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Alternative Conformers of 5S Ribosomal RNA and Their Biological Relevance†

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ABSTRACT: Different conformational states of *Escherichia coli* 5S ribosomal RNA that may participate in protein biosynthesis have been either detected experimentally or predicted on the basis of phylogenetic sequence comparisons. The A conformer exists in a high-salt form (A_H) that binds ribosomal proteins and assembles into the 50S subunit and in a low-salt form (A_L), of uncertain biological relevance, that binds at least one ribosomal protein and differs in tertiary structure from the A_H form. Experimentally, the A_H form has been investigated comprehensively and the A_L form partially. There is also a B conformer that exhibits an altered secondary structure and does not assemble with ribosomal proteins. For this conformer to be functionally active, it must be both discrete and universal among 5S RNAs. Here, we examine its structure by employing single and double strand specific ribonucleases and nucleotide-specific chemical reagents. We demonstrate that the B form exhibits a secondary structure only a part of which is both universal and conformationally homogeneous, and we conclude, therefore, that the whole B form cannot participate in protein biosynthesis. We note, however, that progressive structural changes occur during the transitions $A_H \rightarrow A_L \rightarrow B$ and provide evidence that the structural alteration during the transition $A_H \rightarrow A_L$ may be universal, which reinforces the view that the A_L form is of biological relevance.

One of the most important and unresolved questions concerning RNA function is can RNA operate by the interconversion of alternative conformers? A very specific mechanism was suggested for the interaction and relative movement of tRNA and mRNA by Woese (1970), and more general proposals have been made for reversible and putative 5S RNA–tRNA interactions (Fox & Woese, 1975; Weidner et al., 1977). More recently, comparative sequence studies on the large ribosomal RNAs have also revealed alternative and conserved base-pairing schemes, exhibiting approximately similar free energies [reviewed by Noller (1980) and Brimacombe et al. (1983)].

The 5S ribosomal RNA is small; it interacts with a group of proteins and lies in a functionally important region of the 50S subunit. For these reasons, it has been chosen for many seminal studies on RNA structure, RNA–protein interactions, and RNA function [reviewed by Garrett et al. (1981)]. Some of the studies on the RNA structure that employed biochemical and spectroscopic techniques have yielded evidence for major and minor conformational changes occurring in 5S RNA under solution conditions close to physiological, at least some of which were induced by ribosomal proteins [reviewed by Monier (1974) and Garrett et al. (1981)]. The best characterized transition is between the native A and B conformers (A and B forms) of the *Escherichia coli* 5S RNA (Aubert et al., 1968) that occurs in the absence of ribosomal proteins. It requires an activation energy of 65 kcal/mol and involves the disruption and re-forming of an estimated nine base pairs (Richards et al., 1973). A less dramatic change has also been recorded within the tertiary structure of the A form (A_H to A_L) that is salt dependent and occurs close to physiological pH and over a time scale compatible with the translation process (Kao & Crothers, 1980; Kime & Moore, 1982; Rabin et al., 1983).

The native A form has been subjected to both sequence comparison studies and experimental probing, and there is now

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general agreement as to its minimal secondary structure [Fox & Woese, 1975; Delihas & Andersen, 1982; De Wachter et al., 1982; Kjems et al. (1985) and references cited therein]. The B form of the *E. coli* 5S RNA has also been examined with various ribonucleases and chemical reagents [reviewed by Monier (1974) and Noller & Garrett (1979)]. Evidence for a helix, exclusive to the B form, between nucleotides 35–42 and 79–86 was provided by ribonuclease digestion evidence (Jordan, 1971). Sequence comparison results have also revealed that this helix is widely conserved amongst 5S RNAs (Weidner et al., 1977; Trifonov & Bolshoi, 1983). To date, no comprehensive study of the structure of this conformer has been undertaken, and no satisfactory model has evolved; here, we attempt to amend this situation by probing the structure with single and double strand specific ribonucleases (Vassilenko & Babkina, 1965) and nucleotide-specific chemical reagents (Peattie & Gilbert, 1980).

Materials and Methods

The 5S RNA was purified from *E. coli* MRE 600 cells by the procedure of Monier & Feunteum (1971). A total of 10 μg of RNA was 3′-end labeled with [^{32}P]pCp (Amersham) and RNA ligase (P-L Biochemicals) as described by Bruce & Uhlenbeck (1978) or 5′-end labeled with [γ-^{32}P]ATP (New England Nuclear) and polynucleotide kinase (P-L Biochemicals) as described earlier (Douthwaite & Garrett, 1981). The RNA was repurified by electrophoresing in a 12% polyacrylamide gel (15 × 15 × 0.2 cm) in 50 mM tris(hydroxymethyl)aminomethane–borate (Tris–borate) and 1 mM EDTA, pH 7.5. It was extracted and converted into the B form as described by Aubert et al. (1968). The A and B forms were then resolved electrophoretically, on 10–16% polyacrylamide gels containing 40 mM Tris–acetate, pH 8.3, with circulating buffer. Bands were detected by autoradiography, extracted in 0.3 M sodium acetate, pH 5.6, precipitated with 2.5 volumes of ethanol, and washed with cold ethanol. Samples were immediately dissolved, at 0 °C, in either 40 mM Tris-HCl, pH 7.5, 0.5 or 1 mM $MgCl_2$, and 50 mM KCl ($T_{40}M_{0.5}K_{50}$ or $T_{40}M_1K_{50}$) or 30 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, and 300 mM KCl ($T_{30}M_{10}K_{300}$).

A total of 5–10 μg of RNA in 10 μl of buffer was treated, at 0 °C, with ribonucleases A (Sigma), T_1 and T_2 (Sankyo, Japan), and the double helix specific cobra venom ribonuclease (Vassilenko & Babkina, 1965; gift of A. Boutorin and S. Vassilenko). Digestion conditions were 1:10 000 to 1:50 units/μg of RNA for RNase A, 1:5000 to 1:500 units/μg for RNase T_1, 1:5000 to 1:50 units/μg for RNase T_2, and 1:300 to 1:100 units/μg of RNA for the cobra venom ribonuclease. Subsequently, the RNA samples were electrophoresed again on a 10% polyacrylamide gel containing 40 mM Tris-HCl–10 mM $MgCl_2$, pH 8.0, and a selection was made for nicked but intact molecules that coelectrophoresed with the intact B form (Douthwaite & Garrett, 1981).

Accessible adenosines, cytidines, and guanosines were probed with chemical reagents by the procedure of Peattie & Gilbert (1980); adenosines were carbethoxylated with diethyl pyrocarbonate, cytidines were methylated with dimethyl sulfate and treated with hydrazine, and guanosines were methylated with dimethyl sulfate. Reaction buffers were 50 mM sodium cacodylate–1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.3 ($C_{50}E_1$), 70 mM sodium cacodylate, 50 mM KCl, and 0.5, 1, or 2 mM $MgCl_2$, pH 7.3 ($C_{70}M_{0.5-2}K_{50}$), and 70 mM sodium cacodylate, 20 mM $MgCl_2$, and 300 mM KCl, pH 7.3 ($C_{70}M_{20}K_{300}$). Carbethoxylation reactions within the gel piece were performed by soaking the gel piece for 20 min at 0 °C in 200 μL of modification buffer with 10 μg of carrier tRNA and then adding 10 μL of reagent and incubating for 60–90 min. After chain scission at the site of the modified nucleotide, by aniline/acetic acid treatment, chemically modified samples were electrophoresed with enzymically digested samples on 10% polyacrylamide sequencing gels (40 × 40 × 0.03 cm) containing 50 mM Tris–borate and 1 mM EDTA, pH 7.5, and autoradiographed. Water-hydrolyzed RNA (20 min at 100 °C) and chemically sequenced samples (Peattie, 1979) were coelectrophoresed as markers.

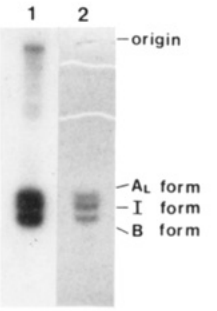

FIGURE 1: Separation of a mixture of conformers of *E. coli* 5S RNA on a 10% polyacrylamide gel in 40 mM Tris–acetate, pH 8.3: (lane 1) an autoradiograph of the gel; (lane 2) the gel was stained for RNA with toluidine blue and dried.

Results

The separation of the conformers after the urea–EDTA treatment is shown in Figure 1; the resolved bands correspond to the A and B forms and to an intermediate I form. Each component was extracted from the gel and probed enzymatically or chemically. Ribonuclease digests were performed on both 3′- and 5′-end labeled samples in order to distinguish primary from secondary cuts (Douthwaite & Garrett, 1981). After enzyme or chemical treatment, each RNA sample was also repurified electrophoretically. This selection for nicked or modified molecules, that were structurally intact, eliminated the gel bands that originated from RNA fragments. All samples were analyzed on sequencing gels. For both 5′- and 3′-end labeled samples, doublet bands were invariably observed due to sequence heterogeneities at the ends of the molecule (Douthwaite & Garrett, 1981).

The B Form. The RNase T_1 digestion pattern, at 1–10 mM Mg^{2+}, shown in Figure 2 emphasizes the high degree of structure within the B form. Under moderate digestion conditions, there was only one main cut at G_{61}; stronger digestion revealed a second major cut at G_{102} and other minor cuts. Digestion with RNase A and the cobra venom nuclease generated more complex patterns; multiple cuts of differing intensities were observed (Figure 3). For each cut, a doublet band was detected due to the sequence heterogeneities at both 5′- and 3′-ends. The intensities of the primary cuts were averaged for each ribonuclease over several experiments, and they were presented in Table I. Secondary cuts, which by definition occur after a primary cut has opened up a site, are summarized in Table II. They were identified on autoradiograms by their absence on the side of the primary cut distal from the end label (Douthwaite & Garrett, 1981), and they denote inaccessible sites in the intact B form.

Differences in the degree of ribonuclease cutting between 0.5 and 10 mM magnesium were small. For the single strand specific RNases A, T_1, and T_2, a general lowering of the kinetics of cutting was detected in the high-magnesium and high-salt buffer. An exception was the strong decrease in susceptibility of G_{16}, G_{100}, and G_{102} to RNase T_1 above 0.5 mM magnesium. The cobra venom nuclease is activated by magnesium, and several of the weaker cuts listed in Table I

Table I: Primary Ribonuclease Cuts and Chemical Modification Sites on the B Form[a]

nucleotide	ribonuclease: cobra venom	A	T_1	T_2	chemical modification: DEP	Me_2SO_4/hydrazine	nucleotide	ribonuclease: cobra venom	A	T_1	T_2	chemical modification: DEP	Me_2SO_4/hydrazine
G_6	+[b]						A_{66}					+	
U_{14}		+					G_{69}	+					
A_{15}				+++	++		C_{70}	(+)					
G_{16}			++[d]	+			C_{71}	(+)					(+)
G_{18}	++						G_{72}	(+)					
C_{19}	(+)						U_{74}	(+)					
U_{25}		+[b]					G_{81}	(+)[c]					
C_{26}				+		(+)	U_{82}	(+)[c]					
C_{27}		++[b]		+		(+)	G_{83}	(+)[c]					
C_{28}	+[b]						G_{84}	(+)[c]					
A_{29}					(+)		G_{85}	(+)[c]					
C_{30}						(+)	G_{86}	(+)[c]					
C_{31}						(+)	U_{87}	+					
C_{38}	++						C_{90}	(+)[c]					(+)
A_{39}	+						C_{91}	(+)[c]					
C_{43}				+		++	C_{92}	(+)					
G_{44}			+				C_{93}	+	+				
A_{45}					++		A_{94}	+[c]				(+)	
A_{46}					+		U_{95}				+		
C_{47}	+[c]					(+)	G_{96}	+[c]		++[d]			
U_{48}	+						C_{97}				+		+
C_{49}		(+)					G_{98}	++					
G_{51}			(+)				A_{99}				+	(+)	
A_{52}					++		G_{100}			+[d]			
A_{53}				(+)	++		A_{101}				++[d]	+	
G_{54}	++		(+)				G_{102}			++[d]			
G_{56}			+				U_{103}		+				
A_{57}					++		A_{104}				+	+	
A_{58}					++		A_{108}				++[d]	+	
C_{60}		+		++			A_{109}					+	
G_{61}			+++	+			C_{110}						(+)
C_{62}		++		(+)		++	C_{114}	+++					
C_{63}						+	A_{115}	++					
U_{65}		+											

[a] The intensities of the bands were estimated visually: +++, strong; ++, moderate; +, weak; (+), very weak. Only positive results are included, and these results are averaged over the following numbers of experiments for each enzyme and reagent: cobra venom nuclease, 10; RNase A, 5; RNase T_1, 8; RNase T_2, 7; DEP, 6; Me_2SO_4/hydrazine, 6. The value for each enzyme includes two experiments performed with 5′-end labeled samples. All guanosines were modified to some degree, and these results are not included. [b] A localization uncertainty of ±1 nucleotide. [c] The effect was detected only at high magnesium concentrations. [d] The effects were weaker at higher magnesium concentration.

Table II: Secondary Cutting Positions[a]

nucleotide	ribonuclease	degree of cut	dependent on primary cut
G_{18}	T_1	(+)	G_{16}
C_{28}	A	+	C_{27}
A_{29}	T_2	+	C_{27}
G_{64}	T_1	(+)	G_{61}
G_{67}	T_1	(+)	G_{61}
C_{95}	A	(+)	C_{93}
G_{105}	T_1	(+)	G_{102}

[a] Secondary cuts are listed that were detected in the 3′-end labeled samples but not in the 5′-end labeled samples (Douthwaite & Garrett, 1981). They were quantified according to the system defined for Table I when approximately half of the 5S RNA molecules remained uncut.

were detected only at the high magnesium concentration.

During the chemical treatments, adenosines and cytidines do not react when involved in double helixes, nor when their respective N-7 and N-3 positions are involved in tertiary interactions. Guanosines, on the other hand, react when base paired but not when the N-7 position participates in tertiary structure or in metal ion binding (Peattie & Gilbert, 1980). The former reactions are, therefore, probes of secondary and tertiary structure, whereas the latter is mainly a probe of tertiary structure.

Adenosines that were carbethoxylated and cytidines and guanosines that were methylated, at 30 °C, are illustrated in Figure 4. The band intensities were quantified visually and averaged over several experiments, and the results are summarized in Table I. Diethyl pyrocarbonate reactions that were performed within the gel pieces yielded essentially the same modification patterns as those done on the extracted conformers. Each guanosine reacted with dimethyl sulfate to some degree, and these results are not listed in the table. The effect of increasing the magnesium concentration was, as for the ribonuclease cutting, general rather than localized, and some very weak adenosine and cytidine bands that are apparent in Figure 4, at low magnesium, but not listed in Table I disappeared at 2–10 mM magnesium.

The I Form. The I form was also probed by both ribonucleases and chemical reagents. Samples of the A_L and B forms were treated under identical conditions and coelectrophoresed on sequencing gels for comparison. Each doublet band produced by the A_L and B forms was present in the I-form tract at reduced intensity, which strongly suggested that the latter was a mixture of the A and B forms (data not shown).

The A_L Form. In order to emphasize the major differences between the A_L and B forms, we show in Figure 5 a comparison of the susceptibilities of the two conformers to RNases T_1 and T_2. The main cutting positions are quite different, although the B-form sample digested by RNase T_1 (track 1B) contains about 5% of renatured A_L form (as judged by the relative intensities of the G_{41} band that does not occur in the pure B conformer, Figure 2). However, the A_L and B forms

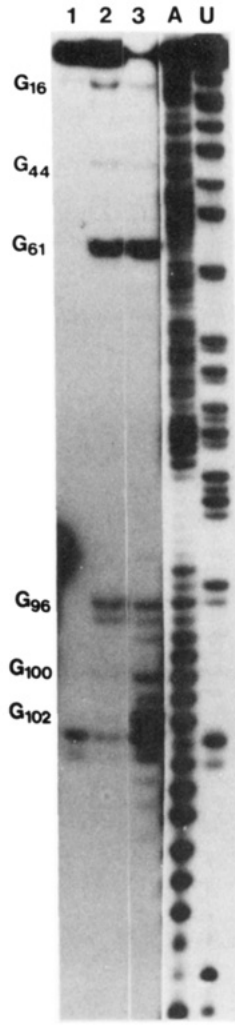

FIGURE 2: Digestion of the B form with RNase T_1: (lane 1) control sample with no enzyme added; (lanes 2 and 3) digested at 1:500 and 1:100 units/μg, respectively, in $T_{40}M_{10}K_{300}$ buffer. All samples were digested for 20 min at 0 °C and run on 10% polyacrylamide sequencing gels.

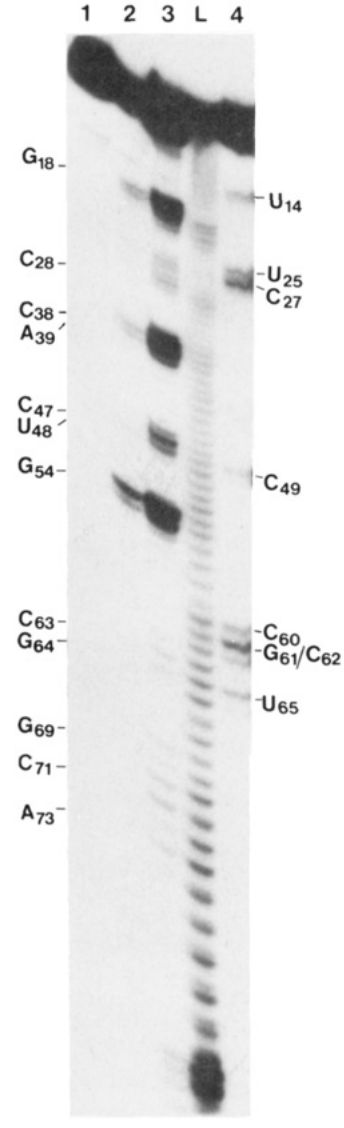

FIGURE 3: Treatment of the B form with the cobra venom nuclease and RNase A in $T_{40}M_{10}K_{30}$ buffer for 30 min at 0 °C: (lane 1) control sample with no enzyme added; (lanes 2 and 3) digested with cobra venom nuclease at 1:300 and 1:150 units/μg, respectively; (lane 4) hydrolysis with RNase A at 1:10 000 μg/μg of RNA; (L) water hydrolysis ladder.

also exhibited some common characteristics in their ribonuclease digestion patterns that differed from those of the A_H form, and these are considered further under Discussion.

Discussion

Secondary Structural Model for the B Form. A model containing five main double-helical segments is presented in Figure 6A. In deriving it, the following strategy was followed: (1) inclusion of the universal helix 4, first characterized for the B form by Jordan (1971), if it was supported by our results; (2) retention of the universal A-form helixes (shown in Figure 6B) where they were compatible with the data; (3) for the rest of the RNA, generation of the most stable base-pair scheme that was consistent with the experimental results. Helix 4 was strongly reinforced by our data. Helixes 1 and 3 were retained from the A form (Figure 6B); the former exhibited identical reactivity patterns in both conformers (Douthwaite & Garrett, 1981). Of the remaining A-form helixes, helix II (16–23/60–68) was incompatible with the highly reactive sequence G_{61}-C_{62} and is rearranged as helix 2, and helix V (70–74/102–106) was incompatible with all of the ribonuclease data and is redrawn as helix 5 (Figure 6A).

With a few exceptions, the sequences that were most accessible to single strand specific ribonucleases and chemical reagents lie in unstructured regions of the model. They include C_{26}–C_{27}, C_{43}–C_{47}, G_{61}–C_{62}, and A_{99}–A_{104}. In addition, the bulged pairs of adenosines A-A_{53}, A-A_{58} and A-A_{109} were accessible to both sets of probes. A few sites, including C_{30}–C_{31}, C_{63}, C_{71} and C_{110}, were chemically modified at 30 °C but not cut by ribonucleases at 0 °C; they occur at less stable sites at the ends of double-helical segments.

Most of the cobra venom nuclease cuts also fall in the putative double helixes (Figure 6A) in accordance with the reported specificity of this enzyme (Vassilenko & Babkina, 1965). Helix 1 incurred the same cuts as in the A form, and helixes 4 and 5 received multiple cuts. Helixes 1 and 4, which are the most stable in the model, were further reinforced by the carbethoxylation results; each of their constituent guanosines was fairly resistant, whereas all of the others in the molecule were reactive (data not shown). Helixes 2 and 5 were also consistent with the cobra venom nuclease results. Helixes 3 and 4 could stack coaxially such that G-A_{34} is paired with either U-C_{49} (Figure 6A) or U-C_{88}; the cobra venom nuclease cuts support the coexistence of both forms.

Limitations of the Model. Evidence at variance with the model is concentrated in, and adjacent to, helix 5. Although

Table III: Comparison of Literature Results on the B Form[a]

nucleotides	glyoxal in 1 mM Mg^{2+} at 20 °C (Aubert et al., 1973)	kethoxal in 0 mM Mg^{2+} at 33 °C (Noller & Garrett, 1979)	ribonuclease T_1 in 20 mM Mg^{2+} at 0 °C (Jordan, 1971)	ribonuclease T_1 in 7 mM Mg^{2+} at 4 °C (Mirzabekov & Griffin, 1972)	ribonuclease T_1 in 0.5–10 mM Mg^{2+} at 0 °C (present data)
G_{13}		+			
G_{16}		+	+	+	+++
G_{18}				+	
G_{23}		+			
G_{24}		+	+		
G_{41}			+		
G_{44}		+	+		+
G_{56}				+	+
G_{61}	+	+	+	+	+
G_{79}			+		
G_{96}			+		++
G_{98}			+		
G_{100}	+	+		+	+
G_{102}		+		+	++
G_{106}			+		

[a] Sites of guanosine modification or enzymic digestion are indicated by a plus. Results were quantified only in the kethoxal modification study and in the present work. Aubert et al. (1973) only detected strongly modified guanosines. Jordan (1971) performed strong digestions, and these data will therefore contain secondary cuts. Mirzabekov & Griffin (1972) did not define their conformer as the B form.

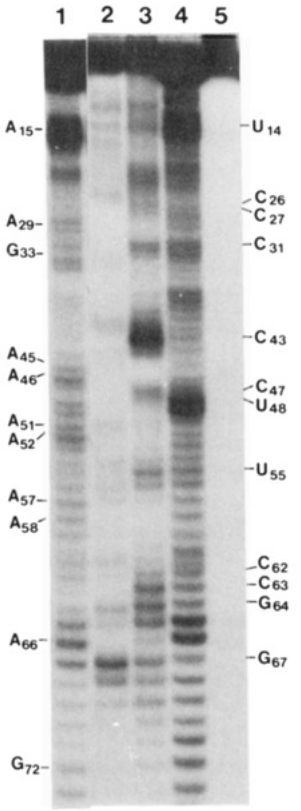

FIGURE 4: Chemical modification of the B-form: (lane 1) carbethoxylation of adenosines for 60 min at 30 °C; (lane 2) methylation of guanosines for 20 min at 30 °C; (lane 3) methylation and hydrazine treatment for cytidines at 30 °C (chain scission was effected by aniline/acetic acid treatment); (lane 4) water hydrolysis ladder; (lane 5) untreated sample of 5S RNA. Samples were modified in $C_{70}M_{0.5}K_{20}$ buffer.

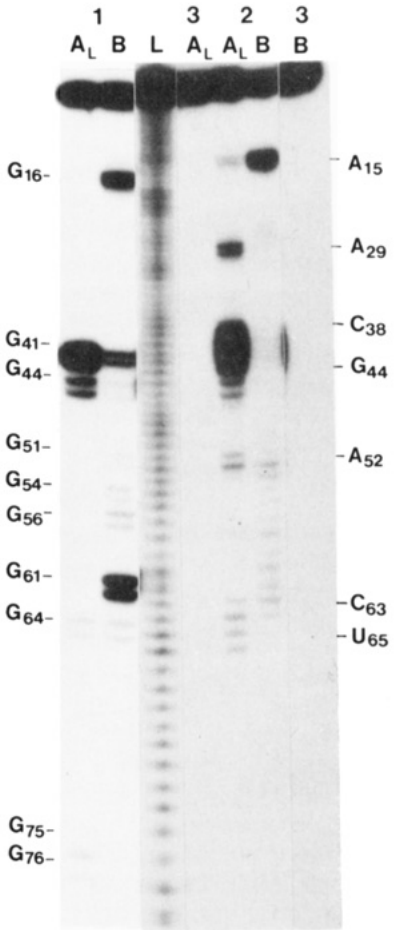

FIGURE 5: Comparison of ribonuclease digestion patterns of the A_L and B forms. (Lane 1) Digestion with RNase T_1 at an enzyme to RNA ratio of 1:1000 units/μg; in this experiment, the B-form sample was cross-contaminated with approximately 5% A form as judged by the relative intensities of the G_{41} band in the A_L- and B- form tracks. (Lane 2) Treated with RNase T_2 at an enzyme to RNA ratio of 1:1000 units/μg; (lane 3) control samples with no enzyme added; (L) ladder produced by water hydrolysis. All digests were performed in $T_{40}M_{0.5}K_{50}$ buffer for 30 min at 0 °C.

cobra venom nuclease cuts occurred along most of its length and single strand specific cuts border the bulged G_{96}, other nucleotides on the 3′-strand (92–98) were also susceptible to the single strand specific probes. This may be due to a conformational heterogeneity in the B form that was detected in an early study (Richards & Lecanidou, 1974). There is clearly, also, additional higher order structuring, especially between helixes 4 and 5, where A_{78} is unreactive and cobra venom nuclease cuts occur in the sequence U_{87}–C_{91}. Moreover, the apparently unstable section of helix 2 is resistant, and this could result from additional tertiary interactions.

Comparison with Earlier Results. Only the accessible guanosines of the B form have been probed, and the results are correlated with ours in Table III. Good agreement exists

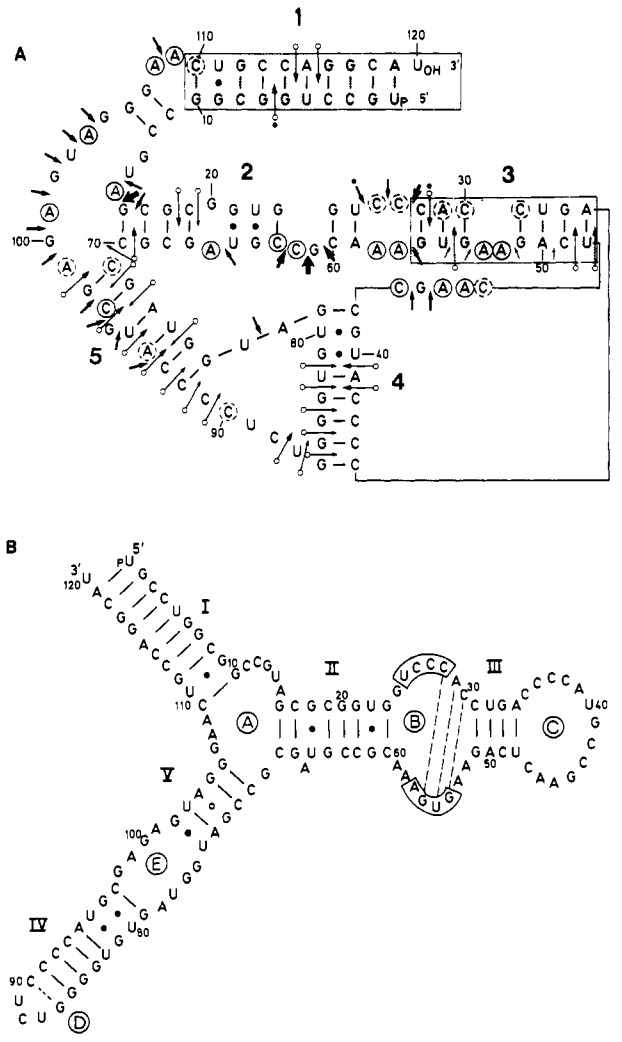

FIGURE 6: (A) Hypothetical model for the secondary structure of the B form. The five double-helical segments are numbered. Helixes 1 and 3 that are common to the A-form model are shaded, and helix 4 is exclusive to the B form. Arrows drawn outside the polynucleotide chain depict the single strand specific ribonuclease cuts, whereas those drawn across the polynucleotide chain, and labeled with a circle, represent cobra venom nuclease cuts; the widths of the arrow heads reflect the intensity of cutting. When weaker ribonuclease cuts were detected at the high magnesium concentrations (see Table I), these intensities are depicted in the figure. Encircled adenosines and cytidines were chemically reactive; full and broken circles indicate strong and weak hits, respectively. (*) Indicates error limits of ±1 nucleotide. Fully quantified data are presented in Table I. (B) Secondary structure of the A form with five universal helixes. The alternative universal base-pairing configurations in loop B, C-A-C_{30}/G-U-G_{56} (stippled) and U-C-C-C_{28}/G-U-G-A_{57} (boxed) (De Wachter et al., 1982) may occur in the A_H and A_L forms, respectively.

for the reactive sites G_{16}, G_{44}, G_{61}, G_{100}, and G_{102} that were exclusive to the B form. Moreover, they all lie in unstructured parts of the model (Figure 6), except G_{16}, which occurs in the terminal base pair of helix 2. The data of Jordan (1971) exhibit additional RNase T_1 cuts that are probably secondary due to the strong digestion conditions employed. The kethoxal-reactive sites at G_{13}, G_{23}, and G_{24} (Noller & Garrett, 1979) were not cut by RNase T_1. However, they lie in unstructured or weakly structured regions of the model, and the reactivity differences may be due to the kethoxal reactions being performed at a higher temperature (33 °C) and in the absence of magnesium.

The model is further reinforced by physical chemical results that suggest that the B form contains more base pairing, but less tertiary structure, than the A_H form (Scott et al., 1968).

Table IV: Differences in Ribonuclease Cutting Characteristics of A_H and A_L Forms[a]

nucleotide	enzyme	A_H	A_L
G_{13}	T_1	+	(+)[b]
A_{29}	T_2		+
C_{35}	T_2	+	*b*
G_{64}	T_1		(+)
G_{75}	T_1		(+)
G_{76}	T_1		(+)
G_{86}	T_1	++	*b*
U_{87}	A	++	*b*
C_{88}	A	++	*b*
U_{89}	A	++	*b*
G_{96}	T_1		(+)[b]
G_{100}	T_1		(+)[b]
A_{101}	T_2		(+)[b]
G_{102}	T_1		(+)[b]
A_{104}	T_2		(+)[b]

[a] The results for the A_H form derive from the study of Douthwaite & Garrett (1981) performed in $T_{30}M_{20}K_{300}$ buffer at pH 7.8 and 0 °C. The results for the A_L form were accrued in the present study in $T_{40}M_{0.5}K_{30}$, pH 7.5, at 0 °C. In both studies, a selection was made for nicked but intact molecules. The band intensities were estimated when 30%–70% of the 5S RNA molecules remained undigested. [b] Effects that were common to the $A_H \rightarrow A_L$ and $A_H \rightarrow$ B transitions.

The model exhibits 42 base pairs compared with about 36 for the A_H form (De Wachter et al., 1982; Delius & Andersen, 1982). Moreover, the low level of tertiary structure is reflected in the accessibility of all the guanosines of the B form to dimethyl sulfate. Finally, helix 4 should constrain the B form into a more compact structure than the elongated and possibly Y-shaped A_H form (Connors & Beeman, 1972; Österberg et al., 1976). This correlates with both the electrophoretic behavior (Figure 1) and the lower gyration radius of the B form (Österberg et al., 1976); the anomalous elution from Sephadex G-100 columns in high salt may be due to aggregation of the B form under these conditions (Aubert et al., 1968).

The I Form. The I-form constitutes a mixture of the A_L and B forms since it exhibited all of the gel bands that were formed by these two conformers. The formation of a separate I band during fractionation on polyacrylamide gels (Figure 1) must reflect the slow equilibrium that occurs between the A and B forms, even at low temperatures (Lecanidou & Richards, 1975; Weidner & Crothers, 1977). This view is reinforced by the observation that only the I band became markedly more diffuse during further electrophoresis.

The A_L Form. The transition $A_H \rightarrow A_L$ form can be induced either by decreasing the temperature from about 40 to 0 °C or by decreasing the Mg^{2+} concentration from about 10 to 0.5 mM at, or below, 25 °C (Kao & Crothers, 1980; Kime & Moore, 1982). Rabin et al. (1983) recently compared the susceptibilities of the A_L and A_H forms to RNase T_1, and they detected seven sites with altered reactivities. Some of these occurred in bands that were very weak (G_{54} and G_{56}) or nondetectable (G_{61}, G_{72}, and G_{79}) in our studies of the A_L form (see Figure 5) and A_H form (Douthwaite & Garrett, 1981). This may be due to our making a preselection for nicked but intact molecules and examining only a range of mild digestion conditions. However, we did confirm the strong decrease in the RNase T_1 cut at G_{13}. Moreover, by comparing our digestion data with RNases A, T_1, and T_2 for the A_L form (Figure 5) with those of the A_H form (Douthwaite & Garrett, 1981), we found further differences that are listed in Table IV and were located at the three main sites described below.

(1) The sequence G_{86}–U_{89} forms a reactive terminal loop in the A_H form, but it was resistant to RNases A and T_1 in the A_L form (although a weak RNase T_2 cut occurred after C_{88}); the RNase T_2 cut at C_{35} was also absent in the latter.

All of these nucleotides participate in helix 4 of the B form where the base pair C_{35}–G_{86} forms (Figure 6). This suggests that a triple helix may form between helix IV (79–86/90–97) of the A_H form (that is conserved in the A_L form; Kime & Moore, 1982) and the first part of the sequence C_{35}–C_{42} (G_{41} is very accessible in the A_L form; Figure 5). This would also render the structure more compact than the A_H form (Kao & Crothers, 1980). (2) The RNase T_2 cut at A_{29} in the A_L form could reflect altered base pairing at the center of the molecule where De Wachter et al. (1982) have emphasized that two alternative and universal base-pairing forms are possible. In the A_H form, the pairing 28–30/54–56 is favored (see Figure 6B), whereas RNase T_2 cut at A_{29} points to the alternative pairing U_{25}–C_{28}/G_{54}–A_{57} in the A_L form. (3) The sequence G_{96}–A_{104} was resistant in the A_H form but reactive in both the A_L form and the B form.

In summary, points 1 and 3 demonstrate that parts of the structure of the A_L form are intermediate between the A_H and B forms. Moreover, points 1 and 2 emphasize that the A_L form may exhibit an altered secondary and tertiary structure from that of the A_H form that could also be universal.

Functional Importance of Alternative Conformers. The main interest in the alternative conformers of the ribosomal RNAs lies in whether they participate in protein biosynthesis. This is of interest because the switching between two RNA conformers could provide a simple mechanism for controlling the reciprocating processes that occur on the ribosome including the opening and closing of the subunits, the binding and release of tRNAs and factors, and the stepwise movement of mRNA relative to the ribosome. Specifically, it has been suggested that alternation between the A and B forms of 5S RNA could control the A-site binding of tRNA (Fox & Woese, 1975; Weidner et al., 1977), and while this particular hypothesis has not been substantiated (Pace et al., 1982), the more general idea may still be valid.

For any conformer to be functionally important, it should satisfy the criteria of being common to all 5S RNAs and being a discrete conformer, at least within the ribosome. The crucial argument in support of a biologically relevant B form is that helix 4 can be formed in all 5S RNAs (Weidner et al., 1977; Trifonov & Bolshoi, 1983). Earlier, when few complete sequences were available, Fox & Woese (1975) had noticed this potential helix but argued that since the sequences involved in helix 4 were fairly conserved, the effect could just be chance. Nevertheless, Trifonov & Bolshoi (1983), with more sequences at their disposal, proposed the existence of a universal conformer (the P form), which contained helix 4 and other helixes that were common to the A form. This hypothesis had the merit of correlating with the experimental estimate, from thermodynamic measurements, of about nine base pairs breaking and re-forming during the A → B transition (Richards et al., 1973). However, our data are incompatible with the presence of the A-form helixes II (16–23/60–68) and V (70–74/102–106) in the B form (see Figure 6).

In summary, there are strong grounds for inferring that the B form is not involved in biological function. First, helix 4 is exceptionally stable in *E. coli* 5S RNA (Figure 6A); it is only three or four base pairs long in many other 5S RNAs. Moreover, most of the sequences involved are conserved, and those nucleotides that change do not normally produce coordinated base pair changes that would provide direct phylogenetic support for a helix. (We thank the referee for emphasizing this point.) Moreover, helixes 2 and 5 in our B-form model are not universal; therefore, the B form does not satisfy the first criterion for functional relevance. In a similar vein, digestion studies with one enzyme, for example RNase T_1 or T_2 (Figure 2 and 5), suggest a highly structured and discrete conformer. However, the mosaic of results (Table I, Figure 6) reveals structural heterogeneities especially within helix 5. The second criterion for functional relevance is, therefore, also not completely satisfied. The negative conclusion is further reinforced by the observation that while two stable conformers have been isolated for many 5S RNAs [e.g., Böhm et al. (1981) and Toots et al. (1982)] they do not, apparently, form in all 5S RNAs (unpublished results). We do, however, provide some experimental support for the hypothesis (De Wachter et al., 1982) that a functionally important structural change occurs in loop B (Figure 6B) during the $A_H \rightarrow A_L$ form transition.

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Acid Lability of the Hydrocarbon–Deoxyribonucleoside Linkages in 7,12-Dimethylbenz[*a*]anthracene-Modified Deoxyribonucleic Acid†

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ABSTRACT: DNA containing bound radioactive 7,12-dimethylbenz[*a*]anthracene was isolated from mouse fetal cell cultures exposed to this carcinogen. The carcinogen–deoxyriboside adducts within the DNA were found to be sensitive to acid-catalyzed hydrolysis. Adducts derived from reaction of a *syn*-dihydrodiol epoxide with deoxyadenosine residues in DNA were the most sensitive to acid and were hydrolyzed to yield a 1,2,3,4-tetrahydrotetraol of 7,12-dimethylbenz[*a*]anthracene under mild conditions. The structure of this tetraol was established by synthesis and mass spectrometry. Although definitive structures cannot be assigned at present to the nucleic acid adducts of this potent carcinogen, the present findings confirm and extend earlier work assigning partial structures to the major adducts.

Since 7,12-dimethylbenz[*a*]anthracene is one of the most potent tumor initiators among the polycyclic aromatic hydrocarbon carcinogens, we have been anxious to discover whether there are significant differences in the DNA interactions for this carcinogen and those for less potent, but more extensively studied, carcinogens such as benzo[*a*]pyrene. The metabolic activation of benzo[*a*]pyrene was shown by Sims et al. (1974) to involve formation of a vicinal dihydrodiol epoxide, and this was found to react almost exclusively with the exocyclic amino group of guanine residues in nucleic acids (Jeffrey et al., 1976b; Koreeda et al., 1976; Osborne et al., 1976). Minor products attributed to reaction with adenine residues have also been described (Jeffrey et al., 1979), though in mouse skin, these represent less than 3% of total DNA adducts (Ashurst & Cohen, 1981).

Metabolic activation of 7,12-dimethylbenz[*a*]anthracene is similarly believed to occur through a dihydrodiol epoxide (Baird & Dipple, 1977; Moschel et al., 1977; Vigny et al., 1977; Ivanovic et al., 1978), but in this case, three major adducts are formed in similar amounts, resulting from reaction of an *anti*-dihydrodiol epoxide to similar extents with deoxyguanosine and deoxyadenosine residues and from selective reaction of a *syn*-dihydrodiol epoxide with deoxyadenosine residues in DNA (Sawicki et al., 1983; Dipple et al., 1983). We now report that the 7,12-dimethylbenz[*a*]anthracene–deoxyadenosine adducts in DNA exhibit a remarkable instability in that they are hydrolyzed readily under mildly acidic conditions to yield hydrocarbon tetraols. Such reactions have not been reported for benzo[*a*]pyrene–DNA adducts.

Experimental Procedures

[G-^{3}H]-7,12-Dimethylbenz[*a*]anthracene was purchased from Amersham/Searle Corp. (Arlington Heights, IL) and purified on silicic acid as described earlier (Dipple et al., 1979).

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