COMMENTARY

Transcription factor IIIA (TFIIIA) in the second decade

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SUMMARY

Transcription factor IIIA is a very extensively studied eukaryotic gene specific factor. It is a special member of the zinc finger family of nucleic acid binding proteins with multiple functions. Its N-terminal polypeptide (280 amino acid residue containing peptide; finger containing region) carries out sequence specific DNA and RNA binding and the C-terminal peptide (65 amino acid residue containing peptide; non-finger region) is involved in the transactivation process possibly by interacting with other general factors. It is a unique factor in the sense that it binds to two structurally different nucleic acids, DNA and RNA. It accomplishes this function through its zinc fingers, which are arranged into a cluster of nine motifs. Over the past three years there has been considerable interest in determining the structural features of zinc fingers, identifying the fingers that preferentially recognize DNA and RNA, defining the role of metal binding ligands and the linker region in promotor recognition and the role of C-terminal amino acid sequence in the gene activation. This article briefly reviews our current knowledge on this special protein in these areas.

Key words: Transcription, Factors, 5 S RNA, Genes

INTRODUCTION

Transcription factor IIIA (TFIIIA) from *Xenopus* is the first eukaryotic gene-specific factor to be isolated and extensively characterized (Wolffe and Brown, 1988; Geiduschek and Tocchini-Valentini, 1988; Shastry, 1991; Hanas et al., 1992; Shastry, 1993; Pieler and Theunissen, 1993). This 38.5 kDa zinc metalloprotein is a highly abundant polypeptide in oocytes compared to several other transcription factors from different sources. It plays a major role in the regulation of transcription of 5 S RNA genes by binding to the internal control region (ICR). It is a unique positively acting transcription factor. In addition to promotor recognition, it binds to 5 S RNA, the transcript of the 5 S RNA gene. It carries out its specific functions of binding two kinds of structurally different nucleic acids through its unusual structural features. It contains nine consecutive zinc finger domains (Miller et al., 1985), with each domain consisting of about 30 amino acids. The zinc metal is coordinated by pairs of highly conserved cysteine and histidine residues. Although intensive studies on the structure and function of TFIIIA have been conducted in many laboratories around the world, which have been discussed in detail, the multifunctional property of this protein still remains to be elucidated. Since the last review (Shastry, 1993), there has been considerable interest in determining the structural features of zinc fingers, identifying the critical fingers necessary for the DNA and RNA recognition, understanding the consequences of altering the ligands to the metal, and defining both the role of linker regions between the fingers and role of C-terminal amino acids in the activation of the gene. In this article an attempt is made to summarize these aspects briefly. Because of space limitations the number of references is restricted and the readers are directed to previous reviews (Wolffe and Brown, 1988; Geiduschek and Tocchini-Valentini, 1988; Shastry, 1991, 1993; Hanas et al., 1992; Pieler and Theunissen, 1993) for more details on the structure and functional aspects as well as a comprehensive list of references.

TFIIIA has been either partially or highly purified (Ogilvie et al., 1993; Moorefield and Roeder, 1994; Ottonello et al., 1994; Smith et al., 1995; Stunkel et al., 1995) from a variety of sources or even expressed in the recombinant form. However, their extensive characterization with respect to the structure and function is not available for biochemical comparison. TFIIIAs from different sources do contain zinc fingers, trans-activate 5 S RNA genes and, in some cases, heterologous factors functionally substitute for a homologous TFIIIA in an in vitro transcription system (*Xenopus* TFIIIA can be substituted for the human factor). They are transcription complex assembly factors (responsible for the assembly of a transcription complex) similar to *Xenopus* TFIIIA, but exhibit a significant sequence variation, even within the species (*Xenopus*) particularly in N- and C-terminal regions (see ref., Shastry, 1991, 1993, for details). The yeast protein is only 20% identical to the frog TFIIIA (Archambault et al., 1992). Since *Xenopus* TFIIIA has been extensively studied at the level of both protein and gene, the following brief discussion is restricted to frog TFIIIA.

ROLE OF INDIVIDUAL BASE-PAIRS IN PROMOTER RECOGNITION

One of the major functions of TFIIIA is to program the 5 S RNA gene for transcription by binding directly to the ICR,

which subsequently directs an ordered assembly of general factors to form a functional transcription complex. Previous studies have demonstrated a tripartite structure (Pieler et al., 1985, 1987) for ICR and the importance of the box C element (Fig. 1A) in TFIIIA interaction (Pieler et al., 1987). Studies involving a substitution mutation within the box C and intermediate element (IE) have further shown (Veldhoen et al., 1994) that TFIIIA interaction is strong with box C element (nt. 80-91). Base-pairs 78-79 and 92-96 are not essential for the high-affinity interaction. However, substitution at GC70 has a major effect, while CG67 to TA69 substitution has no effect and GC71 has a moderate influence on TFIIIA binding. Similarly, substitutions at GC81, 85, 89 and CG91 have a dramatic effect, while 83, 88 and 90 have varying degrees of influence, but substitution at TA84 reduces the affinity by 70%. No effect has been found at 80, but a slight effect has been reported on substitution at GC82, 86 and 87. Thus, it appears that every base-pair within the box C region plays an essential role in binding of TFIIIA.

ROLE OF INDIVIDUAL ZINC FINGERS AND THEIR LIGANDS IN PROMOTER RECOGNITION

Although in recent years many DNA binding proteins are found to contain a zinc finger type structural motif (Coleman, 1992), TFIIIA still remains a unique factor, since it recognizes both DNA and RNA. It accomplishes this unique function through its zinc fingers. On the basis of proteolytic digestion and footprinting studies (Vrana et al., 1988), it was proposed that the fingers can be aligned in order over the ICR of the 5 S RNA gene with N-terminal fingers interacting with the 3'-end and C-terminal fingers with the 5'-end (see Fig. 1A). A variety of studies (Christensen et al., 1991; Liao et al., 1992; Clemens et al., 1993, 1994; Bogenhagen, 1993) with truncated protein have further shown that the N-terminal three fingers are necessary and sufficient for much of the specificity and affinity of binding. To assess further the contribution of each finger as to its function, second, third or fourth ligands (Fig. 1B) that coordinate the zinc atom were individually replaced (Smith et al., 1991; Rollins et al., 1993; Del Rio et al., 1993) and each substitution mutant ('broken finger') was assayed for its ability to activate the endogenous 5 S RNA genes as well as for their susceptibility to proteolytic digestion. As expected, such mutations introduced localized structural perturbations of each mutant protein, suggesting the importance of cysteine and histidine residues to give a properly folded structure of the zinc fingers. Consistent with the earlier data using truncated proteins, the broken finger analysis shows that the zinc fingers are more or less aligned in order along the ICR. DNase I protection analysis using singly disrupted fingers further indicates that mutations in fingers 1 and 9 perturb the protection at the 3'- and 5'-ends of the control region, respectively. In addition, mutations in fingers 3 and 8 results in loss of protection over much of the 3'- and 5'-ends of the ICR, respectively (Del Rio et al., 1993). However, another study showed that mutation in finger 1 alone leads to complete loss of binding (Smith et al., 1991). The differences between the two studies may be due to different amino acid substitution. No difference in the protection pattern was observed with the 4th finger mutant protein. However, it has a large affect on DNA binding energy. These results demonstrate a complex and irregular mode of interaction of TFIIIA. It also appears that the binding of the N-terminal fingers influence the binding of the other fingers. It is interesting to note that the zinc finger protein isolated from fish ovarian tissue (Ogilvie et al., 1993) contains cysteine in the fourth position of one of its fingers, suggesting that this ligand is interchangeable with histidine. The thermodynamic measurements using mutant proteins further suggest that fingers 3 and 4 are energetically more important than fingers 1 and 2. When the mutant proteins are assayed for their ability to direct transcription, fingers 1, 2 and 3 gave reduced level of transcription while substitution in 5 and 7 has no apparent effect on the level of transcription. Mutation in finger 8 and 9 abolish the activation of 5 S gene (fingers 7, 8 and 9 have a weak affinity for DNA), confirming their role in promoting transcription. Surprisingly, a mutation in finger 4 or 6 has stimulated 5 S RNA gene transcription and finger 6 showed a reduced binding to 5 S RNA relative to wild-type. This has also been confirmed by proteolytic footprinting, which suggests that fingers 4-7 are tightly associated with 5 S RNA (Bogenhagen, 1993). Since the finger 6 mutant does not bind the 5 S RNA well, it is possible that it might be more available to bind DNA and hence activate transcription better than the wild-type gene.

The contact point of each finger was previously described in detail and will not be discussed here (see Shastry, 1993). While fingers 1-3 dominate the DNA recognition event, recombinant TFIIIAs containing 3 fingers, 4 fingers or 5 fingers also bind specifically and stoichiometrically to the ICR of the 5 S RNA gene protecting the region between the nucleotides +77 to +96 on both strands (fingers 3 or 4 fingers) and +63 to +96 (containing 5 fingers) on the coding strand (Hansen et al., 1993). The first 3 zinc fingers interact in the major groove with +81 to +89. There is less intimate contact between TFIIIA and the ICR at +66 and +72 (coding strand) and +70 and +77 (noncoding strand), indicating that TFIIIA skips part of the ICR at the border of the binding site for finger 3. These results, together with those of other experiments (Kochoyan et al., 1991; Hayes and Tullis, 1992), demonstrate the discontinuity in the immediate contact between TFIIIA and 5 S RNA gene in the center region of the ICR. Furthermore, the hydroxy radical footprinting (Tullis et al., 1987; Vrana et al., 1988; Hanas et al., 1989; Churchill et al., 1990) and missing nucleotide experiments (Hayes and Tullis, 1992) support the notion that TFIIIA traverses the minor grove about the nucleotide +74 to +76 (coding strand) and +77 to +79 (noncoding strand) and fingers 4 and 5 span across the major groove. This may explain the existence of a discontinuity in TFIIIA contact with DNA and ICR and the extent of 33 bp protection by 5 fingers. The models proposed by Klug and co-workers (Miller et al., 1985) and Pavletich and Pabo (1991) indicate that each finger interacts with 5.5 and 3 bp DNA, respectively. However, the protection observed by the first five fingers (33 bp) cannot be interpreted by these proposals. The model proposed by Kochoyan and co-workers (1991), although supported by hydroxy radical footprinting, also fails to explain the site of footprinting alterations observed with mutant fingers 6, 7, 8 and 9. Therefore, these models should be interpreted with caution. It is also interesting to note that zinc ions by themselves show a strong interaction with the sequence TGGGA, which is in the binding region of TFIIIA (Martinez-
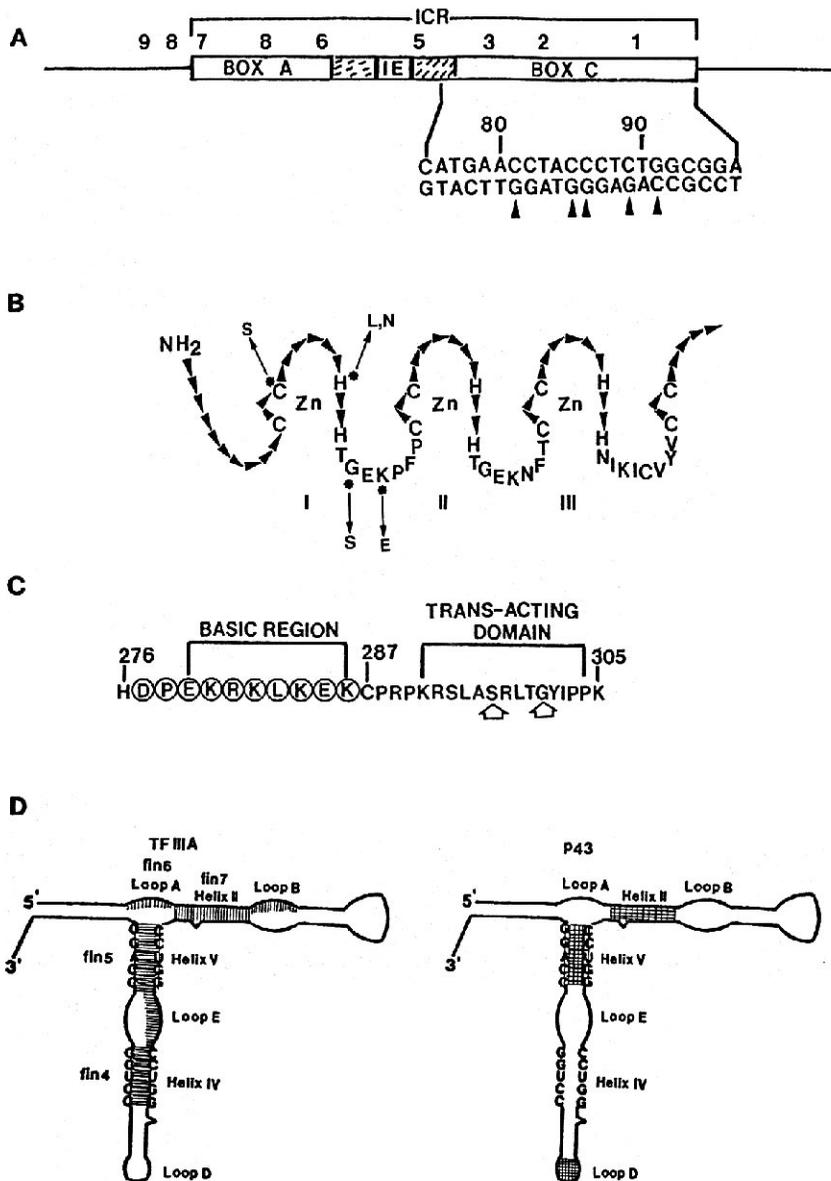
Fig. 1. Contribution of individual base-pairs to TFIIIA binding. (A) The three regulatory elements (box C, intermediate element and box A) of the internal control region of the 5 S RNA gene are shown as a rectangular box (Pieler et al., 1985, 1987). The nucleotide sequence of the C box region is shown below the rectangular box. The substitutions of base-pairs that reduce the TFIIIA binding are indicated by arrowheads (Veldhoen et al., 1994). The sequence protected by the first N-terminal three fingers are enclosed in a zig-zag bracket (Christensen et al., 1991). The numbers 1-9 above the rectangular box refer to the approximate location of 1-9 zinc fingers. (B) The schematic structure of the N-terminal first three fingers and their linker sequence. The asterisks represent the replaced ligands for the zinc ions and mutated highly conserved linker region amino acids. The replacements are shown by the arrows. (C) The C-terminal region of the TFIIIA showing the basic amino acid region and the trans-acting domain. The amino acid residue insensitive to change are circled. The open arrowheads denote that substitution at this site results in complete loss of transcription (Mao and Darby, 1993). Amino acid residues 291-304 are indispensable for transcription. (D) A schematic representation of the 5 S RNA secondary structure and binding of TFIIIA fingers 4-7 (left side of the Fig.). The binding region is shown by a shaded area. Recognition elements for p43 protein are shown on the right side of the Figure. The structure of stems II and V are commonly required for both TFIIIA and p43 proteins.

Balbas et al., 1995). In addition, the 5 S RNA gene may also contribute some structural features for binding, since RNA-DNA heteroduplexes fail to bind TFIIIA (Nightingale and Wolffe, 1995). It is possible that the zinc fingers not only serve as structural components of the proteins, but also may introduce conformational change on the DNA by binding to its bases. Since there is a high degree of non-uniformity and functional interdependency among zinc fingers (Del Rio et al., 1993; Smith et al., 1991), it is not possible at present to propose a detailed structural model for TFIIIA:DNA interaction. Such a three-dimensional structure of TFIIIA interaction ultimately requires crystallographic analysis.

ROLE OF ZINC FINGER LINKER SEQUENCE IN PROMOTER RECOGNITION

Each zinc finger of TFIIIA is connected by an invariant short peptide termed 'linker' (Fig. 1B). The role of this conserved sequence in zinc finger function has not been understood until

recently. Studies involving single and multiple amino acid substitution (Smith et al., 1991; Choo and Klug 1993; Clemens et al., 1994) in the first three N-terminal fingers have been reported. When these mutant fingers were assayed for their ability to bind DNA no appreciable binding was detected. Interestingly, when the first linker of TFIIIA was replaced with the linker connecting the 3rd and 4th fingers, DNA binding was abolished, suggesting that the relationship between finger 1 to finger 2 is not the same as that between fingers 3 and 4. Although these short peptides are conserved between the first three fingers, their function appears to be different from one another. A full understanding of the functional significance of these linkers awaits further experiments.

ROLE OF LINKER SEQUENCE BETWEEN 9TH FINGER AND ACTIVATION DOMAIN

The carboxyl-terminal domain (amino acid residues 295 to 313) of TFIIIA does not bind DNA, but is required for trans-activa-

tion. To determine the precise role of this region, studies involving a series of deleted and substituted mutants have been reported (Mao and Darby, 1993). When assayed for their transcriptional activity, a region containing 18 amino acid residues (287-304) is found to be indispensable for the activation of the 5 S RNA gene (Fig. 1C). Substitution mutations of the conserved serine 296 to alanine and glycine 300 to glutamic acid completely abolished transcription, indicating their essential role for the activity of the protein. On the other hand, the basic amino acid region, which is predicted to have an α-helical structure, when substituted by a diverse range of amino acids did not affect the transcription, suggesting that they are not required for transactivation. However, deletion of amino acids in this region (279-286) completely abolished the transcriptional activity, but not the DNA binding property. Interestingly, when the distance between the DNA binding domain and the trans-acting domain was increased by an insertion mutation, transcriptional activity is reduced by 5-fold. This suggests that the distance between the 9th zinc finger and the transacting domain plays a critical role in the productive interaction of TFIIIA with its target. Further mutational analyses within the C-terminal region are necessary to define the critical amino acids and their functions in carrying out the trans-activation.

ROLE OF ZINC FINGERS IN RNA RECOGNITION

As mentioned above, one of the unique features of TFIIIA is that it recognizes both DNA and RNA. It has been previously shown (Shastry, 1991; Christensen et al., 1991; Lio et al., 1992; Clemens et al., 1993; Shastry, 1993) that the N-terminal fingers 1-3 dominate in DNA recognition and the fingers 4-7 preferentially participate in 5 S RNA recognition. In addition, a truncated polypeptide consisting of fingers 4-7 has been shown to have a higher affinity for RNA when compared to the full-length TFIIIA (McBryant et al., 1995), supporting the notion that these fingers contained the necessary information for RNA recognition. This is also consistent with the idea that the primary role of native TFIIIA is to activate the gene for transcription, since the presence of the first 3 fingers reduces the affinity for RNA. Studies on mutations covering the entire structure of 5 S RNA and truncated TFIIIA containing zinc fingers 4-7 further suggest that fingers 7, 6, 5 and 4 recognize helix II, loop A, helix V and region E of the 5 S RNA gene (Fig. 1D), respectively (McBryant et al., 1995). Furthermore, region E appears to provide crucial structural features for this interaction. This has been further substantiated by using truncated 75mer 5 S RNA, which provided all the structural requirement for this binding of TFIIIA.

The 5 S RNA in Xenopus is also complexed with another protein termed p43 in the form of 42 S ribonucleoprotein particles (RNP). This protein is structurally similar to TFIIIA in its zinc finger region and contains 9 zinc fingers of which 7 of these have TFIIIA-type structure. However, they are functionally different, in that p43 does not bind the 5 S RNA gene, but exclusively binds to 5 S RNA. Studies involving a series of substitution and deletion mutations of 5 S RNA have demonstrated that TFIIIA and p43 require different structural features of 5 S RNA for recognition (Zhang and Romaniuk, 1995). Xenopus p43 requires the structural features and base sequences of stems II, V and loop D (Fig. 1D) of 5 S RNA, which are slightly different than those required for TFIIIA binding (stems II, V and loop A). Furthermore, loop D may be directly involved in p43 binding. Although TFIIIA and p43 proteins are similar in their zinc finger structure and in their function of storage of 5 S RNA, their mode of interaction with 5 S RNA is significantly different. Further studies, possibly using TFIIIA from other sources, are needed to understand the mechanistic aspects of these structurally or functionally similar proteins and their interactions with 5 S RNA. It is also not presently known how many fingers in p43 participate in RNA recognition.

CONCLUDING REMARKS

Xenopus TFIIIA is one of the best-studied transcription factors at the level of both the protein and its gene. This, in part, is due to its natural abundance in oocytes and its unique property of DNA and RNA binding. Although a detailed model of TFIIIA:DNA and TFIIIA:RNA interactions awaits crystallographic analysis, we are still ignorant of some of the other intriguing aspects. First, the reasons for the massive amount of TFIIIA (Shastry et al., 1984) in immature oocytes are not known (10^7 molecules more than the total number of 5 S RNA genes). Secondly, regulation of the TFIIIA gene (Martinez et al., 1994) to produce such an extraordinary amount of TFIIIA is not understood. Since most other transcription factors isolated and characterized to date are present in the cell in exceedingly small amounts, it is important to know the biological significance of a large amount of TFIIIA in oocytes. One possible explanation is that 10^{12} molecules of TFIIIA will be responsible for the synthesis of 10^{12} molecules of 5 S RNA that are transported and stored in the form of 7S RNP. Since there are approximately 10^{12} ribosomes in mature Xenopus oocytes (Davidson, 1986), and since each ribosome contains one molecule of 5 S RNA, it seems to be justified to produce such a large amount of TFIIIA to ensure that each ribosome is functional during protein synthesis.

Apart from this, it is also not understood whether TFIIIA has functions other than binding to the DNA and 5 S RNA. Although TFIIIA from yeast does not appear to have any other functions than the transcription of 5 S RNA genes (Camier et al., 1995), it is possible that Xenopus TFIIIA is a unidirectional RNA transport protein. The formation of 7 S RNP particles not only serves the purpose of storage of 5 S RNA (and thereby controlling the expression of 5 S genes), but also transport the RNA from nucleus to cytoplasm. If this is true, then what mechanisms are involved in the transport of the free TFIIIA synthesized in the ribosome to the nucleus. Does the formation of 7 S RNP in the nucleus and its dissociation in the cytoplasm provide the signal to the protein to travel back and forth from the nucleus to cytoplasm? In the next few years, answers to many of these questions will undoubtedly bring more exciting news regarding this unique multifunctional protein.

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Note added in proof

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