Review of Literature
REVIEW OF LITERATURE

2.1 RNA – THE VERSATILE BIOMOLECULE

RNA molecules possess interesting double property of being depository of genetic information, like DNA, and of displaying catalytic activities, like protein enzymes. The functional versatility of RNA molecules is evident in all steps of gene expression and in many other biological activities. Like proteins, RNAs achieve those biological functions by adopting intricate three-dimensional folds and architectures. Further, as in protein sequences, RNA sequences contain signatures specific for three-dimensional motifs, which participate in recognition and binding. In regulatory pathways, RNA molecules exhibit equilibria between transient structures differentially stabilized by effectors such as proteins or cofactors.

2.1.1 The RNA Structure

The secondary structure of any RNA molecule is defined by Watson-Crick base pairs and wobble pairs (G.U) forming right handed helices of standard ‘A’ conformation (Brion and Westhof, 1997). The helical domains formed, combine together to constitute the tertiary structure, which in turn is maintained through long-range interactions (Batey et al., 2001). The secondary structure thus features helices, hairpin loops, internal and asymmetric loops, bulges and junctions. Non-Watson-Crick base pairs stabilized by non-canonical hydrogen bonding provide distinct alterations and
together with base triples, lead to the formation and stabilization of the tertiary structure of the RNA. Interactions among non-canonical base pairs, triples etc., are conserved among most three-dimensional RNA architectures (Batey et al., 2001; Hermann, 1999). Non-canonical base pairs widen the major groove, thereby making it accessible to ligands (Battiste et al., 1996). RNA-ligand accessibility is essential for vital functions such as RNA self-assembly, RNA-protein, RNA-metal ion or other molecular interaction crucial for RNA functioning in key cellular processes. The assembly of RNA into a particular structure is also ruled by certain other factors like the metal ions in the milieu. The metal ion requirement was recognized in studies of tRNA folding (Cole et al., 1972; Römer and Hach 1975; Stein and Crothers 1976a) when the ability of millimolar Mg\(^{2+}\) concentrations to stabilize RNA tertiary structures that are otherwise only marginally stable in the presence of high monovalent cation concentrations was observed. Thus both monovalent and divalent metal ions are essential for correct folding and formation of the RNA tertiary structure (Pyle, 2002; Draper, 2004). Functional RNAs such as tRNA, rRNA, snRNPs and most catalytic RNAs adopt complex three-dimensional folds stabilized by tertiary interactions. However mRNAs and snoRNAs have no direct relationship between extensive folded structures and function. The mRNAs exhibit distinct transient structural motifs in their 5′- and 3′-untranslated regions (UTR) for various important functions (Pesole et al., 1997; Gray and Wickens, 1998; Hazelrigg, 1998). Structured RNAs achieve their active states by traversing complex, multidimensional energetic landscapes. Given the versatile biological functions that RNAs perform, it is important to understand the mechanisms by which they form complex three-
dimensional structures (Cech, 1993; Herschlag, 1995; Brion and Westhof, 1997). Considering the molecular properties of RNA (Sigler, 1975; Herschlag, 1995), it is generally held that folding landscapes for RNAs are more uneven than those for proteins (Treiber and Williamson, 1999; Woodson, 2000; Treiber and Williamson, 2001). RNA folds by multiple paths and the folding kinetics of large molecules is dominated by non-native or misfolded intermediates (Pan et al., 1997).

2.1.2 The ‘RNA World’ Hypothesis

The dual property of RNA of being genetic depository and catalyst, led to the hypothesis of an ‘RNA world’ (Woese, 1967; Crick, 1968; Orgel, 1968; Sharp, 1985; Pace and Marsh, 1985; Lewin, 1986; Gilbert, 1986; Cech 1986). Thus ‘RNA world’ is a hypothetical stage in the evolution of earth where RNA performed all biochemical reactions and where RNA was capable of undergoing Darwinian evolution. RNA is capable of self-folding and bring about long-range and short range interactions as evinced from studies on its physiochemical properties (reviewed in Spirin 1960 and Spirin 1963). The existence of a self-replicating RNA entity was suspected to have formed a part of a RNA world. The self-replicating systems as a part of Darwinian evolution, would have introduced variants, which would have again undergone replication, marking the beginning of life. Although there is no direct physical evidence for an ‘RNA world’, studies have been carried out recreating the prebiotic earth in the laboratories. Ribose, phosphate, purines and pyrimidines all may have been available, although the case for pyrimidine is less compelling (Ferris et al., 1968; Robertson and Miller, 1995). These
might have combined to form nucleotides (Lohmann and Orgel, 1968; Fuller et al., 1972) and these nucleotides would have combined to form polymers. Through a series of biased synthesis, fractionation and other enrichment processes (Müller et al., 1990; Ferris and Ertem, 1992; Krishnamurthy et al., 1999), the formation of an RNA pond might have been established. Studies in the laboratory reflect the emergence of RNA molecules capable of catalyzing reactions including RNA synthesis (Unrau and Bartel, 1998), RNA polymerization (Johnston et al., 2001), aminoacylation of transfer RNA (Lee et al., 2000) and peptide bond formation (Zhang and Cech, 1997). Since then, advances have been made towards exploring the possibility of developing self-replicating RNAs similar to the hypothesized early ancestor. In vitro evolution studies identified an RNA enzyme that catalyses the template-directed condensation of an oligonucleotide 3'-hydroxyl and an oligonucleotide 5' triphosphate leading to formation of 3', 5'- phosphodiester linkage similar to the reaction catalyzed by RNA-dependent RNA polymerase (Bartel and Szostack, 1993). A related RNA enzyme was used to catalyze template-directed polymerization of mononucleoside 5' triphosphates (NTPs), in a reaction that preceeds with remarkable accuracy (Ekland and Bartel, 1996). Aminocylation of RNA precursors must have provided the assistance in eventual development of translation machinery. These precursor RNAs could have been just short stem-loop structures capable of molecular interactions. More studies by way of recreation of the prebiotic earth and in depth studies on present day RNA molecules have provided more support to the ‘RNA world’ hypothesis. The reported crystal structure of ribosome (Ban et al., 2000; Wimberly et al., 2000; Yusupov et al., 2001) for example is one
of them, which has exposed the face of the RNA world in the active role that the modern RNA has in cellular activities. Various RNA molecules performing diverse functions are still being dissected in an effort to trace the ‘RNA world’.

2.1.3 Therapeutic Potential of RNA

Characteristic folds and motifs serve as specific drug binding target within the RNA molecule for many RNA binding effectors. Aminoglycosides for example, consists of compounds binding to many RNA folds and motifs (Walter et al., 1999; Schroeder et al., 2000). RNA folds serving as targets for aminoglycosides include the eubacterial 16S rRNA (Moazed and Noller, 1987), Group I introns (von Ahsen et al., 1991), RNase P RNA (Mikkelsen et al., 1999), hammer head and HDV ribozymes (Rogers et al., 1996). Such RNA based drugs act by translational miscoding in rRNAs and by inhibiting the catalytic activity in ribozymes. The RNA motifs are the recognition sites for proteins, which means drugs binding to these motifs, interfere the RNA – protein complex formation. However RNA folds are difficult to predict and hence recognition by the effectors might be in the packing interfaces between individual motifs as in the case of viomycin by RNA pseudoknots (Wallis et al., 1997), aminoglycosides by hammerhead ribozyme (Hermann and Westhof, 1998; Tor et al., 1998) etc. In vitro selection studies and NMR studies have revealed some of the RNA-antibiotic interactions. Tobramycin for example binds to the RNA major groove at a stem-loop junction site with part of the antibiotic clasped between the major groove and a looped out cytosine residue (Jiang et al., 1997). Neomycin prefers a widened major
groove as its target site (Wallis et al., 1995). High-resolution NMR structures of aptamers selected for citrulline, arginine, FMN, ATP, aminoglycoside antibiotics, and theophylline have been determined and they reveal a wide range of specific tertiary interactions and previously unforeseen distortions in backbone topology (Yang et al., 1996; Jiang et al., 1996; Jiang et al., 1997; Zimmerman et al., 1997). In all of these cases, RNA helices are used to present either an internal bulge or a hairpin loop that is principally responsible for binding. The antibiotic recognition by RNA is so specific that while streptomycin inhibits the Group I intron RNA of T4 thymidylate synthase gene, its analogue bluensomycin does not (von Ahsen and Schroeder, 1991). The difference between the two antibiotics is that streptomycin has a guanidino group in its para position but bluensomycin has a carbamido group in its para position. Aminoglycosides also specifically recognize some motifs such as an internal loop in the 5' -UTR of the human thymidylate synthase (TS) mRNA (Tok et al., 1999) and regulatory domains in the RNA genome of HIV, namely TAR (Mei et al., 1995) and RRE (Zapp et al., 1993). Since each of these RNA motifs is a recognition site for a protein (TS itself for the TS 5' -UTR, Tat for TAR, and Rev for RRE), binding of aminoglycosides interferes with the formation of the RNA-protein complex. Other small molecule recognition interactions include thiostrepton recognition in the tertiary folded GTPase center of eubacterial 23S rRNA (Ryan et al., 1991; Conn et al., 1999; Wimberly et al., 1999), recognition of the cyclic peptide antibiotic viomycin by RNA pseudoknots (Wallis et al., 1997) etc. The recognition elements in mRNAs are mostly hairpin motifs in combination with bulges, internal loops etc. Non-Watson-Crick base pairs also serve as
recognition elements for small molecules such as drugs and proteins (Hermann, 2000). Antibiotics act as modulators of RNA function in some cases. *In vivo* selection studies on RNA aptamers have identified the role of RNA itself in drug resistance. Mutation studies revealed that in *E. coli*, a C to U at base position 912 or an A to C change at 523 results in a streptomycin-resistant phenotype (Montandon *et al.*, 1986; Melancon *et al.*, 1988). Studies have revealed a class of natural small RNAs whose expression induced spectinomycin resistance in *E. coli* (Zimmerman and Maher, 1998). These small RNAs were shown to fold in such a manner to produce binding sites to allow direct binding of spectinomycin and thereby preventing them to reach their target sites. The study demonstrated the competitive binding of non-ribosomal transcripts to the drug preventing them to reach their target sites in the ribosomal surface.

The structural flexibility of RNA has also led to RNA-based therapeutics where the RNA molecules themselves are used as effectors against other RNA molecules. Regulation of gene expression using antisense RNA is recognized as a naturally occurring process in prokaryotes (Green *et al.*, 1986). Inhibition of gene expression through antisense expression cassettes was first reported in bacteria (Pestka *et al.*, 1984; Coleman *et al.*, 1984) and then also in eukaryotic cells (Izant and Wientraub, 1985). Short RNA decoys are being studied as inhibitors of HIV replication *in vivo* (Sullenger *et al.*, 1990; Kohn *et al.*, 1999). The use of small, structured RNAs to inhibit the activity of viral proteins was first explored employing a short transcript corresponding to the TAR RNA sequence (Sullenger *et al.*, 1990).
This "decoy" RNA competes with the virally encoded TAR sequence for binding to tat. By sequestering the tat protein from the real TAR RNA sequence present in the HIV viral RNA, the decoy RNA can inhibit HIV RNA transcription and thus reduce viral replication. Expression of these TAR decoys in CD4+ T-cell lines and in bulk populations of transduced immortalized T lymphocytes renders these cells highly resistant to HIV replication. It has been reported that a large fraction of vertebrate mRNAs has conserved regions in their 39 and 59 UTRs (Duret et al., 1993). These conserved regions comprise unique sequences in the genome and show sequence conservation only between corresponding regions of orthologous mRNAs in other species. A very interesting model has been suggested where the long stretches of conserved regions in 39 UTRs might actually be involved in regulation of RNA stability via the formation of long, perfectly matched sense-antisense duplexes with complementary RNAs (Lipman, 1997). In this model, duplexes with perfect matches could be targets of cellular regulatory machinery designed to destabilize or modify the sense transcripts, as a novel mode of regulation. The structure feature of the target RNA also plays a key role in determining the efficiency of the antisense oligos. The rat TNF α mRNA was cleaved more efficient by antisense oligonucleotides designed against target site that included a GGGA motif (Tu et al., 1998). Similar action of antisense oligos were reported for mouse TNF α mRNA also (Matteucci and Wagner, 1996). It was suggested that cellular RNAs might have had strong specificity towards the GGGA motif either in the RNA strand of DNA-RNA duplex or in the single RNA strand adjacent to DNA-RNA hybrid or the GGGA motif was situated at a site near easily
accessible for antisense oligonucleotides and RNase H providing binding energy for antisense hybridization.

2.2 RIBOZYMES

The discovery of Group I introns from *Tetrahymena thermophila* (Kruger *et al.*, 1982) led to the coining of the word ‘ribozyme’ meaning RNA with an enzyme-like activity. The existence of catalytic RNAs was further confirmed by the discovery of catalytic activity in the RNA component of a ribonucleoprotein complex RNase P (Guerrier-Takada *et al*., 1983). More RNA molecules like the hairpin and the hammerhead ribozymes were added to this class. Ever since their discovery, these molecules have been and are still being dissected to obtain in depth knowledge on their structural organization and functional importance for their therapeutic potential and the credibility they offer towards the hypothesis of an ‘RNA world’.

The discovery that RNA can act as a biological catalyst, as well as a genetic molecule, indicated that there was a time when biological reactions were catalysed in the absence of protein-based enzymes. It also provided the platform to develop those catalytic RNA molecules, called ribozymes, as trans-acting tools for RNA manipulation. Viral diseases or diseases due to genetic lesions could be targeted therapeutically through ribozymes, provided that the sequence of the genetic information involved in the disease is known. The hammerhead ribozyme, one of the smallest ribozymes identified, is able to induce site-specific cleavage of RNA, with ribozyme and substrate being two different oligoribonucleotides with regions of complementarity. Its ability
to down-regulate gene expression through RNA cleavage makes the hammerhead ribozyme a candidate for gene therapy. This could be particularly useful for dominant genetic diseases by down-regulating the expression of mutant alleles. The group I intron ribozyme, on the other hand, is capable of site-specific RNA trans-splicing. It can be engineered to replace part of an RNA with sequence attached to its 3' end. Such application may have importance in the repair of mutant mRNA molecules giving rise to genetic diseases. However, to achieve successful ribozyme-mediated RNA-directed therapy, several parameters including ribozyme stability, activity and efficient delivery must be considered.

2.2.1 Hammer head ribozymes

The hammerhead, is the smallest of the naturally occurring ribozymes, consists of around 40 nucleotides and mediates rolling-circle replication within circular virus-like RNAs that infect plants. The hammerhead motif is the most efficient self-cleaving sequence that can be isolated from randomized pools of RNA, suggesting that it may have arisen multiple times during the evolution of functional RNA molecules (Salehi-Ashtiani and Szostak, 2001). Three short helices are connected at a conserved sequence junction. The hammerhead catalyses site-specific cleavage of one of its own phosphodiester bonds via nucleophilic attack of the adjacent 28-oxygen at the scissile phosphate. The simplicity of the hammerhead secondary structure lent itself to the design of two-piece constructs in which the strand containing the cleavage site was separated from the rest of the self-cleaving RNA. By treating one strand as the substrate and the other as the
enzyme, multiple-turnover cleavage occurred with a typical rate of 1 molecule per minute at physiological salt concentrations, consistent with a substantial 109-fold rate enhancement over the unanalyzed rate of nonspecific RNA hydrolysis. Studies at much higher ionic strength (4-M monovalent salt) has showed that the hammerhead as well as the hairpin and VS ribozymes could react nearly as fast in the absence of divalent ions (Murray et al., 1998). This discovery suggested two distinct possibilities: either these ribozymes use a different catalytic mechanism in the presence of high, nonphysiological concentrations of monovalent salts, or the divalent metal ion requirement at low salt concentrations serves a structural rather than a purely chemical function. Recently, molecular modelling and kinetic analysis of the hammerhead cleavage reaction in the presence of monovalent versus divalent salts support the idea that divalent metal ions are not essential to the catalytic step, but instead stabilize the active ribozyme structure (Murray and Scott, 2000; O’Rear et al., 2001; Curtis and Bartel, 2001; Murray et al., 2002).

2.2.2 Human Delta Virus ribozyme and Hairpin ribozymes

The HDV and hairpin ribozymes catalyse the same chemical reaction as that of the hammerhead, and they are likewise responsible for cleaving intermediates generated during rolling-circle replication of the HDV and a plant virus satellite RNA respectively. Crystal structures of these ribozymes showed that in each case the RNA forms an enclosed cleft in which strand scission takes place (D’Amare et al., 1998; Rupert and D’Amare, 2001). Furthermore, neither ribozyme seems to coordinate a divalent metal ion at the site of catalysis, but instead positions functionally essential
nucleotides proximal to the substrate in a configuration suggesting the possibility of their direct role in catalysis.

2.2.3 Group II introns

Group II introns, found in bacteria and in organellar genes of eukaryotic cells, catalyze precise self-excision and ligation of the flanking RNA sequences to form a mature transcript. In a mechanism distinct from that of the group I introns, the group II reaction involves nucleophilic attack by the 28-hydroxyl of a specific adenosine within the intron - the ‘branch site’ - to form a branched or lariat-type structure. Magnesium ions coordinated within the intron are thought to have a direct role in catalysis (Gordon et al., 2000; Sigel et al., 2000; Gordon and Piccirilli, 2001), and several studies have revealed aspects of the intron tertiary structure that are essential to catalytic function (Jestin et al., 1997; Boudvillain et al., 2000; Zhang and Doudna, 2002). Interestingly, some group II introns encode proteins that assist RNA splicing and can also enable efficient integration of the intron RNA into double-stranded DNA by reverse splicing and reverse transcription (Yang et al., 1996). This reverse splicing activity promotes intron mobility by enabling insertion into targeted genes.

2.2.4 Group I Introns

The coding regions of genes are often interrupted by non-coding sequences referred to as intervening sequences or the introns. These introns need to be removed via a cleavage-ligation reaction to form mature, functional RNA molecules. Group I introns, the self-splicing introns were first
discovered in the pre-ribosomal RNA (rRNA) of the ciliated protozoan *Tetrahymena thermophila* (Kruger *et al.*, 1982). Group I introns are found within genes encoding mRNA, rRNA and tRNA in extraordinarily diverse genetic systems - nuclear, mitochondrial, chloroplast and bacteriophage. Nuclear group I introns are limited to rDNA genes, where as in the organelles, they are found in rRNA, tRNA and protein coding regions.

**Mechanism of self-splicing**

The self-splicing of the *Tetrahymena thermophila* Group I intron from the precursor RNA occurs through a two-step trans-esterification reaction (Cech, 1987). A guanosine or one of its 5'-phosphorylated forms (GMP, GDP, or GTP) attacks the phosphorus atom at the 5' splice site and forming a 3', 5'-phosphodiester bond to the first nucleotide of the intron. The 5' exon, terminating in a free 3' hydroxyl group, then attacks the phosphorus atom at the 3' splice site resulting in ligation of the exons and excision of the intron. The mechanism of self-splicing was found to be similar for group I introns of precursor RNAs from diverse sources. The excised intron usually undergoes an intramolecular cyclization again by transesterification where the 3'-terminal G-OH of RNA attacks the phosphorous atom near the 5' end of the molecule (Grabowski *et al.*, 1981; Zaug *et al.*, 1983). Group I RNA self-splicing has an obligatory divalent cation requirement commonly met by Mg$^{2+}$. Studies with the *Tetrahymena* system have shown that Mn$^{2+}$ can substitute, but not Ca$^{2+}$, Zn$^{2+}$, Co$^{2+}$ or Pb$^{2+}$ (Cech and Bass, 1986). Although the self-splicing reactions in many cases occurs in the absence of proteins *in vitro*, the self-splicing reactions *in vivo* occur with the assistance of protein
factors that, in some cases (Lambowitz and Perlman, 1990), are encoded within the intron itself. More recent evidence comes from *Saccharomyces cerevisiae* in which case Mss116p, one of the three DexH/D-box proteins is required for efficient splicing of all the nine *S. cerevisiae* mitochondrial Group I introns (Huang *et al.*, 2004).

**Secondary structure and the catalytic core**

The Group I introns fold to form active sites that accomplish the auto-catalysis (Kruger *et al.*, 1982; Peebles *et al.*, 1986; van der Veen *et al.*, 1986). Comparative analysis of group I introns has revealed that they share a common core secondary structure (Burke and RajBhandary, 1982; Michel *et al.*, 1982; Davies *et al.*, 1982). The secondary structure comprises paired stem segments P1 through P10, loops that are numbered according to the stem that closes them (Waring and Davies, 1984; Burke *et al.*, 1987) and the joining segments - the nucleotides located between pairing segments but are not loops numbered according to the pairing segments they connect, from 5′ to 3′ (Waring and Davies, 1984; Burke *et al.*, 1987).

The paired segment comprises stems P1 through P10, paired predominantly through Watson-crick base pairs although non-Watson-crick base pairs also occur in a conserved pattern. The folded secondary structure of a Group I intron shares a conserved common core structure formed of four short sequences P, Q, R and S (Waring and Davies, 1984; Cech, 1988). This core defines a set of nucleotides that are necessary for catalytic activity, the paired stems P3, P4, P5, P6, P7, P8 and P9, common to all known group I
introns (Michel and Westhof, 1990). The base paired stems P1 and P10 bring together the intron and exon nucleotides and consists of the 5′ and 3′ splice sites. The P1 which is present in all Group I introns either alone or along with P10 which is found in most Group I introns forms the “substrate” for the catalytic activity. The P1 and P10 even when synthesized behave as a substrate when incubated with the core enzyme (Doudna and Szostak, 1989). Within the intron lies the IGS or the ‘Internal Guide Sequence’, which is a short stretch of nucleotides that recognizes the binding sequences at the 5′ exon.

The catalytic core of almost all Group I introns are conserved. Yet, each Group I intron consists of at least one peripheral base-paired region, formed of extra nucleotides, often including long open reading frames, that is usually variable. Although the presence of peripheral elements is universal, they are not usually conserved either in sequence or structure (Doherty and Doudna, 2000). Based on the sequence composition and structural features, Group I introns are classified into 5 major subgroups and 10 minor subgroups – IA, IB, IC, ID and IE and IA1, IA2, IA3, IB1, IB2, IB3, IB4, IC, IC1 and IC3 respectively (Michel and Westhof, 1990; Suh et al., 1999).

**GNRA tetraloops**

The paired stems of Group I introns often end in terminal loops. The most common terminal loop in ribosomal RNAs is a four-base loop or a tetraloop, which are either a GNRA (where G= Guanine, N=any nucleotide, R= Purine and A= adenine) or a UNCG (Woese et al, 1990). Loops with
GNRA consensus sequences is a prominent feature of most self-assembling RNAs. A major feature forming the periphery of group I introns is the GNRA tetraloops. GNRA tetraloops are found in high frequency in natural RNAs (Jaeger et al., 1994). GNRA loops constitute up to one third of the total in some molecules (Woese et al., 1990) and at least half the tetraloops in catalytic RNAs are GNRA loops (Michel et al., 1989; Michel and Westhof, 1990). Most Group I introns have GNRA tetraloops at L2 and L9. The periphery secondary structure almost always includes a loop L9 occurring between P7 and the 3' extremity most often is also a GNRA tetraloop (Michel et al., 1982; Michel and Westhof 1990). More than one third of known Group I introns mostly of subgroups IA, IB and IC have a GNRA tetraloop at L2. Some Group I introns also exhibit a GNRA tetraloop at P5b (). Many Group I introns position their P1 substrate by means of an L2 (GNRA)-P8 interaction (Michel and Westhof 1990) even though GAAA loops are rare at the tip of P2. The tetraloops exhibit local structure including base stacking and hydrogen bonding within the loop sequence (Westhof et al., 1989; Cheong et al., 1990; Heus and Pardi, 1991; de Stevenson et al., 1991). GNRA tetraloops contribute to long-range tertiary interactions in Group I introns. They are directly involved in the long-range tertiary interaction facilitating the P4-P6 domain structure (Michel and Westhof 1990).

Although the L2 terminal GNRA loop was reported to separate the tetraloop from the universal G (of U.G) of P1 helix by exactly 12 nucleotides in majority of Group I introns (Michel and Westhof 1990), in some cases like the *T. thermophila* and closely related Group I introns, the number of
nucleotides separating the loop from the U.G varies (Peyman, 1994). The L2
y interacting with some conserved component of the core enzyme
ontributes to the correct recognition and positioning of the catalytic site of
J.G pair at the intron- 5’exon junction. The conserved component was found
ω be P8 bp 4, a C.G (Michel and Westhof 1990). Studies on *Tetrahymena
hermophila* Group I intron showed that a GAAA tetraloop and an adenosine-
ich bulge stabilizes domain tertiary structure in a sequence-specific manner.
A single base change in the GAAA tetraloop disrupted Fe (II)-EDTA
tection both locally and in P6a, and a specific base-pair substitution in P6a
imilarly disrupted protection locally and in the tetraloop (Murphy and Cech,
1994). The *Tetrahymena thermophila* Group I intron crystal structure
revealed two specific long-range interactions clamping together the two core
omains of which a GAAA binding to a conserved 11 nucleotide internal loop
one (Cate *et al.*, 1996). The interaction of these GNRA loops with their
ceptors stabilizes the catalytic core and has an enhancing activity on the
catalysis. The GNRA loop-receptor sets identified include the L5b-P6a, L9-
P5 and L2-P8 (Costa and Michel, 1995). The L5b-P6a pair is found only in IC
 subgroup of Group I introns whereas the other two pairs are commonly
The GNRA receptor sequences include CC-GG pairs, CU-AG pairs,
CUAAG-UAUGG motif (Michel and Westhof, 1990). A CCC-GGG P8
motif is a characteristic feature of IC3 Group I introns (Ikawa *et al.*, 1999).
The catalytic activity of Group I intron involves the binding of either a guanosine or an internal G residue. The major component of the guanosine-binding site corresponds to a universally conserved GC pair in P7 (Michel et al., 1989). Guanosine was initially proposed to interact with this base pair via formation of a base triple, but the contribution of neighbouring nucleotides and the binding of analogs are also consistent with a model in which guanosine binds axially to the conserved and flanking nucleotides (Michel and Westhof, 1990).

Tertiary Structure

The tertiary structure is the deciding feature for an actual catalysis to take place. Studies involving 3D models and crystal structures have provided useful insights into the tertiary structure of Group I introns. A three-dimensional model of the Group I intron core enzyme identifies two main, elongated structural domains formed of P5-P4-P6 and P7-P3-P8 respectively assembled in such a way to form a cleft on which the “substrate” is bound (Michel and Westhof, 1990; Kim and Cech, 1987). To accomplish substrate helix docking and catalysis (Cech et al., 1992; Bevilacqua et al., 1992), the *Tetrahymena* ribozyme folds into a complex tertiary structure with a close-packed catalytic core (Latham and Cech, 1989) in which several structural elements come together to jointly recognize and interact with the P1 substrate helix. These elements include the J4/5 internal loop (Wang et al., 1993; Strobel et al., 1998), the J8/7 strand (Pyle et al., 1992; Szewczak et al., 1998),
and the attacking guanosine nucleophile bound within the P7 helix (Michel et al., 1989). This complex interaction of multiple closepacked helical and single-stranded RNA elements (Cech and Herschlag, 1996) requires several layers of interactions to create the active site of the molecule. An array of hydrogen bonding interactions that stabilize the interaction between the P1 substrate helix and the highly conserved J8/7 strand within the intron core were reported (Szewczak et al., 1998; Ortoleva-Donnelly et al., 1998). In conjunction with a G.U wobble receptor in the J4/5 region (Wang et al., 1993; Strobel et al., 1998), the J8/7 strand creates a complementary surface for recognition of the P1 substrate helix (Pyle et al., 1992; Szewczak et al, 1998).

The peripheral elements of Group I introns assist in the formation of the core structure and stabilize the ribozyme core structure (Golden et al., 1998; Doherty and Doudna, 2000; Lehnert et al., 1996; Engelhardt et al., 2000; Adams et al., 2004). Stabilizing the ribozyme core is replaceable by magnesium in many cases. Yet, such a function might be essential for the ribozyme activity in living cells where the magnesium concentrations are usually lower than the levels needed to replace the requirement for these peripheral elements (Khvorova et al., 2003). Peripheral elements interacting with themselves or with conserved structural elements form important tertiary interactions significantly contributing to the ribozyme activity such that they even modulate the folding pathway of the ribozyme (Pan and Woodson, 1999; Treiber and Williamson, 2001; Russell et al., 2002). Eventhough the cellular environment or reaction conditions govern self-splicing activity, the folding pathway remains intrinsic to intron tertiary structure (Tinoco and Bustamante,
1999; Hanna and Doudna, 2000; Treiber and Williamson, 2001; Woodson, 2002). The conserved core region, where the actual catalysis occurs, is formed of two helical domains- the P3-P7 and P4-P6- connected via “base triples” evinced as short triple-helices. In some introns conserved A residues not participating in the helix formation, participate in A-minor motif interactions, the most abundant tertiary interaction in 23 rRNA contributing to the structural stability of the RNA (Gutell et al., 2000; Nissen et al., 2001). The peripheral element P2.1 is essential in folding of the compact tertiary structure of the Candida ribozyme that is required for intron self-splicing. This essential function, however, is not performed by the terminal loop of P2.1 that participates in the formation of the P13 tertiary interaction. Unexpectedly, the very short P2.1 stem linking P2 and P3 performs the essential role, through fostering the triple helical interaction with both P3 and P6 (Xiao et al., 2005).

A- rich bulge

An A-rich bulge is conserved and frequent in IC1 and IB4 class of Group I introns. The bulge is highly responsible for activating the ribozyme (Naito et al., 1998). The bulge forms a corkscrew turn that permits A183 and A184 to specifically interact with G110–C211 (the 4th pair of the P4 helix) and C109–G212 (the 3rd pair of the P4 helix) in the minor groove of P4, respectively, as observed in the crystal structure (Cate et al., 1996). These interactions are likely to be responsible for ribozyme activation (Doherty et al., 2001).
Base Triples

Base triples first observed in tRNAs (Robertus et al., 1974) are also involved in forming higher-order structure of domains of Group I intron (Flor et al., 1989; Michel and Westhof, 1990; Michel et al., 1990; Murphy and Cech, 1994; Cate et al, 1996). Known base triples include the third base pair of P4 and the second nucleotide of J6/7, the second base pair of P4 and the first nucleotide of J6/7, the first base pair of P6 and the third nucleotide of J3/4, the second base pair of P6 and the second nucleotide of J3/4 (Michel et al., 1990a). The base triples between J3/4 and P6, J6/7 and P4 are highly conserved further tethering domains P4-P5-P6 and P8-P3-P7-P9-P9.1-P9.2 in IE introns (Li and Zhang, 2005).

Non-Watson Base pairs

Another important feature of the intron structure is the prevalence of non-Watson base pairs. Occurrence of non-Watson Crick base pairs facilitate forming a multi-layer core Complex (Walczak et al., 1998) non-canonical base pairs in group-I intron occur at specific region of helices or stems. These non-canonical base pairs in group-I intron are functionally important in: (i) recognition of guanosine cofactor, (ii) binding to metal ions, thereby reducing the electrostatic repulsive interactions due to polynucleotides folding, and (iii) facilitating domain–domain interaction involving tertiary hydrogen bonds. Furthermore, the wobble pair G.U is the most conserved in the group-I intron contributing to the folded structure and base pairs of the type GA and AA provides additional stabilization to the active core structure.
During the core organization, the L2 of P2 stem are found to interact with L5c of P5c thereby creating a tertiary contact at the junction of catalytic core. For undergoing this type of loop–loop interaction, the P2 stem is provided with an unusual base pair of type UG which provides flexibility for the domain without creating any helix distortion. A high degree of conservation of non-Watson base pairs at conserved positions has been observed from comparative analysis of group I introns (Chandrasekar and Malathi, 2003).

2.2.4.1 Group I Introns And The ‘RNA World’

The hypothesis of the existence of an RNA world probably around 4 billion years ago (Joyce, 1991) elicited exciting search for the ‘pre-biotic RNA’ molecule. The ‘pre-biotic RNA’ of an RNA world needed to contain and transfer information or replicate, all the reactions catalyzed by the RNA itself. The universal RNA three-dimensional architecture and the ability to catalyze biochemical reactions both in vitro and in vivo has projected catalytic RNAs as the possible molecular fossils of an ‘RNA world’. The primary task of its own replication would require an RNA-dependent RNA polymerase like activity. The catalytic function of Group I intron has been suggested as a model for the primordial replicase (Sharp, 1985; Lewin, 1986; Gilbert, 1986; Cech, 1986; Szostak, 1986). The sequence-specific transesterification, the presence of an internal template and the specificity for a complementary substrate and the flexibility of template sequence has rendered the Group I introns the characteristics of an RNA-dependent RNA polymerase including the ability to catalyze template-directed nucleotidyl transfer reactions (Joyce, 1991). The early RNA molecules, would have formed short stem-loop
structures which in turn would have interacted with each other through long range RNA-RNA interactions to form higher order structures. The stem-loop structure of Group I introns support their ancestral nature. The short stem-loop structures in the Group I intron in the form of stems with GNRA tetraloops and their receptors interactions are remnants of long-range RNA-RNA interactions that existed in the RNA world.

Comparing ribozymes that have been vertically inherited for millions of years gives an insight into the evolution of structure-function of RNA. Conserved sequences of Group I introns can be used to infer the evolutionary significance using sequence alignments. These sequences provide potential examples of intron-host co-evolution and are candidates for introns that might have evolved a function that is beneficial to the host. Two outstanding examples of long-term vertical inheritance are the ancient group I intron inserted in the tRNA-Leu gene of cyanobacteria and plastids (Kushel et al., 1990; Xu et al., 1990; Simon D et al., 2003). The similarity of distribution pattern of group I introns in cyanobacteria tRNA$^{Leu}$ genes and chloroplast tRNA$^{Leu}$ genes remains a major evidence for the ancestral origin of Group I introns (Kushel et al., 1990; Xu et al., 1990; Paquin et al., 1997). Group I introns could have existed prior to the origin of chloroplasts as endosymbionts and probably evolved from structurally similar tRNAs (Caprara et al., 1996b). Phylogenetic relationships of green algal nuclear-encoded small subunit rDNA group I introns, has been analyzed for members of the Trebouxiophyceae (common phycobiont components of lichen) and the Ulvophyceae (Bhattacharya et al., 1996). Phylogenetic analyses using a
weighted maximum-parsimony method showed that most group I introns form distinct lineages defined by insertion sites within the SSU rDNA. Phylogenetic analyses of nut-SSU-rDNA and mitochondrial SSU-rDNA sequences suggest that *Clavicorona* and *Lentinellus* are closely related, but that *Panellus* is not closely related to these. The distribution of the introns have been twice independently gained via horizontal transmission, once on the lineage leading to *Panellus* and once on the lineage leading to *Lentinellus* and *Clavicorona* (Hibbett, 1996). Phylogenetic analyses of intron sequences suggest that the mushroom introns are monophyletic, and are nested within a clade that contains four other introns that insert at the same position as the mushroom introns, two from different groups of fungi and two from green algae. Important structural features such as distribution and conservation of the non-Watson-Crick base pairs in the stem regions of group I introns have been analysed to deduce their evolutionary significance (Chandrasekhar K and R. Malathi 2003). The structure conservation and the position has been associated with evolutionary and functional significance (Jackson *et al.*, 2002).

### 2.2.4.2 Therapeutic Potential of Group I Introns

Group I introns have a sporadic and highly biased distribution in nature and many microbial eukaryotes are rich in these sequences (Haugen *et al.*, 2005). Group I introns are found in many pathogenic fungi like *Naegleria* (Embley *et al.*, 1992), *Pnemosystis carinii* (Liu and Leibowitz, 1993), *Candida albicans* (Mercure *et al.*, 1993), *Acanthamoeba* (Gast *et al.*, 1994), *Candida dubliniensis* (Boucher *et al.*, 1996) etc. Interestingly the Group I
introns are present in essential genes of these organisms. Group I introns are absent in mammalian genes and assays on self-splicing can be easily carried out (Zaug and Cech, 1986; Disney et al., 2001). This has led to studies on potential inhibitors of Group I intron self-splicing as antimicrobial agents.

The self-splicing reaction of group I introns has been shown to be inhibited by a number of small molecules. The antibiotics streptomycin (von Ahsen and Schroeder, 1991) and viomycin (Wank et al., 1994) are competitive inhibitors of the self-splicing via their guanidino groups, which they have in common with the cofactor guanosine. However, the pseudosaccharide lysinomycin (Rogers and Davies, 1994) which does not contain a guanidino group also inhibits the self-splicing by a competitive interaction. Aminoglycoside antibiotics like neomycin B, gentamicin, kanamycin and tobramycin (von Ahsen et al., 1991), tetracycline, pentamidine (Liu et al., 1994), and spectinomycin (Park et al., 2000) which do not have guanidino groups are non-competitive inhibitors of the group I intron splicing. The susceptibility of C. albicans to the base analogs 5 Fluorouracil and 5 Fluorocytosine has been shown to correlate with the presence of Group I introns (Mercure et al., 1993) and later these base analogs were shown to inhibit the self-splicing of Group I introns (Mercure et al., 1997). Hoechst 33258 has been shown to possess antimicrobial activity and to inhibit the self-splicing of Group I intron of C. albicans (Disney et al., 2004) and P. carinii (Disney et al., 2005). Since the Group I introns are not found in mammalian genes, design of inhibitors is feasible. With the development of antisense technology, antisense oligonucleotides have been
directed against the 5' exon of *C. albicans* Group I intron to achieve inhibition of self-splicing (Disney *et al.*, 2001).

The ability of group I ribozymes to perform trans-splicing reactions *in vitro* suggests the possibility of therapeutic modification of disease-relevant RNA targets *in vivo*. In a model system, truncated lacZ transcripts have been corrected in *Escherichia coli* (Sullenger and Cech 1994) and mammalian cells (Jones *et al.*, 1996). In these studies the ribozyme was able to repair the mutant RNA by recognizing the target transcript by base pairing with it followed by cleaving off the mutant sequences and ligating the wild sequences. Since the efficiency of the trans-splicing group I ribozymes can approach 25–50% of targeted transcripts (Jones and Sullenger, 1997), this approach has the potential to correct inherited and other diseases caused by expression of mutant mRNAs. The L-21 group I ribozyme has been used to correct mutant β^S^ globin mRNA transcripts by trans-splicing with the γ-globin 39-exon in sickle cell anemia-derived erythroid lineage precursor cells (Lan *et al.*, 1998).

**2.2.5** RNase P RNAs

The RNase P ribozyme is an ubiquitous endoribonuclease formed of a 14kDa protein unit and a 377 nucleotide RNA sub unit called the M1RNA (Guerrier -Takada *et al.*, 1983). This ribozyme is involved in the tRNA 5' end processing and also processing of 5S rRNA and signal recognition particle RNA (Frank and Pace, 1998; Morl and Archfelder, 2001). Like group I introns, RNase P ribozymes also require a divalent metal ion for
catalytic activity (Guerrier-Takada et al., 1986). Bacterial RNase P RNAs examined so far fall into two main structural classes. These RNAs share a common ‘core’, and synthetic minimal RNase P RNAs consisting only of these core sequences and structures are catalytically proficient (Waugh et al., 1989; Siegel et al., 1996). Type A is the usual form of RNase P RNA in Bacteria, where as type B RNAs are found only in the low G+C Gram-positive Bacteria, such as *Bacillus subtilis* (Haas et al., 1996). Structural variation between type A and B RNase P RNAs, and between the instances of each structure type, is predominated by variation in the presence or absence of helical elements and in variation of the size of the distal regions of these helices. Eubacterial RNase P RNA consists of two domains: a specificity domain or S-domain and a catalytic domain or C-domain (Loria and Pan, 1996; Pan, 1995). The S-domain alone can bind pre-tRNA directly (Qin et al., 2001). The distinct features of the secondary structure of the S-domain include a) a cruciform formed by the stacked P7, P10 and P11 and the stacked P8 and P9 helices, b) the packing of the P10.1 and P12 helices through a GAAA tetraloop-tetraloop receptor interaction, and c) an unusually folded module linking P11 and P12 (J11/12-J12/11), which contains a large number of universally conserved nucleotides, and is stabilized without canonical Watson-Crick base pairing. While the S-domain forms a well-packed and compact structure, it is important to note that the P11 and P9 helices together with the J11/12-J12/11 module form a clamp-like opening that contains nucleotides involved in pre-tRNA binding (Krasilnikov et al., 2003).
RNase P RNA AND THE RNA WORLD

RNase P is present and essential in all cells and subcellular compartments that synthesize tRNA, but catalytic proficiency by the RNA alone has been demonstrated only for the bacterial RNAs (and in all such RNAs tested). Although recognizable RNase P RNAs are present in all organisms, they are more variable in both sequence and secondary structure than are the ribosomal RNAs and transfer RNAs. Bacterial and archaeal RNase P RNAs are more similar in sequence and structure than either is to the eukaryal RNAs (Brown and Haas, 1996; Haas et al., 1996; Chen and Pace, 1997). Studies have shown the high versatility of RNase P as a potential marker for the phylogeny and molecular ecology of *Prochlorococcus* (Schon et al., 2002). Comparisons between RNase P enzymes from diverse organisms reveals substantial sequence variation; however, there are similar core sequences and secondary structures that likely represent the catalytic domains (Frank and Pace 1998). The cyanelle RNase Ps have an essential RNA component which is encoded in the plastidome and almost conforms the bacterial consensus, but have no ribozyme activity. Although the overall composition of the Cyanophora RNase P is more similar to the eukaryote than to the bacterial-type enzymes, a fully active RNase P holoenzyme can be reconstituted from the corresponding organelle RNA and a cyanobacterial protein subunit. This suggests that, despite of the high structural diversity, the catalytic activity still resides in the RNA subunit.
2.2.5.2 Therapeutic Potential of RNase P and the EGS Technology

The ubiquitous nature of RNase P, the lack of a requirement for specific nucleotide sequences for cleavage, and the inherent efficiency of utilizing a cellular enzyme, have created interest in directing RNase P mediated cleavage to therapeutic target mRNAs. Both *E. coli* and human RNase P have been shown to cleave a target RNA that resembles a tRNA substrate in a bimolecular reaction (Yuan *et al.*, 1992). The 39-proximal sequence of the stem functions to identify the target and is considered to act as an external guide sequence (EGS) that can be targeted to essentially any target RNA. The sequence RCCA is included in the 3’end of the EGS to mimic the 3’end of precursor tRNAs, the natural substrates for *E. coli* RNase P. The antisense EGS molecule is in a complex with the target RNA forming a stem like structure (typically with 13–16 bp). EGSs of this type are referred to as “stem” EGS. Direct expression of EGSs directed against a bacterial resistance gene (Guerrier-Takada *et al.*, 1997) or essential viral genes (Plehn-Dujowich and Altman, 1998) has proven effective in functional cellular assays. When *E. coli* harboring a chloramphenicol resistance gene (chloramphenicol acetyltransferase, *cat*) was transformed with a plasmid encoding a stem EGS specific for the *cat* gene, the *cat* mRNA was selectively destroyed by endogenous RNase P, and consequently expression of chloramphenicol acetyltransferase was decreased (Guerrier-Takada *et al.*, 1997). These cells were therefore rendered sensitive to chloramphenicol and resulted in phenotypic conversion of drug resistant bacteria to drug sensitivity. Another study demonstrated that microbial viability can be
decreased to less than 10% of the wild type strain if the EGS-mediated approach is employed to reduce the level of expression of essential proteins such as gyrase A and the protein subunit of RNase P (McKinney et al., 2001). The investigation also showed additivity of combined use of EGSs and that a three nucleotide mismatch between target and EGS can be tolerated with no loss in efficiency. However, an EGS specific for the gyrase A mRNA in *Salmonella typhimurium* was ineffective in targeted cleavage of the gyrase A mRNA in *E. coli* because there were six nucleotides that differed in sequence between the two homologous mRNAs. The success of the RNase P-mediated approach *in vivo* depends on (i) stable expression of the EGSs (using either constitutive or regulated promoters), (ii) co-localization of the target mRNA substrate (the EGS and RNase P within the same subcellular compartment), and (iii) accessibility of the target mRNA to binding by the EGS. A fusion between the M1 RNA and a sequence complementary to the herpes simplex virus I thymidine kinase (TK) mRNA was shown to efficiently cleave TK mRNA *in vitro* and reduce TK mRNA and protein levels by 80% in transfected cells (Liu and Altman, 1995). Deletion studies have defined that an EGS of only 30 nucleotides is sufficient for efficient cleavage in a biomolecular reaction *in vitro* (Werner et al., 1999).

**CANDIDA ALBICANS AND DRUG RESISTANCE**

*C. albicans* a common opportunistic fungi can cause life threatening infections in immunocompromised individuals like HIV infected and cancer patients and has been identified as a major cause for mortality in such patients (Dixon et al., 1996). Three classes of antifungals, polyenes, azoles and
flucytosine, are widely used. However the incidence of the acquisition of resistance to most of these drugs by *Candida albicans* cells has increased considerably in recent years, which has posed serious problems in successful chemotherapy. This has led to a search for newer and effective, alternate targets (Sternberg 1994). Although the molecular basis of azole resistance in *C. albicans* is not very clear, accumulated evidence suggests that multidrug resistance (MDR) is a multifactorial phenomenon, with some of the most common mechanisms being the failure of drug accumulation mediated by drug extrusion pumps such as *CDR1, CDR2* (ATP-binding cassette [ABC] family), and *CaMDR1* (MFS family), alterations in Erg11p, and upregulation of *ERG11*. A combination of different resistance mechanisms has been reported to be responsible for fluconazole resistance in clinical isolates of *C. albicans* (Lopez-Ribot et al., 1999; Prasad et al., 2002; Prasad et al., 2002; Sanglard et al., 2002). Recent evidence (White et al., 1998; Kohli et al., 2002) also suggests that the generation of a resistant strain from a highly susceptible strain is the result of multiple mechanisms, each of which probably contributes partially to the resistant phenotype. Amphotericin B mediates its antifungal property through interference in the ergosterol pathway. Development of effective antifungals requires identification of selective and efficient targets and also the virulence factors involved. Several putative virulence factors of *C. albicans* have been described, including secreted hydrolytic enzymes (Calderone and Fonzi, 2001). Two types of secreted enzyme seem to be the most important: phospholipases and secreted aspartyl proteinases (Ghannoum, 2000; De Bernardis et al., 2001). Secreted aspartyl proteinases are capable of degrading epithelial and mucosal barrier proteins.
such as collagen, keratin and mucin, as well as antibodies, complement and cytokines. Cloning and disruption of the genes for these enzymes have shown their involvement in *Candida* virulence (Hube *et al*., 1997; Sanglard *et al*., 1997; De Bernardis *et al*., 1999).

**BACTERIAL DRUG RESISTANCE**

Drug resistance in pathogenic bacteria is a major cause of concern. Several reasons are attributed to this resistance property. Multiple mechanisms may be involved in the development of resistance like phosphorylation of the antibiotic, mutations in the bacteria leading to changes in membrane permeability etc. β-lactam antibiotics have been used as a major antibacterial drug for a long time. Resistance to β-lactam drugs developed with the evolution of β-lactamases. β-Lactamases are enzymes that recognize the β-lactam ring in the antibiotics and cleave them leaving the antibiotics non-functional (Then and Angehrn, 1982; Vu and Nikaidio, 1985). Structural analogs of β-lactam antibiotics are being studied as β-lactamase inhibitors or as modified drugs. Extensive use of broad spectrum antibiotics, prolonged hospitalisation, indwelling devices and severe underlying diseases have been reported as factors that have led to spread of ESBL and difficulties in managing severe infections in many parts of the world (Nathisuwan *et al*., 2001; Lin *et al*., 2003). Extended spectrum β-lactamase (ESBL) enzymes have been reported in a number of species in Gram-negative bacteria. The ESBL enzymes are usually plasmid mediated and are capable of hydrolysing and inactivating a wide variety of β-lactams, including third-generation
cephalosporins, penicillins and aztreonam, but are susceptible to β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam (Sturenburg and Mack, 2003; Gniadkowski, 2001). Many ESBL producers also carry other genes that confer resistance to other antimicrobial agents such as aminoglycosides and fluoroquinolones (Spanu et al., 2002; Sekowska et al., 2002).

**SPECTINOMYCIN AND VANCOMYCIN**

Spectinomycin is an aminocyclitol antibiotic derived from *Streptomyces spectabilis* and is bacteriostatic in nature. It is a selective inhibitor of protein synthesis *in vivo* and it has also been reported to inhibit the *in vitro* synthesis of polypeptides by binding to the 30S ribosomal subunit (Sammes, 1977). Spectinomycin has been reported to inhibit the self-splicing of the Group I intron RNA through interaction with specific and functional Mg$^{2+}$ binding sites within the RNA causing displacement of the Mg$^{2+}$ necessary for the self-splicing activity (Park et al., 2000). It can selectively bind to certain short RNA transcripts as in the case of some bacteria, altering the RNA folding and thereby rendering them the spectinomycin resistance property (Zimmerman and Maher, 1998). Vancomycin is a peptide antibiotic derived from *Nocarida orientalis* (previously *Streptomyces orientalis*). It can alter the membrane permeability and selectively inhibit RNA synthesis.

**BLEOMYCIN**

The bleomycins are a family of naturally occurring, structurally related, glycopeptide-derived antitumor antibiotics discovered from cultures
of *Streptomyces verticillus* in 1966 (Umezawa et al, 1966), which have more than 200 members, such as A2, A5 and B2 (Claussen and Long, 1999). BLMs consist of an unusual linear hexapeptide, a disaccharide and a terminal amine. Mixtures of BLMs are presently used for the clinical treatment of a variety of cancers, notably squamous cell carcinomas, testicular tumors and non Hodgkin’s lymphoma (Claussen and Long, 1999). The therapeutic effect of BLM is believed to result from its ability to induce single- and double-strand breakage of DNA molecules by oxidation of the deoxyribose moiety in the presence of oxygen and a redox-active metal ion, e.g. Fe and Co (Claussen and Long, 1999; Goodisman et al, 1997; Stubbe and Kozarich, 1987; Burger, 1998). Bleomycin also mediates oxidative RNA degradation in a highly site-selective manner at sites adjacent to Guanosine residues (Holmes et al, 1993; Carter et al., 1990). It has been found that BLM-induced autoxidation of ferrous iron follows the Michaelis–Menten kinetics (Caspary et al, 1979; Caspary et al, 1981).

**5 BROMOURACIL**

Of the few nucleic acid targeting drugs known currently, base analogs such as 5 Fluorocytosine and 5 Fluorouracil etc have been known to have effective inhibitory activity on fungal pathogens resulting from incorporation of fluoro-substituted nucleotides derived from these base analogs (Grunberg et al., 1964). One such base analog, 5 Bromouracil has the ability to get incorporated into DNA forming base mispairs (Lasken and Goodman, 1984).