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Commentary–Hypothesis

A model for the interaction of nucleic acids with transcription factor IIIA

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Based on published biochemical evidence which examines the interaction of *Xenopus* transcription factor IIIA (TFIIIA) with 5 S RNA genes and 5 S RNA, this paper proposes that the formation of a 5 S RNA type stem-loop structure in the DNA occurs during the binding of TFIIIA to 5 S genes.

Transcription factor IIIA; 5 S RNA; 5 S RNA gene; (*Xenopus laevis*)

1. INTRODUCTION

Eukaryotic transcription factor IIIA (TFIIIA) is necessary for accurate initiation during transcription of 5 S ribosomal RNA genes by RNA polymerase III in *Xenopus laevis* [1]. The protein binds specifically to an intragenic transcriptional control region (ICR) (positions 50–96) [2–4]. Once bound to the 5 S gene, TFIIIA quickly induces a gyrase activity found in oocyte extracts, which results in the appearance of negative supercoils in the DNA [5]. TFIIIA also participates with another transcription factor, TFIIIC, in the formation of stable transcription complexes [6,7]. Further, TFIIIA binds specifically to the gene product, 5 S RNA, to form the 7 S ribonucleoprotein (RNP) particle which is ubiquitous in the cytoplasm of *X. laevis* immature oocytes (Xlo) [8,9]. While there are other proteins which interact with both types of nucleic acids, the specificity of TFIIIA's interaction with both 5 S DNA and the gene product, 5 S RNA, makes it a unique protein.

It has been suggested that the interaction of TFIIIA with 5 S DNA may be similar in some aspect to its interaction with 5 S RNA [10]. Data from two independent studies support the notion that a structure analogous to an A-form double helical structure (RNA prototype) may exist within the ICR [11,12]. Other investigators have proposed that certain base-paired sequences found in both the DNA and RNA form a recognition signal for the protein [13]. Based on the existing published data concerning the interaction between TFIIIA and nucleic acids, I propose that a stem-loop structure represented by helix V in the RNA is crucial for the interaction of TFIIIA with both types of nucleic acids and that the formation of the stable transcription complex is concomitant with the formation of that stem-loop structure in the DNA.

2. THE INTERACTION OF TFIIIA WITH 5 S RNA

The stem of 5 S RNA that contains helices IV and V has been implicated in the binding of TFIIIA in a number of studies (fig.1). Footprint analysis by Huber and Wool [13] shows protection of this stem from digestion with α-sarcin in the 7

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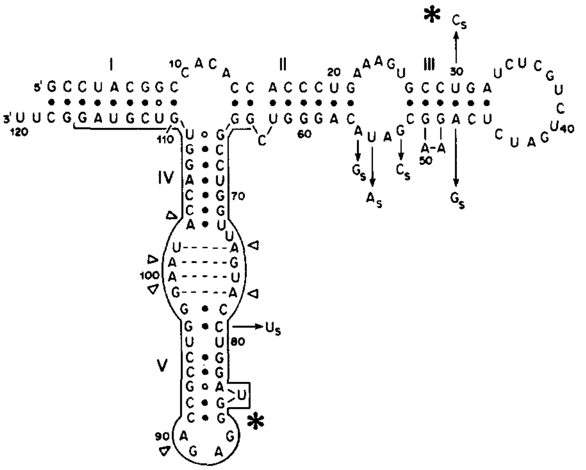

Fig.1. The sequence of Xlo 5 S RNA folded according to the generalized secondary structural model [20]. Arrows directed away from the structural model point to the base differences found in *X. laevis* somatic 5 S RNA. The line drawn around positions G64–G116 represents the region on the RNA that is protected by TFIIIA from digestion with α-sarcin [13]. Open triangles point to adenosines that are protected from chemical modification by TFIIIA [18]. Large asterisks designate helical regions that undergo conformational change when 5 S RNA is complexed with TFIIIA [16].

S RNP particle. Similar findings were reported using partial ribonuclease T_1 and T_2 digestions [14]. When the RNA is complexed in the RNP particle, residues 74, 77, 90, 100, 101 and 103 are protected from (single-stranded specific) chemical modification by diethylpyrocarbonate [15]. The protected adenosines (all phylogenetically conserved) are clustered between helices IV and V in 5 S RNA. Helix V is also one of the regions to undergo protein-induced conformational change when the RNA is complexed in the particle [16]. Therefore, TFIIIA appears to bind primarily to the region of 5 S RNA that contains the stem-loop structure of helices IV and V, although other regions of the RNA may also interact with the protein [14–16].

In in vitro transcription competition assays, a variety of eukaryotic 5 S RNAs [17,18] and 5 S RNAs from eubacteria and archebacteria [18] inhibited the transcription of *X. laevis* 5 S RNA genes. In another study, several eukaryotic and eubacterial 5 S RNAs have been shown to interact specifically with TFIIIA in an RNA exchange assay [14,19]. In fig.2, sequences of the eukaryotic 5 S RNAs from the latter study have been superimposed on each other illustrating only the common nucleotides. Phylogenetically conserved nucleotides (designated by bold type in fig.2) in 5 S RNA are predominately situated in single-stranded regions and at the end of helical regions in the molecule, but generally are not within the helices [20]. The base-paired sequence predicted to be necessary for TFIIIA interaction with 5 S RNA (CCUGG/GGACC) [13] is not conserved among all the 5 S RNAs that interact with TFIIIA and therefore this sequence does not appear to be a recognition signal for 5 S RNA by TFIIIA. What is shared by all the RNAs is the generalized secondary structure for 5 S RNA [20]. Experimental evidence reveals that maintenance of the higher order structure of Xlo 5 S RNA is essential for the RNA to complex with the protein [19]. Universal 5 S RNA secondary structural elements and not contiguous sequences appear to be required for TFIIIA-RNA interactions [18,19], although it still needs to be determined whether any conserved residues in 5 S RNA interact directly with TFIIIA.

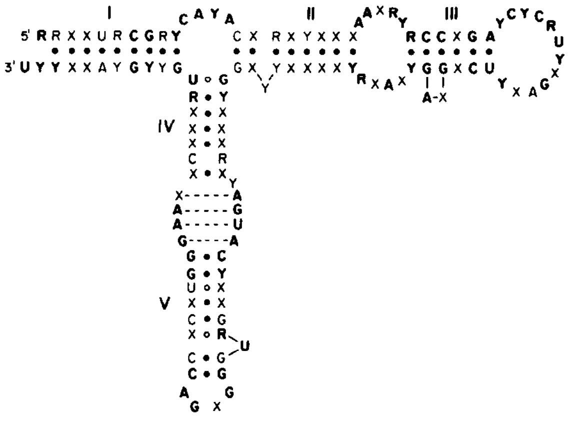

Fig.2. The sequences of five eukaryotic 5 S RNAs known to interact with TFIIIA [19] were superimposed to determine common residues and these residues are presented using the 5 S RNA secondary structural model as a template. The bold-face residues are conserved residues in eukaryotic 5 S RNAs [20]; A, adenine; G, guanine; C, cytosine; U, uracil; R, purine; Y, pyrimidine and X, any base.

3. THE INTERACTION OF TFIIIA WITH 5 S DNA

The fact that 5 S RNA can compete with the 5

S genes for TFIIIA in in vitro transcription assays (see above) suggests that the interactions between TFIIIA and the nucleic acids may be related. The uniformity of the 'finger-like' structures of TFIIIA's nucleic acid binding domain also lends credence to this notion [21,22]. Thus, if TFIIIA interacts with the hairpin stem of 5 S RNA that includes helices IV and V, it may also interact with a similar structure in 5 S DNA. Such a structure can potentially be formed in the ICR (see below).

The ICR was initially defined by deletion mutant analyses [2,3] and confirmed by DNase I protection studies (footprint analyses) [1,10]. Although footprint analyses show that TFIIIA protects both DNA strands of 5 S RNA genes from nuclease digestion, Sakonju and Brown [10] determined by chemical modification exclusion studies that TFIIIA interacts directly with the non-coding strand in the 3′-region of the gene. Fig.3 summarizes the data obtained from their study depicting the guanines (in bold type) and phosphates (with triangles) that are important to TFIIIA interaction. In accord with the model proposed in this paper, the non-coding strand (+ strand) and the coding strand (− strand) are drawn with stem-loop structures. Note that the guanines and phosphates that apparently interact directly with TFIIIA [10] congregate around the 3′-end of the ICR on the non-coding strand where a putative stem-loop structure analogous to that on the RNA can be drawn (fig.3). Two important guanines and two reactive phosphates are situated on the non-coding strand in the region of the gene that codes for helix IV. Another of these guanines is situated on the coding strand and, as drawn in fig.3, closes the inverted stem-loop by base pairing with a cytosine.

The phosphate contacts of TFIIIA between residues 80 and 90 (see fig.3) have been interpreted to suggest that local unwinding occurs in the DNA [10,23], although the degree of unwinding with purified TFIIIA appears to be insufficient for hairpin formation [23,24]. However, TFIIIA-induced gyrase activity found in oocyte extracts [5] could facilitate local unwinding in this region and allow for hairpin formation. Supportive of the notion that hairpin formation can follow supercoiling of DNA are the data of Müller and Wilson [25]. They detected, by Bal 31 cleavage, the transient extrusion of small cruciform structures (7 bp or less) after physiological supercoiling of ϕX174 replicative form DNA.

Fairall et al. [26] have conducted a comprehensive analysis of the protection conferred on the ICR by TFIIIA from dimethylsulfate and micro-

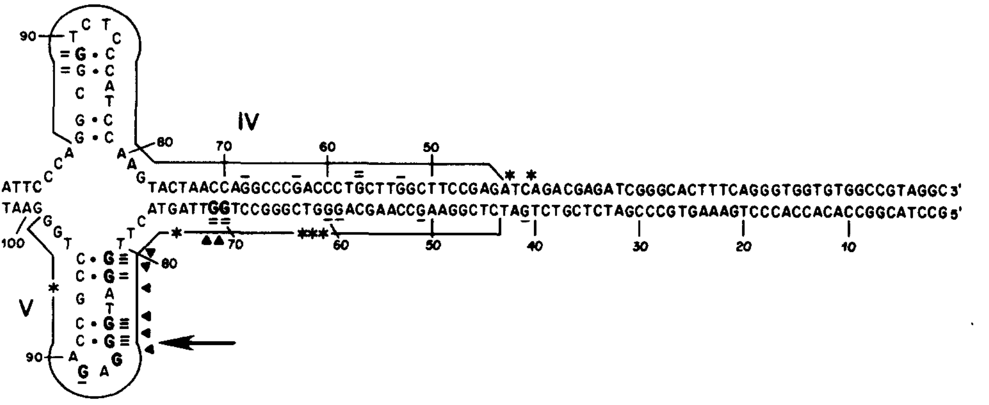

Fig.3. *X. laevis* somatic 5 S RNA gene depicted with the potential stem-loop structure analogous to helix V in 5 S RNA formed on the non-coding strand (+). The inverted stem-loop is also shown on the coding strand (−). The lines cover the regions of the gene that are protected from DNase I digestion by TFIIIA while the asterisks show positions of enhanced cleavage during DNase I digestion [10]. The solid triangles mark the positions of phosphates and the bold-type guanines that if chemically modified prevent TFIIIA from binding to the gene [10]. The dashes mark the guanines that are protected from dimethylsulfate modification by TFIIIA [26]. Low protection (−0.5 to −1 units) is shown by one dash; moderate protection (−1 to −2 units) by two dashes; greatest protection by three dashes (< −2 units) [26]. The large arrow points to position G86 which if changed to an adenosine in *Drosophila* 5 S genes, reduces transcription to 2% of wild type [27].

coccal nuclease. The guanines protected from dimethylsulfate are shown in fig.3. TFIIIA confers a gradient of protection on the DNA which is maximal at the 3′-end of the non-coding strand of the ICR and protection of some residues at the 5′-portion of the ICR (i.e. guanine 41) is dependent on the TFIIIA:DNA ratio [26]. Much less protection from dimethylsulfate is seen on the coding strand. Although TFIIIA protects both strands of a small DNA fragment (52 bp) containing the ICR from digestion with micrococcal nuclease, maximal protection occurs at the 3′-half of the ICR. These protection data, therefore, reiterate the major role of the 3′-end of the ICR to TFIIIA binding.

In another study [27], a single base substitution in *Drosophila melanogaster* 5 S DNA at conserved position 86 (G → A) (see the large arrow in fig.3) reduced the level of in vitro transcription of the 5 S DNA to 2% of wild-type levels. As drawn in fig.3, G86 forms a base pair with C91 to close the helix adjacent to the loop. In addition, Pieler et al. [28] show that a point mutation at position 81 (G → C) abolishes TFIIIA's ability to protect the DNA from DNase I. One explanation for the reduction in transcription of the mutant and the lack of TFIIIA protection due to the point mutation is that the stem-loop structure cannot form and thus TFIIIA cannot bind stably.

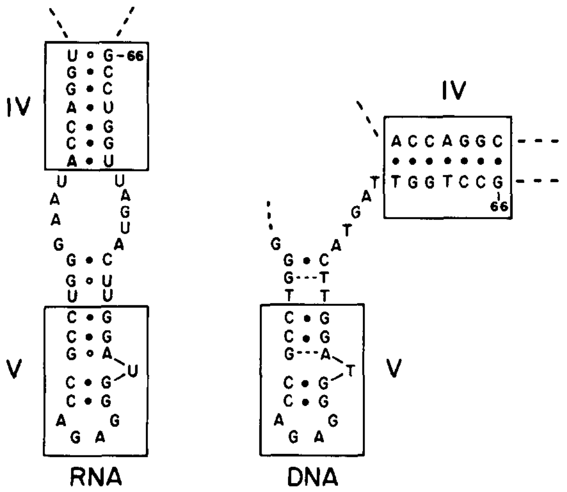

Fig.4. Structural elements in 5 S RNA and potentially in 5 S DNA that, according to the model presented in this paper, are important for the tight binding of TFIIIA. These elements consist of a stem-loop structure analogous to helix V in the RNA separated from a perfect helical structure by an open region. The ΔG^0 for helix V from position 78 to 98 on 5 S RNA is calculated to be −2.3 kcal/mol [46].

The transcriptionally active Xlo 5 S RNA pseudogene [29], a natural mutant, has several base substitutions that disrupt all potential helical regions except helix V in a resulting transcript [30]. The pseudogene could not be transcribed if the DNA needs to form a complete 5 S RNA type structure for transcription, however, the region of the gene that codes for helix V in the RNA is conserved and the analogous stem-loop structure can still potentially be formed in the DNA. In accord with this observation, fig.4 depicts the similarity of the potential stem-loop structures and adjacent helical regions in 5 S RNA and 5 S DNA that may play a role in TFIIIA interactions.

4. CONCLUSION

Existing biochemical evidence is consistent with a model for TFIIIA-nucleic acid interaction whereby the presence of a stem-loop structure in both nucleic acids is important to the binding of the protein. Another model for TFIIIA binding to 5 S DNA has been proposed by Fairall et al. [26], which involves successive TFIIIA 'fingers' making contact with guanine residues placed equidistant along the entire length of the ICR. The model proposed in the present paper is not inconsistent with that of Fairall et al. in that both types of interactions may occur at different stages of the dynamic process of transcription initiation. TFIIIA may interact with the double helix at the 5′-portion of the ICR in a manner described by Fairall et al. [26], while its interaction at the 3′-end of the ICR may occur with the hairpins depicted in fig.3.

The model of TFIIIA interaction with DNA presented in this paper can be used to decipher the contrasting work of Bogenhagen [31] and Ciliberto et al. [32]. In the former study [31], analysis of the transcriptional activity of deletion mutants prompted the author to conclude that two blocks in the ICR must be properly aligned for TFIIIA to bind and transcription to be supported. In the latter study [32], a 16 base-pair (bp) insertion was made between the two blocks but transcription of the altered gene was not inhibited. The authors concluded from these and other data that the distance between the two blocks is not critical. Examination of the sequence of the 16 bp insertion that rendered the gene transcriptionally active shows that, in addition to having a possible conserved recognition sequence [26], it has the potential to form a hairpin structure. This potential hairpin could substitute for the endogenous one analogous to helix V and be sufficiently aligned with the upstream block so that TFIIIA could bind. Recent data indicate that single-stranded DNA oligomers capable of forming stem-loop structures (one being analogous to helices IV and V in 5 S RNA) will specifically bind to TFIIIA [33]. It appears that stem-loop structures in DNA as well as in RNA are important to TFIIIA interaction in vitro.

The formation of hairpin structures in DNA has been postulated for other transcriptional control regions [34–38]. The most pertinent of these proposals involves the ICR of eukaryotic tRNA genes also transcribed by RNA polymerase III. Although biochemical evidence [39,40] does not support the original concept that the A and B boxes of the tRNA gene ICR form hairpin structures [34], it does support a model whereby, during transcription, the non-coding strand of the DNA assumes a stem-loop structure similar to the anticodon loop of the resulting transcript [39].

One might visualize the formation of transcription complexes in 5 S DNA as a step-wise process. As described for other DNA-binding proteins [41], TFIIIA probably binds non-specifically to double helical DNA until it finds a specific binding site. It is believed that the protein initially interacts with the 5′-portion of the ICR in a lower ‘intrinsic’ affinity interaction [31] before complexing with the 3′-portion. Gyrase activity induced by TFIIIA in oocyte extracts [5] causes negative supercoils to be formed in the DNA. Negative supercoiling can allow for the strand separation expected to occur during the process of transcription and also can lower the thermodynamic barrier to stem-loop formation [25,42–45]. The formation of a stem-loop structure at the 3′-portion of the ICR where TFIIIA makes direct contact with the DNA [10] may stabilize the interaction of TFIIIA with 5 S DNA by increasing the affinity of TFIIIA for the DNA. The TFIIIA-DNA hairpin complex could serve as a recognition signal for TFIIIC and thus, promote the formation of a stable transcription complex. At the start of transcription, the 5′-end of the gene must be unwound and the presence of a hairpin at the 3′-end stabilized by protein interactions may be important to this event.

ADDENDUM

After completion of this manuscript, Christiansen et al. [47] published additional data showing the protection of 5 S RNA by TFIIIA from several RNases and chemical probes. They concluded that TFIIIA forms contacts with 5 S RNA at clustered positions scattered over much of the RNA molecule. These data also support the concept that TFIIIA recognizes features of secondary and tertiary structure in 5 S RNA and not repeated nucleotide sequence [47].

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