

Role of the Pseudoautosomal Region in Sex-Chromosome Pairing during Male Meiosis: Meiotic Studies in a Man with a Deletion of Distal Xp

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Summary

Meiotic studies were undertaken in a 24-year-old male patient with short stature, chondrodysplasia punctata, ichthyosis, steroid sulfatase deficiency, and mild mental retardation with an inherited cytologically visible deletion of distal Xp. Molecular investigations showed that the pseudoautosomal region as well as the steroid sulfatase gene were deleted, but telomeric sequences were present at the pter on the deleted X chromosome. A complete failure of sex-chromosome pairing was observed in the primary spermatocytes of the patient. Telomeric approaches between the sex chromosomes were made at zygotene in some cells, but no XY synaptonemal complex was formed. The sex chromosomes were present as univalents at metaphase I, and germ-cell development was arrested between metaphase I and metaphase II in the vast majority of cells, consistent with the azoospermia observed in the patient. The failure of XY pairing in this individual indicates that the pseudoautosomal sequences play an important role in initiating XY pairing and formation of synaptonemal complex at meiosis.

Introduction

The human X and Y chromosomes pair at their distal short arms during male meiosis, the telomeres first approaching each other during the zygotene stage of meiotic prophase. As pachytene progresses, a paired segment is formed, and a synaptonemal complex (SC), which, on the average, extends to about one-third of the total length of the Y chromosome, can be seen (Solari 1980; Chandley et al. 1984). The human pseudoautosomal region (PAR) consists of approximately 3 Mb of DNA on the X and Y chromosomes (for review, see Ellis and Goodfellow 1989). DNA sequences within the PAR are identical between X and Y chromosomes and are maintained so by recombination between X and Y during spermatogenesis (Buckle et al. 1985; Cooke et al. 1985; Simmler et al. 1985).

It is thought that the pseudoautosomal sequences are important in initiating XY pairing, while the pairing observed proximal to the PAR in the sex bivalent is considered to be nonhomologous (Chandley et al. 1984).

The studies reported here were undertaken to determine the consequences of an inherited cytologically visible deletion of distal Xp for the pairing of X and Y chromosomes in male meiosis. The Xp deletion was identified in a male with ichthyosis, steroid sulfatase (STS) deficiency, and other abnormalities (Curry et al. 1984). The finding that pairing is completely inhibited between the sex chromosomes in this individual points to the importance of the PAR in the initiation of XY pairing in male meiosis.

Subject and Methods

Clinical Findings

Patient D.R. was a 24-year-old male at the time of this study, with short stature, chondrodysplasia punctata, ichthyosis, STS deficiency, and mild mental retardation. He was initially evaluated at age 13–19

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years, and detailed clinical findings have been reported elsewhere (Curry et al. 1984, individual III-14 in family A). An inherited deletion of distal Xp was identified in D.R., and his karyotype was designated 46,del(X)-(p22.32),Y (Curry et al. 1984). D.R. had undergone normal pubertal development and had adult-male secondary sexual characteristics. Serum-testosterone, follicle-stimulating-hormone, and luteinizing-hormone levels were within normal limits. His ejaculate contained no mature spermatozoa. A testicular biopsy was performed, and the tissue was used for meiotic chromosome investigations and testicular histology.

Somatic Cell and Cytogenetic Studies

Fibroblasts from a skin biopsy had been obtained from D.R. as part of his earlier work up. Fibroblasts were fused with mouse A9 cells deficient in hypoxanthine phosphoribosyltransferase, and hybrid clones were isolated in hypoxanthine-aminopterin-thymidine medium as described elsewhere (Mohandas et al. 1986). CF126, a clone retaining the del(X) chromosome, was identified and used for molecular definition of the deletion in D.R.

To determine whether the del(X) has telomeric sequences at its pter, fluorescence in situ hybridization (FISH) was carried out using biotinylated human telomere probe (Oncor P5097-B.5). Chromosomes were simultaneously hybridized to an X-specific alpha satellite probe (DXZ1; Oncor P5060-B.5), to identify the X chromosome. Metaphase chromosome preparations were obtained from the fibroblast culture by using standard procedures. Chromosomes were denatured in 70% formamide, $2 \times$ SSC at 70°C for 2 min. After ethanol dehydration of the slides, 30 μ l of human telomere probe (0.5 μ g/50 μ l) was combined with 0.1 μ l of the alpha satellite probe (0.5 μ g/1,050 μ l) and was hybridized to the chromosomes overnight at 37°C. Slides were washed in 50% formamide, $2 \times$ SSC at 37°C. Signal was detected with fluorescein isothiocyanate-conjugated avidin DCS (5 μ g/ml) (Vector labs) after amplification with biotinylated anti-avidin D (5 μ g/ml) according to the procedure of Pinkel et al. (1986), with minor modifications. Chromosomes were counterstained with propidium iodide in antifade solution and were examined with a Zeiss photomicroscope equipped for fluorescence microscopy.

Molecular Studies

High-molecular-weight DNA was extracted from hybrid clone CF126 containing the del(X) from D.R.,

as described elsewhere (Yen et al. 1984). DNA was digested with *Eco*RI and was hybridized to nine probes by using standard procedures (Sambrook et al. 1989). The probes used were 29C1 (*DXYS14*), 113D (*DXYS15*), p19B (12E7/*MIC2*), 38j (*DXS283*), pSTS311 (*STS*), dic 56 (*DXS143*), p18-55 (*DXS70*), p71-7A (*DXS69*), and pert87 (*DMD*, *DXS164*), all from the distal half of the short arm of the X chromosome (see Davies et al. 1990).

Meiotic Studies

Testicular biopsy from patient D.R. was used for meiotic studies and testicular histology. Prophase spermatocytes were prepared for microspreading according to standard methods (Chandley 1987). SCs were examined at the electron-microscope (EM) level after staining with silver nitrate and phosphotungstic acid. Air-dried slides for examination of metaphase I (MI) spreads by light microscopy were made according to the protocol of Evans et al. (1964), with minor modifications (Chandley 1987). A portion of the testicular biopsy was fixed in Bouin's fluid and sectioned for histological examination.

Results

Cytogenetic Studies

The presence of telomeric sequences at Xpter of the del(X) in patient D.R. was examined by FISH using a commercially available human telomere-specific probe. To identify the human X, chromosomes were simultaneously hybridized to an X-specific α -satellite probe. Thirty metaphases were examined in which the X chromosome could be identified unambiguously (fig. 1). A total of 122 fluorescent spots were observed outside their centromeric regions on these X chromo-

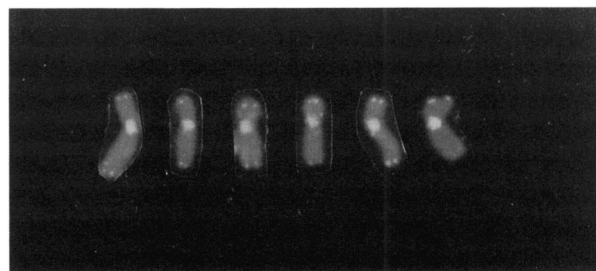


Figure 1 FISH of human telomere and X-specific centromere probes on the deleted X chromosome from patient D.R. Examples of the deleted X from six different metaphases are shown.

somes. Of these, 112 were located at the pter(57) or qter(55) of the del(X). These results indicate that the del(X) has telomeric sequences at the terminal end of its short arm.

Molecular Studies

For a molecular definition of the Xp deletion in D.R., DNA from hybrid clone CF126 was tested for the presence of sequences from distal Xp, by Southern analysis. Results presented in figure 2 show that pseudoautosomal sequences *DXYS14*, *DXYS15*, and *MIC2* are deleted from the X chromosome. In addition, the deletion also encompasses X-specific loci *DXS283* and *STS*; however, *DXS143* defined by probe dic 56 is present on the del(X). As expected, sequences known to be more proximal to *DXS143* (i.e., *DXS70*, *DXS69*, and *DXS164*) are also present on the del(X), as these were detected in the genomic DNA of hybrid

clone CF126 (fig. 2). When this deletion was characterized initially, we determined that *STS* and *MIC2* were missing from the distal short arm, by evaluating the expression of these two loci (Curry et al. 1984). In a second study, CF126 was used to help orient a long-range map of the *STS* region, and the breakpoint on the X in D.R. was determined to lie between *DXS237* (detected by GMGX9) and a more proximal *DXS278* sequence (detected by CRI-S232) approximately 8 Mb from the Xpter (Li et al. 1990). The results presented here show the loss of additional pseudoautosomal sequences, and, taken together with the previous findings, indicate that the deletion in D.R. has resulted in the loss of a contiguous segment of about 8 Mb from distal Xp.

Meiotic Prophase Analysis by Microspreading

A total of 50 zygotene-pachytene spermatocytes were analyzed at the EM level. Many other prophase spermatocytes that could not be analyzed showed degenerative changes and a high background level of nucleolus-organizing region (NOR)-like silver-positive material that obscured the SC structure. This particular feature has been noted previously in infertile patients showing germ-cell-maturation impairment (Speed and Chandley 1990). In the 50 healthier-looking cells, the morphology of the X and Y axes allowed them to be classified into stages described by Solari (1980). This indicated a large degree of maturation delay in germ-cell development, with 88% of all cells examined being in the zygotene-early pachytene stages (types O-II) and with only 12% showing thickened axes indicative of progression to the later pachytene stages (types III-V). The relative proportions of these two categories in men with normal spermatogenic development are about 30%:70% (Speed and Chandley 1990).

The cardinal feature in patient D.R. was, however, the total failure of XY-pairing initiation. None of the 42 cells examined at zygotene/early pachytene (types O-II) showed SC formation between the sex chromosomes. In about half the cells, the X and Y axes lay far apart, often on opposite sides of the cell, whereas for the remainder, although an approach for pairing appeared to have been made, pairing itself had not been initiated (fig. 3a and b). In some of these cells, the distal tips of the X and Y axes were closely associated, but no SC was ever formed (fig. 3c and d). A variety of end-to-end associations were in fact seen both within and between the sex chromosome axes, as illustrated in figure 3b, e, and f and shown diagrammati-

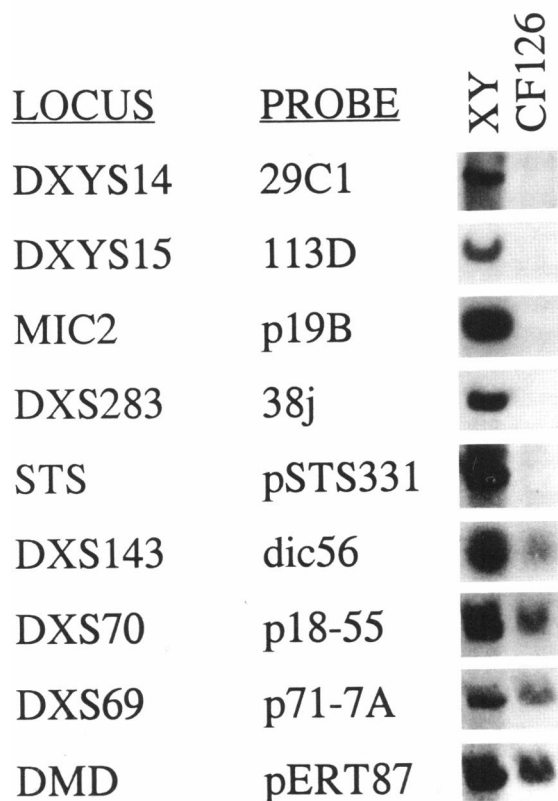


Figure 2 Southern analysis of DNA from hybrid clone CF126, using nine different probes, compared with that from a normal male control. Sequences identified by probes 29C1, 113D, p19B, 38j, and pSTS331 are absent from the deleted X of D.R., which is retained in hybrid clone CF126.

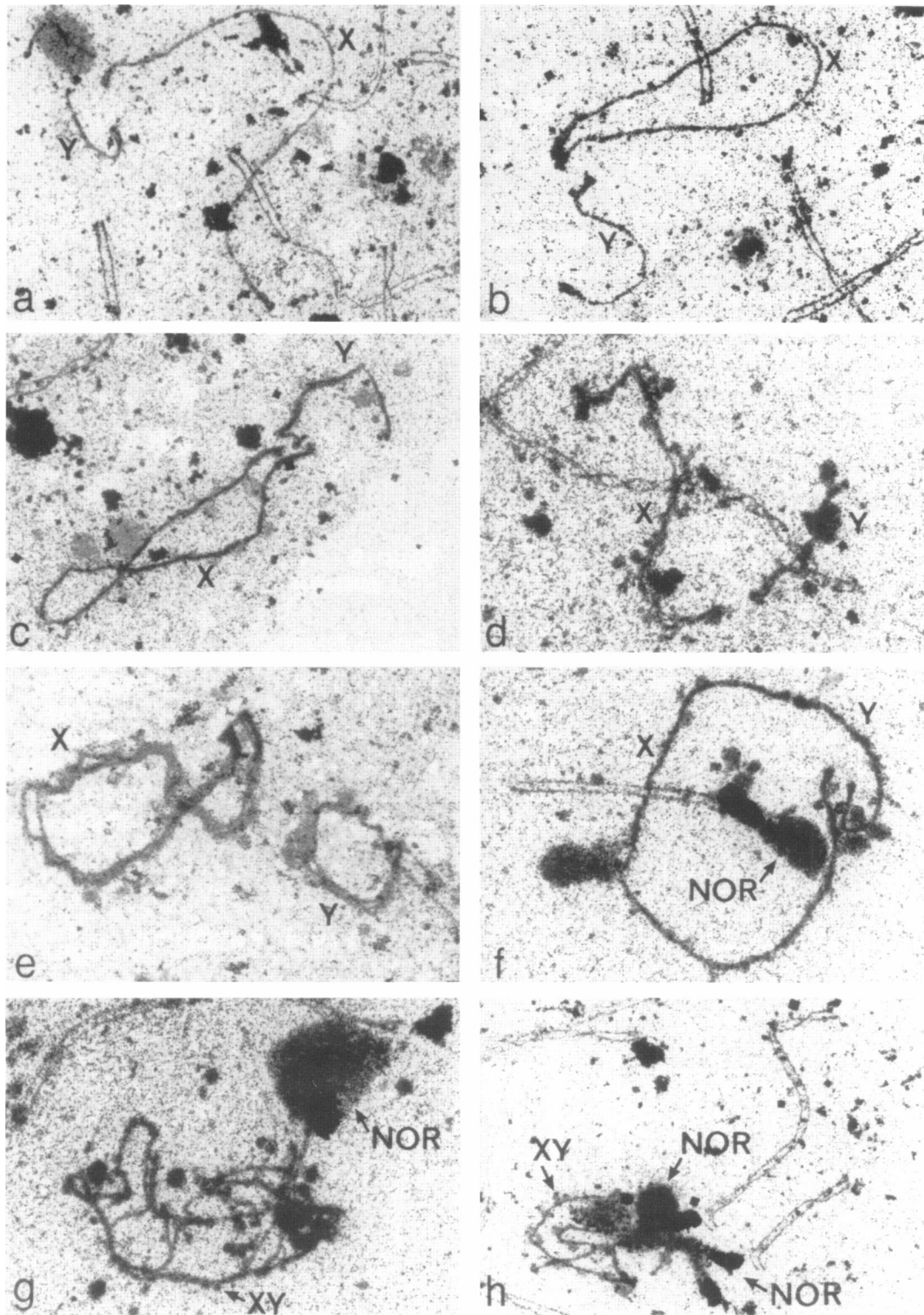


Figure 3 Electron micrographs of microspread preparations of zygotene–early pachytene spermatocytes from D.R. The X and Y chromosome axes are indicated. *f–h*, Associations between acrocentric NORs and the sex chromosomes.

cally in figure 4. Altogether 34/42, or 81%, of all types O-II cells examined showed some form of intra- or interchromosomal association between ends, e.g., XpXq (figs. 4c and d), YpYq (figs. 4b and d), and even triple or quadruple associations involving XpYpYq (fig. 4f), XpXqYp (fig. 4g), and XpXqYpYq (figs. 4h and i).

In this patient one final observation of interest was the very high level (72%) of XY-acrocentric NOR contacts seen at prophase (fig. 3f-h). In normal spermatocytes prepared by spreading, only about 2% of such contacts are seen (Speed and Chandley 1990), although, in men with severe oligospermia or azoospermia attributable to spermatogenic arrest, the frequency can rise to more than 60%, as observed in our patient (for review, see Johannisson et al. 1987).

Metaphase I Analysis by Air-drying

A distribution of 100 dividing cells in the air-dried preparations showed 12% in spermatogonial metaphase, 86% in MI, and 2% in MII. Compared with the distribution for normal men, these figures indicate an arrest in development between the first and second meiotic divisions, for the vast majority of spermatocytes. Examination of 50 MI spermatocytes showed the X and Y chromosomes to be present as univalents in every cell (fig. 5). The 22 autosomal bivalents appeared normally paired in every cell, and chiasma counts made on 10 cells showed values within the normal range (mean count/cell 52.0; range 47-59).

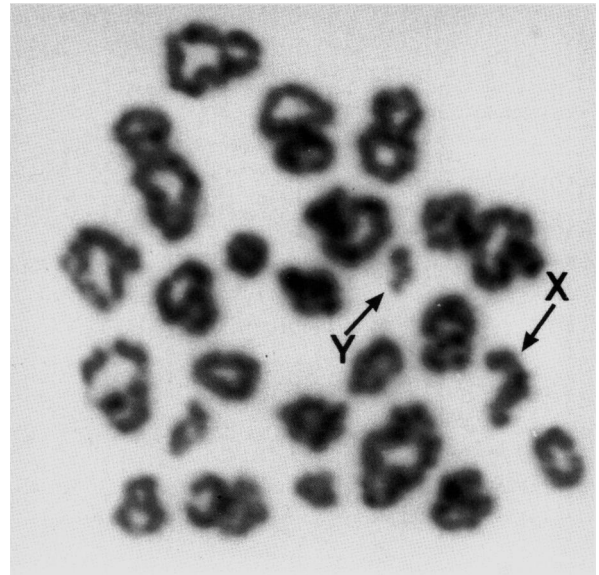


Figure 5 Air-dried MI preparation showing the univalent X and Y chromosomes.

Testicular Histology

In section, the seminiferous tubules were mostly of normal dimension, but one or two were small and had slightly thickened basement membranes. The cells lining the tubules were spermatogonia and spermatocytes with a rare spermatid. Spermatozoa were not identified. There were no other abnormalities of note.

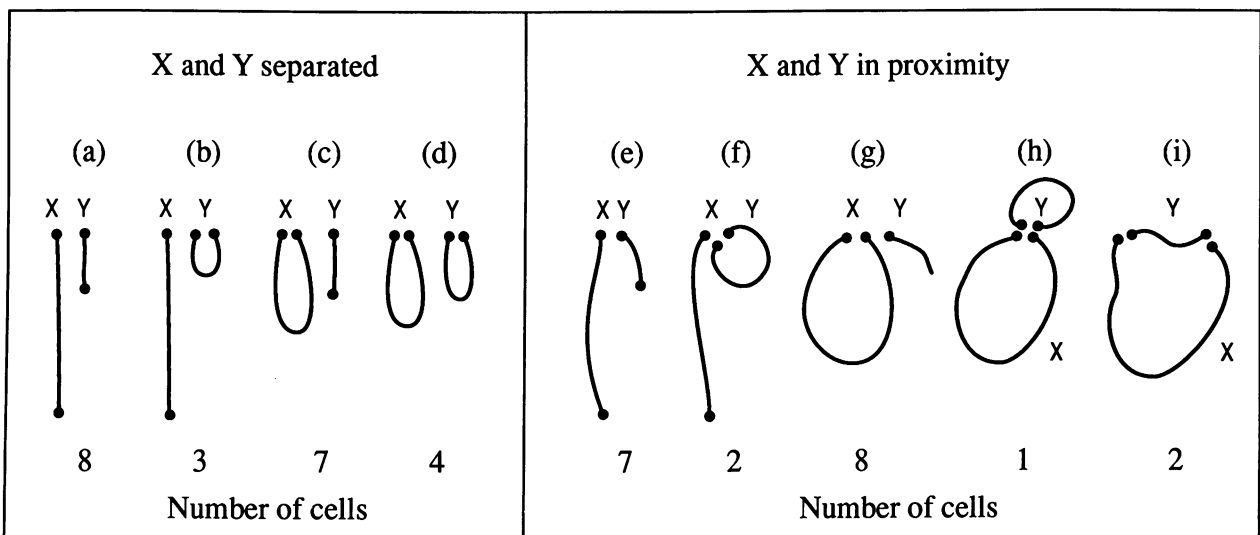


Figure 4 Classification of X and Y axes according to location in 42 cells analyzed at the zygotene-early pachytene stage, and the variety of telomeric associations seen both within and between axes.

The features were those of arrested spermatogenesis (fig. 6).

Discussion

The molecular studies clearly show that the majority, if not all, of the PAR is deleted from the X chromosome in patient D.R. *DXYS14* (detected by 29C1) is estimated to be within 25 kb of the Xp telomere (Cooke and Smith 1986) and is deleted from the X. On the proximal side, the breakpoint was determined to be between *DXS237* (detected by GMGX9) and a more proximal *DXS278* sequence (detected by CRI-S232), indicating that the size of the deletion is approximately 8 Mb and includes *STS* (Li et al. 1990). However, the del(X) does appear to have telomeric sequences, suggesting that the deletion may be interstitial rather than terminal. Alternatively, this may be a terminal deletion to which telomeric sequences have been added, deriving a healed end. Recent studies have shown that true terminal deletions in human chromosomes can be stabilized by addition of telomeric sequences (Wilkie et al. 1990; Morin 1991). Thus the molecular investigations do not resolve whether the deletion in D.R. is terminal or interstitial. The pres-

ence of telomeric sequences on the del(X) is consistent with the observed stability of this chromosome through multiple generations.

The meiotic studies in D.R. show failure of XY pairing and spermatogenic arrest. The arrest of spermatogenesis noted between MI and MII in D.R. is consistent with other reports of an association between XY-pairing failure and arrest of spermatogenesis, both in man and mouse (Chandley and Edmond 1971; Beechey 1973; Chandley 1973; Miklos 1974; Matsuda et al. 1991). The high frequency (72%) of contacts between the acrocentric NORs and the sex chromosomes seen in D.R. has also been observed among other sterile males, notably those carrying structural rearrangements involving acrocentric chromosomes, with the frequencies of contacts increasing as reductions in the sperm count become more severe (Chandley 1981). The contacts in our patient could have arisen as a secondary consequence of the initial XY-pairing failure. XY-autosome contacts are seen in about 7% of cells at meiotic prophase in fertile men (Speed and Chandley 1990).

Molecular and meiotic investigation in D.R. indicates that the PAR is necessary for effective pairing between X and Y chromosomes. These results are con-

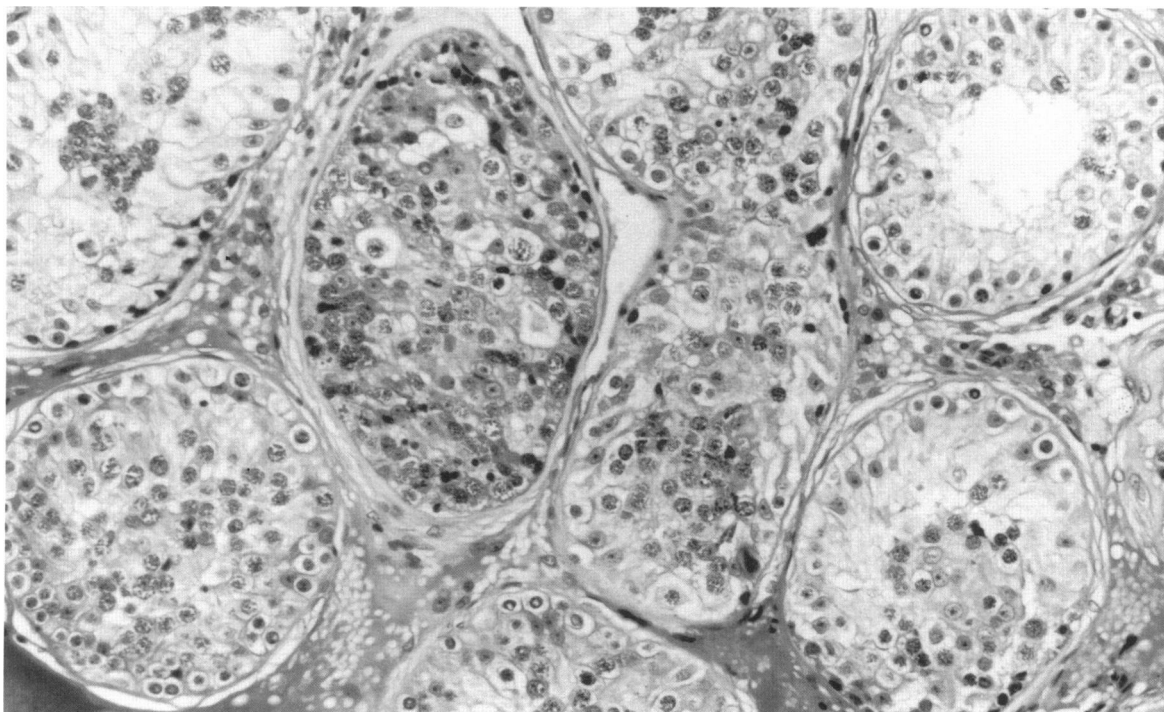


Figure 6 Histological section of the testis from D.R., showing spermatogenic development arrested at the spermatocyte level

sistent with the observations of Gabriel-Robez et al. (1990), who also found failure of XY pairing in a patient with a karyotype of 46,der(X),t(X;Y)(p22.3;q11),Y. The X/Y translocation chromosome was shown to be missing the majority of PAR, by dosage studies and by in situ hybridization. In the study reported by Gabriel-Robez et al. (1990), a second patient with an independent X/Y translocation (missing PAR) was also shown to have spermatogenic arrest. It was of interest that in these two cases no pairing took place between the derived X chromosome and the normal Y. Inhibition of pairing in Yq heterochromatin has previously been seen in meiotic prophase of an XYY male (Speed et al. 1991), and the same inhibitory effect of heterochromatin on Yqter synapsis is apparent in the X/Y translocation cases. Unlike the X/Y translocation cases, meiotic studies in D.R. allow an evaluation of the role of distal Xp on XY pairing, in the absence of any inhibitory effect introduced by the translocation of Yqh to distal Xp. The findings in D.R. reported here are also in keeping with the observations by Matsuda et al. (1991), who recently showed that the sterility in F1 males of the mouse interspecific cross, *Mus musculus* × *M. spretus*, is due to dissociation of the X and Y chromosomes in meiosis. Matsuda et al. (1991) further showed that the XY dissociation resulted from divergence of the XY-pairing region between the two *Mus* species. Taken together, these findings suggest that the PAR is important in initiating and maintaining effective pairing between mammalian X and Y chromosomes.

Telomere sequences are present at the pter of the del(X) in D.R., as demonstrated by the FISH studies. It was observed that, in many cells, the ends of the X and Y axes did approach each other and came together in close association, as if in preparation for pairing. However, the presence of telomeres at Xpter and Ypter alone appear insufficient for XY synaptic initiation. There was no evidence of nonhomologous pairing between Xp and the Y axis, even though pairing and SC formation can occur in regions of genetic dissimilarity (Moses 1977; for review, see Gillies et al. 1984). To explain the total lack of pairing observed, consideration must therefore be given to the deleted region itself and to the DNA sequences normally contained in it.

Although numerous cytological descriptions of meiotic pairing have been given (for review, see von Wettstein et al. 1984), the underlying molecular mechanisms are not yet understood. Nevertheless, meiotic pairing is generally considered to take place in two

phases, initial alignment occurring perhaps by matching of special sequences or blocks of sequences before precise DNA-DNA pairing for recombination is established (for review, see Chandley 1986). Molecular studies have revealed that both the PAR of the XY pair and the subtelomeric regions of autosomes in man are rich in repetitive sequences, including minisatellites (Cooke and Smith 1986; Royle et al. 1988) and subtelomeric interspersed repeat elements (Simmler et al. 1987; Rouyer et al. 1990). It has been suggested that these repeat sequences in human chromosome protermini, including the PAR, might act as promoters of initial pairing alignment (Chandley 1989).

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