynthesis of a specific compound are designated orphan clusters; of which most fungal

biosynthetic gene clusters are placed in this class. Numerous fungal biosynthetic gene clusters are ostensibly silent because they are not transcribed at optimum level to form a detectable chemical structure. Fungi as eukaryotes where a single structural gene encode as a particular biosynthetic enzyme regulated by its own promoter. These promoters are activated in, albeit, unique and ambiguous conditions. Certain silent fungal biosynthetic gene clusters are involved in the encoding of a transcriptional regulator. In a few instances as observed in the aspyridon A1 biosynthetic gene cluster, it was revealed that "biosynthesis" is switched on by overexpressing the transcription factor. The resultant impact is the activation of the promoters of the structural genes, culminating in the formation of the biosynthetic processes of their own, and the synthesis of an emergent compound. As not all silent biosynthetic gene clusters possess a transcription factor, there is limitation in this procedure. The induction of transcription factors is liable to activate undeciphered genes extraneous to the biosynthetic gene cluster forming unpredictable chemical impacts.

pH:

A system for gene expression regulation by extracellular ambient pH was first detected in Aspergillus nidulans. The system comprises products of the pacC and paIA, B, C, F, H, and I genes. pacC functions in the coding of a zinc finger transcription factor, with thesepal genes coding components of an ambient pH signal transduction pathway. pH regulatory systems were also detected in certain fungi. Units of these regulatory systems are homologous to those in A. nidulans (Denison 2000). As demonstrated, several fungi grow over an expansive pH range with their gene gene expression compatible to the ambient pH. In A. nidulans, it is clear that the transcription factor, pacC that constitutes gene activator expressed in alkaline states is involved in dual processing proteolysis, with the initial aspect being pH signal dependent and the other being proteasomal (Panalva et al 2008). Signal transduction comprises an intermediary connection of two complexes, one of which constitutes two plasma membrane proteins and an arrestin as well as the other that is constituted of PacC, a cysteine protease, a scaffold and edosomal units. The Saccharomyces cerevisiae PacC orthologue, Rim101p is different because it is not involved in the second round of proteolysis, and it acts directly only as a repressor. It was observed that PacC/Rim101 pH regulation is vital to the pathogenicity of fungi.

Genetics demonstrated that proteolysis of the transcription factor PacC at alkaline pH is pertinent for its function; thus suggesting that the complete form is not an active molecular aspect of PacC. It is implied that the transcriptional regulator PacC acts at both alkaline and acidic environmental pH as either the full-length or proteolyzed state, if it bears a pal-dependent molecular tag. It was observed that the pal gene products are associated in a metabolic pathway that culminated in

effector molecular synthesis that tagged the pacC product with probable enhancement of its proteolysis (Rossi et al 2013).

Comparative analysis:

Data obtained by the comparative analysis of the cre1 homologous gene creA from A. nidulansa conducted with pH-deregulated mutants revealed that ambient pH regulates the expression status of the carbon repressor genes, and that this regulation is independent on the pH transcription factor PacC (Vautard-Mey & Fevre 2003); thus indicating that enzyme synthesis is pH-dependent and amenable to carbon catabolite repression.

Comparative analysis of genes, operons and regulatory components was conducted for the lysine biosynthetic pathway in presenting bacterial genomes (Rodionov et al 2003). The study identified a lysine-specific element, the LYS element in the regulatory ambient of bacterial genes associated in lysine biosynthesis and transport. As regards other RNA regulatory elements for the vitamins riboflavin, thiamine, and cobalamine including purine and methionine regulons, the regulatory RNA structure is increasingly conserved on the levels of sequence and structure (Rodionov et al 2002). The LYS element encompasses areas of lysine-constitutive mutations as identified in E. coli and B. subtilis. It is suggested that the mechanism of the lysine-specific riboswitch is identical to the erstwhile stated mechanisms for other metabolite-specific riboswitches, and is associated either by transcriptional and translational attenuation in diverse bacterial groups (Vitreschak et al 2002). Detection of LYS elements in Gram-negative y-proteobacteria, Gram-positive bacteria of the Bacillus/Clostridium group, and the Thermotogales depicted the uncharacterized lysine regulon in the narrated bacterial species. Positional analysis of LYS components resulted in the detection of certain nascent candidate lysine transporters, viz, LysW, YvsH, and LysXy. The preferential candidates for lysine biosynthesis genes not detectable in Gram-positive bacteria were identified by employing the genome context analysis.

Vegetative incompatibility in fungi restricts the production of viable heterokaryons. It emanates from the coexpression of incompatible genes in the heterokaryotic cells and culminates in a cell death reaction. In podospora anserine, an alteration of gene expression occurs during this reaction as well as a significant reduction in total RNA synthesis, and the presence of a new protein assemblage. By employing in vitro mRNA translations and disaggregation of protein products via two-dimensional gel electrophoresis, it was demonstrated that the mRNA content of cells undergoes qualitative modification in the course of the incompatibility reaction (Bourges et al 1998). Invariably, gene expression is regulated during vegetative incompatibility, at the minimum, by variation of the mRNA concentration of specific genes. A subtractive cDNA library embedded

in sequences with preferential expression in the course of incompatibility was formulated. The library was employed for the identification of genome loci relating to genes of which the mRNA was induced during incompatibility. Three identical genes were featured and identified as IDI genes for gene induced within incompatibility. It is suggested that their expression profiles are involved in disparate steps of the incompatibility reaction. The putative IDI proteins encoded by these genes are detectable small proteins having signal peptides IDI-2 amd IDI-3 proteins present certain similarities in a tryptophan-rich ambient (Bourges et al 1998).

Aptamers comprise artificial nucleic acids which selectively bind small molecules. RNA sensors have been detected in both Gram-negative and Gram-positive bacteria which act as molecular switches elicited by direct structural binding of disparate metabolites. These riboswitches function as natural RNA aptamers which are entrenched in the hierarchical sequences of multiple metabolic genes. Riboswitches have the propensity in the repression or activation of their cognate genes during transcription and translation (Nudler & Mironov 2004). Riboswitches constitute genetic components resident in non-coding locales of certain mRNA which are available in all three life domains. The ligand binding to ligands which causes conformational alterations in the mRNA molecule culminating in gene transcription modulation, or RNA splicing, translation or stability. This control mechanism is specifically expansive in bacteria, and permits a direct response to diverse metabolic alterations. A vast majority of riboswitches have been detected in recent years which are suggestive of extant enormous diversity of regulatory ligands and genetic regulatory mechanisms (Bastet et al 2011).

DISCUSSION AND CONCLUSION

In bacterial and fungal cells, enzymatic reactions may be controlled or regulated, usually by dual unregulated modes, viz. control or regulation of (i) enzyme activity or end product inhibition that operates in the control or regulation of biosynthetic pathways; and (ii) enzyme synthesis and end product repression that functions in biosynthetic pathways, enzyme induction and catabolite repression which predominantly regulate degradative pathways. This study of protein-nucleic acid interactions in the regulation of amino acid biosynthetic pathways in bacteria and fungi reveals the unique regulatory mechanisms in these organisms.

It is perspicuous that allosteric control or regulation by end product inhibition is a vital pathway-specific regulatory mechanism for diverse biosynthetic pathways in bacteria and fungi. Repression and attenuation in response to end product presence transcriptionally regulate the biosynthetic genes and are pertinent to pathway-specific regulation in bacteria (Yanofsky & Crawford, 1987). In addition to pathway-specific regulation in fungi, a cross-pathway regulatory mechanism

referred to as general control or cross-pathway control is available to regulate amino acid biosynthesis (Hinnebusch, 1992). Starvation of yeast for a single amino acid leads to an increased steady state concentrations of mRNAs for a minimum of three dozen enzymes of amino acid and purine biosynthesis in at least a dozen pathways.

Feedback inhibition is consistent with a role In the regulation of amino acid and accumulation in microbes. A feedback-insensitive mutant of anthranilate synthase (ASA), the first enzyme in the tryptophan, Trp biosynthetic pathways, accentuates accumulation of three times greater free Trp than is available for the wild type. (Li & Last, 1996). The reaction reaction catalysed by A. thaliana AS may be limiting for the tryptophan pathway, and that accumulation of tryptophan biosynthetic enzymes is not reversed by a 3-fold excess of end product. The tryptophan, Trp biosynthetic pathway results in the formation of numerous secondary metabolites with various activities, and its control is foreseen to respond to the requirements for both protein synthesis and secondary metabolism. It is suggested that Trp regulation pathway enzymes in amino acid deprivation status is mainly a stress response to give the latitude for augmented secondary metabolite biosynthesis (Zhao et al, 1998).

There is ample evidence that RNA-based regulatory mechanisms are commonly employed in the control of gene expression in numerous organisms. These mechanisms make provision for the exploitation of relatively short, unique RNA sequences in the alteration of transcription, translation and/or mRNA stability in response to the availability of signal molecules. The capability of a mRNA segment to fold and produce alternative hairpin bodies with each compatible to a disparate control function allows for the selection of defined sequences which can influence transcription and/or translation (Yanofsky, 2007). The availability of rapid and frugal genome sequencing of fungi and bacteria has exposed numerous biosynthetic gene clusters which encode secondary metabolite synthesis. A vast majority of the gene clusters are amenable to accelerated annotation, for instance, by employing bioinformatic tools, such as Anti Smash; and the category of compounds specified by them are identifiable, but it is far-fetched to foresee the exact structure of the encoded metabolite for any extant natural product biosynthetic gene cluster (Wasil et al., 2013).

As regards the biosynthetic pathways whereby the end product functions as a corepressor, the problem is surmountable by determining if the complex binds to operator DNA. Another problem relates to aoprepressor levels in cells because low concentrations would present arduous task for any assay reliant on binding of corepressors to aporepressors or of the extant complex to DNA. There must be present either operator-constitutive mutants or strains containing a deletion of the operator region. The non-availability of appropriate mutants could present difficulty to observe the

specificity of any relevant protein-DNA interaction. (Calvo & Finki, 1971. In taking into consideration the relative merits of non-dispensable and dispensable proteins as repressors, there need be predictability of less limitations on dispensable repressor evolutionary presentation than on proteins with both enzyme and repressor functions. On the contrary, some enzymes such as, feedback-sensitive enzymes or aminoacyl-tRNA synthetases could appropriate a repressor activity during evolution since there are extant binding sites for readily available corepressors. In certain other instances, dispensable protein repressors evolved from enzymes which contained required binding sites, and culminated in the dissipation of enzyme function.

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