Review of Literature
2.1 Aminoacyl-tRNA synthetases

Aminoacyl-tRNA synthetases are a class of canonical enzymes catalyzing a very crucial step in protein biosynthesis and thus maintaining the fidelity of transfer of the genetic message at the translational level (Schimmel, 1987). These enzymes found in all life forms (Ofengand, 1977) were first described in 1955 (Hoagland, 1955). Initially they were thought to be aminoacyl-adenylate generating enzymes (Berg, 1956) but later it was shown that, the same enzyme also catalyzes the transfer of aminoacid to the cognate tRNA (Berg and Ofengand, 1958).

2.1.1 Occurrence and Number

Aminoacyl-tRNA synthetases are ubiquitous and found in all life forms like the archaebacteria, prokaryotes and eukaryotes. Unlike prokaryotes which possess only a single ARS for each aminoacid, irrespective of the presence of isoacceptors, eukaryotes possess multiple ARSs for a given aminoacid because of the presence of organellar enzymes and in a few cases (Leveque et al., 1990; Clark et al., 1990) two enzymes in the cytoplasm itself. When two forms of synthetases are present in the cytoplasm, it is suggested that the aminoacyl-tRNAs synthesized are channeled towards two different functions (Sivaram and Deutscher, 1990).

2.1.2 Molecular Structure

The molecular structure of ARSs varies from enzyme to enzyme and for the same enzyme, from species to species. They can be either monomers,
dimers or tetramers with identical or dissimilar subunits, giving rise to 4 types of quaternary structures namely $\alpha_1$, $\alpha_2$, $\alpha_4$ and $\alpha_2\beta_2$ (Schimmel, 1987). *E. coli* CysRS, a protein of 461 amino acids is the smallest monomeric synthetase known to date (Hou et al., 1991). TrpRS is a homodimer of 37,000 daltons each (Hall et al., 1982). On the other hand AlaRS (Putney et al., 1981) is an example for $\alpha_4$ tetramer (Mr. 380,000), while PheRS and GlyRs are $\alpha_2\beta_2$ tetramers (Dessen et al., 1990).

The variance for the same enzyme in different species can be noted from the findings that AlaRS, a tetramer in *E. coli* is a monomer of 115 KDa in *Bombyx mori* (Nishio and Kawakami, 1984a; Dignam and Dignam, 1984); GlyRS a heterotetramer with $\alpha_2\beta_2$ structure in *E. coli* exhibits a dimeric structure of Mr. 166,000 in *Bombyx mori* (Dignam and Dignam, 1984) and is twice the size of the bacterial enzyme in humans (Fett and Knippers, 1991).

### 2.1.3 Multienzyme complexes

In higher eukaryotes the aminoacyl-tRNA synthetases occur as high molecular weight complexes, an assembly of multiple synthetase activities. The first such complex isolated was from rat liver which contained all the ARSs and a large portion of the tRNAs (Bandyopadhyay and Deutscher, 1971). In sheep liver, extensive purification lead to the isolation of a complex with 7 ARSs (Kellerman et al., 1979) with apparent molecular weight $10^6$ on agarose, which failed to dissociate at high salt concentrations (Brevet et al., 1979). Complexes containing 9 ARSs were also reported (Norcum, 1989).
Molecular weights of the specific subunits of the complexes from rabbit and sheep liver were measured and assigned to major polypeptide components viz LeuRS, MetRS, GlyRS, LysRS, ArgRS and the unassigned was presumed to be IleRS (Johnson and Yang, 1981; Mirande et al., 1982a,b).

Some of the enzymes like AspRS in rat liver exist in both free form and in the complex (Mirande and Walter, 1989). ArgRS which also exists in two forms exhibits an extra -NH₂ terminal extension in the complex which creates the basicity and is presumed to contribute to the polyanion binding properties (VelleKamp and Deutscher, 1987).

Some ARSs form complexes with enzymes other than ARSs and protein factors like elongation factor (Filonenko et al., 1989; Sarisky and Yang, 1991; Motorin et al., 1991). Other than being in complex form, ARSs show a distinct character of being associated with non-protein components. Arg and LysRS complexes isolated from rat liver contain mannose and N-acetyl glucosamine (Dang et al., 1982). TrpRS though not associated with the complex also showed the presence of monosaccharides like mannose, fucose, galactose and N-acetyl glucosamine (Kovaleva et al., 1992). Similarly, homogenous yeast cytoplasmic and mitochondrial PheRS exhibited the presence of carbohydrates (Gabius et al., 1983a). High molecular weight complexes were also found to contain lipids (Saxholm and Plot, 1979), 5srRNA (Ogata, 1991a) etc. This 5srRNA acts as an activator of methionyl, arginyl and isoleucyl-tRNA synthetases (Ogata, 1991b).

Work on the role of lipids suggested that delipidation affects the functional and structural properties of individual synthetases which include
sensitivity to salts, detergents, protein digestion and temperature inactivation
(Sivaram et al., 1988; Sivaram and Deutscher, 1990).

Contrary to the general belief that high molecular weight complexes are
found only in higher eukaryotes, similar complexes were also isolated from
bacterial cells which may have escaped earlier detection due to fragility during
isolation. The same complex also seems to contain tRNA modification enzymes
leading to the speculation that final steps in tRNA modification might occur
just before aminoacylation (Harries, 1987; 1990).

Finally multienzyme complexes are implicated in the fidelity of
aminoacylation as it was observed that, ARS complex can recognize tRNA\textsuperscript{Ile}
from the same species more specifically than single chain IleRS (Eguchi et al.,

2.1.4 Structure-function relationship of aminoacyl-tRNA synthetases

As is evident from the varied molecular structures of ARSs, though they
exhibit diversity in their quaternary structure and size, there is a common
theme which runs through the organization of the structure of the entire class
of enzymes. This is an arrangement of functional units along the aminoacid
sequence such that the synthesis of aminoacyl-adenylate is determined by
sequences located within the amino terminal halves of the protein, RNA
recognition is determined by these sequences and those that are located on the
carboxyl side of the sequences that are required for adenylate synthesis (Jasin
et al., 1983; Schimmel, 1987). The carboxyl-end residues of the peptide chain
are believed to be involved in the structural conformation rather than in catalytic function which was later proposed by producing a series of C-terminal truncated MetRSs (Mellot et al., 1984).

In the last decade, there has been a spurt in studies on sequence homologies and their functional significance. In most of the enzymes there is a region of 11 aminoacids called 'signature sequence' located in the aminoterminal part. Out of 11, there are 10 identities and one conservative substitution between the E. coli Ile and Met enzymes, which is the strongest homology observed between two synthetases (Webster et al., 1984).

Apart from the signature sequence, other smaller sequences like HIGH, KMSKS and other motifs were found in some of the synthetases, based on which recently a comprehensive classification of synthetases with a clear partition in to two families of equal size called class I and class II with functional correlation was given. Each class is subdivided into three subclasses (Eriani et al., 1990a; Cussack et al., 1991; Schimmel, 1991a and Moras, 1992).

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoacylation site on terminal ribose</td>
<td>2'OH</td>
<td>3'OH</td>
</tr>
<tr>
<td>Characteristic motifs</td>
<td>1. HIGH</td>
<td>1. ... P ....</td>
</tr>
<tr>
<td></td>
<td>2. KMSKS</td>
<td>2. ... FRXE</td>
</tr>
<tr>
<td></td>
<td>3. ... GXGXGXER</td>
<td></td>
</tr>
<tr>
<td>Subclasses</td>
<td>a   b   c</td>
<td>a   b   c</td>
</tr>
<tr>
<td></td>
<td>Leu  Tyr  Arg</td>
<td>His  Asp  Gly</td>
</tr>
<tr>
<td></td>
<td>Ile  Trp  Gln</td>
<td>Phe  Pro  Asn</td>
</tr>
<tr>
<td></td>
<td>Val  Trp  Glu</td>
<td>Ala  Ser  Lys</td>
</tr>
<tr>
<td></td>
<td>Cys  Met</td>
<td>Thr</td>
</tr>
</tbody>
</table>

Bold type in the motifs are conserved residues.
In class I enzymes, the first consensus sequence HIGH, where I can be leucine, valine or methionine forms part of the signature sequence, however out of four residues only two are strictly conserved (the first histidine and the glycine). The two histidine residues were shown to be involved in ATP binding. The second motif KMSKS first identified by affinity labeling was later shown in all class I ARSs by sequence analysis (Hountondji et al., 1986). In this motif the strict conservation is limited only to the second lysine, among all known sequences, which was shown to be essential for the aminoacid activation catalyzed by MetRS in the E.coli system (Mechulam et al., 1991a,b). Other than these two motifs, there is a dinucleotide binding fold called Rossmann fold which was first characterized 15 years ago from the structural analysis of the enzymes of the glycolytic pathway (Rossmann et al., 1974). This domain contains a parallel β-sheet with a βαβ structural motif as the repeating unit, with the consensus HIGH sequence located at the turn between the first β-strand and the first α-helix, the KMSKS in the loop connecting the fifth β-strand and the fifth α-strand.

The class I enzymes are chiefly monomeric except for Tyr and Trp which are dimeric, are again divided into three subclasses as given in the table I (Moras, 1992). In all the enzymes except ArgRS the first half of the Rossmann fold contains the signature sequence occurring with in the first 70 aminoacids.

The major difference among the three subclasses is dependant on the connective polypeptides (CP₁ and CP₂). The Met family (CIa) contain a second connective polypeptide (CP₂) between the first and second strands, the Gln
family has CP$_2$ between the seconds and third strands, and the Tyr family has no identifiable CP$_2$ (Burbaum and Schimmel, 1991).

IleRS of *Methanobacterium thermoacetotrophicus marbarg* which also contain HIGH, KMSKS sequences, has homology of 30% with the yeast enzyme and 32% with that from *E. coli* thus fitting into the class I family (Jenal et al., 1991).

Class II enzymes, where all 10 ARSs are oligomeric lack both HIGH and KMSKS motifs but contain a mutually exclusive set of motifs identified from data obtained on ProRS sequence (Eriani et al., 1990a, 1991), structure determinations of SerRS (Cussack et al., 1990) and the AspRS : tRNA$^{Asp}$ complex (Ruff et al., 1991). These class II enzymes are again divided into three subclasses.

Motif I, a signature peptide of 18 residues (+G (F/Y) XX (V/I/L) XX P@, where + is a positively charged and @ is a hydrophobic residue) surrounds an invariant proline. Motif II (+@XXFRXE) contains an invariant arginine. Motif III (G@G@G@ER@@@) also involves an invariant arginine fitting the pattern and appears in all 10 class II synthetases and is the only one of three to be found in GlyRS β subunit (Carter, 1993). Motif II has tentatively been identified in AlaRS (Cussack et al., 1991) and in HisRS (Raben et al., 1992). Motif I is present in at least seven class II enzymes.

Though ARSs are divided into two entirely different classes, it can be observed from the various crystallographic and site specific mutagenic studies
that in both the classes, respective signature sequences and motifs present are involved in their catalytic activity.

One of the best worked out examples for class I enzymes is TyrRS from \textit{B.stearothermophilus}. The most striking feature of the subunit surface, is a deep cleft which forms the adenylate binding site. Opening from the bottom of this cleft, is a narrow pocket which is the tyrosine binding site, where the aromatic ring of tyrosine lies besides strands $\beta$ and $\epsilon$ of the six stranded sheet. The pocket has two polar groups which are favourably oriented to form hydrogen bonds with the hydroxyl group (Brick \textit{et al.}, 1987, 1988). Other residues like HIS45, Lys48 of motifs 225, 230 and 233 of pattern III, positively charged residues 265, 268 and Asp194 of GSDG sequence are all important for the catalytic activity of aminoacyl adenylate formation (Sokalski \textit{et al.}, 1991). Similarly K230, K233 and T234 of the KMSKS loop in \textit{E. coli}, help in adopting a highly constrained conformation during transition state complex formation (First and Fersht, 1995), histidyl residues of TyrRS in bovine liver are involved in catalysis (Gnatenko, \textit{et al.}, 1991a), four lysine residues per enzyme dimer in cattle liver were implicated in tRNA recognition (Gnatenko, \textit{et al.}, 1991b). In \textit{E. coli} TrpRS; HIGH is present as \textit{T}$^{17}$IGN and \textit{T}$^{17}$ and Lys195 in \textit{K}$^{195}$MSKS were found to be involved in aminoacyl adenylate formation (Chan and Krepp, 1995).

MetRS from the extreme thermophile \textit{T. thermophilus} HBB show low homology (25\%) with those from \textit{E. coli}, cytoplasm and mitochondria of yeast. However, the binding site for ATP, the anticodon and the 3'terminal of \textit{tRNA}^{Met} are highly conserved among all four MetRS stressing on the
implication of signature sequences in catalytic activity of the enzyme. Lys61 and Lys335 part of the dinucleotide binding fold are involved in the catalytic activity of the MetRS (Hountondji and Blanquet, 1985; Hountondji et al., 1990).

Arg52, Arg233 of MetRS in E. coli is also conserved in the ARSs specific for Ile, Leu and Val (Ghosh et al., 1991a) His 301 and Trp 305 (Fourmy et al., 1991) are important in direct interaction with the bound aminoacid, while Lys142, Tyr 358 and Tyr 359 are involved in the binding of ATP and enhancement of rate of intermediate formation by bringing about a significant conformational change which supports the induced fit mechanism proposed for class I enzymes (Ghosh et al., 1991c).

The tRNA binding domain in the enzyme was determined to be at a site close to Lys465 (Leon and Schulman, 1987; Schulman et al., 1987), Trp 461 (Schulman and Pelka, 1988; Ghosh et al., 1990), Lys 402, 439, K596, 461 (Brunie et al., 1990); phylogenetically conserved residue 395 of the sequence 391-395 (Ghosh et al., 1991b; Meinnel et al., 1991b and Kim et al., 1993).

E. coli CysRS shows striking homology with MetRS, to a lesser extent with Ile, Leu and ValRSs (Eriani et al., 1991), and has segments common with the cytoplasmic LeuRS of Neurospora crassa, TrpRS of B. steaothermophilus; PheRS of Saccharomyces cerevisiae and is close to the equivalent acceptor binding domain of the GluRS of E. coli and surprisingly a further resemblance to SerRS, a class II enzyme. This has led to the belief that both classes had a common origin and this was the ancestor of the CysRS (Avalos et al., 1991).
The crystallographic data showed that, Asp 235 contacts with the 2-aminogroups of guanine via the tRNA minor groove in the acceptor stem at G$_2$ and G$_3$. The central domain of this synthetase binds ATP, glutamine and the acceptor end of tRNA, and makes specific interactions with the acceptor stem, supporting the belief that aminoacid and ATP binding sites are together and the tRNA binding site is nearby (Rould, et al., 1989). Arg130 and Glu131 are important for accuracy of aminoacylation as it was found that mutation here lead to misacylation with tRNA$^{\text{Glu}}$ (Durasevic et al., 1993a).

Coming to class II enzymes in human HisRS, all three motifs have been demonstrated (Raben et al., 1992). Another enzyme showing all three motifs is ThrRS of human, which shows extensive homology with yeast cytoplasmic, mitochondrial and E. coli ThrRSs (Cruzen and Arfin, 1991).

SerRS, the first example of an ARSs that does not contain a dinucleotide binding fold has an active site on top of the central part of the antiparallel $\beta$-sheet (Cussack, et al., 1990). Similar catalytic site domains are formed by the signature motifs built around the antiparallel $\beta$ sheet and flanked by 3 $\beta$ helices that form the pocket in which ATP and the -CCA end of the tRNA bind. The loop between 320 and 342 in motif2 of AspRS interacts with the acceptor stem and brings about a conformational change in tRNA to facilitate anticodon binding (Ruff et al., 1991) where as in the case of SerRS, the anticodon arm does not bind to the protein, though the tRNA binds across the dimer (Price et al., 1993).
Tyr 426 of *E. coli* AsnRS is involved in ATP binding, (Anselme and Hartlein, 1991) while Pro231 is implicated in a structural role of positioning the loop formed by motif 2 (Madern *et al.*, 1992).

Yeast mitochondrial, cytoplasmic and *E. coli* LysRS show several regions of high primary sequence conservation. The fact that these domains are seen in the AspRS and AsnRS confirms the notion that all three present day enzymes originated from a common ancestral gene. The most conserved motif in this group of enzymes is characterized by a cluster of glycines and is homologous to the carboxyl terminal region of *E. coli* ammonia dependant asparagine synthetase which uses aspartate as a substrate and catalyzes the hydrolysis of ATP to AMP and PP_i. This region is implicated in ATP binding and formation of aminoacyladenylate intermediate (Gatti and Tzagoloff, 1991; Eriani *et al.*, 1991).

In the case of GlyRS, β-subunit sequences are required for the tRNA dependant step (Toth and Schimmel, 1990a), while the α subunit and aminoterminal half of the β-subunit are sufficient for the adenylate synthesis (Toth and Schimmel, 1990b).

In *E. coli* AlaRS, the first 360 aminoacids encode a domain for adenylate synthesis, followed by the domain required for tRNA^Ala^ binding. Lys 72 (Hill and Schimmel 1989), Gly171 and Gly 174 (Miller *et al.*, 1991a), Arg 69, Asp 76 and Phe 90 (Davis *et al.*, 1994) are implicated in catalytic function. A 76 aminoacid polypeptide outside the catalytic centre, is a structural appendage
that folds back to the catalytic centre to make contact with the bound acceptor stem of the tRNA (Buechter and Schimmel, 1995).

This enzyme contains the retroviral like Cys-Xaa$_2$-Cys-Xaa$_6$-His-Xaa$_2$-His motif recognized as a potential metal binding site (Berg, 1986), starting at position 178 and essential for enzyme structure and activity (Miller et al., 1991b). Although, motifs in the two classes of enzymes for recognition of tRNA may be in part idiosyncratic to each enzyme, it is worth noting that nine enzymes have potential Cys/His metal binding site (Miller and Schimmel, 1992).

Affinity labelling studies with PheRS have identified potential tRNA$^{\text{Phe}}$ binding peptide sequence in the amino terminal region of the enzyme (Hountondji et al., 1987). Gly 191, part of motif II and residue 294 of motif III (Kast et al., 1991a) in E. coli enzyme are implicated in the formation of amino acid binding site (Kast and Hennecke, 1991b), while Ala 294 was shown to be a determinant of the size of the aminoacid binding pocket (Ibba et al., 1994).

Earlier it was believed that sulphydryl groups are necessary for catalytic activity of ARSs, but later it was demonstrated that, the sulphydryl groups are not actually involved in the catalytic function, but they are involved only in the conformational or stearic changes (Profy and Schimmel, 1986).

In certain cases, synthetases have more resemblances to some proteins and enzymes other than ARSs. One of the best known examples is beef pancreatic TrpRS. This enzyme which has little homology (limited to conserved
sequences) with prokaryotic and yeast enzymes exhibits a 90% homology with rabbit peptide chain release factor (eRF) (Lee et al., 1990; Garret, 1991). KMSKS is present as KMSAS in eRF and human and bovine TrpRS (Leatherbarrow et al., 1985). The previously unidentified mRNA constituent which corresponds to SerRS has a sequence motif comparable both to EF-1α and GlnRS (Miseta et al., 1991). Residues 1-42 of E.coli TyrRS, are comparable to 293-334 of the estrogen receptor, both being the ATP binding sites (Baker, 1989).

2.2 tRNA Identity

The highly specific selection of tRNA substrates by ARSs is an intriguing problem in RNA-protein interactions. By virtue of the fact that they must function interchangeably on the ribosome during protein biosynthesis, all tRNAs have similar primary, secondary and tertiary structures. Within these constraints distinct elements, that mediate correct recognition by the ARS are called identity elements of tRNA (Normanly and Abelson, 1989).

Identity elements can be both positive and negative. Positive elements are those features of the tRNA that the cognate ARS recognizes directly, without which, the enzyme cannot recognize the cognate tRNAs. The term tRNA recognition is also used for positive identity (Schulman, 1991).

Negative elements are those features that block the recognition by other ARSs (Schimmel, 1989). The reason for poor heterologous charging in some systems is also sometimes due to these identity elements (Durasevic et al.,
They are dispersed in the tRNA tertiary structure, while some identity elements for different tRNAs overlap, some are located at entirely different positions (Frugier et al., 1993).

There are two different approaches in studying the tRNA identity. In the first method called identity swap, tRNAs with few specific base changes, still retaining the biological activity were used to determine the tRNA identity. The second way is to synthesize tRNA or small helices of known sequences as substrates of aminoacylation (Shi et al., 1992; Francklyn et al., 1992a, 1992b).

2.2.1 Role of anticodon in tRNA identity

Since, the anticodon is directly correlated with the identity of any tRNA species, it is the most logical identity element and was shown to be so for tRNA

Schulman et al., 1983). Study on derivative of E.coli tRNA

containing single base substitution at wobble position or a variant of tRNA containing only CA of the anticodon, decreased the rate of aminoacylation drastically indicating that the nucleotide bases of anticodon sequences are important for recognition (Shulman et al., 1983; Chattopadhyay et al., 1990).

Even stronger evidence was obtained by substituting the anticodon of non-cognate tRNAs with the anticodons corresponding to those of E.coli tRNAs for Arg, Met, Thr and Val which leads to $10^4$ to $10^6$ times increase in mischaring by corresponding synthetases (Schulman, 1991).

The importance of anticodon in tRNA identity was also shown for Asp, Ile, Met and Val tRNAs by constructing genes for amber suppressor derivatives
of tRNAs where the aminoacylation activity was completely abolished and with partial decrease for derivative of Arg, Glu, Gly and Thr (Klevia et al., 1990; Normanly et al., 1990).

Among the anticodon residues also there are variations. In some cases only the base at wobble position plays an important role in identity, while in some it is the first position, in some, the second position and in others all three bases were found to be important for recognition. While the anticodon acts as a major determinant like in val tRNA (Dreher et al., 1992), it is indicated that the entire inside of the L-shaped molecule from the acceptor stem to the anticodon interacts with ValRS (Chu and Horwitz, 1991). Similar observations, where along with anticodon being a major determinant, other discriminator bases at different positions on the acceptor stem were found to be important for the tRNA identity like in tRNAArg (Tamura et al., 1992), tRNAAsn (Jahn et al., 1991); tRNALys (Tamura et al., 1992); tRNAAsp from E.coli (Nameki et al., 1992), tRNAAsp from yeast (Putz et al., 1991; Frugier et al., 1994), tRNAAsn from E.coli (Li et al., 1993).

A C35 - T mutation in an E.coli tRNATrp gene creates an amber suppressor which efficiently inserts glutamine in response to UAG codons in vivo, but the same change in S.cerevisiae tRNATrp does not alter the tryptophan charging activity, indicating that inspite of substantial structural similarities between yeast and E.coli ARSs, fundamental differences can exist with regard to tRNA recognition (Yesland et al., 1993).
Lot of work has been going on in the last decade on the tRNA identity with many groups demonstrating the importance of anticodon in tRNA identity. Some of the examples come from the work on turnip yellow mosaic virus tRNA like structure (Florentz et al., 1991), tRNA\textsuperscript{Cys} (McClain et al., 1990); \textit{E.coli} tRNA\textsuperscript{Val} and tRNA\textsuperscript{Tyr} (Himeno et al., 1990; Tamura et al., 1991a), tRNA\textsuperscript{Glu} of \textit{E.coli} (Sylvers et al., 1993); tRNA\textsuperscript{Met} (Schulman and Pelka, 1990), tRNA\textsuperscript{Ile}, tRNA\textsuperscript{Phe} (Pallanck and Schulman, 1991); tRNA\textsuperscript{Phe} from yeast, mammalian and \textit{E.coli} system (Peterson and Uhlenbeck, 1992).

Aminoacylation of the oligo molecule with attached - CCA sequence (G(U)20 - CCA) by yeast and mammalian LysRS which is anticodon dependant was also demonstrated (Khvororova et al., 1992b).

In some of the tRNAs, the anticodons are shown to be not important for recognition as in tRNA\textsuperscript{Ser} (Himeno et al., 1990b; Asahara and Shimizu, 1991 and Schartz et al., 1991), and in tRNA\textsuperscript{Ala} (Hou and Schimmel, 1988) and tRNA\textsuperscript{Leu} (Asahara et al., 1991).

2.2.2 Role of the discriminator bases

Studies with mutants of tRNA\textsuperscript{Ala} amber suppressor, carrying a mutation in G-U base pairs at position 3-70 failed to insert alanine (Hou and Schimmel, 1988). The significance of G3-U70 base pairs was verified by identity swap experiments where in amber suppressor of tRNA\textsuperscript{Cys} and tRNA\textsuperscript{Phe} were synthesized each containing the G3-U70 base pairs. The altered cystein
suppressor inserted entirely alanine while altered Phe suppressor inserted alanine predominantly.

The ability of the G3-U70 pair to direct tRNA$^{\text{Ala}}$-AlaRS recognition has been tested in other tRNAs with mixed results. Superimposition of G-U pair at position 3-70 of a lysine suppressor, resulted in complete conversion to alanine identity, while the same alteration in tRNA$^{\text{Gly}}$ suppressor resulted in the insertion of predominantly glutamine, a small amount of glycine and no detectable levels of alanine indicating that, there are some more discriminator bases which confer positive or negative identity (Normanly and Abelson, 1989).

From then on, there is a spurt in the amount of work on the tRNA identity, based on the various discriminator bases in different tRNAs. With time, anticodon was shown to have no effect on the tRNA identity using AlaRS (Hou and Schimmel, 1988; Tamura et al., 1991b) and tRNA$^{\text{Leu}}$ (Asahara et al., 1993). The unique G3-U70 base pair present in tRNA$^{\text{Ala}}$ in E.coli is conserved in evolution appearing in eukaryotic cytoplasmic tRNA$^{\text{Ala}}$ (Hou and Schimmel, 1989).

In tRNA$^{\text{Ala}}$ this single pair alone appears to be the major identity element (Park et al., 1989; Hou and Schimmel, 1992a,b) which works as a G3-U70 base pair, but not alone. In addition using RNA hair pin mini helix it has been shown that A73 also enhances aminoacylation (Shi et al., 1990).

Apart from the vast amount of work done on tRNA$^{\text{Ala}}$ AlaRS recognition, other tRNA identities were also worked out. G73 and U73 in the case of E.coli
tRNA\textsuperscript{Met} (Lee et al., 1992; Senger et al., 1992), position 1-72 and 3-70 in the case of tRNA\textsuperscript{Trp} (Pak et al., 1992), discriminator base A73 conserved in all eukaryotic cytoplasmic and archebacterial tRNA\textsuperscript{Tyr} C1-G72 in yeast tRNA\textsuperscript{Tyr} (Lee and Raj Bhandary, 1991; Perret et al., 1989), certain discriminator bases in the D-Stem and/or in the anticodon stem in the case of tRNA\textsuperscript{Ilc} and tRNA\textsuperscript{Glu} from E.coli (Nureki et al., 1991a,b), A73, G18-G19 sequence in the D-loop, the A15-U48 base pair and stem pairing pattern of the long variable arm in the case of tRNA\textsuperscript{Leu} from E.coli (Asahara et al., 1993), 2 and 3 of tRNA\textsuperscript{Gln} (Jahn et al., 1991), G1-G72 or C2-G71 base pair in the acceptor stem of tRNA\textsuperscript{Thr} (Hasegawa et al., 1992) U20 and U59 in the variable pocket of tRNA\textsuperscript{Phe} in E.coli, the acceptor end (A73), the tRNA central core G10, G25, A26, G44 and U45 (Tinkle and Uhlenbeck, 1991; Peterson and Uhlenbeck, 1992; Sampson et al., 1992), G-1 in the G-1:C73 base pair of tRNA\textsuperscript{His} (Himeno, 1989; Yan and Francklyn, 1994), tRNA\textsuperscript{Cys}, tRNA\textsuperscript{Asp} and tRNA\textsuperscript{Gly} (McClain et al., 1990a; Shimizu et al., 1992) have been shown to be essential identity elements either independent or dependent on the anticodon residues.

Construction of mini and microhelices containing the discriminator bases was also used to determine the identity elements (Schimmel, 1991). Eight base changes located in the acceptor stem and D-stem of tRNA\textsuperscript{Leu} confers serine accepting activity (Normanly et al., 1992). The extra arm also appears to be an essential part of the contact area of the yeast tRNA\textsuperscript{Ser} with SerRS studied by footprinting experiments (Bergeon et al., 1990).

Available data suggest the discriminator bases rather than the anticodon as the major identity elements in class II enzymes. With regard to class I
enzymes, the first one to be studied was MetRS (Kim and Schimmel, 1992). Earlier work had shown the C terminal domain of this enzyme to be responsible for the tRNA$^\text{Met}$ binding (Valenzuela and Schulman, 1986; Ghosh et al., 1990, Meinnel et al., 1991a,b) and also the conserved segment containing Tyr461 to be the best candidate for anticodon binding (Brunie et al., 1990; Perona et al., 1991; Despons et al., 1990 and Ghosh et al., 1991b). Later it was shown that internal deletion in the carboxy terminal domain that disrupt the anticodon interaction has no effect on the kinetic parameters for aminoacylation. Moreover, this internally deleted enzyme can aminoacylate an RNA microhelix which represents the acceptor stem of the tRNA$^\text{Met}$, with the same efficiency as the native protein, independent of the anticodon binding.

Latest example of class I enzymes where discriminator base (U73) is found to be a positive determinant which confers the acceptor activity to a mini or microhelix has come from the work on CysRS in E.coli (Hamnan and Hou, 1995).

More thorough knowledge of all the enzymes and tRNA-enzyme recognition is required, before any conclusions can be drawn.

2.2.3 Role of tRNA conformation in identity

In addition to the different identity elements in different tRNAs, efficient aminoacylation is also dependent on a precise conformation of the tRNA (Perret et al., 1992). The size of the T loop and the base pairing of the D stem play a role in the stability of the aminoacylation (Puglisi et al., 1993;
Hou et al., 1993). Pseudouridines also play an important role in the stabilization of the tRNA tertiary structure, which is required for aminoacylation (Arnej and Steitz, 1994). Nucleotides in the D loop especially G15 and A14 and backbone functional groups in the D stem appear to be critical for maintaining a tRNA structure that is optimal for recognition by ProRS in vitro (Liu and Forsyth, 1994). Moreover, according to the recent reports, the identity of a tRNA in vivo is determined by competition among ARSs (Schulman, 1991).

### 2.3 Aminoacylation

Aminoacylation is a process in which the aminoacids are covalently attached to their cognate tRNAs. This process occurs in general in two steps except in GluRS, GlnRS, and ArgRS systems (Schimmel, 1987). The first step comprises of the formation of an aminoacyl-adenylate with the liberation of pyrophosphate (PPi)

\[
AA + ATP + E \rightleftharpoons E.AA - AMP + PPi
\]  

... (1)

In the second step, the aminoacyl group is transferred to the 2’OH or 3’OH of terminal adenosine of the -CCA residue on the acceptor stem of the specific tRNA to form an aminoacyl-tRNA with the release of AMP and the free enzyme which is recycled.
E.AA - AMP + tRNA $\rightarrow$ AA - tRNA + AMP + E

The total reaction is represented as

$$\text{AA + ATP + E} \rightarrow \text{E.AA - AMP + PPi}$$ ... (1)

$$\text{E.AA - AMP + tRNA} \rightarrow \text{AA - tRNA + AMP + E} \rightarrow \text{PPi}$$ ... (2)

$$\text{AA + ATP + tRNA} \rightarrow \text{AA - tRNA + AMP + PPi}$$ ... (3)

The AA-tRNA formed is at as high energy level as ATP since the overall equilibrium constant of (3) is less than 1, being 0.32 for Val-tRNA, 0.37 for Thr-tRNA and 0.25 for Arg-tRNA (Ofengand, 1977).

2.3.1 Assay methods for tRNA aminoacylation

The different assay methods available can be divided into radioactive and non radioactive assays. One of the oldest, but commonly used, highly sensitive method is based on the incorporation of radio active aminoacids into the TCA insoluble material (Mans and Novelli, 1961). Incorporation of the aminoacids into the hydroxamate was measured in one method (Loftfield and Eigner, 1963), while in another, the measurement is based on the aminoacid dependent incorporation of $^{32}\text{PPi}$ into ATP (Drake et al., 1979). Yet another method developed is based on the stoichiometric conversion of PPi to ATP and subsequent analysis of the ATP formed using a heterokinase/glucose-6-phosphate dehydrogenase system.
A method to measure the concentration and colorimetric determination of nanomoles of PPI was developed later (Heinonen et al., 1981). A simple and more recent economical colorimetric assay is based on the green colour developed between PPI and ammonium molybdate in the presence of β-ME (Chang et al., 1983). A continuous spectrophotometric assay has been developed but it is not applicable to all synthetases (Roy, 1983). Yet another continuous spectrophotometric method developed is based on the quantities of PPI formed correlated with NADH oxidation after coupling with four enzymes (Chang et al., 1984). A similar, but more recent method for AlaRS is based on coupling the AlaRS dependent formation of AMP to the lactate dehydrogenase oxidation of NADH to NAD⁺ (Wu and Hill, 1993).

Not only for quantitation, but also for localization, a method has been developed. It measures the ARS activity in polyacrylamide gel based on the white precipitation of Ca-PPI (Chang et al., 1985).

2.3.2 Effect of cations, salts and diol containing agents on aminoacylation

Mg²⁺ is required in the first step for the formation of aminoacyl adenylate (Yaniv and Cros, 1969). Spermine cannot replace Mg²⁺ completely, but can be used in its absence where a small amount of Mg²⁺ is present (Fasiolo and Ebel, 1974; Lovegren et al., 1978). Presence of Mg²⁺ and spermine also alters the mechanism of the enzyme in certain cases. In the presence of spermine only, substrate binding to the IleRS is random, before any product
is released, while Ile-tRNA formation in the presence of spermine and/or Mg\(^{++}\) proceeds through a bi-uni-uni-bi ping pong mechanism (Igarashi et al., 1978).

These enzymes are metalloproteins and removal of the metal ions inactivates them (Mayaux et al., 1982). Increasing concentrations of salt inhibit the reaction and this effect is suppressed in the presence of ammonium sulfate. In fact, ammonium sulfate brings about an increase in valylation in the absence of other salts; such an effect is thought to be due to stronger RNA/protein interactions (Florentz et al., 1990).

During \textit{in vitro} incubation, addition of glycerol, sucrose, etc substantially increased the rate of hydrolysis of the aminoacyl-ester bond (Johnson and Adkins, 1984).

\subsection*{2.3.3 Effect of pH}

The different assay conditions including the pH affect the reaction. The pH optima was found to be 7.65 to 7.5. The pH of the reaction not only changes the rate of the reaction, but also the order of addition of substrates (Friest, 1989).

\subsection*{2.3.4 Substrate specificity}

\subsubsection*{2.3.4.1 Amino acid}

\(\alpha\)-amino group is essential both for substrate and inhibitor activity. The carboxyl group on the other hand, is not necessary. Though it was. D-amino acid in the primitive protein synthesis, with evolution, the specificity of
ARSs changed to the L-isomer of the aminoacid which exist biologically (Lacey Jr., 1992). Acylation of unnatural aminoacid like parachloro.Phe or parabromo.Phe and their incorporation into protein possible only with mutant enzymes, with reduced activity in protein synthesized (Ibba and Hennecke, 1995).

2.3.4.2 ATP

Only ATP and with higher Km and lower $V_{\text{max}}$ dATP are active. Other nucleotide triphosphates are inactive (Mitra and Mehler, 1969).

2.3.4.3 AA-AMP

All the synthetases have affinity only for their own cognate AA-AMP. Usually the non-cognate AA-AMPs formed are hydrolyzed in what is called a pre-transfer proof reading mechanism (Degetyarev, 1983).

2.3.4.4 tRNA

The ARSs are highly specific for their cognate tRNAs. They can recognize the cognate tRNAs by the presence of identity elements (Normanly and Abelson, 1989).

2.3.5 Order of substrate addition

Among the different ARSs, the addition of substrates and release of products vary widely. It can be either sequential or random and for the same enzymes, different mechanisms were proposed due to the variance in the assay
conditions. Based on the different results obtained, 4 models were put forth (Friest, 1989).

A. **Sequential random**

B. **Sequential Ordered**

C. **Random bi-uni-uni-bi** (Ping pong)

D. **Bi-bi-uni-uni** (Ping pong)
In model D, the AA-tRNA released before the addition of tRNA suggests that the tRNA must be complexed with the enzyme in the preceding transfer step itself (Friest and Cramer, 1983).

It was observed that, binding of aminoacid, ATP enhances the binding affinity of either ligands during simultaneous association with LeuRS and PheRS in the presence of Mg^{2+}, while spermine was inhibitory to this reaction (Holler et al., 1975).

Binding of the tRNA triggers a rearrangement of the protein chain yielding a fully active catalytic centre. Such a multistep adoption process of tRNA and ARS may have been selected so as to allow a fine tuning of the rate of aminoacylation and is to ensure the fidelity of the reaction (Bacha et al., 1982).

2.3.5.1 IleRS

This enzyme has a peculiar property of forming valyl adenylate as well as isoleucyl-adenylate, though only enzyme bound isoleucyl-AMP serves as intermediate in the syntesis of aa-tRNA (Baldwin and Berg, 1966a), while the valyl adenylate is hydrolyzed by the tRNA^{lle} (Baldwin and Berg, 1966b). The class I enzymes exhibited the bi-bi-uni-uni mechanism while for aminoacylation with valine, a bi-uni-uni-bi mechanism has been identified (Friest and Cramer, 1983; 1986).
Crystallization studies revealed the formation of H-bonds between enzyme and aminoacids. ATP behaves as an activator at a low Mg$^{++}$ concentration and in the presence of spermidine. The binding of several molecules of ATP increases the rate of dissociation of Ile-tRNA from the enzymes (Airas, 1988). Presence of increased amount of Mg$^{++}$ and spermidine affects the tRNA binding to the enzyme. An increase in Mg$^{++}$ concentration shifts the rate limitation from the transfer reaction towards dissociation of the product (Airas, 1992). Spermine stimulation of rat liver Ile-tRNA formation correlates with the structural change of the acceptor stem by spermine (Eguchi et al., 1991a,b).

### 2.3.5.2 MetRS

Each subunit of the dimeric enzyme is capable of binding 1 molecule each of methionine and ATP - Mg$^{++}$. In contrast, occupation of one tRNA site
brings about an asymmetric conformational transition at the inter subunit domain of the two subunits in MetRS (Ferguson and Yang, 1986). However this effect is attenuated upon occupation of the methionyl-adenylate site. Mg\textsuperscript{++} relieves the asymmetry induced by the tRNA (Meinnel \textit{et al.}, 1990). The role of enzyme bound metal is to render the $\alpha$-proteins more acidic, through coordination of the carboxylate group, suggesting that the enzyme bound zinc may have a catalytic role as well as a possible structural role in MetRS (Williams and Rosevear, 1991).

\textbf{2.3.5.3 ValRS}

This enzyme again follows a two step (bi-bi-uni-uni) ping pong mechanism. Presence of Mg\textsuperscript{++} accelerates the reaction tremendously. Binding of ATP enhanced the enzymes specificity towards the aminoacid substrates, while the binding of the L-valine and ATP to the enzyme followed random mechanism (Kakitani \textit{et al.}, 1989).

\[
\text{E.val} \rightarrow \text{tRNA}_{\text{val}}^{\text{val}} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ ATP} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ ATP-val}
\]

\[
\text{E.val - tRNA}_{\text{val}}^{\text{val}} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ AMP} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ val-AMP-PPi} \rightarrow \text{PPi}
\]

\[
\text{E.val - tRNA}_{\text{val}}^{\text{val}} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ val - AMP} \rightarrow \text{E.val - tRNA}_{\text{val}}^{\text{val}} \text{ val - tRNA}_{\text{val}}^{\text{val}}
\]
2.3.5.4 TyrRS

The enzyme which consists of two identical subunits binds a single molecule of L-tyrosine per dimer, in contrast to the binding of two tRNA\textsuperscript{Tyr} molecules (Bodshard \textit{et al.}, 1975). Later, TyrRS from \textit{B. stearothermophilus} was found to be a classical example of an enzymes with half of the sites activity which results from an inherent asymmetry of the enzyme. It appears that 2 molecules of tRNA\textsuperscript{Tyr} bind sequentially to the same site. The binding of the second tRNA\textsuperscript{Tyr} perhaps aids the dissociation of Tyr-tRNA\textsuperscript{Tyr} by displacing the tyrosyl moiety from its binding site (Ward and Fersht, 1988). The hydrolysis and transfer of activated tyr to the tRNA\textsuperscript{Tyr} can be considered as a single step (Garcia, \textit{et al.}, 1990). Mg\textsuperscript{++} an essential requirement for all ARSs could be substituted by Co\textsuperscript{++} or Ca\textsuperscript{++} though with lower preference in TyrRS. The tRNA\textsuperscript{Tyr} interact with the N-terminal region of one subunit and the C-terminal region of the other subunit in the dimer (Carter \textit{et al.}, 1986); spanning both the subunits and exhibit the half of the sites activity (Fersht, 1987).
2.3.5.5 TrpRS

The dimeric TrpRS from beef pancreas has been found to activate two tryptophan molecules/mol enzyme, but by using quenched flow and stopped flow methods under presteady-state conditions, it was shown that only one enzyme subunit operates at a time in the aminoacylation of tRNA\textsuperscript{Trp} (Trezequet et al., 1983). Four models were proposed for the possible mechanism for such half of the sites reactivity.

a) two independent and non equivalent subunits
b) single active subunit presenting absolute half of the sites activity.
c) alternate functioning of the subunits (flip-flop mechanism)
d) random functioning of the subunits with half of the sites reactivity.

Further work and analysis favored model D. In this, the enzyme-adequate complex is symmetrical and tRNA\textsuperscript{Trp} can bind of any to the two subunit. However the binding of tRNA molecule prevents a second tRNA molecule from binding, until the reaction product tryptophanyl-tRNA departs and next cycle starts (Trezequet et al., 1986).

While in the prokaryotic system, the two subunits nearly present half of the sites reactivity, but exhibit kinetic positive cooperativity toward tryptophan, the eukaryotic system exhibit negative cooperativity toward tryptophan (Merle et al., 1986).
A group of three ARSs, namely ArgRS, GlnRS and GluRS seem to undergo only a single step mechanism, where the binding of all the three substrates viz aminoacid, ATP and the cognate tRNA takes place before the first product could be released in contrast to the two step mechanism followed by the other ARSs (Ofengand, 1977). This conclusion was drawn due to lack of (P$^{32}$) PPI-ATP exchange in absence of tRNA.

Work carried out earlier, to isolate aminoacyl-adenylate, an intermediate usually formed in the two step mechanism failed to yield any such intermediate complex in brewer's yeast (Theibe, 1983a,b), but recent work (Lin et al., 1988a) showed formation of aminoacyl-adenylate in *N. crassa* and bakers yeast, while no such intermediate was identified in *B. stearothermophilus* and brewer's yeast. An intermediary complex of ArgRS with tRNA, ATP and Arg leads to loading of the aminoacyl moiety on the tRNA by the enzyme without adenylate formation suggesting a transformation due to initial binding of tRNA and ATP which renders favourable situation for the rest of the substrate, Arg to bind. An important conformational change of the enzyme is its dimerisation in the presence of tRNA. The enzyme is further modified on binding of ATP and the thermostability of the enzyme is increased upon binding of the aminoacid. Due to dimerization of the enzyme, the original single site of all substrates is increased (Lin et al., 1988b).

However, a random sequence was seen in ArgRS isolated from *E.coli*, bakers yeast and human placenta (Papas and Peterofsky, 1972).
2.3.5.7. **GlnRS**

Another monomeric enzyme that does not catalyze the tRNA independent \( P^{32} \) PPI-ATP exchange reaction is GlnRS. Fluorescence spectroscopic studies on GlnRS from *E. coli* were attempted to work out the mechanism of the enzyme (Bhattacharya *et al.*, 1991). They concluded that, the lack of tRNA independent PPI exchange activity in this enzyme is not due to lack of binding of either aminoacid or ATP in the absence of tRNA. Formation of glutaminyl-adenylate was ruled out as attempts to locate glu-adenylate by sephadex gel filtration did not succeed. No significant conformational change in the size, and shape of the synthetase was observed upon binding of tRNA. Hence it was suggested that, the tRNA dependent \( ^{32} \)PPI exchange may result in limited conformational changes. More work is to be carried out before a conclusion could be drawn on the exact mechanism of the enzyme.

2.3.5.8 **GluRS**

This is another synthetase which follows a single step mechanism and shows tRNA\(^{Glu}\) requirement for \( ^{32} \)PPI-ATP exchange reaction. Moreover, the \( ^{32} \)PPI exchange reaction proceeds at a much faster rate when compared to tRNA charging and its reversal. However, there are arguments though not definitive that, the three enzymes including GluRS follow the general two step mechanism. The evidence in favour of the two step mechanism was obtained from the observation that, the GluRS catalyzes:
1. an equilibrated AMP-dependent and PPI independent deacylation of Glu-tRNA\textsuperscript{Glu}.

2. the initial rate of this reaction is similar to that of overall reversal of tRNA\textsuperscript{Glu} charging, the deadenylation being AMP and PPI dependent.

3. the reversal of tRNA\textsuperscript{Glu} charging generates an enzyme intermediate exhibiting properties similar to those of the enzyme adenylate isolated for other synthetases (Kern and Lapointe, 1980).

Thus, it is believed that, though these 3 enzymes show a single step reaction, they are not an entirely separate group of enzymes but form the border line cases.

2.3.5.9 SerRS

SerRS from Saccharomyces carispergensis C386 seems to be an intrinsically symmetric enzyme, with an $\alpha_2$ subunit structure. Results of binding and kinetic experiments were in agreement with this assumption. It has two sites each for tRNA\textsuperscript{Ser}, L-serine, and Mg\textsuperscript{++}-ATP, both of which are involved in aminoacylation. Under normal conditions, the activity of SerRS was shown to be regulated mainly by tRNA\textsuperscript{Ser} as seryl-tRNA\textsuperscript{Ser} is liberated upon the association of a second tRNA\textsuperscript{Ser} molecule to the enzyme showing second order dependency of the enzyme on tRNA\textsuperscript{Ser}. However under high serine concentrations, the reaction shifts to first order via serine dependance (Pachmann and Zachau, 1978).
2.3.5.10  ThrRS

Rat liver ThrRS forms an ATP - enzyme complex when incubated with ATP and Mg**.

The nature of kinetic patterns obtained on Thr-\text{tRNA}^{\text{Thr}} formation catalyzed by ThrRS in RL system showed that threonylation follows a bi uni uni bi ping pong mechanism.

\[
\begin{array}{cccc}
\text{ATP} & \downarrow & \text{Thr} & \downarrow & \text{PPI} & \downarrow & \text{tRNA} & \downarrow & \text{aa-tRNA} & (\text{or AMP}) & \uparrow & \text{AMP} & (\text{aa-tRNA}) \\
E & E-\text{ATP} & ( ) & E-\text{AMP-Thr} & ( ) & ( ) & & & & & & &
\end{array}
\]

ATP binds to the enzyme prior to aminoacid was confirmed by incubation of the enzyme with ATP in the absence of aminoacid and isolation of the complex on Sephadex G-50 (Allende, 1970)

2.3.5.11  AspRS

The kinetics of the aspartylation of tRNA by human AspRS was found to be consistent with the usual two step reaction. The dissociation of Asp-\text{tRNA}^{\text{Asp}} from the enzyme was found to be rate limiting, stimulated by elongation factor 1\alpha (EF 1\alpha) and GTP (Reed and Yang, 1994).

\textit{E.coli} AspRS can very accurately distinguish between the \(\alpha\)- and \(\beta\)-carbonyl groups of aspartic acid. Hence this leads to very low amount of isoaspartic acid incorporation into cellular proteins by misacylation (Momand and Clarke, 1990).
2.3.5.12 PheRS

The aminoacylation of tRNA$^{\text{Phe}}$ by PheRS is one of the best investigated mechanisms among the ARSs, which is believed to follow a two step mechanism.

The activity of the PheRS is stimulated by low concentration of Mg$^{++}$, organic diamines, spermine and spermidine (Robinson and Zimmerman, 1971).

The $\alpha_2\beta_2$ tetrameric enzyme was shown to be a functional dimeric enzyme with two active sites for aminoacylation (Fasiolo and Ebel, 1974). In the beginning, it was believed that each protomer can bind twice all the substrates, the intermediate Phe-AMP and at least Phe-tRNA$^{\text{Phe}}$. However, under catalytic conditions it exhibits half of the sites reactivity following a flip-flop mechanism (Thiebe, 1982).

Later it was shown that yeast PheRS shows a symmetric behaviour, when activation of Phe by ATP in the absence of tRNA$^{\text{Phe}}$ is catalyzed simultaneously at the same rate by the two protomers (Baltzinger, et al., 1983). However further studies confirmed the theory of half of the sites activity by showing that, nonacylated tRNA$^{\text{Phe}}$ induces an asymmetric behaviour of PheRS, which is expressed by a reduced affinity of one of the two enzyme protomers for phenyl - adenylate (Baltzinger and Remy, 1985).

In the case of yeast PheRS, tRNA$^{\text{Phe}}$ may induce polarization of the ATP in complex with the enzyme to stimulate the formation of Phe - AMP in the presence of Phe and hydrolysis of ATP in the absence of Phe. The PPi released
during the reaction binds tightly to the yeast PheRS inactivating the enzyme in the heterologous system of \textit{E.coli} tRNA$^{\text{Phc}}$ (Thiebe, 1984). This behaviour is an exclusive feature of enzymes which undergo self inactivation in the course of the reaction (Khvororova, 1992).

2.4 Editing activity of ARSs

Despite the high fidelity of ARSs, misacylation does occur, though rarely. To maintain this high fidelity, the enzyme has an inherent correction mechanism called the 'chemical proof reading'.

This can occur at two different stages

a) Pretransfer proof reading, in which, before the transfer reaction, the non-cognate aminoacyl adenylate complex breaks down in the presence of tRNA.

b) In the post transfer proof reading mechanism the misacylated tRNA is hydrolyzed enzymatically.

The capacity of the enzyme to proof read was first shown with IleRS, wherein, a tRNA$^{\text{Ile}}$ induced hydrolysis of valyladenylate was shown (Baldwin and Berg, 1966b). It was suggested that the mechanism of inter-site interaction is used to distinguish between correct and wrong adenylates (Degetyarev, 1983). On the other hand PheRS with the same subunit structure and molecular weight differs considerably in the mechanism used to maintain fidelity. While the enzyme from \textit{E.coli}, \textit{S.cerevisiae} and \textit{N.crassa} follow the
path of post transfer proof reading, the turkey enzyme was tRNA dependant
pretransfer proof reading with natural aminoacids (Gabius, et al., 1983b).

Some synthetases prefer pretransfer proof reading such as the IleRS (Friest and Cramer, 1983), MetRS (Jacobowski, 1990), yeast LeuRS (Englisch, et al., 1986), while post transfer proof reading is preferred by ValRS (Englisch, et al., 1990), LeuRS from E.coli (Englisch, et al., 1986). In bakers yeast ARSs for Ser, Asp, Asn, Val, Leu, His, Phe, Lys and Trp prefer a post transfer proof reading, while IleRS shows proof reading at both the stages.

2.5 Other catalytic functions of ARSs

In addition to the main function in aminoacylation, these enzymes have been implicated in a number of other cellular activities. Aminoacyl-tRNA synthetases are capable of converting 5' ATP to 5'-5' diadenosine tetraphosphate which is supposed to play a role in the proliferation activity of the animal cells, and in turn has been implicated in tumor cells (Brevet, et al., 1989).

They also catalyze the deacylation reaction, which is 100-200 times faster than chemical hydrolysis and the hydrolysis of misacylated tRNA is much faster than that of the correctly acylated tRNA (Ofengand, 1977).

These enzymes are involved in RNA splicing in fungal mitochondria. It has been suggested that these enzymes have two separate functions, one in splicing and another as a synthetase, the functions being performed by two separate domains (Benne, 1988). A group I intron that in not self splicing in
*vitro* undergoes reverse splicing in a reaction promoted by the *N. crassa* mitochondrial TyrRS, which is required for splicing the intron *in vivo*. It is suggested that, proteins that promote splicing could contribute to intron mobility by promoting reverse splicing *in vivo* (Lambowitz and Pearlman, 1990; Mohr and Lambowitz, 1991). It was confirmed that, a small amino-terminal domain not found in bacterial TyrRS is required for splicing activity along with the carboxy terminal tRNA-binding domain. The protein functions by binding to the precursor RNA and facilitating formation of the correct RNA structure. Regions required for splicing are distributed throughout the protein, but are not identical to regions required for TyrRS activity (Kittle, *et al.*, 1991).

Another novel role assigned to these enzymes is in the regulation of peptide chain initiation, by the regulation of eIF2 function mediated through phosphorylation of the α-subunit of this factor (Pollard, *et al.*, 1989).

TrpRS shows an anomalous distribution among mammalian organs: its content is far greater in the exocrine part of the ruminant animal pancreas in comparison with other organs (liver) or with other mammalian orders; suggesting that it may play a role in the digestive function of ruminant animals. It is also found in considerable quantities in diffuse chromatin of mammalian cell nuclei, indicating that this enzyme may participate in such processes in the nucleus as transcription, processing, transport etc. (Beresten, *et al.*, 1991).