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REVIEW

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Mitochondrial transcription initiation: promoter structures and RNA polymerases

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Introduction

The well-conserved features of mitochondria across the eukaryotic kingdoms have led to the widely-held (but not universal) consensus that mitochondria are of eubacterial ancestry, and that all present-day mitochondria can be traced to a single endosymbiotic event (reviewed in Gray 1989, 1992). However, under closer scrutiny it is clear that significant divergence has occurred since this event, as there is remarkable variability in genome size and structure, gene content and alternative biochemical functions, between and even within the four kingdoms (reviewed in Tzagoloff and Myers 1986; Chomyn and Attardi 1987; Attardi and Schatz 1988). In light of these findings, it is not surprising that eukaryotic species also vary in their modes of mitochondrial gene expression (previous references: Simpson 1987; Levings and Brown 1989; Clayton 1991; Shadel and Clayton 1993; Jang and Jaehning 1994). In an effort to find common themes among the apparent idiosynchracies of plants, animals and fungi, this review summarizes what is known about mitochondrial transcription initiation in each kingdom. Emphasis has been placed on the analysis of promoter structures and RNA polymerases, and the review has been subdivided to facilitate such comparisons.

The first attempts to characterize mitochondrial promoters entailed comparisons of sequences surrounding transcription initiation sites. Initiation sites were commonly identified by S1 nuclease protection analysis or primer extension of mitochondrial transcripts synthesized in vivo (Montoya et al. 1982). In these cases, primary transcripts could be distinguished from products of endonucleolytic processing by their ability to be end-labeled with vaccinia-virus guanylyltransferase in vitro (Christianson and Rabinowitz 1983; Yoza and Bogenhagen 1984). In plant systems, run-on transcription experiments in conjunction with filter hybridizations have been employed to determine the relative transcriptional activities of mitochondrial genes (Carlson et al. 1986; Finnegan and Brown 1990; Mulligan et al. 1991). Further characterization of promoter structures and RNA polymerases became possible with the development of in vitro transcription systems. Such systems have now been reported for human (Walberg and Clayton 1983), mouse (Chang and Clayton 1986 a), rat (Yaginuma et al. 1982), *Xenopus laevis* (Bogenhagen and Yoza 1986), yeast (Edwards et al. 1982), *Neurospora crassa* (Kennell and Lambowitz 1989), *Crithidia oncopelti* (Zaitseva et al. 1985), wheat (Hanic-Joyce and Gray 1991), maize (Rapp and Stern 1992), and pea (S. Binder and A. Brennicke, personal communication) mitochondria.

With the exception of yeast, all these systems utilize partially purified enzymes, yet their activities have revealed different modes of transcription initiation between organisms, and some unexpected relationships between mitochondrial transcription factors and known proteins of both prokaryotic and nuclear origin. In consideration of this increasing complexity, the simplified nomenclature of Xu and Clayton (1992) will be used in this review to designate the proteins involved in mitochondrial RNA synthesis.

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Mitochondrial promoter structure

Human

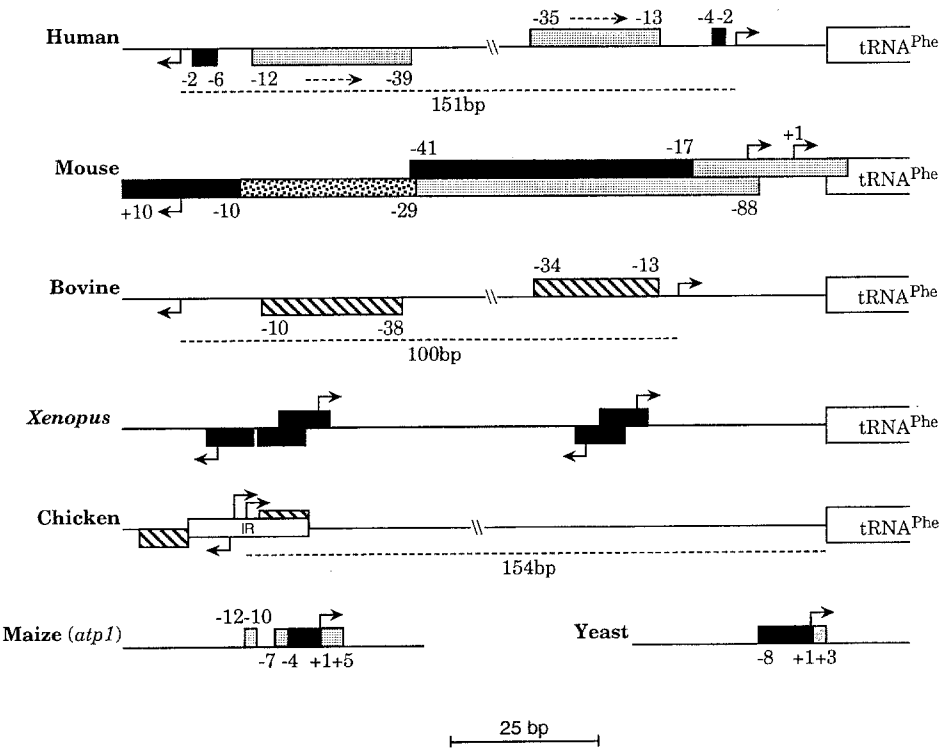
The mitochondrial genomes of vertebrates are highly compact and, with the exception of a variable 1–2 kb

non-coding region, are largely colinear. It is these variable sequences which contain the origins of replication and the promoters for both the light (LSP) and heavy (HSP) strands of mitochondrial DNA (mtDNA) (Fig. 1). Replication from the H-strand origin stalls shortly after initiation and creates a triple-stranded displacement loop (D-loop) for which this control region is named (reviewed in Clayton 1991). Transcription initiation from these promoters also gives rise to long polycistronic messages which are cleaved endonucleolytically to produce the individual RNA species. The situation of these control sequences within the D-loop may not be simply coincidental since the mitochondrial RNA polymerase appears to play a role in both transcription and DNA replication, as discussed in a later section.

Inspection of the human LSP and HSP sequences revealed 15-bp consensus motif that includes the initiation sites (Fig. 2). Furthermore, studies conducted in vitro have shown that this consensus sequence can support a minimal amount of accurate transcription and that of the nucleotides most critical for promoter function are located just upstream of the H- and L-strand initiation sites (Chang and Clayton 1984; Hixson and Clayton 1985). Each of these studies also identified additional upstream sequences required for optimal transcription. Although these upstream sequences share limited similarity, they were later shown to include orientation-independent binding sites for a transcription factor.

A low level of transcription initiation has also been reported from a third match to the 15-bp consensus sequence (Montoya et al. 1983; Chang and Clayton 1984). Located downstream from the major HSP, within the 3′-end of the $tRNA^{Phe}$ gene, the 2% (when measured in vitro) of H-strand transcripts initiating at this promoter extend the entire length of the H-strand template and therefore include rRNAs, mRNAs and

Fig. 1 The organization of the mitochondrial promoters of selected organisms. Elements that have been shown by in vitro mutagenesis to be essential for promoter function are indicated by *black boxes*, sequences shown to enhance transcriptional activity are depicted with *gray boxes*, and *arrows* indicate the position and direction of transcription initiation. The minor H-strand initiation site in human mtDNA, located within the $tRNA^{Phe}$ gene (Chang and Clayton 1984), is not shown. In the animal systems, HSP transcription is rightward and LSP transcription is leftward. In human mtDNA, the orientation of the h-mtTFA-binding sites are indicated by the *dashed arrows*; in mouse mtDNA, sequences proposed to be important in the formation of a pre-initiation complex are represented by a *stippled box*. The *hatched boxes* in bovine mtDNA are the locations of protein:DNA interactions as determined by *in organello* DNase-I protection analysis. The *hatched boxes* in chicken are sequences identified solely by their dyad symmetry and sequence similarity to the *Xenopus* consensus sequence; the box labeled *IR* represents the location of the inverted repeat that can potentially fold into a cruciform structure. Each figure has been drawn to the scale shown, with the discontinuities and total length of DNA given for the human, bovine and chicken promoters. References are as follows: human (Hixson and Clayton 1985; Fisher et al. 1987); mouse (Chang and Clayton 1986 b, c); bovine (Ghivizzani et al. 1993); *Xenopus* (Bogenhagen and Yoza 1986); chicken (L'Abbé et al. 1991); maize (Rapp et al. 1993); and yeast (Biswas and Getz 1986)



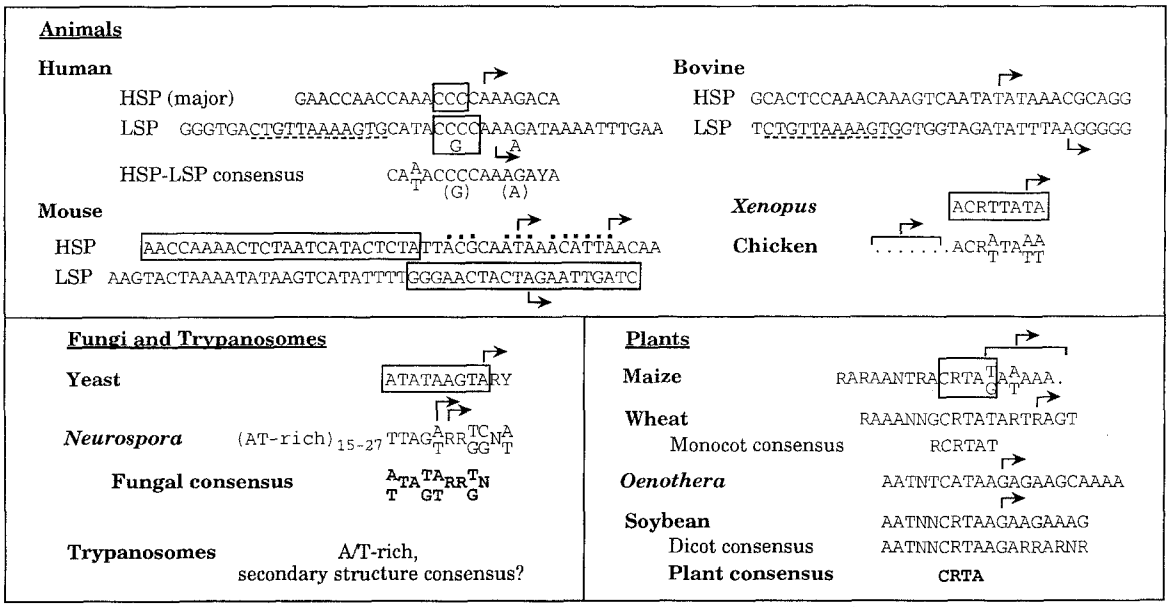


Fig. 2 Mitochondrial promoter sequences for several organisms grouped according to kingdoms. The major initiation sites are indicated by the *arrows* above the sequences; in chicken and maize, transcription initiates at variable distances from the promoter sequence and these sites are enclosed by *brackets*. *Boxed* sequences have been shown to be essential for promoter activity in vitro. Sequences common to the human and bovine LSPs are underscored by a *broken line*. The two sets of sequences in the HSP of mouse with similarity to the consensus sequences of *Xenopus* and chicken are indicated by *small filled squares*. *HSP* = heavy strand promoter; *LSP* = light-strand promoter; *R* = purine; *Y* = pyrimidine; *N* = any base. References are as for Fig. 1 with the following additions: *Neurospora* (Kennell and Lambowitz 1989); trypanosomes (Michelotti and Hajduk 1987); maize (Mulligan et al. 1991); wheat (Covello and Gray 1991); *Oenothera* (Binder and Brennicke 1993 a); and soybean (Brown et al. 1991)

tRNAs. In contrast, transcripts initiating from the more active upstream HSP terminate after synthesis of the rRNAs and adjacent tRNAs. The discovery of the two HSPs has provided explanations for the two H-strand transcripts and the higher rate of rRNA synthesis, relative to that of mRNAs, seen in vivo. The mechanism underlying this phenomenon require further study; they may include differential control of these promoters and/or the action of a recently-identified transcription termination factor, mTERF (Daga et al. 1993).

Mouse

Analysis of in vivo- and in vitro- synthesized mouse mtRNA has revealed that transcription initiates at a single site in the L-strand and at two closely-spaced sites in the H-strand (Chang and Clayton 1986 a). Although not similar in sequence to its human counterpart (Fig. 2), the structure of the mouse LSP is similar in that a small region surrounding the initiation site supports a minimal amount of accurate transcription, and upstream sequences are required for optimal transcription in vitro (Fig. 1). A variety of in vitro experiments have shown that these upstream sequences are sites of transcription factor binding and participate in pre-initiation complex formation.

The structure of the mouse HSP contrasts with that of the human promoters and the mouse LSP. Deletion mutagenesis studies have revealed that a single HSP controls both H-strand start sites. However, the HSP does not include the initiation sites but is instead located upstream and overlaps with sequences of the LSP (Chang and Clayton 1986 b, c). In fact, in 3′-deletion clones that lack the H-strand initiation sites, sequences within the vector serve as alternate start sites with comparable efficiency. In light of the different positioning of the promoters and the lack of a mouse LSP/HSP consensus sequence, it is difficult to imagine that each employs an identical model of transcription initiation. However, the fact that the same transcription factors binds within each of the promoter regions suggests a role of higher-order DNA structure and/or other factors.

Bovine

In bovine mitochondria, the locations of the HSP and LSP are comparable to those of other mammals (Fig. 1), yet little sequence similarity exists either between the two bovine promoters, or with their human and mouse counterparts (Fig. 2; King and Low 1987). The one exception is a 12-nt motif upstream of the bovine LSP, which resembles the human LSP

enhancing region in both sequence and location. Footprinting in isolated organelles has revealed that this motif, along with a similarly located sequence at the bovine HSP, are, as in human mitochondria, sites of protein binding (see below).

The following general conclusions may be drawn with regard to mitochondrial transcription in mammals. First, promoters are primarily unidirectional and transcription initiates from relatively well-conserved locations within the D-loop. Second, sequences upstream from these sites enhance transcription rates and are sites of protein:DNA interaction. Third, primary sequence conservation between mammalian promoters is relatively low. This mechanistic similarity in the absence of primary sequence identity suggests that DNA secondary structures and homologous, but species-specific, transcription factors serve to position the transcriptional apparatus.

Xenopus laevis

The D-loop region of *X. laevis* mtDNA contains two bidirectional promoters, which have been identified by the mapping of both in vivo- (Bogenhagen et al. 1986), and in vitro-synthesized transcripts (Fig. 1; Bogenhagen and Yoza 1986). Transcription initiates within an octanucleotide consensus sequence (Fig. 2) which is found at seven sites within the D-loop region; four of these sites are active in vitro and in vivo. The fact that these octanucleotide sequences are arranged symmetrically and are in two clusters accounts for the observation of bidirectional transcription from two locations. Deletion mutagenesis studies have shown that the octanucleotide sequence is sufficient to direct accurate transcription initiation in vitro; however, unlike the situation in mammals, these promoters do not rely on upstream sequence elements. Point mutagenesis has identified the residues + 1T and − 6A of the consensus sequence as most critical for promoter function in vitro. The fact that mutations in one consensus sequence can also diminish transcription from adjacent promoters suggests that a cooperative relationship may exist between the octanucleotide sequences (Bogenhagen and Romanelli 1988).

Although the transcription initiation sites in *Xenopus* are at analogous locations to those in mammals, there is little sequence similarity apart from matches between the *Xenopus* octanucleotide consensus and two sequences near the H-strand initiation sites in mouse (Fig. 2). It is likely that this homology is either fortuitous or an evolutionary remnant, as these sequences are not important for transcription initiation in mouse (Chang and Clayton 1986 b), nor are they found in bovine or human promoters.

Chicken

Chicken mtDNA is thus far unique in having a single, major, bidirectional promoter (L'Abbe et al. 1991). Analysis of in vivo-synthesized transcripts revealed that the L-strand initiation site lies within the D-loop, at a location similar to that of other vertebrates (Fig. 1). In contrast, two closely-spaced H-strand initiation sites are found approximately 135 bp farther upstream than in other vertebrates, and are located immediately adjacent to the L-strand initiation site. A nearly perfect inverted repeat surrounds the initiation sites, and has the potential to form a cruciform structure and H- and L-strand initiation sites within the loops. Such a structure could function as a recognition signal for the transcription machinery. The H- and L-strand initiation sites are flanked on their 3′-ends by an octanucleotide sequence matching the *Xenopus* consensus, and therefore also sequences in the mouse HSP (Fig. 2). A clear assessment of the significance of this potential secondary structure and these sequence similarities awaits the analysis of other avian initiation sites and the development of a homologous in vitro system.

Yeast

Mitochondrial promoters in fungi differ from their animal counterparts in several important respects: each genome contains numerous promoters, despite only an approximately five-fold difference in genome size; the promoters are not multipartite and their primary sequences are conserved both within and between species. In the well-studied mitochondrial genome of the yeast *Saccharomyces cerevisiae*, transcription initiates from at least 20 promoters, and all transcripts appear to be composed of two or more coding sequences (reviewed in Tzagoloff and Myers 1986). Each promoter consists of a highly-conserved sequence of nine nucleotides, with transcription generally initiating at the terminal adenine residue (Fig. 2). Deletion mutagenesis studies have shown that the consensus sequence alone is sufficient to promote high-efficiency transcription in vitro (Biswas et al. 1985; Schinkel et al. 1986). Although no upstream elements analogous to those found in animal mitochondrial promoters are present in yeast, the occurrence of a purine at position + 2 and a pyrimidine at position + 3 has been reported to enhance transcription rates (Biswas and Getz 1986). Within the consensus sequence, saturation point mutagenesis has shown an absolute requirement for − 2G, − 4A, − 6A and − 7T, but little constraint on the nucleotides that occupy positions + 1 and − 8 (Biswas et al. 1987). However, the fact that promoter activity in vitro was compromised to some degree by all mutations suggests that the optimal promoter sequence is that which is found in vivo.

Neurospora crassa

In *Neurospora crassa*, transcription initiation sites were initially defined following the development of a homologous in vitro transcription system (Kennell and Lambowitz 1989). Subsequent comparisons of several initiation sites defined an 11-nucleotide promoter consensus (Fig. 2), and deletion mutagenesis revealed its importance for promoter activity in vitro (Kubelik et al. 1990). Closer inspection of matches to this consensus sequence revealed that functional promoters also have upstream regions containing sequences with an (A + T) content 81–100%. A comparison of the promoters of *Neurospora* and yeast reveals matches at 6 of 9 positions; however, the *Neurospora* promoter differs in the relative locations of its initiation sites and in the degree of sequence divergence between promoters. These results raise the possibility that, unlike animal systems, a universal consensus sequence may exist for fungal mitochondrial promoters. In support of this notion, exact copies of the yeast nonanucleotide sequence have been found at putative transcription initiation sites in the mtDNA of the yeasts *Kluyveromyces lactis* (Osinga et al. 1982) and *Torulopsis glabrata* (Clark-Walker 1985).

Trypanosomes

Perhaps the most extensively studied mitochondrial genomes in protists are the maxicircle DNAs of trypanosomes (reviewed in Benne 1985; Simpson 1987), particularly with respect to the post-transcriptional process of RNA editing (reviewed in Benne 1994). Studies of primary transcripts have shown that transcription initiates at multiple sites in the maxicircle genome and, in this respect, transcription in protists bears similarity to that in fungi. However, inspection of the sequences surrounding these initiation sites have not revealed an obvious consensus other than the presence of inverted repeats. It may be that DNA structures such as cruciforms play a more dominant role than primary sequence in determining the sites of transcription initiation (Michelotti and Hajduk 1987). In spite of the apparent absence of "traditional" promoters, transcription in trypanosome mitochondria is nevertheless complex and highly regulated (Bhat et al. 1992). From a evolutionary perspective, a unique mode of transcription initiation in trypanosome mitochondria would be consistent with their early branching from the other eukaryotes (Sogin et al. 1989).

Plants

Within the plant kingdom, analysis of mitochondrial promoters has revealed limited similarity, both within and between species (reviewed in Gray et al. 1992). Analysis of mitochondrial RNAs from maize (Mulligan et al. 1988 a, b), wheat (Covello and Gray 1991), *Oenothera berteriana* (Binder and Brennicke 1993a, b), and soybean (Brown et al. 1991) has demonstrated that transcription initiates from multiple promoters within the mitochondrial genome. However, unlike fungal promoters, transcription initiation can occur at variable and multiple sites relative to the loosely conserved plant promoters (Fig. 2). So far, the only identifiable commonality between the mitochondrial promoters of all plant species is the consensus sequence CRTA at or near the transcription initiation site, with slightly more homology evident in interspecies comparisons within the monocotyledonous or dicotyledonous plants. Even to this rule, however, exceptions have been noted (Mulligan et al. 1991; Lizama et al. 1994). Studies in maize have revealed a correlation between a promoter's match to the consensus and its activity in run-on transcription experiments, suggesting that the primary sequence may be an important determinant of promoter strength, and that it could serve a regulatory role in mitochondrial gene expression (Mulligan et al. 1991).

Inferences derived from the inspection of sequences have been largely confirmed by in vitro transcription experiments, although only a small number of promoters have been analyzed to-date. Initially, a wheat *cox2* promoter was used to program an in vitro transcription system derived from wheat mitochondria (Hanic-Joyce and Gray 1991). An analogous system was subsequently developed in maize and used to analyze the maize mitochondrial *atp1* promoter in detail (Rapp and Stern 1992; Rapp et al. 1993). Linker-scanning and point mutagenesis suggested that the *atp1* promoter extends at least 17 bp, and have shown that mutations within this region have varying effects on promoter activity in vitro. These results led to a model in which the *atp1* promoter consists of a 12-bp central domain, with an essential core (-4 to $+1$) containing the CRTA motif and an initiating nucleotide, and a small upstream element centered around -12 (Fig. 1). The importance of the core sequence has been confirmed by mutagenesis of the maize *cox3* and *atp6* promoters (A. Caoile, S. Lupold and D.B. Stern, unpublished results). The compact nature of the core sequence is reminiscent of the fungal promoters; however, the tolerance of some base substitutions within the central domain, and the apparent importance of a separate, upstream, region, contrasts with the strictly limited extent, and nearly invariant sequence, of yeast mitochondrial promoters. Although this model was developed for maize *atp1*, with refinement by additional experimentation it should eventually lead to the establishment of a paradigm for most plant mitochondrial promoters.

Recently, however, a novel promoter was identified in the mitochondrial *cox2* gene of *Zea perennis*, a distant relative of maize (Newton et al. 1995). There is no similarity to the *atp1*-type promoter, and the

Z. perennis promoter cannot be transcribed by the maize in vitro system. Initiation at this *cox2* promoter is dependent on a maize nuclear gene product coded by the *MCT* gene, which could be a sigma-like or other transcription factor. Whether such promoters also exist in maize mtDNA has not yet been determined.

Mitochondrial RNA Polymerases

Mammals

Biochemical dissection of crude human and mouse mitochondrial preparations has revealed an absolute requirement for two proteins for efficient transcription initiation: a core RNA polymerase and a specificity factor (reviewed in Clayton 1991). Neither the human nor mouse core RNA polymerase (core h-mtRNA or m-mtRNA polymerase, respectively) have been purified to homogeneity and each could conceivably consist of multiple polypeptides. Alone, the core mtRNA polymerases are not capable of sequestering promotor-containing DNA in a pre-initiation complex, and exhibit only a minimal amount of promoter selectivity (Fisher et al. 1987, 1989). Formation of the pre-initiation complex and high-efficiency transcription are conferred by the 25-kDa specificity factor, h-mtTFA and m-mtTFA from human and mouse mitochondria, respectively (mtTFA was formerly termed mtTF1) (Fisher and Clayton 1988; Fisher et al. 1989). Gel-mobility shift and DNase-I protection assays have shown that multiple mtTFA molecules can interact both non-specifically with promoterless DNA, and specifically with the previously described enhancing regions of mouse and human promoters (Fig. 1; Fisher and Clayton 1988; Fisher et al. 1987, 1989). Although there is no sequence similarity between the mtTFA-binding sites of the two species, there does exist a loose consensus between the HSP and LSP sites in humans. Because these two sequences are found in opposite orientations with respect to the direction of transcription, h-mtTFA may function bidirectionally. Recently, data from *in organello* DNase-I protection analysis of bovine mtDNA revealed that protein:DNA interactions occur upstream of the bovine promoters in locations similar to mtTFA-binding sites in human and mouse promoters (Ghivizzani et al. 1993). This is the first evidence that the mtTFA-binding sites identified in vitro may accurately reflect in vivo conditions.

The mtTFA proteins are also capable of inducing a conformational change in promoter-containing DNA; this suggests that promoter conformation may play an important role in mammalian transcription initiation (Fisher et al. 1992). In human mitochondria, efficient transcription apparently requires a single h-mtTFA molecule at the LSP, which bends the DNA, and multiple h-mtTFA molecules at the HSP, which wraps the DNA (Fisher and Clayton 1988). Furthermore, results obtained both in vitro and in vivo have indicated that the steady-state level of L-strand transcripts is higher than that of H-strand transcripts. For these reasons, it is possible that, at least in humans, the efficiency of transcription may also be related to the stoichiometry of h-mtTFA-binding, which in turn influences promoter conformation. A general model for mitochondrial transcription initiation in mammals states that the DNA-binding activity of mtTFA increases the efficiency with which core mtRNA polymerase initiates transcription. Due to the positioning of mtTFA-binding sites adjacent to the promoter regions, one role of DNA binding is to lead the core polymerase to the sites of transcription initiation. The conformational change subsequent to DNA binding may further act as a recognition signal for the core enzyme and facilitate the initial melting of the DNA for transcript synthesis. The ability of protein factors to activate transcription through DNA bending is now a common theme in both prokaryotic and eukaryotic systems, and has been recently reviewed for prokaryotes (Van der Vliet and Verrijzer 1993; Perez-Martin et al. 1994).

Recently, a cDNA corresponding to the nuclear gene encoding h-mtTFA was isolated (Parisi and Clayton 1991). A search for possible homologs revealed two domains in h-mtTFA that are characteristic of high-mobility group (HMG) proteins. Of this protein family, the HMG-box domains of h-mtTFA are most closely related to those in hUBF, a human HMG protein that plays an important role in RNA polymerase-I transcription. These highly basic domains contribute to the general DNA-binding ability of HMG proteins, and their presence in h-mtTFA may explain its ability to bind DNA non-specifically. In addition, HMG proteins are also characterized by an acidic carboxy-terminal tail that has been postulated to interact with positively charged histones, which mitochondria appear to lack. Indeed, preliminary mutagenesis studies of h-mtTFA have shown that the HMG-box domains possess DNA-binding activity. However, the presence of the C-terminal domain is required for specific recognition of the promoter regions and transcriptional activation (D. Dairaghi and D. Clayton, personal communication).

The discovery of mtTFA in both mouse and human mitochondria indicates conservation of the mammalian transcriptional machinery, in spite of the diversity in their promoter structures. In support of this idea, heterologous in vitro transcription experiments have shown that a combination of mtTFA and core mtRNA polymerase from mouse is capable of accurate initiation from rat promoters, but not from those of human, gorilla or bovine mitochondria (Chang and Clayton 1986 b). It has also been reported that h-mtTFA can

substitute for m-mtTFA in transcription of the mouse promoter, but only if the core m-mtRNA polymerase fraction is provided (Parisi and Clayton 1991). Together these results suggest that the mtTFA protein is flexible in sequence recognition that and additional factors, or perhaps the core mtRNA polymerase itself, are the critical determinants of promoter recognition in mammalian mitochondria.

Xenopus laevis

The *Xenopus* mitochondrial RNA polymerase also consists of two separable components (Bogenhagen and Insdorf 1988). A protein of approximately 140 kDa, with sedimentation coefficients and chromatographic properties similar to the core mtRNA polymerases of human and yeast, has been shown to exhibit non-specific transcription and may be the core xl-mtRNA polymerase. At this time a dissociable factor required to confer specificity has yet to be purified. Limited characterization of the *Xenopus* RNA polymerase has suggested a preference for supercoiled DNA (Barat-Gueride et al. 1989) and the possibility that it acts cooperatively between adjacent initiation sites (Bogenhagen and Romanelli 1988). Because the *Xenopus* promoter lacks an upstream enhancing region, it is questionable how closely any specificity factor(s) will resemble the mtTFA proteins of mammals. Based on the compact nature of the *Xenopus* promoter, a specificity factor might bind the core enzyme directly and thus resemble the situation in yeast mitochondria.

Yeast

As in mammals and *Xenopus*, transcription of the yeast *S. cerevisiae*) mitochondrial genome is performed by a core RNA polymerase and a specificity factor which have been designated core sc-mtRNA polymerase and sc-mtTFB, respectively. Both components of the yeast holoenzyme have been purified to homogeneity (Kelly and Lehman 1986; Schinkel et al. 1987) and their cognizant nuclear genes cloned and sequenced (Kelly et al. 1986; Lisowsky and Michaelis 1988). The sequence of the gene encoding the 145-kDa core sc-mtRNA polymerase (termed RPO41) has revealed a number of amino-acid domains with significant homology to the RNA polymerases of bacteriophages T7 and T3 (Masters et al. 1987). The sequence of the gene encoding the sc-mtTFB protein has revealed regions of obvious homology with eubacterial sigma factors (Jang and Jaehning 1991). These regions in the bacterial proteins are involved in promoter recognition, promoter melting, interaction with the core RNA polymerase, and holoenzyme stability.

Mutagenesis studies have confirmed the importance of several regions of the sc-mtTFB gene. Only two mutations have effects on transcription both in vivo and in vitro. These mutations map to conserved residues in sigma factor-like domains. However, a larger number of mutations alter other highly conserved residues in these regions and have no effect on transcription in vivo or in vitro. Finally, mutations that map outside the sigma factor-like regions have effects on transcription, at least in vitro, suggesting that other regions of the protein are important for function (Shadel, Clayton 1995).

Mobility shift assays have shown that, individually, the sc-mtRNA polymerase and sc-mtTFB proteins are capable of only weak and non-specific interactions with DNA (Schinkel et al. 1988 a). Formation of a stable pre-initiation complex requires the combination of both core and factor in the presence of promoter-containing DNA (Schinkel et al. 1987). These results, taken together with the compact nature of the yeast promoter, suggest that sc-mtTFB binds the core enzyme directly, as would be expected of a sigma-like factor, and stabilizes the holoenzyme. Consistent with this notion, over-expression of sc-mtTFB is capable of suppressing a mutation in the core sc-mtRNA polymerase (Riemen and Michaelis 1993). Once bound to the promoter, the yeast holoenzyme induces a bend in the DNA, the degree of which has been shown to be correlated with promoter activity in vitro (Schinkel et al. 1988 b). Thus, as in the mammalian systems, promoter conformation appears to play a direct role in yeast transcription initiation.

DNase-I footprinting revealed that the initiation complex protects approximately 30 bp surrounding the transcription site on both the coding and non-coding strands (Schinkel et al. 1988 b). These studies have also shown that synthesis of the first eight nucleotides increases the size of the footprint at the 3′ border, and suggest that a second conformational change occurs as the complex shifts from an initiating to an elongating mode. Interestingly, a similar pattern of changes is seen in the footprint of T7 RNA polymerase during the early steps of transcription (Ikeda and Richardson 1986), again consistent with a common origin for these two RNA polymerases. These and other studies have led to a working model for transcription initiation in yeast mitochondria. First, direct binding of the sc-mtTFB protein to the core sc-mtRNA polymerase permits the formation of a non-specific complex on mtDNA. Next, the holoenzyme scans along the DNA until it reaches its target sequence, the nonanucleotide promoter. At this point, a stable pre-initiation complex is formed and this induces the conformational change required for the initiation of transcription (Schinkel et al. 1988 a). Finally, mtTFB is released from the core polymerase shortly after promoter clearance, and become available for a subsequent round of transcription initiation (Mangus et al. 1994).

Both mammalian and yeast mitochondria contain mtTFA proteins

In addition to sc-mtTFB, a second nuclear-encoded protein in yeast has been implicated in mitochondrial transcription. This 19-kDa protein, originally designated ABF2, HM or p19/HM, was identified by its ability to selectively bind autonomously replicating sequences (Diffley and Stillman 1991), and by its ability to bind a promoter-containing fragment (Fisher et al. 1991). Yeast cells lacking this protein became rho$^-$, indicating that it is required for the maintenance of mtDNA. This protein was later renamed sc-mtTFA when analysis of its predicted amino-acid sequence revealed HMG-boxes similar to those of h-mtTFA (Fisher et al. 1992). Other properties shared by the two proteins include phased DNA binding, generalized wrapping and unwinding of DNA, specific binding to and bending of promoter-containing DNA (Diffley and Stillman 1992; Fisher et al. 1992), and the ability to enhance transcription in vitro (Fisher and Clayton 1988; Xu and Clayton 1992). It was subsequently shown that h-mtTFA can functionally replace sc-mtTFA both in vitro and in vivo (Parisi et al. 1993). In spite of its similarities to the human specificity factor and its essential role in the maintenance of mtDNA, sc-mtTFA is not an alternative transcription factor in yeast; it is unable to confer promoter recognition to the core sc-mtRNA polymerase in vitro and therefore cannot substitute for mtTFB (Xu and Clayton 1992). Instead, it is more likely that sc-mtTFA functions in genome organization and its binding near regulatory regions facilitates the access of other *trans*-acting factors involved in DNA replication and transcription. The specialization of mtTFA in yeast and animal mitochondria raises the question of whether an mtTFB-like protein, with an as-yet unidentified function, remains to be found in animal mitochondria.

Plants

The plant mitochondrial transcriptional machinery is less well-characterized than that of yeast or animals. Promoter characterization has so far been limited to interpretations arising from in vitro transcription of the maize *atp1* promoter with the partially purified maize RNA polymerase. The fact that this extract has shown accurate initiation from several maize promoters (Rapp and Stern 1992), as well as from the wheat *cox2* and soybean *atp9* genes (W. Rapp and D. Stern, unpublished data), may indicate less restrictive primary sequence requirements for this enzyme than its counterparts in other organisms, and suggests some degree of conservation may exist between the promoters and transcription machineries of the different plant species. However, the observation that the maize system is unable to transcribe 18s rDNA (Rapp and Stern 1992), in spite of run-on assays which indicate that this promoter is perhaps the strongest in maize mitochondria (Mulligan et al. 1991), may suggest the presence of more than one RNA polymerase in plant mitochondria, and/or the involvement of gene-specific transcription factors. One potential alternate transcription factor is the product of the maize *MCT* locus, as discussed above (Newton et al. 1995). The presence of multiple RNA polymerase activities would be analogous to the transcription of chloroplast rRNA genes by a RNA polymerase activity that is biochemically distinct from that which transcribes tRNA and protein-coding genes (Lakhani et al. 1992; Lerbs-Mache 1993; reviewed in Igloi and Kössel 1992), or to the apparent existence of both nuclear- and plastid-encoded forms of RNA polymerase that transcribe subsets of protein-coding genes (Hess et al. 1993).

Other organisms

RNA polymerases have also been partially purified from the mitochondria of the rat (Yaginuma et al. 1982), *N. crassa* (Kennell and Lambowitz 1989), and one trypanosome species, *Crithidia oncopelti* (Zaitseva et al. 1985). Studies with the rat mtRNA polymerase indicated that there may be more than one type of enzyme activity capable of producing transcripts from the D-loop region in vitro, but it was not determined if these transcripts are also found in vivo. The RNA polymerase from *Neurospora* mitochondria has been isolated by methods similar to those used in the human system and has a sedimentation rate comparable to that of the human, yeast and *Xenopus* enzymes. Characteristics of the trypanosome mtRNA polymerase include a preference for denatured DNA, a greater activity on mtDNA over nuclear-derived DNA, and optimal activity on single-stranded poly(dAT).

Mitochondrial polymerases may also function in the replication of mtDNA

There are indications that the mtRNA polymerases of some organisms play the role of a primase for mtDNA replication. This view is generally accepted for vertebrates and has been proposed for yeast (reviewed in Schinkel and Tabak 1989; Clayton 1991). In vertebrates, H-strand replication begins with transcription initiation at the LSP; a transition to DNA synthesis occurs at a series of conserved sequence blocks through the action of a ribonucleoprotein termed RNAse MRP (Clayton 1991). In yeast, the facts that the origins of replication were adjacent to promoters and that the disruption of the RPO41 gene resulted in a loss of mtDNA first led to this proposal. More recently, candidate enzymes with similar activity to the human RNAse MRP have been isolated from bovine (Dairaghi

and Clayton 1993), *Xenopus* (Bennett et al. 1992) and yeast mitochondria (Schmitt and Clayton 1992; Stohl and Clayton 1992) and, although not studied in as much detail, lend further credence to this proposal. The yeast (and possibly other) MRP is also involved in nuclear pre-5.8S rRNA processing (Schmitt and Clayton 1993), a fact which helps explain the relatively abundant nuclear (rather than mitochondrial) localization of MRP (Kiss and Filipowicz 1992; Kiss et al. 1992; Topper et al. 1992). A conservation of the dual role of mitochondrial RNA polymerases in transcription and replication in animals and fungi may reflect a conservation in polymerase structure and transcriptional strategies that would not be expected based on the dissimilarity of promoter structures. A detailed analysis of additional mitochondrial RNA polymerases will clarify whether this is universal feature of mitochondrial systems; there already exists evidence for such a replication mechanism among viral-like linear mitochondrial genomes, such as that of the green alga *Chlamydomonas reinhardtii* (Ma et al. 1992; Vahrenholz et al. 1993).

Summary

A diversity of promoter structures

It is evident that tremendous diversity exists between the modes of mitochondrial transcription initiation in the different eukaryotic kingdoms, at least in terms of promoter structures. Within vertebrates, a single promoter for each strand exists, which may be unidirectional or bidirectional. In fungi and plants, multiple promoters are found, and in each case, both the extent and the primary sequences of promoters are distinct. Promoter multiplicity in fungi, plants and trypanosomes reflects the larger genome size and scattering of genes relative to animals. However, the dual roles of certain promoters in transcription and replication, at least in yeast, raises the interesting question of how the relative amounts of RNA versus DNA synthesis are regulated, possibly via *cis*-elements downstream from the promoters.

Mitochondrial RNA polymerases

With respect to mitochondrial RNA polymerases, characterization of human, mouse, *Xenopus* and yeast enzymes suggests a marked degree of conservation in their behavior and protein composition. In general, these systems consist of a relatively non-selective core enzyme, which itself is unable to recognize promoters, and at least one dissociable specificity factor, which confers selectivity to the core subunit. In most of these systems, components of the RNA polymerase have been shown to induce a conformational change in their respective promoters and have also been assigned the role of a primase in the replication of mtDNA. While studies of the yeast RNA polymerase have suggested it has both eubacterial (mtTFB) and bacteriophage (RPO41) origins, it is not yet clear whether these characteristics will be conserved in the mitochondrial RNA polymerases of all eukaryotes.

mtTFA–mtTFB; conserved but dissimilar functions

With respect to transcription factors, mtTFA has been found in both vertebrates and yeast, and may be a ubiquitous protein in mitochondria. However, the divergence in non-HMG portions of the proteins, combined with differences in promoter structure, has apparently relegated mtTFA to alternative, or at least non-identical, physiological roles in vertebrates and fungi. The relative ease with which mtTFA can be purified (Fisher et al. 1991) suggests that, where present, it should be facile to detect. mtTFB may represent a eubacterial sigma factor adapted for interaction with the mitochondrial RNA polymerase. In plants, sigma-like factors capable of interacting with a eubacterial polymerase are found in chloroplasts (Lerbs et al. 1988; Tiller and Link 1993; Troxler et al. 1994) raising the possibility that a gene family in plants contributes transcriptional factors to both mitochondria and chloroplasts. In coming years, we can expect a more detailed analysis of RNA polymerases, accessory factors and promoter structures, which will lead to a better understanding of the different modes of mitochondrial transcription initiation in eukaryotic species and the evolutionary relationships between them.

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