FMR1 CGG-Repeat Instability in Single Sperm and Lymphocytes of Fragile-X Premutation Males

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Summary

To determine the meiotic instability of the CGG-triplet repeat in the fragile-X gene, FMR1, we examined the size of the repeat in single sperm from four premutation males. The males had CGG-repeat sizes of 68, 75, 78, and 100, as determined in peripheral blood samples. All samples showed a broad range of variations, with expansions more common than contractions. Examination of single lymphocytes indicated that somatic cells were relatively more stable than sperm. Surprisingly, the repeats in sperm from the 75- and 78-repeat males had very different size ranges and distribution patterns despite the similarity of the repeat size and AGG interruption in their somatic cells. These results suggest that *cis* or *trans* factors may have a role in male germline repeat instability.

Introduction

Fragile-X syndrome (fragile X; MIM 309550) was the first triplet-repeat disorder identified that is caused by the expansion of a trinucleotide repeat. The fragile-X gene (FMR1) (Oberlé et al. 1991; Verkerk et al. 1991; Yu et al. 1991) contains a CGG repeat in the 5′ UTR that is unstably transmitted in families affected by fragile X. The repeat is polymorphic in the normal population and has a range of ~10–50 repeats, with 30 being the most commonly observed allele (Brown et al. 1993; Snow et al. 1993). Males and females with ~60–200 repeats are described as "premutation carriers," whereas those with >200 repeats are described as having the "full mutation." Because no clear boundary separates the car-

rier individuals from the normal population, repeats in the 50–60 range have been referred to as "gray zone" or "borderline" alleles (Eichler et al. 1994). Although premutation carriers are clinically normal, virtually all males and some of the females with the full mutation are mentally retarded. Full-mutation males also have physical and behavioral abnormalities and an inducible cytogenetic fragile site at Xq27.3 (Brown 1992).

One of the hallmarks of trinucleotide disorders is the unstable nature of the repeat, which increases in size from one generation to the next. In fragile X, expansion of the repeat is governed by several factors. The first factor is the gender of the transmitting parent. Expansion from a premutation to a full mutation occurs exclusively in transmission through females. Second, the larger repeats carried by premutation females are associated with greater risks of expansion to the full mutation in the next generation (Fu et al. 1991; Heitz et al. 1992; Yu et al. 1992). Third, the presence of AGG interruptions within the CGG-triplet array may stabilize the repeat region on transmission. Alleles in families affected by fragile X generally contain long arrays of CGG repeats with either one or no AGG interruption, whereas alleles in the normal population generally carry AGG repeats interspersed at regular intervals within the CGG repeat (Eichler et al. 1994; Kunst et al. 1994; Snow et al. 1994; Zhong et al. 1995).

There seems to be a strict limitation on the repeat size in the germline of males. Premutation males have only premutation daughters. More surprisingly, daughters of full-mutation males always carry the premutation and are clinically normal (Moric-Petrovic et al. 1983; Van Roy et al. 1983; Voelckel et al. 1988; Willems et al. 1992). Reyniers et al. (1993) examined sperm from four full-mutation males and identified only premutation alleles in all samples. Thus, from the pedigrees of families affected by fragile X and the presence of premutation alleles in sperm of full-mutation males, it is clear that fragile-X males do not transmit the full mutation, even when it is present in their somatic cells and they are affected by the syndrome. Nevertheless, daughters frequently carry a repeat size that differs from that carried by their premutation fathers. We previously analyzed the

repeat inherited by daughters of premutation fathers in 27 families affected by fragile X (Nolin et al. 1996). A majority (62%) of the daughters had larger repeats than their premutation fathers, whereas the remainder had either the same repeats (16%) or smaller ones (22%). Surprisingly, daughters with smaller repeats were observed only among fathers with $\geqslant$80 repeats. Analysis of paternal transmissions reported by other investigators (Fisch et al. 1995; Murray et al. 1997; Ashley et al. 1998) reveals a similar pattern of repeat contraction associated primarily with premutation males carrying larger repeats.

The CGG-repeat studies in premutation and full-mutation males raise several questions. Is the repeat more unstable in sperm than in somatic cells? What repeat sizes are present in the sperm of premutation males? Does a limit in repeat number exist in sperm? Do factors other than repeat size influence CGG stability in sperm? We approached these questions with an analysis of sperm from premutation males. Single-cell analysis of sperm (Jeffreys et al. 1988; Leeflang et al. 1994) or small-pool PCR (Jeffreys et al. 1994) permits the study of hundreds of meiotic events as an alternative to pedigree analysis, in which limited numbers of meiotic events occur in each generation. Single-sperm and small-pool analyses have been performed for several trinucleotide-repeat disorders, including spinal and bulbar muscular atrophy (Zhang et al. 1995), Huntington disease (Leeflang et al. 1995; Chong et al. 1997), and myotonic dystrophy (Monckton et al. 1995). Although, because of its GC composition, the fragile-X repeat is more difficult to amplify than other trinucleotide repeats, there are studies of the FMR1 repeat in sperm that include small-pool studies (Mornet et al. 1996), as well as analysis of two males with a normal allele size of 39 repeats (Kunst et al. 1997). Here we present a study of single sperm from premutation males that shows that the FMR1 CGG repeat is much more unstable in the germline than in somatic cells and that *cis* or *trans* factors may be an additional influence on repeat instability.

Subjects and Methods

Subjects

Sperm and peripheral blood samples were obtained from four unrelated premutation fragile-X males. The study was approved by the Institutional Review Board at the New York State Institute for Basic Research in Developmental Disabilities, and informed consent was obtained from the subjects.

Isolation of Single Cells

Sperm were prepared according to the procedures described by Leeflang et al. (1994). Leukocytes were iso-

lated from whole blood, with a modification of the technique described by Boyum (1968). Four milliliters 6% dextran in 0.9% saline were added to 10 ml heparinized whole blood. The blood was mixed by inversion and allowed to sit at room temperature for 30–45 min. The clear upper layer was transferred to a 15-ml tube and centrifuged for 5 min at 300 × g. The supernatant was discarded, and the pellet was resuspended. Six milliliters of cold solution (0.83% NH_4Cl, 0.1% $KHCO_3$, and 0.004% NaEDTA) were added for 6 min. After centrifugation as above, the supernatant was discarded, and the pellet was resuspended and washed in 10 ml cold 0.9% saline. The saline wash was repeated once. After repeat centrifugation, the pellet was resuspended in 0.5 ml PBS. The cells were vortexed, and 5 ml cold 70% ethanol was added during vortexing. The cells were passed through a syringe several times, placed on ice for 30 min, and stored at 4°C. Both cell types were fixed in 70% ethanol, treated with 0.3% Triton X-100, RNAse A, and stained with propidium iodide (10 mg/ml). The cells were stored at 4°C prior to fluorescence-activated cell sorting (FACS) by E. Yurkow at the Flow Cytometry and Image Analysis Facility of the Environmental Occupational Health Sciences Institute of Rutgers University. A sort gate encompassing the entire population of individual sperm was set on red-integrated and red-peak fluorescence to ensure that the cells selected by FACS represented a random sample. This gate also functioned to exclude sperm doublets and clumps/debris from analysis and sorting. One or two sperm were sorted into each well of a 96-well plate. Lymphocytes were selected from the prepared leukocytes, and one lymphocyte was sorted into each well. Four or eight wells were left blank in each plate for both sperm and lymphocytes as a negative control. The cells were lysed in 200 mM KOH, 50 mM DTT at 65°C for 20 min and were neutralized with 250 mM tricine (Leeflang et al. 1995).

PCR Analysis

Nested PCR was employed to amplify the FMR1 CGG repeat from single cells. These nested primers amplify premutation alleles without difficulty but do not amplify full mutation alleles efficiently. First-round primers were FRXPST283 (forward), 5'-AGG CGC TCA GCT CCG TTT CGG TTT CAC TTC-3' (Levinson et al. 1994); and primer 2 (reverse), 5'-TCC TCC ATC TTC TCT TCA GCC CT-3' (Brown et al. 1993). Second-round primers were primer 1 (forward), 5'-GAC GGA GGC GCC GCT GCC AGG-3'; and primer 3 (reverse), 5'-GTG GGC TGC GGG CGC TCG AGG-3' (Brown et al. 1993).

First-round PCR used 50-μl reactions in each well of a 96-well plate. The reactions consisted of 100 μM each of dATP, dCTP, dTTP, and 7-deaza-dGTP; 1 × Strata-
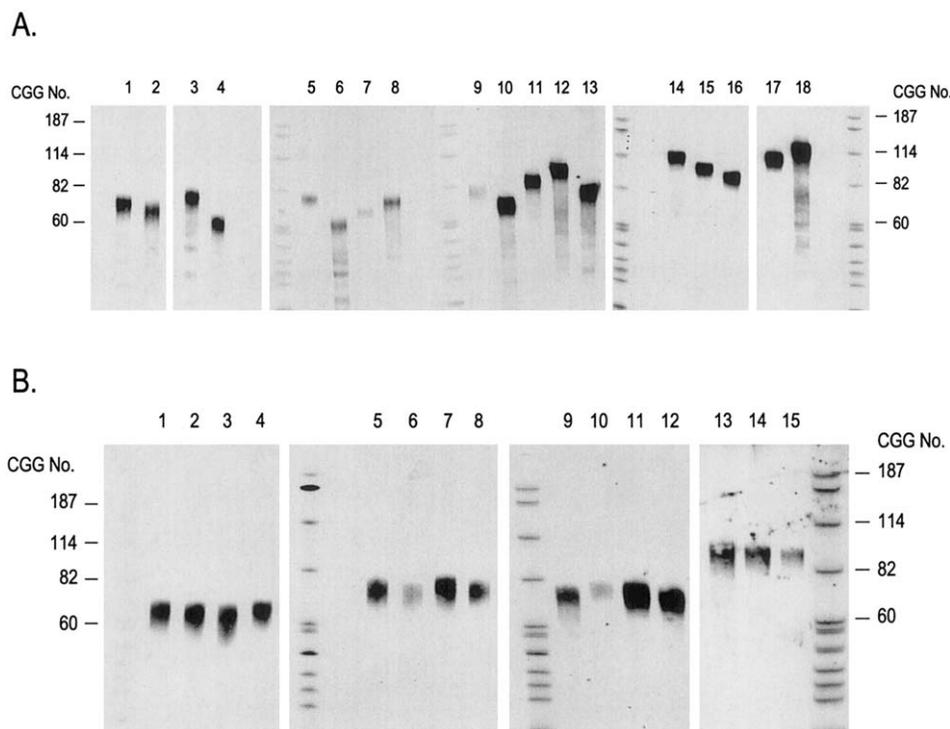
Figure 1 FMR1 CGG repeat in single sperm and lymphocytes from premutation males with 68, 75, 78, and 100 repeats. *A,* Single sperm. Lanes 1–4, 68-repeat male. Lanes 5–8, 75-repeat male. Lanes 9–13, 78-repeat male. Lanes 14–18, 100-repeat male. *B,* Single lymphocytes. Lanes 1–4, 68-repeat male. Lanes 5–8, 75-repeat male. Lanes 9–12, 78-repeat male. Lanes 13–15, 100-repeat male. End-labeled markers (pBR322 digested with *Msp*I) indicate the number of CGG repeats.

gene cloned *Pfu* DNA polymerase reaction buffer (20 mM Tris-HCl pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, and 100 μg BSA/ml), 2 mM $MgCl_2$; 0.1 mM each of outer primers FRXPST283 and 2; 12.5% dimethyl sulfoxide (DMSO); 1.25 U Cloned *Pfu* polymerase (Stratagene). The cycling was performed in a MJ Research PTC-100: 94°C for 4 min, followed by 20 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min. In second-round PCR, 1.3 μl first-round reaction product was added to each well of a 96-well plate containing 24 μl second round reaction mixture: 1 × Perkin-Elmer/Applied Biosystems Ampli-*Taq* DNA polymerase buffer II (10 mM Tris-HCl pH 8.3 and 50 mM KCl), 0.75 mM $MgCl_2$, 200 μM each of dATP, dCTP, dTTP, and 7-deaza-dGTP, 0.5 μM each inner primer 1 and 3, 10% DMSO, and 0.625 U Perkin-Elmer/Applied Biosystems Ampli*Taq* DNA polymerase. The cycling conditions consisted of 94°C for 4 min, followed by 25 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 2 min. Three milliliters of reaction product per well were analyzed by PAGE, transferred to nylon membrane (Biotrans-Plus Nylon; ICN Biomedicals) by electroblotting (semidry electroblotter; Integrated Separation Systems) with 2.8 × Tris-borate EDTA for 30 min at 1.5 mA/cm², and hybridized with $(CGC)_n$ probe

(Lifecodes) and a chemiluminescent detection system (Lifecodes). Autoradiography was performed at 37°C for 2–4 h.

Results

To investigate instability of the FMR1 CGG repeat in premutation males, we analyzed single sperm and lymphocytes from four unrelated males who carried 68, 75, 78, or 100 repeats in their blood. Aliquots of each sample were analyzed by nested PCR. Figure 1 shows examples of the single-cell studies of sperm (panel *A*) and lymphocytes (panel *B*) from the four premutation males. The single sperm from each male showed significant variations in repeat size and were distributed over a broad range, demonstrating a high degree of instability in the germline. In contrast, the respective single lymphocytes generally had very similar repeat sizes, with most of the repeats concentrated within a narrow range.

The results of the sperm and lymphocyte typing are shown in figure 2. In table 1, the CGG-repeat sizes of the single cells are compared with the repeat sizes in blood. For the male with 68 repeats (fig. 2*A*), 18% of sperm analyzed exhibited no change in repeat size, 57% carried expansions, and 24% carried contractions. The
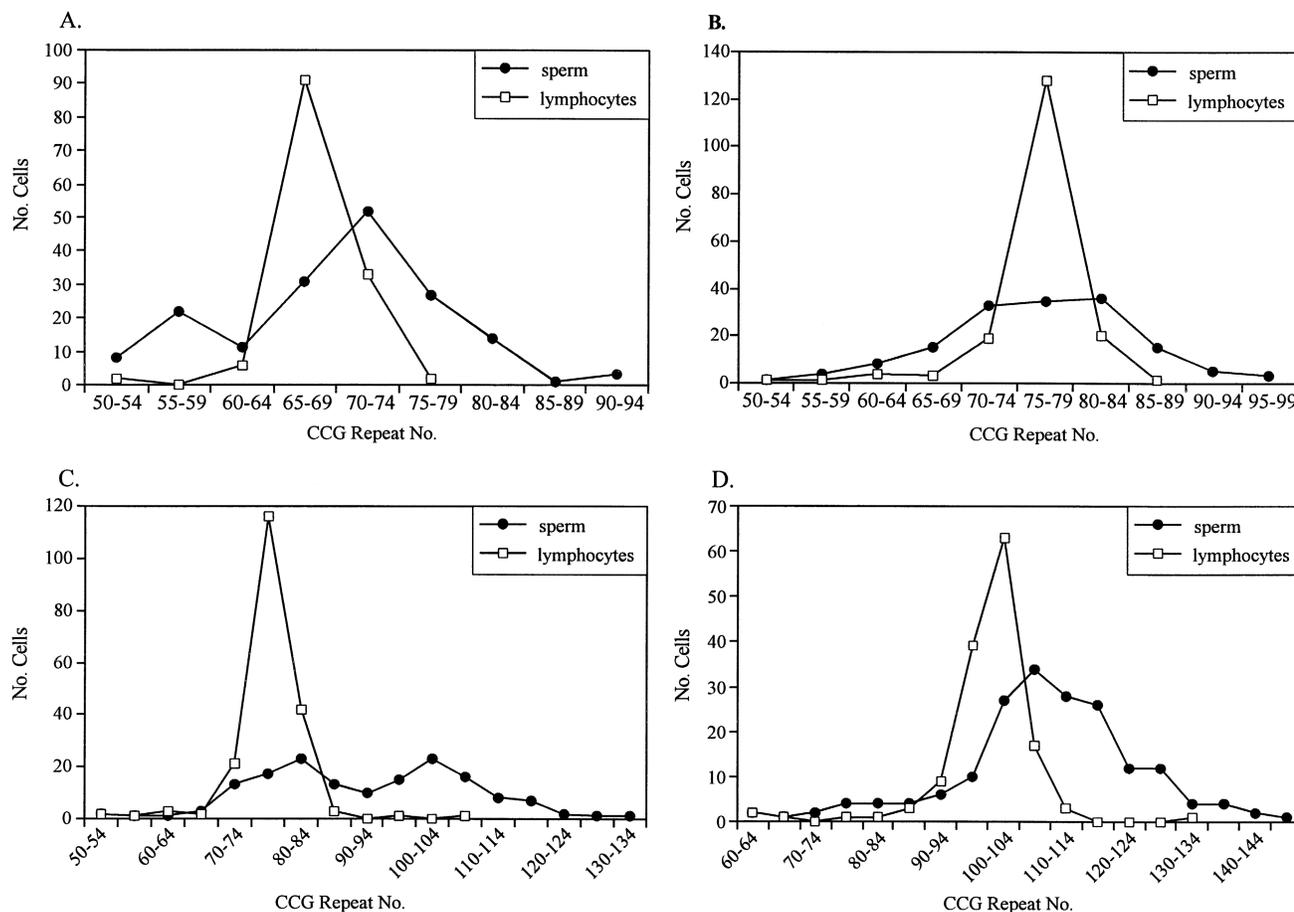
Figure 2 CGG-repeat distribution in single sperm and lymphocytes from four premutation males with 68 (*A*), 75 (*B*), 78 (*C*), and 100 (*D*) repeats. The CGG-repeat sizes are shown in intervals of five repeats. The number of cells in each interval is shown on the Y axis.

range in the number of repeats was 50–94. In lymphocytes, 68% of cells were unchanged, 26% expanded, and 6% contracted. Although the range in lymphocytes was 50–79 repeats, 97% were concentrated within 15 CGGs, as compared with 55% of sperm in the same interval. The means were similar, however, with 68 for the lymphocytes and 70 for sperm.

Analysis of single sperm from the male with 75 repeats (fig. 2*B*) showed 23% unchanged in size and nearly equal percentages of sperm expanded (38%) and contracted (39%), with a repeat range of 50–99. In the lymphocytes, 72% were unchanged, 12% expanded, and 16% contracted. In addition, 95% of lymphocytes from the 75-repeat male had repeats in a range of 15 CGGs, in contrast to 67% of sperm in the same interval. The means for both sperm and lymphocytes were 75.

Unlike the other samples, single sperm from the male with 78 repeats (fig. 2*C*) showed a bimodal distribution with peaks at 80–84 and 100–104 repeats. Approximately 11% of sperm were unchanged in size, whereas >76% of sperm showed an expansion—the greatest per-

centage observed in any of the samples. Contractions were observed in 13% of sperm, and the range in repeat sizes was 50–134. For single lymphocytes from the same male, 60% were unchanged, 24% had expansions, and 16% had contractions. The range of repeats was 50–109, with 93% of lymphocytes within a range of 15 CGGs, compared with 53% of sperm in the same interval. The means for this patient's lymphocytes and sperm, 76 and 90, respectively, showed the greatest difference among the patients analyzed.

Among the single sperm examined from the male with 100 repeats (fig. 2*D*), 15% were unchanged, 67% expanded, and 18% contracted. The range in repeat sizes was 60–149. In the single lymphocytes, 45% were unchanged, 15% expanded, and 40% contracted. Whereas the repeats ranged from 60–134, 91% of lymphocytes were grouped within 20 repeats, and 42% of sperm were grouped within the same interval. The mean repeat size in the sperm, 106, was 8 repeats greater than the mean in the lymphocytes (98).

The variances for both cell types from each male are

Table 1

Single-Sperm and Lymphocyte Typing from Four Premutation Fragile-X Males

CGG-REPEAT SIZE IN BLOOD AND CELL TYPE	NO. OF CELLS ANALYZED	MEAN CGG	NO. (%)			VARIANCE
			Unchanged	Expanded	Contracted	
68 Repeats:						
Sperm	169	70	31 (18)	97 (57)	41 (24)	66.64
Lymphocyte	134	68	91 (68)	35 (26)	8 (6)	10.17
75 Repeats:						
Sperm	155	75	35 (23)	59 (38)	61 (39)	70.90
Lymphocyte	177	75	128 (72)	21 (12)	28 (16)	18.17
78 Repeats:						
Sperm	156	90	17 (11)	119 (76)	20 (13)	239.88
Lymphocyte	193	76	116 (60)	47 (24)	30 (16)	34.46
100 Repeats:						
Sperm	183	106	27 (15)	123 (67)	33 (18)	212.19
Lymphocyte	140	98	63 (45)	21 (15)	56 (40)	60.22

shown in table 1. The variances indicate that the variation in repeat sizes was found to be highly significant (*F*-test, $P < .001$) between sperm and lymphocytes from the premutation males.

The repeat sizes of the single sperm were considered with respect to the ages of the four males, the repeat organization, and the CGG-repeat sizes of their daughters (table 2). Whereas the 68-repeat male was 32 years old, the age range of the remaining subjects was 64–72 years, which precluded conclusions relating age and repeat instability. The repeat organization of the premutation males was determined by sequencing from the 5′ and 3′ ends (data not shown). No AGG interruptions were present in the male with 68 repeats. The males with 75 and 78 repeats both had one AGG interruption at the 10th position, with 65 or 68 uninterrupted CGG repeats, respectively, at the 3′ end. The male with 100 repeats had two AGG interruptions, at the 10th and 20th positions, with 80 uninterrupted CGG repeats. The repeat sizes of the daughters of all males were well within the repeat ranges observed in the single-sperm analysis of their respective fathers, indicating that the experimental and the clinical data were consistent. These results suggest that repeat-size differences between father and daughter can be accounted for by instability in the sperm rather than by postzygotic instability.

Discussion

Our studies of the FMR1 CGG repeat in single sperm from four premutation males revealed a high degree of instability in the germline. Analysis of single lymphocytes exhibited a more modest instability, both in the frequency and in the range of repeat sizes. The differences observed indicate that the results accurately reflect the CGG repeats present in the two tissue types from the males and that the variations are not a consequence of PCR artifacts. The analysis of single lymphocytes and

sperm in our study was the first to examine germline instability of the FMR1 CGG repeat in premutation males from fragile-X families. Other investigators have examined the stability of the FMR1 repeat in sperm from males with high-normal or gray-zone alleles. The sperm of a male with 55 repeats showed greater variation than that of a 29-repeat male (Mornet et al. 1996). Kunst et al. (1997) compared repeat instability in two males with 39 repeats—one of whom carried 19 and the other of whom carried 29 uninterrupted CGGs—and observed a modest instability in sperm from the latter. Our results are consistent with the previous studies and add information about the stability of the repeat.

Our findings indicate that the patterns of repeat sizes in sperm can show surprising differences. The single-sperm studies of the 75- and 78-repeat males, for example, were particularly striking because they had virtually identical somatic repeat structure, but the patterns of expansions and contractions were very different. The sperm of the male with 75 repeats had a unimodal distribution, a mean of 75 CGGs, and a peak at 80–84 CGGs, with approximately equal numbers of sperm showing contractions and expansions. The largest repeats observed were ~95 CGGs. In contrast, the sperm of the male with 78 repeats had a bimodal distribution with peaks at 80–84 and 100–104 and a mean of 90. In addition, most of the sperm carried expansions, whereas few carried contractions. The largest repeats observed were ~130 CGGs. Analysis of the repeat structure revealed that both males carried a single AGG interruption at the 5′ end. Thus, the differences observed in the single sperm from these two males do not appear to be a function of AGG interruptions or of repeat size. This suggests that repeat size and structure are not the sole determinants of CGG-repeat instability in the male germline and that additional *cis* or *trans* factors may play a role in repeat transmission in the germline.

There is some evidence for the existence of *cis* and

Table 2

Age of the Premutation Males and Repeat Sizes of their Daughters

CGG-Repeat Size of Father	Age (years)	Repeat Organization	CGG-Repeat Size of Daughter(s)
68	32	$(CGG)_{68}$	None
75	64	$(CGG)_9AGG(CGG)_{65}$	75
78	72	$(CGG)_9AGG(CGG)_{68}$	78
100	65	$(CGG)_9AGG(CGG)_9AGG(CGG)_{80}$	105 and 120

trans elements affecting repeat instability in other tri-nucleotide-repeat disorders as well. In single-sperm studies of intermediate alleles in Huntington disease, Chong et al. (1997) observed a higher degree of instability associated with a *cis* element flanking the repeat. Studies in transgenic mice with constructs including the repeat and 5′ regions from affected individuals with myotonic dystrophy (Gourdon et al. 1997) and Huntington disease (Mangiarini et al. 1997; Monckton et al. 1997) have suggested the existence of both *cis* and *trans* factors in repeat instability in those diseases.

The single-sperm analyses reported here show evidence of meiotic instability in repeat size, with a broad range of contractions and expansions. Meiotic instability in premutation males has also been observed in many fragile-X pedigrees. As many as 66%–93% of daughters of premutation males have repeat sizes different from those of their fathers (Snow et al. 1993; Fisch et al. 1995; Nolin et al. 1996; Murray et al. 1997). Although expansions in daughters of premutation males have been seen in all size categories, contractions have been most commonly observed in daughters of males with ≥80 repeats. In our previous study (Nolin et al. 1996), 39.5% of daughters from fathers with ≥80 repeats inherited contractions, 18.4% inherited the same number of repeats, and 42.1% inherited expansions. Our single-sperm studies show that a majority of sperm from four premutation males underwent expansions to larger sizes. Sperm from the 100-repeat male, the one individual with >80 repeats, had more expansions than contractions. These results are consistent with our previous study, since as many daughters of premutation males with >80 repeats inherit expansions as contractions. The frequency of contractions may vary between individuals, just as expansions in the 75- and 78-repeat males do. The 100-repeat male has two daughters, one with an increase of 5 repeats and another with an increase of 15 repeats. This is consistent with analysis of his sperm, which supports the idea that contractions are not a common occurrence in this individual. Additional studies of single sperm from premutation males known to have daughters with contractions would clarify this question.

One of the most interesting questions in fragile X is the exclusive expansion to the full mutation through premutation females—and its corollary, the apparent in-ability of premutation and full-mutation males either to "create" the full mutation or to maintain it in sperm. Reyniers et al. (1993) first reported the presence of premutation alleles in the sperm of full-mutation males and suggested that the FMR1 protein might be required for germ-cell proliferation, with a resultant selection for those sperm expressing the protein. Development of the fragile-X knockout mouse (Dutch-Belgian Fragile-X Consortium 1994) with normal fertility, however, indicated that the protein is not essential for sperm maturation. Malter et al. (1997) analyzed gonads from two full-mutation male fetuses to determine the repeat size present in that tissue. Immunological staining with a FMR1 protein-specific antibody revealed no protein expression in the testis of a 13-wk male fetus, but revealed limited expression in the 17-wk male fetus.

We suggest that gametogenesis differences between males and females may explain the presence of premutation repeats in the sperm of full-mutation males. The differentiation of primordial germ cells into oogonia in females and into gonocytes in males occurs at an early embryonic stage. In the female, the ovarian stem cells undergo rapid mitotic divisions, emerging as oocytes from the 2nd mo of gestation. By 5 mo of gestation there are an estimated 6.8×10^6 germ cells, which decrease in number to 2.6×10^6 by birth. These cells have undergone ~22 mitotic divisions, with 2 additional meiotic divisions occurring after puberty, for a total of 24 divisions (Vogel and Rathenberg 1975). In spermatogenesis, the germ cells undergo numerous mitotic divisions from stem cells, resulting in an estimated 1.2×10^9 cells by the onset of puberty. With sexual maturity, the stem cells undergo continuous mitotic division, with each maturing spermatocyte going through two final meiotic divisions as well. By the age of 35 years, the number of cell divisions during spermatogenesis may be as high as 540 (Vogel and Rathenberg 1975). We hypothesize that contractions in repeat size occur in sperm because the long FMR1 CGG repeats are unstable over numerous mitotic divisions, resulting in a reduction from full-mutation– to premutation-size alleles.

If repeat contractions in sperm are the consequence of selection, at the DNA level, against long repeats over repeated mitotic divisions in the germline, then males affected by other trinucleotide disorders with long re-

peats should also show contractions in sperm. Of these disorders, FraXE is most similar to fragile X, because of both its location on the X chromosome and a CGG repeat, in the 5′ region of the gene, that is methylated in individuals with the full mutation. Carbonnell et al. (1996) examined the lymphocytes and sperm of one full-mutation male and observed that sperm carried a smaller repeat than that present in lymphocytes. Friedreich ataxia, the only autosomal recessive trinucleotide disorder identified to date, has >200 GAA repeats, in the affected state. Analysis of sperm and lymphocyte DNA from three carrier males (Pianese et al. 1997) revealed that the repeats in sperm were strikingly smaller and more variable in repeat number than were those in the respective lymphocytes. Finally, the congenital form of myotonic dystrophy, which is the most severe form and is associated with the largest amplifications, is inherited exclusively through maternal transmission (Tsilfidis et al. 1992; Lavedan et al. 1993; Mulley et al. 1993; Ashizawa et al. 1994). Analysis of males with myotonic dystrophy (Jensen et al. 1994; Monckton et al. 1995) indicates that, although males with smaller repeats may have repeat expansions in sperm, males with larger repeats carry smaller repeat sizes in sperm. Thus, analysis of these other trinucleotide-repeat disorders suggests a trend of contractions in sperm.

Two models for repeat instability have been proposed: DNA-polymerase slippage, or displacement of Okazaki fragments on the lagging strand during DNA replication. In both models, the formation of secondary structures (such as hairpins) within the repeats on the lagging strand may escape repair and cause expansion (Gordenin et al. 1997). Conversely, the formation of hairpins on the template strand could result in contractions. Although triplet-repeat instability is likely to evolve from a series of events, several studies in yeast have suggested that FEN1 endonuclease, an enzyme that functions in the processing of Okazaki fragments, may have a role (Freudenreich et al. 1998; Schweitzer et al. 1998).

Previous studies of sperm in full-mutation males and of transmission of the repeat from premutation father to daughter support a model of repeat contractions in the sperm of fragile X–affected males, as a result of selection at the DNA level in the male germline. The zygote begins with a single repeat that is subject to unstable replication at some point in development. The variable repeat numbers observed in Southern analysis of full-mutation males and mosaic full-mutation males (Rousseau et al. 1991; Nolin et al. 1994) demonstrate that mitotic instability occurs. Nevertheless, Wöhrle et al. (1993) observed that full-mutation alleles in fibroblast clones from an adult male and a 13-wk fetus were stable over serial passages. The authors suggested that repeat instability is limited to an early developmental stage. The single-cell results presented here are consistent with their conclusions. That is, the sperm, in an early developmental stage, exhibit instability, whereas lymphocytes show less significant instability.

Acknowledgments

Electronic-Database Information

The URL for data in this article is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for fragile-X syndrome)

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