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Wheat Germ Initiation Factors 4F and (iso)4F Interact Differently with Oligoribonucleotide Analogues of Rabbit α-Globin mRNA†

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ABSTRACT: The binding of capped oligoribonucleotide analogues of the 5′ terminus of rabbit α-globin mRNA to wheat germ protein synthesis initiation factors eIF-4F and eIF-(iso)4F was measured by direct fluorescence techniques. An analysis of the equilibrium association constants (K_{eq}) indicates that both eIF-4F and eIF-(iso)4F recognize primarily the m^7G cap structure but differ in the recognition of other structural features. eIF-4F is sensitive to the position and sequence of hairpin structures within the oligoribonucleotide, while eIF-(iso)4F shows a preference for linear sequences. These differences suggest that wheat germ eIF-4F and eIF-(iso)4F may have discriminatory activity for mRNA recognition.

The cap-binding protein eIF-4E,[1] a 25-kDa polypeptide, has been shown to bind directly to the m^7G cap of mRNA (Tahara et al., 1981; Sonenberg, 1981; Sonenberg et al., 1981; Hellmann et al., 1982; Webb et al., 1984; Carberry et al., 1989, 1990; Goss et al., 1990). However, a recent study on the interaction of human eIF-4E with oligoribonucleotide analogues of rabbit α-globin mRNA has shown that eIF-4E is also capable of interacting with other structural features of the globin mRNA (Carberry et al., 1991b).

Two cap-binding proteins have also been isolated from wheat germ (Lax et al., 1986a,b): eIF-(iso)4F (previously designated eIF-4B) which consists of a 28- and a 82-kDa polypeptide in

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[1] Abbreviations: m^7G, 7-methylguanosine; eIF, eukaryotic initiation factor; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; kDa, kilodalton.

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a 1:1 molar ratio (Lax et al., 1985), and eIF-4F, which consists of a 26- and a 220-kDa polypeptide in a 4:1 molar ratio (Lax et al., 1985, 1986b). We have recently characterized the binding of methylated cap analogues to these wheat germ factors (Carberry et al., 1991a). The differences in the affinities of the two wheat germ factors for these cap analogues suggested that eIF-4F and eIF-(iso)4F may have different mechanisms for recognizing mRNA.

In order to investigate whether the differences in the binding characteristics of wheat germ eIF-4F and eIF-(iso)4F are based solely upon differences in recognition of the m^7G cap structure or if recognition of other structural features of the mRNA could contribute to the interaction, as in the case of eIF-4E, we measured the binding of rabbit α-globin oligoribonucleotide analogues (which contained a variety of primary and secondary structural features) to wheat germ eIF-4F and eIF-(iso)4F by direct fluorescence titration experiments. A comparison of the magnitudes of the equilibrium association constants (K_{eq}) allowed us to assess the mRNA features that may be discriminatory for the interaction with wheat germ eIF-4F and eIF-(iso)4F.

Materials and Methods

The oligoribonucleotide analogues of rabbit α-globin mRNA were prepared by cell-free transcription in a T7 RNA polymerase system as described elsewhere (Carberry et al., 1991b).

Wheat germ initiation factors eIF-4F and eIF-(iso)4F were purified according to the protocol of Lax et al. (1986a,b) with certain modifications as described previously (Carberry et al., 1991a).

The solutions for the fluorescence studies were prepared in 20 mM HEPES–KOH/1 mM DTT, adjusted to the pH optimum of 7.6 for samples containing eIF-(iso)4F and pH 8.0 for samples containing eIF-4F as previously reported (Carberry et al., 1991a); solutions containing 100 mM KCl and 2 mM $MgCl_2$ were all pH 7.6. Fluorescence measurements were carried out at 23 ± 0.2 °C, and data were collected and analyzed as previously described (Carberry et al., 1989, 1990).

Results

eIF-4F and eIF-(iso)4F have very different binding characteristics and affinities for methylated cap analogues (Carberry et al., 1991a): eIF-(iso)4F has 1.3- and 1.9-fold *greater* affinity for $m^{2,7}GMP$ and $m^{2,7}GpppG$, respectively, than for m^7GpppG, whereas eIF-4F has 3.3- and 1.7-fold *lower* affinity for $m^{2,7}GMP$ and $m^{2,7}GpppG$, respectively, than for m^7GpppG. Therefore, we wanted to determine whether or not these protein factors would be discriminatory for different structural features of mRNA as well. In order to investigate this point, two categories of oligoribonucleotides (Figure 1) were prepared. Oligoribonucleotides V–IX were prepared as analogues of rabbit α-globin mRNA and contain variances in the position and sequence of the hairpin structure. Oligoribonucleotide VI is the closest analogue of the 5′ terminus of α-globin mRNA (Baralle, 1977); however, in order to ensure a homogeneous transcript, several modifications of the α-globin mRNA structure were necessary, including the omission of a single-base bulge in the hairpin stem and eight nucleotides in the hairpin loop found in the α-globin structure. Linear oligoribonucleotides I–IV correspond to oligoribonucleotide VI with the hairpin portion removed. In addition, the 3′ terminus of the linear oligoribonucleotides has been varied in order to study the effects of length as well as sequence on the RNA–protein interaction. The structure and stability of these oligoribonucleotides has been confirmed as described elsewhere

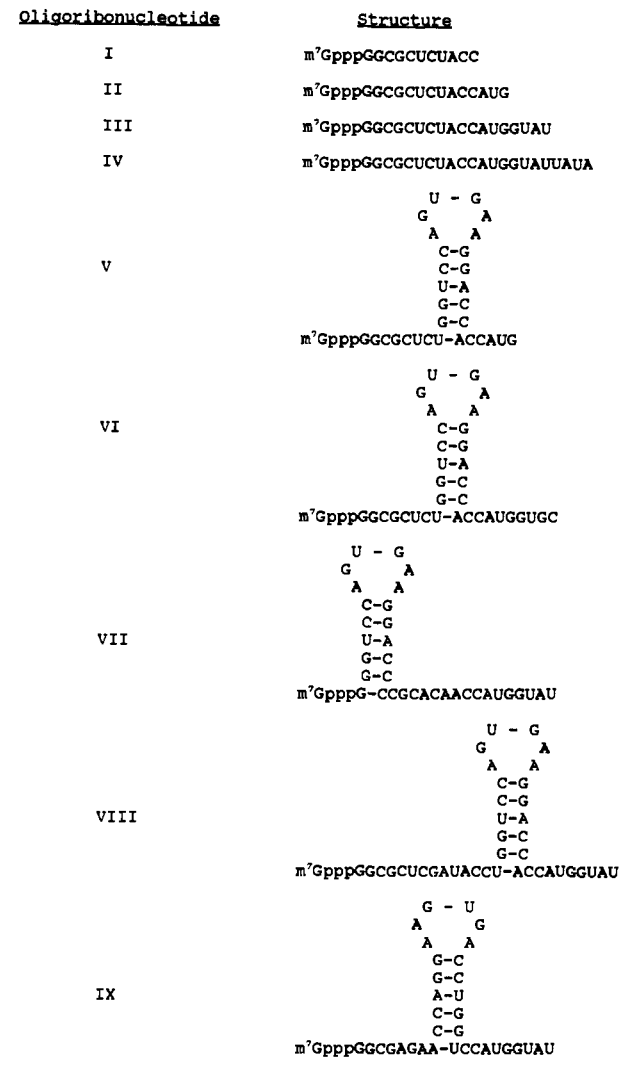

FIGURE 1: Structures of the oligoribonucleotides used in this study.

(Carberry et al., 1991b). The K_{eq} values for the interaction of the oligoribonucleotides with wheat germ eIF-4F and eIF-(iso)4F have been determined, in the absence of salt, at the pH optima of 7.6 for eIF-(iso)4F and 8.0 for eIF-4F. The binding of mRNA to eIF-4F at pH 8.0 was found to be slightly (1.3-fold) stronger than at pH 7.6, in agreement with previous work (Carberry et al., 1991a). Measurements were also carried out under physiological conditions (100 mM KCl, 2 mM $MgCl_2$, pH 7.6) for comparison; these values are summarized in Table I.

Comparison with Cap Analogues. Oligoribonucleotides or α-globin mRNA have greater affinity for eIF-4F and eIF-(iso)4F than cap analogues. In the absence of salt, the affinity of oligoribonucleotide VI for either eIF-(iso)4F or eIF-4F is 3.5–3.8-fold greater than the cap analogue m^7GpppG. In the presence of salt, pH 7.6, eIF-(iso)4F and eIF-4F have 4.3- and 3.2-fold greater affinity for oligoribonucleotide VI and 5.3- and 7.1-fold greater affinity for rabbit α-globin mRNA, respectively, relative to m^7GpppG. These results are similar to a previous report that eIF-4E interacts 5 times more strongly with oligoribonucleotide VI than with the m^7GpppG cap analogue (Carberry et al., 1991b). In order to determine which mRNA structural features, other than the cap, are recognized by wheat germ eIF-4F and eIF-(iso)4F, we proceeded to analyze the individual structural features of the two categories of oligoribonucleotides.

Effects of Length. In the absence of added salt, eIF-4F binding increases with the length of the oligoribonucleotide

Table I: Summary of the Binding of Oligoribonucleotides to Wheat Germ Factors eIF-4F and eIF-(iso)4F

	$K_{eq} \times 10^{-5}$ (M^{-1})			
	eIF-4F		eIF-(iso)4F	
oligoribonucleotide	0 mM KCl, 0 mM $MgCl_2$, pH 8.0	100 mM KCl, 2 mM $MgCl_2$, pH 7.6	0 mM KCl, 0 mM $MgCl_2$, pH 7.6	100 mM KCl, 2 mM $MgCl_2$, pH 7.6
m^7GpppG	2.32 ± 0.10	1.40 ± 0.08	1.21 ± 0.10	0.62 ± 0.03
I	2.77 ± 0.33		1.64 ± 0.15	
II	2.14 ± 0.66	2.37 ± 0.17	5.95 ± 0.12	2.65 ± 0.08
III	6.58 ± 0.26		4.91 ± 0.29	
IV	6.95 ± 0.56	2.49 ± 0.03	3.16 ± 0.16	4.34 ± 0.57
V	4.25 ± 0.12		3.13 ± 0.16	
VI	8.69 ± 0.17	4.44 ± 0.58	4.19 ± 0.17	2.68 ± 0.45
VII	3.79 ± 0.63	3.68 ± 0.29	5.06 ± 0.25	3.95 ± 0.36
VIII	3.77 ± 0.19	3.17 ± 0.63	8.17 ± 0.33	4.50 ± 0.37
IX	4.13 ± 0.52	2.67 ± 0.56	6.80 ± 0.14	3.22 ± 0.32
globin mRNA		7.47 ± 0.37		4.41 ± 0.18

up to about 18 bases. A comparison of the binding affinity of the shortest linear oligoribonucleotide I with that of oligoribonucleotide IV shows that the shorter oligoribonucleotide has a 3.0-fold lower affinity for eIF-4F than the longer one. A less pronounced trend is seen in the presence of salt. eIF-(iso)4F shows this length dependence in the presence of salt, with oligoribonucleotide IV binding 1.6-fold more tightly than oligoribonucleotide II. In the absence of salt, there is also a length dependence but there is some reduction in binding for longer oligoribonucleotides.

The addition of AUG to oligoribonucleotide I (oligoribonucleotide II) increases the affinity of oligoribonucleotide II for eIF-(iso)4F 3.6-fold and decreases the affinity for eIF-4F 1.3-fold, relative to oligoribonucleotide I. The replacement of AUG by GUA in oligoribonucleotide III (sequence not shown) results in a similar (2.8-fold) increase in affinity for eIF-(iso)4F relative to oligoribonucleotide I (data not shown). Therefore, for eIF-(iso)4F, the effect of adding the AUG appears to be due to length rather than to sequence.

The addition of four bases to the 3′ side of the AUG (oligoribonucleotide III) results in a 1.2- and 3.1-fold increase in affinity of this oligoribonucleotide for eIF-(iso)4F and eIF-4F, respectively, relative to oligoribonucleotide II. The effect of the 3′ bases can also be determined by comparing the affinities of hairpin-containing oligoribonucleotides V and VI. Addition of these bases (oligoribonucleotide VI) results in a 2.3- and 2.1-fold increase in affinity for eIF-(iso)4F and eIF-4F, respectively, relative to oligoribonucleotide V. The addition of a further four bases to oligoribonucleotide III (oligoribonucleotide IV) results in a slight (1.1-fold) increase in affinity for eIF-4F and a 1.6-fold decrease in affinity for eIF-(iso)4F relative to oligoribonucleotide III. Thus, these additional bases in oligoribonucleotide IV do not dramatically affect the stability of the oligoribonucleotide–protein interaction.

In the presence of salt, the longest linear oligoribonucleotide IV has 1.6-fold greater affinity for eIF-(iso)4F than oligoribonucleotide II, which had optimal binding to eIF-(iso)4F in the absence of salt (Table I). Oligoribonucleotides IV and II have similar affinity for eIF-4F in the presence of salt. The differences in the relative affinities of oligoribonucleotides II and IV for eIF-(iso)4F and eIF-4F under conditions of high salt may be due to the additional stability imparted to the oligoribonucleotides under such conditions (Holder & Lingrel, 1975), which could modulate the efficiency of eIF-4F and eIF-(iso)4F binding.

Effects of the Position and Sequence of the Hairpin. The presence of the hairpin itself influences the binding affinity to eIF-4F and eIF-(iso)4F. A comparison of the K_{eq} values for hairpin-containing oligoribonucleotide VI and linear oligoribonucleotide III, which corresponds to oligoribonucleotide VI with the hairpin segment removed, indicates that the presence of the hairpin *decreases* the affinity of the oligoribonucleotide for eIF-(iso)4F by 1.2-fold and *increases* the affinity for eIF-4F by 1.3-fold.

Table II: Summary of the ΔG Values for Structural Features of mRNA for the Interaction of Oligoribonucleotides with Wheat Germ eIF-4F and eIF-(iso)4F[a]

	ΔG (kcal/mol)	
structural feature	eIF-4F	eIF-(iso)4F
cap	−7.3	−6.9
linear	−0.11	−0.18
AUG	+0.15	−0.76
3′ bases (linear)	−0.67	+0.11
hairpin	−0.41	+0.38
hairpin adjacent to cap	+0.49	−0.11
hairpin moved to 3′ side	+0.50	−0.40
hairpin sequence inverted	+0.44	−0.29

[a] ΔG values for the individual features were calculated from contributions to the total ΔG of the oligoribonucleotides obtained from the K_{eq} values reported in Table I (no salt) as described in the text.

Changes in the position or sequence of the hairpin structure influence the affinity of oligoribonucleotides for eIF-(iso)4F and eIF-4F. In the case of eIF-(iso)4F, moving the hairpin adjacent to the 5′ cap (oligoribonucleotide VII) increases the binding 1.2-fold while moving the hairpin six bases downstream increases the affinity 2.0-fold (oligoribonucleotide VIII) and inverting the sequence of the hairpin (oligoribonucleotide IX) increases the binding affinity 1.6-fold relative to oligoribonucleotide VI. In the presence of salt, pH 7.6, a similar trend for eIF-(iso)4F binding is observed (Table I). In contrast, oligoribonucleotides VII–IX have a 2.1–2.3-fold *lower* affinity for wheat germ eIF-4F than oligoribonucleotide VI in the absence of salt and a 1.3–1.7-fold lower affinity for eIF-4F under physiological salt conditions, pH 7.6 (Table I).

Discussion

The K_{eq} values in Table I for the interaction of the oligoribonucleotide analogues with wheat germ eIF-4F and eIF-(iso)4F can be used to calculate ΔG values for the contribution of the individual mRNA structural features to the binding. These ΔG values were obtained by subtracting combinations of the total ΔG values for the protein–oligoribonucleotide interactions obtained from the K_{eq} values cited in Table I. For example, the subtraction of ΔG for oligoribonucleotide II from that of oligoribonucleotide V yields a ΔG value for the hairpin contribution; similarly, the subtraction of ΔG for oligoribonucleotide I from II yields the value for the AUG. The values

obtained in this manner for the individual ΔG's are summarized in Table II. The ΔG value for the m^7G cap contribution to the interaction with eIF-4F and eIF-(iso)4F is similar in magnitude to that previously reported for eIF-4E (−7.6 kcal/mol; Carberry et al., 1991b). This represents 90–93% of the total binding of the oligoribonucleotide. Omission of the m^7G cap from an oligoribonucleotide results in an approximate 20-fold reduction in eIF-4F or eIF-(iso)4F binding (data not shown). Therefore, the m^7G cap is the major contributor to the stability of the protein–oligoribonucleotide complex, although other features modulate this interaction.

In the case of eIF-4F, the hairpin structure as defined in oligoribonucleotide VI contributes 5% to the *stability* of the oligoribonucleotide–eIF-4F interaction. Modification of the position or sequence of the hairpin structure results in a *positive* contribution to the ΔG, indicating that these structures contribute to the *destabilization* of the oligoribonucleotide–eIF-4F complex. In particular, oligoribonucleotide VIII, which would appear to have a more exposed cap, does not bind as well as oligoribonucleotide VI. This is similar to the effect of the hairpin on human eIF-4E binding (Carberry et al., 1991b). In addition to the effects of the hairpin structure, wheat germ eIF-4F is also sensitive to the length of the oligoribonucleotide. The AUG destabilizes the interaction by about 2%, while the addition of four bases to the 3′ side of the AUG results in an 8% stabilization of the complex. Addition of more basis to the 3′ side of the AUG did not significantly alter the affinity of eIF-4F for the oligoribonucleotide.

Wheat germ eIF-(iso)4F is also sensitive to the length of the linear portion of the oligoribonucleotide. Increasing the length of the oligoribonucleotide beyond 11 bases (oligoribonucleotide I) by addition of AUG (oligoribonucleotide II) or GUA (data not shown) results in an increase in affinity for eIF-(iso)4F (Table I); this addition contributes 11% to the overall stability of the complex, whereas inclusion of additional bases to the 3′ side of the AUG resulted in a slight decrease in affinity (Table I). The data presented here indicate that the addition of the AUG to oligoribonucleotide I (oligoribonucleotide II) is a length effect and not due to specific recognition of the AUG, since the binding of an oligoribonucleotide with a sequence identical with that of oligoribonucleotide III, except that the AUG was replaced by GUA, binds with almost equal affinity to eIF-(iso)4F as does oligoribonucleotide III (see Results). One interesting result is that the optimum α-globin hairpin structure in oligoribonucleotide VI decreases the interaction; modification of the sequence or position of this hairpin also contributes a small (1–4%) portion to the stability of the complex. This result is consistent with the interpretation that the hairpin structure itself does not contribute significantly to the stability of the interaction with eIF-(iso)4F. Of all the hairpin-containing oligoribonucleotides, oligoribonucleotide VIII, in which six bases were added to the linear portion on the 5′ side of the hairpin, has the greatest affinity for eIF-(iso)4F (Table I). This is probably the oligoribonucleotide with the most exposed cap. This result is consistent with results observed with the linear oligoribonucleotides I-IV: that eIF-(iso)4F favors interaction with oligoribonucleotides having a linear sequence of at least 12 nucleotides following the m^7G cap.

Even though wheat germ eIF-4F and human eIF-4E have different pH dependence and salt dependence of binding (Carberry et al., 1991a), eIF-4F and eIF-4E recognize similar mRNA structural features, including the m^7G cap. For the oligoribonucleotides tested, the optimal structure for both eIF-4E and eIF-4F binding is the α-globin analogue (oligoribonucleotide VI; viz. Table I and Table II in Carberry et al. (1991b)). Modification of either the sequence or position of this α-globin hairpin reduces the interaction between the oligoribonucleotide and eIF-4F or eIF-4E. In contrast, wheat germ eIF-(iso)4F was found to have a similar mechanism to human eIF-4E for interaction with the cap analogue m^7GpppG but preferred interaction with hypermethylated cap analogues (Carberry et al., 1991a). eIF-(iso)4F also prefers to interact with oligoribonucleotide analogues containing linear sequences. These results suggest that human eIF-4E and wheat germ eIF-4F prefer similar mRNA structures for binding, while eIF-4E and eIF-(iso)4F may have similar *mechanisms* for interaction with mRNA. The differences in the specificity of eIF-4F and eIF-(iso)4F for globin mRNA analogues suggest that these factors have discriminatory activity at the level of mRNA recognition.

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