Expansion of CTG repeat sequences is associated with several human genetic diseases. We have examined the consequences of CTG repeat expansion for nucleosome assembly and positioning. Short CTG repeats are found within the most favored DNA sequences yet defined for nucleosome assembly. We find that as few as six CTG repeats will facilitate nucleosome assembly to a similar extent as the 50 or more repeats found in disease genes. Thus an increase in nucleosome stability on expansion of existing triplet repeats is unlikely to explain the acquisition of the disease phenotype. However, the CTG repeat sequence is efficiently wrapped around the histone octamer, preferring to associate with histones at the nucleosomal dyad. Thus short segments CTG repeat sequence will facilitate the assembly of a stable positioned nucleosome which might contribute to the expansion phenomenon and the functional organization of chromatin.

Expansions of CTG trinucleotide repeat sequences are now associated with several human genetic diseases. These include myotonic dystrophy, spinal and bulbar muscular atrophy, Huntington’s disease, spinocerebellar ataxia, and dentatorubral-pallidoluysian atrophy (La Spada et al., 1991; Harley et al., 1992; Brook et al., 1992; Mahadevan et al., 1992; Fu et al., 1992; Orr et al., 1993; Huntington’s Disease Collaborative Research Group, 1993; Koide et al., 1994; Nagafuchi et al., 1994a; Burright et al., 1995; reviewed by Bates and Lehrach, 1994). The CTG trinucleotide repeats are found associated with certain genes whose products are necessary for correct cell function. Aberrant gene expression or the synthesis of a mutant protein from the gene following expansion of the number of CTG trinucleotide repeats, leads to the disease phenotype. The molecular mechanisms leading the number of CTG repeats to increase from the range of 6 to 50 found in normal individuals, and that expansion of CTG repeat sequences within the nucleosome dyad. Thus there is an apparent contradiction between the reduction in DNA flexibility and/or curvature in expanded CTG repeats (Chastain et al., 1995) and the increase in affinity for the histone octamer within the nucleosome (Wang and Griffith, 1995).

An increase in nucleosomal stability over expanded CTG repeats could potentially explain the increased protection of DNA segments containing triplet repeat expansions from DNase I cleavage observed in vivo (Otten and Taspov, 1995), the decline in expression of disease genes containing such expansions within the transcription unit (Carango et al., 1993; Fu et al., 1993; Hoffman-Radvanyi et al., 1993), and how CTG repeats might repress transcription from positions outside the transcription unit (Imagawa et al., 1995). Nucleosomes are known to protect DNA from nucleases (Noll, 1974), to potentially impede the progression of RNA polymerase II (Izban and Luse, 1991; Verdin et al., 1993), and to repress transcription initiation over promoters (Knezetic and Luse, 1986; Workman and Roeder, 1987; Lorch et al., 1987).

In this paper we examine the number of CTG repeats necessary to facilitate association of the histone octamer and the influence of flanking DNA sequences. We determine the structural features of this DNA in the nucleosome and the favored position of CTG repeat sequences within the nucleosome. We find that the phenomenon of nucleosome stability (Wang and Griffith, 1995) is conferred by relatively few CTG repeats such as found within normal individuals, and that expansion of CTG repeats does not increase the stability of histone-DNA interactions. The preference of CTG repeats to associate with core histones at the dyad axis of the nucleosome may contribute to the increased affinity of these sequences for interaction with core histones and the phenomenon of nucleosome positioning (Wang et al., 1995). DNA at the dyad axis of the nucleosome is known to have a relatively straight path in spite of histone association and to adopt an unusual helical periodicity (Hayes et al., 1990, 1991b; Arents and Moudrianakis, 1993).

**MATERIALS AND METHODS**

DNA Constructs—Plasmids containing a portion of the human DRPLA1 (dentatorubral-pallidoluysian atrophy) gene were a kind gift of Dr. Shoji Tsuji of the Brain Research Institute, Niigata University. The abbreviations used are DRPLA, dentatorubral-pallidoluysian atrophy; PCR, polymerase chain reaction; bp, base pair(s).
structure might facilitate the highly constrained wrapping of leads to the hypothesis that pre-existing elements of DNA (Hayes et al., 1993). The precise determinants of preferential histone association and the mechanism by which the histone octamer is positioned within the DRPLA patients contain 55 and 62 CTG repeats; incorporated into plasmids pCTG55 and pCTG62, respectively. Bluescript plasmid (Stratagene) containing the Xenopus histone gene cluster (Xh3a-B5) (Perry et al., 1985) was a kind gift of R. Roeder. The Xenopus borealis 5 S RNA gene was contained in plasmid XP-10 (Wolffe et al., 1988).

Competitive Reconstitutions—Plasmids were cut with the indicated restriction enzymes, labeled using a fill-in reaction, and the appropriate fragments were gel-isolated. Reconstitution was as described (Shrader and Crothers, 1989) except that 10 ng of labeled DNA, 1 μg of cores, and 1 μg of sonicated salmon sperm DNA was used. All volumes were scaled down appropriately and incubation was at 37°C instead of room temperature. Separation on non-denaturing gels was as described (Godde et al., 1995) and quantitation was by densitometeric analysis.

Translational Mapping—DNA from 3 × 100 μl PCR reactions (10 μg) was purified using a PCR clean-up kit (Promega) and used to reconstitute nucleosomes by dialysis (Camerini-Otero et al., 1976). Histones were purified from HeLa cells (O'Neill et al., 1992) and were used at 1 μg of histones per μg of DNA. The histone core was mixed with 2 M NaCl (100 μl total) and dialyzed overnight at 2 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM 2-mercaptoethanol. After this step, dialysis was into 1.2, 1, and 0.75 M NaCl in the above buffer with changes occurring every 2 h. Finally, the sample was dialyzed overnight into the above buffer without salt or phenylmethylsulfonyl fluoride added. Sixteen μl of the resulting sample was made 1 M CaCl₂ and digested for 5 min at room temperature with 0.075-0.15 units of micrococcal nuclease (Worthington). The reaction was stopped with a 2.5 × nolar excess of EDTA and made 0.25% SDS and 0.3 mM sodium acetate followed by phenol-chloroform extraction and ethanol precipitation. The resulting fragments were labeled with kinase and run on a 6% polyacrylamide gel for isolation of the 46-50 bp reflecting DNA within the nucleosome core that represents a stable kinetic intermediate in the digestion of nucleosomal DNA (Nil, 1974; van Holde, 1989). Isolated DNA was cut with restriction enzymes as indicated and run on a denaturing 6% polyacrylamide gel for analysis (see Godde et al. (1995)).

DNase I and Hydroxyl Radical Cleavage Reactions—Nucleosome particles used for footprinting were assembled by the exchange reaction as described previously (Tatchell and van Holde, 1977; Godde et al., 1995). The isolation of the chicken erythrocyte mononucleosomes used in this reaction as well as the details of DNase I footprinting were also as described previously (Hayes et al., 1991b). Hydroxyl radical reactions were as described (Wolff and Hayes, 1993). Autoradiographs were scanned using a Molecular Dynamics Personal Densitometer; data was read into an Excel spreadsheet using Crick Graph, plotted, and fitted in its final form using MacDraw II. The display of rotational setting was created by assigning peak values to densitometer plots, aligning these to the gene sequence using the G-reaction data, and by graphing this information using Microsoft Excel.

RESULTS

A Region Downstream of the Xenopus Histone H4 Gene Is the Strongest Known Naturally Occurring Nucleosome Positioning Sequence: Effects of Trinucleotide Deletions—One of the most preferred natural DNA fragments for association with the histone octamer and one of the strongest natural nucleosome positioning sequences is found within the X. borealis somatic 5 S RNA gene (Rhodes, 1985; Hayes et al., 1990; Schild et al., 1993). The precise determinants of preferential histone association and the mechanism by which the histone octamer is positioned relative to the 5 S RNA gene sequence are not entirely understood (FitzGerald and Simpson, 1985; Shrader and Crothers, 1989; Hayes et al., 1991a). However, hydroxyl radical cleavage has shown that the naked 5 S DNA contains a region of intrinsic structure that is located 15–70 bp downstream of the region of dyad symmetry and which is retained once DNA is wrapped in a nucleosome (Hayes et al., 1990). This leads to the hypothesis that pre-existing elements of DNA structure might facilitate the highly constrained wrapping of DNA within the nucleosome (Hayes et al., 1991a, 1991b). It has recently been described how DNA sequences containing trinucleotide repeats in the form of CTG position nucleosomes with greater affinity than any previously described DNA sequence, including the X. borealis 5 S RNA gene (Wang and Griffith, 1995).

We have tested whether the segment of the Xenopus H4 gene containing the six CTG repeats would preferentially assemble nucleosomes. The standard assay for preferential formation was pioneered by Shrader and Crothers (1989) and involves reconstitution of nucleosomes by an exchange reaction in the presence of both the labeled sequence of interest and unlabeled competitor DNA, followed by polyacrylamide gel electrophoresis. The association of the histone H4 gene fragment containing the six CTG repeats with the histone octamer is favored (Fig. 1A, lanes 2 and 4) compared to the formation of nucleosomes on the 5 S RNA gene (Fig. 1A, lanes 1 and 9) (see Table I for a quantification of the results). Comparable high efficiencies of nucleosome formation are obtained with a fragment of the human DRPLA gene containing 10 CTG repeats (Fig. 1A, lanes 3 and 5). Thus relatively short tracts of CTG repeats can facilitate nucleosome assembly.

We next examined the consequences of deleting the trinucleotide repeat sequences from the Xenopus H4 gene on preferential nucleosome assembly. The Xenopus histone H4 gene contains not only a stretch of CTG trinucleotides as in the DRPLA gene but also a stretch of CGG repeats upstream of the CTG trinucleotides. Interestingly, this almost exactly mimics the arrangement of the triplet repeats found in the human gene which causes Huntington’s Disease (Huntington’s Disease Collaborative Research Group, 1993). Deletion of the CTG repeats from the Xenopus H4 gene reduced the efficiency of nucleosome assembly (Fig. 1A, lane 7). The relative free energy of nucleosome formation on the H4 gene fragment following deletion of the CTG repeats is comparable to that of the 5 S RNA gene (Table I). We next deleted the CGG repeats as well as the CTG repeats. This deletion reduces the efficiency of nucleosome assembly of the H4 gene fragment to the level of the 5 S RNA gene (Fig. 1A, lane 8; Table I). We conclude that the CTG and CGG repeats make a major contribution toward the preferential association of the H4 gene fragment with the histone octamer.

Expansions of CTG Regions in the Human DRPLA Gene Do Not Lead to a Greater Affinity for Histone Octamers—Since a deletion of trinucleotide repeats within the Xenopus H4 gene leads to a decrease in the efficiency with which the histone octamer associates with this DNA (Table I), we next wished to investigate the effects of nucleosome formation of an expansion of these repeats. It has been reported that expanded repeats in the myotonic dystrophy gene lead to nucleosome formation efficiencies roughly twice that observed (Table I) for the “normal” DRPLA gene containing 10 CTG repeats (Wang and Griffith, 1995). We next determined the effects of increasing the number of CTG repeats from 10 to either 55 or 62 repeats. This was done using DNA derived from patients exhibiting disease symptoms (Nagafuchi et al., 1994b), guitho Shoji Tsujii. The propensity to form nucleosomes is not increased as the length of the trinucleotide repeats is expanded (Fig. 1B, lanes 2–4; see Table I). The Xenopus 5 S RNA gene was again used as a standard for nucleosome affinity (Fig. 1B, lane 1). We conclude that within the DRPLA gene as few as 10 CTG repeats
enhance nucleosome assembly and that further expansion of the number of CTG repeats to that found in disease states does not contribute to a direct increase in the efficiency of nucleosome assembly.

The Xenopus Histone H4 Gene Directs Nucleosome Position to a Single Location—We wished to understand the determinants of favored nucleosome assembly on DNA fragments containing CTG triplet repeats. We first examined the potential positioning of a nucleosome including a fragment of the Xenopus histone H4 gene containing CTG triplet repeats at high resolution. Rotational settings of DNA on the histone octamer within the reconstituted nucleosomes were examined using hydroxyl radical cleavage or DNase I digestion. A clear 10–11 bp repeat in hydroxyl radical cleavage is apparent over the intact histone H4 gene fragment following nucleosome reconstitution (Fig. 2, H4, lane 2) as are DRPLA genes containing expansions of the CTG repeat that were cut with Apal and BamHI to produce fragments of 249 and 256 for CTG tracks of 55 and 62 bp, respectively (lanes 3 and 4).

### Table I

Comparative free energies of nucleosome formation

| DNA                        | Free energy (cal/mol) |
|----------------------------|-----------------------|
| 5 S RNA gene               | -1750                 |
| Histone H4 gene            | -3206                 |
| H4 ΔCTG                    | -1958                 |
| H4 ΔCTG/CGG                | -1885                 |
| CTG<sup>10</sup>           | -2942                 |
| CTG<sup>25</sup>           | -2707                 |
| CTG<sup>52</sup>           | -2801                 |
| Mononucleosomal DNA        | 0                     |

DNA (compare lane 2 with lane 3 for each of the constructs). Although the CTG repeats are rotationally positioned on the surface of the histone octamer this analysis does not reveal the CTG repeats as naked DNA to have any intrinsic structure that is detected by the hydroxyl radical (Fig. 2, histone H4 gene, lane 5). Moreover the CTG repeats are clearly not essential for the rotational positioning of this DNA sequence on the surface of the histone octamer.

The DNase I and hydroxyl radical cleavage patterns due to the rotational positioning of DNA on the surface of the histone octamer were examined more closely (Fig. 3). The densitometer tracings clearly reveal the differential accessibility of hydroxyl radical and DNase I to different regions of the wild type and mutant H4 gene fragments including the CTG repeats (Fig. 3A, open box). An interesting feature apparent on the tracings is a region of DNA structure within naked DNA falling to one side of the nucleosome repeat boundaries (Fig. 3A, solid bar). This structural region spans an area that encompasses over four helical turns of DNA. The modulation in hydroxyl radical cleavage is consistent with variations in minor groove width (Hayes et al., 1991a), these variations are maintained and exaggerated following incorporation of this DNA segment into a nucleosome (Fig. 3A, compare Oct-DNA and Naked DNA tracings for hydroxyl radical cleavage). The presence of this element of intrinsic DNA structure may explain why even a deletion of both nucleotide repeat sequences from the H4 gene does not entirely eliminate rotational positioning of DNA on the surface of the histone octamer (Fig. 3, B and C) and does not reduce the free energy of nucleosome formation to the level of bulk mononucleosomal DNA (see Fig. 1 and Table I). Although the segment of intrinsic structure is not as apparent in the scans of hydroxyl radical cleavage of the deletion mutants (Fig. 3, compare A with B and C), expansions of the scans reveal the modulations in hydroxyl radical cleavage to be maintained (not shown). However, the modulations of hydroxyl radical cleavage for nucleosomal DNA assembled using the deletion constructs are reduced relative to the cleavage patterns obtained with nucleosomes assembled using the intact histone H4 gene segment (Figs. 2 and 3).

DNase I and hydroxyl radical cleavage data have been aligned with the sequence of the histone H4 gene (Fig. 4). A DNA frag-
Nucleosome Assembly on CTG Triplet Repeats

Our next experiments mapped the translational positions of nucleosomes reconstituted on the histone H4 gene. Translational position refers to exactly where DNA-histone contacts begin and end with respect to DNA sequence. This is a much more stringent test of the nucleosome positioning ability of a given sequence, since it is not uncommon for DNA sequences associated with genes to have a rotational preference with respect to the surface of the histone octamer within a nucleosome, but DNA sequences which lead to the assembly of a single translationally positioned nucleosome upon in vitro reconstitution are rare (Meersseman et al., 1991; Wolfe, 1995). Such translational positioning of nucleosomes has been suggested to occur from analysis at the electron microscopic level on DNA fragments containing CTG repeats within the myotonic dystrophy gene (Wang et al., 1995). Our experiments make use of controlled digestion with micrococcal nuclease to prepare nucleosome core particles from our synthetic chromatin template (Dong et al., 1989; Dong and van Holde, 1991). Mapping of translational positions was achieved by PCR amplification of the desired sequence, followed by nucleosome reconstitution by salt dialysis using purified histones. The nucleosome core particles are enzymatically trimmed using micrococcal nuclease, digestion products are end-labeled using \([\gamma^{-32P}]ATP\) and polynucleotide kinase, and the 146-bp kinetic intermediate of digestion is isolated from a gel. The isolated DNA is then subjected to restriction enzyme cleavage to reveal the boundaries of strong histone-DNA interaction. We find that a unique translational position for the histone octamer relative to DNA sequence results from nucleosome reconstitution on the intact histone H4 gene fragment containing both the CTG and CGG repeat sequences (Fig. 5A, histone H4 gene). A single lower band (40 bp) is present after digestion with the restriction endonuclease BstYI (Fig. 5A, lane 3), together with a tight cluster of bands migrating at approximately 100 bp, reflecting a single major translational position. Deletion of the CTG repeats does not affect the predominance of a single translational position, although the background of additional boundaries does appear somewhat higher and more core size DNA is resistant to digestion with the BstYI enzyme (Fig. 5A, ΔCTG). The ability to retain positioning may be due to the continued presence of the structured DNA region immediately adjacent to the CTG repeats (see Fig. 3). Deletion of both repeats, however, gives a greater heterogeneity in the positions of the histone octamer relative to DNA sequence (Fig. 5A, ΔCTG/CGG). Although some nucleosomes still appear to be present at the original position, considerable variation is now detected and a great deal of core sized DNA is not digested by the enzyme. In our experience the failure to digest is due predominantly to variability in the extraction of DNA from contaminants present in polyacrylamide gels that inhibit restriction endonucleases. It is also possible that a portion of the histone octamers assemble nucleosomes with this DNA fragment at positions that do not contain the BstYI site.

Analysis of the translational positioning data (Fig. 5B), reveals that nucleosomes prefer to form with the CTG regions directly abutting the nucleosomal dyad (see also Fig. 4B). The CGG repeats, however, prefer to associate with histones toward the edge of the nucleosome. We suggest that any preference of the CTG repeats for association with the histones depends on their location at the dyad axis of the nucleosome.

Expansion of CTG Tracts in the DRPLA Gene Maintain Rotational Settings of DNA Sequence within the Nucleosome. But Loses the Single Translational Position—We have established that CTG repeats can contribute to the preferential interaction with the histones in the context of a positioned nucleosome in vitro containing a fragment of the Xenopus histone H4 gene (Figs. 1–5). We now examine the positioning capabilities of the human DRPLA gene. Periodic (10–11 bp) cleavage of DNA by DNase I (Fig. 6A) or hydroxyl radical (Fig. 6B) is clearly visible in nucleosomes reconstituted both on the normal gene containing 10 CTG repeats and the expanded “disease” gene containing 55 repeats. Mapping of translational positions reveals that the normal DRPLA gene containing 10 CTG repeats assembles a single positioned nucleosome (Fig. 7, page 15225)
However, mapping of translational positions on the disease gene containing the expanded repeats reveals a number of nucleosome positions, spaced by 3 bp (Fig. 7, A and B, CTG\textsuperscript{10}). A significant amount of undigested core sized DNA is also recovered. This undigested DNA reflects both the difficulty of digesting this DNA extracted from polyacrylamide gels to completion (data not shown), and the potential presence of nucleosomes at positions that do not include the Apal restriction site, or that have this site close to the end of the DNA fragment (e.g. the shaded nucleosome in Fig. 7B). The relative positions of the nucleosomes are shown (Fig. 7B). Additional digestions were performed to eliminate any additional orienta-

**Fig. 3.** Densitometer tracings of Xenopus H4 gene footprints. Line graphs of the data contained in Fig. 2. 3' and 5' ends of the DNA strand are marked at the top of each group of tracings as is an open box representing the area of CTG repeats and a solid bar indicative of a region of intrinsically structured DNA. An asterisk indicates a common G-residue as an aid in comparison. Oct-DNA denotes nucleosomal DNA.

**Fig. 4.** Rotational settings of Xenopus H4 gene constructs. A, data from Fig. 3 is plotted relative to the sequence of the histone H4 gene constructs used in hydroxyl radical footprinting of nucleosomal DNA. Dotted lines are drawn through the regions of maximal cleavage in the H4 gene in order to facilitate comparison. The region of deletion in the H4 ΔCTG construct has been divided to maintain the alignment. Sequence is shown below the cleavage data as are 5' and 3' indicators. B, rotational settings of CTG and CGG trinucleotide repeats found in a reconstituted nucleosome on the histone H4 gene. Histone contacts are also shown, using translational position data from Fig. 5. Histone contacts are derived from histone octamer crystal structure (see Pruss et al. (1995), for details). Different core histones are marked at their amino- and carboxy-terminal extension, a dotted line being an approximate position of a flexible tail not defined in the crystal structure.
tions for the nucleosome other than that described: constructs CTG10 and CTG55 were mixed with the endonucleases NdeI and BamHI, respectively, however, no cleavage of the core sized DNA was detected (data not shown). The nucleosome assembled using the CTG10 DNA, like that incorporating the CTG repeat of the Xenopus histone H4 gene, has the trinucleotide repeats positioned directly adjacent to the nucleosome dyad. Interestingly, both of these DNA fragments position nucleosomes with the last “G” of the CTG tract adjacent to the dyad. This may suggest that trinucleotide repeats are unidirectional in their positioning capabilities. The expanded repeat, however, appears to be unable to occupy a single position; although all of the positions detected have a portion of the repeat in contact with the nucleosomal dyad.

**DISCUSSION**

Our experiments were designed to examine the determinants of preferential nucleosome assembly on CTG repeat sequences (Wang et al., 1995; Wang and Griffith, 1995). We have confirmed the basic observation that CTG repeats favor the association of DNA with the histone octamer (Wang et al., 1995; Wang and Griffith, 1995). Our major conclusion is that this preference is conferred by as few as six CTG repeats within the X. laevis histone H4 gene and 10 CTG repeats within the human DRPLA gene (Fig. 1). Thus an increase in the stability of nucleosomes postulated to occur on expansion of the CTG repeats appears unlikely to contribute to the establishment of a disease state (Figs. 1–7). Our results help explain how the presence of short stretches of CTG repeats have a profound effect on the local architecture and function of chromatin (Otten and Tapscott, 1995; Imagawa et al., 1995).

The Influence of CTG Repeats on Nucleosome Assembly—
The properties of DNA that contribute to selective interactions with the histone octamer are incompletely defined. Intrinsic DNA curvature (Drew and Travers, 1985; Wolffe and Drew, 1989), anisotropic flexibility (Shrader and Crothers, 1989, 1990), and ease of local unwinding (Hogan et al., 1987; Pruss et al., 1994) all might contribute to the efficiency of nucleosome assembly. Recent experimental work indicated that expansions of CTG repeats within the myotonic dystrophy gene would also facilitate nucleosome assembly (Wang et al., 1995; Wang and Griffith, 1995). This preference appears paradoxical since earlier work demonstrated that the triplet CTG has no rotational preference in mapping around the histone octamer (Satchwell et al., 1986) and by inference will not inhibit intrinsic DNA curvature or anisotropic flexibility. Consistent with these observations DNA fragments containing CTG repeats exhibit anomalous electrophoretic mobility reflecting a reduction in DNA flexibility and/or curvature (Chastain et al., 1995).

Our results indicate that the lack of intrinsic curvature/flexibility in CTG repeat DNA presents no impediment to the dominant constraint of DNA path by the histone octamer within the nucleosome (Figs. 2, 3, and 6; Hayes et al. (1991a, 1991b)). Exactly why no impediment to nucleosome assembly is presented by this DNA segment remains unclear, however, the favored position of the CTG repeat DNA within the nucleosome is at the dyad axis of the nucleosome (Figs. 4B, 5, and 7). At this position nucleosomal DNA has a special structure, it is understand to a helical periodicity of 10.7 bpturn (Hayes et al., 1990; 1991b; Arents and Moudrianakis, 1993) and has a relatively straight path with respect to the histone surface
The relatively straight CTG repeats (Chastain & Wolffe, 1993; Pruss et al., 1994), thus an extension of CTG repeats beyond 10 will lead the additional DNA to being more severely distorted around the core histones with a 10.0 bp/turn helical periodicity. This might account for the reduction in energy data in favor of preferential assembly (Shrader and Moudrianakis, 1993). Thus short segments of six CTG repeats are remarkably prevalent in the eukaryotic genome: more than 500 known genes in 1996 contain such sequences. More than 50 genes contain segments of 10 CTG repeats. Such repeat sequences are found in 5′, 3′, and coding sequences. All represent potential architectural features of regulatory significance within the chromosome. Nucleosomes of unusual stability containing such repeats might impede the progression of RNA and DNA polymerase through chromatin (Izban and Luse, 1991; Verdin et al., 1993). Inhibition of RNA polymerase might contribute to a reduction in transcriptional efficiency. Inhibition of DNA polymerase might facilitate expansion of the existing triplet repeats leading to a disease phenotype (Gacy et al., 1995). Stable positioning of nucleosomes can influence chromatin organization over extended DNA sequences (Roth et al., 1990). This transmissibility of chromatin organizational change due to the phasing of adjacent nucleosomes could account for the capacity of CTG repeats to repress transcription from positions that lie within and outside of the promoter region (Carango et al., 1993; Fu et al., 1993; Hoffman-Radvangy et al., 1993; Imagawa et al., 1995). Promoter activity is very sensitive to the exact context of histone-DNA interactions (Straka and Horz, 1991). Thus short CTG repeats might have the capacity to influence gene expression at a distance from the site of transcription initiation. Future experiments will explore the significance of these repeat at the 3′ end of the Xenopus histone H4 gene for chromatin organization and gene function in vivo.
