Hum Genet (1984) 66 : 267–271

Human Genetics

Complete pachytene chromomere karyotypes of human spermatocyte bivalents

J. M. Luciani, M. R. Guichaoua, and M. R. Morazzani

Department of Embryology and Cytogenetics, Faculty of Medicine, Blvd. P. Dramard, F-13015 Marseilles, France

Summary. Well-spread human pachytene spermatocyte bivalents were obtained allowing specific identification of each bivalent within its total complement according to its chromomere sequence combined with further staining of its centromeric heterochromatin. The total number of chromomeres was found to be related to the degree of bivalent contraction: 396 in condensed bivalents and 511 in decondensed bivalents. A striking correspondance between chromomeres and mitotic G-bands was observed; on account of the variability of bivalent contraction, condensed bivalents corresponded to prometaphase somatic chromosomes and decondensed bivalents to mid/late prophase chromosomes.

Introduction

Recent progress in the study of human pachytene spermatocytes led to the establishment of complete karyotypes of the 22 autosomal bivalents originating from the same nucleus (Luciani et al. 1975; Jagiello and Fang 1982; Jhanwar et al. 1982). Compared to early investigators' attempts, this progress was achieved by obtaining well-spread pachytene bivalents following the introduction of prolonged hypotonic treatment. Then specific identification of the autosomal bivalents was obtained using the G-banding technique (Luciani et al. 1975) and more recently according to the number and distribution of the chromomeres (Jagiello and Fang 1982; Jhanwar et al. 1982). However, some disagreements exist between these reports about the precise identification of each bivalent. They are due to the difficulty in analyzing the chromomere pattern of the bivalents with the lack of visualization of the centromere as a primary constriction. Though centromeric heterochromatin is spontaneously present on pachytene bivalents and appears like a chromomeric structure, it sometimes can be confused with the other chromomeres. Thus, in order to obtain optimal identification of the 22 autosomal bivalents within their total complement each bivalent was accurately identified according to its chromomere pattern combined with the further staining of its centromeric heterochromatin. This investigation was extended to the determination of the number of chromomeres on male pachytene bivalents which was found to be in good agreement with that previously reported in meiotic cells (Jagiello and Fang 1982; Jhanwar et al. 1982) and with the number of G-bands described on prometaphase and late prophase chromosomes (Yunis et al. 1978, 1979; Francke and Oliver 1978; ISCN 1981).

Offprint requests to: J. M. Luciani

Materials and methods

Testicular biopsy specimens were obtained from 12 infertile subjects, aged 27 to 41 years, presenting with a varicocele and normal somatic karyotype. In order to obtain both well-spread pachytene bivalents and preservation of their chromomere structure, the testicular samples were treated according to a technique briefly described previously (Luciani et al. 1975). This technique which has the advantages of being very simple and allowing mailing of the samples needs to be detailed here. After removal, the testicular fragments were immediately immersed in 10 ml of 0.88% KCl and kept at room temperature for 8 to 10 h. The samples were then transferred in fixative (methanol/glacial acetic acid: 3/1 vol.); they were fixed for 12 to 18 h, usually overnight, at room temperature. The next day the fragments were shredded into the fixative solution. The cell suspension was pipetted into a conical vial and centrifuged at 800 rpm for 7 min. The pellet was resuspended in 5 ml of 45% glacial acetic acid, then immediately centrifuged at 800 rpm for 5 min. Spreads were made on clean precooled slides and gently dried over a low gas flame. The preparations were stained with 4% Giemsa solution, diluted in distilled water. The pH was adjusted to 6.7 with sodium phosphate buffer.

For the identification of the whole autosomal bivalents within their total complement, 10 well-spread pachytene cells with well-preserved chromomeres were selected and photographed using a Zeiss photomicroscope with a 100 × Plan apo objective. Negatives were printed at a magnification of × 2000. In each of the 10 complete pachytene nuclei, identification of the autosomal bivalents was carried out on the basis of their relative length and chromomere pattern. The position of the centromere could be deduced from the morphological aspect of the centromeric heterochromatin which usually appeared as a more spherical and darker-stained mass than the chromomeres. However sometimes the centromeric heterochromatin could not be distinguished from the chromomeres and precise identification of the bivalent was difficult. For this reason, after examination of these selected pachytene nuclei, Giemsa-stained slides were washed in methanol and treated for obtention of centromeric heterochromatin. The delay between examination of the slides and application of the C-banding technique did not exceed three weeks. The slides were placed in N HCl for 5 min at room temperature, then carefully washed in water and treated for 3 min in 5% barium hydroxide solution at 58° C. After washing the slides were placed for 20 min in 2 × SSC at 58° C and the pH was adjusted to 7.0. The slides were rinsed again and stained with Giemsa solution. The same pachytene nuclei were photographed again using a Zeiss

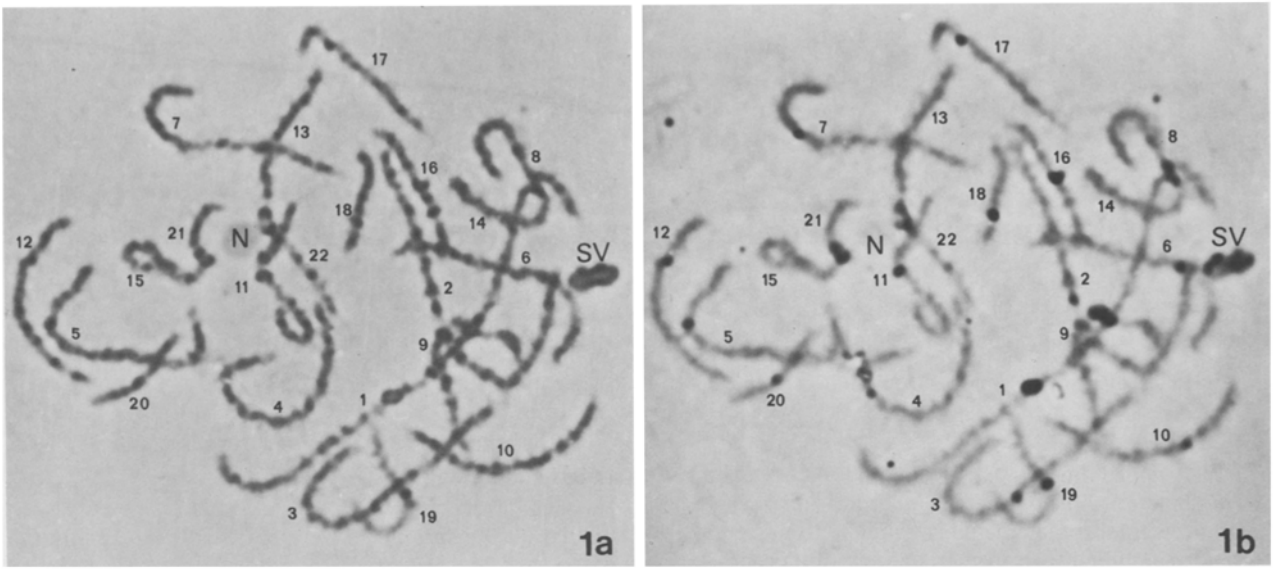

Fig. 1a, b. Human pachytene spermatocyte in which each of the 22 autosomal bivalents can be identified on the basis of its chromomere pattern (**a**) and centromere position following C-banding (**b**). N, nucleolus; S.V., sex vesicle (×1500)

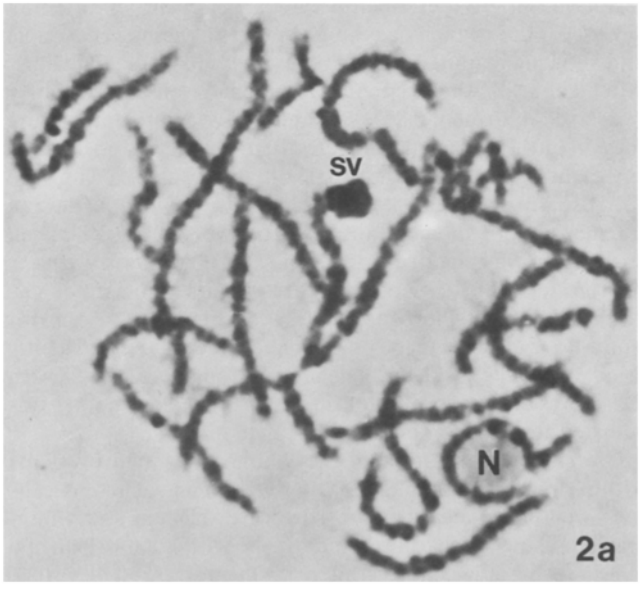

Fig. 2. a Human pachytene spermatocyte showing well-spread bivalents with good preservation of their chromomeres. N, nucleolus; S.V., sex vesicle (×1800). **b** Complete chromomere karyotype of the cell shown in (**a**) combined with a chromomere idiogram prepared from 20 selected bivalents of each type

microscope and a 100×Neofluar phase contrast objective. Negatives were printed at the same magnification as previously. The two prints originating from the same nucleus were then compared and the position of the centromeric heterochromatin was marked on the print with the chromomere pattern. Three out of ten selected nuclei were available for both examinations. Complete karyotypes were realized, then each autosomal bivalent was identified according to its chromomere sequence and centromere position.

Numbering of chromomeres was based on the selection of suitable pachytene nuclei which were photographied. Twenty bivalents of each autosomal type, free of overlaps, were analyzed. They were identified on the basis of their length, chromomere pattern, and centromere index. The chromomeres were counted and the average number was calculated for each type of bivalent. The bivalents were then classified as condensed or decondensed type, irrespective of the timing of the cell (early, mid, or late pachytene). Each bivalent whose chromomere number was lower than the mean number was designated as a morphological condensed bivalent. In contrast, each bivalent whose chromomere number was greater than the mean number was designated as a morphological decondensed bivalent. The mean value of chromomeres was calculated within each category of bivalents. Drawings of the chromomere distribution were made and then compared to the human G-band prometaphase mitotic chromosomes from Yunis et al. (1978, 1979), Francke and Oliver (1978), and ISCN (1981).

Results

Specific identification of each autosomal bivalent within its total complement was obtained on the basis of its length, chromomere pattern, and centromere position following C-band staining (Fig. 1). As previously reported (Luciani et al. 1975) stretching of bivalents 17 and 19 was particularly obvious and resulted in an increase of their relative length compared to the other bivalents (Figs. 1 and 2). C-banding performance was then very helpful for accurate identification of these two bivalents and its absence could have given rise to erroneous classification.

The chromomeres observed along 20 bivalents of each autosomal type were counted and their number varied according to the degree of contraction of the pachytene nucleus from which they originated. As shown in Table 1, the total number of chromomeres counted on the autosomal bivalents was 396 in the nuclei with the maximum condensation and 511 in the less contracted nuclei. A good correspondance was found between the chromomere pattern observed along each pachytene bivalent and the Giemsa dark bands obtained from mitotic chromosomes in various degrees of contraction (Yunis et al. 1978, 1979; Francke and Oliver 1978; ISCN 1981). Although the numbers of chromomeres and Giemsa dark bands are approximately the same (Table 1), some differences were noticed concerning the position of the chromomeres along some bivalents: stretching of some interchromomere regions, such as

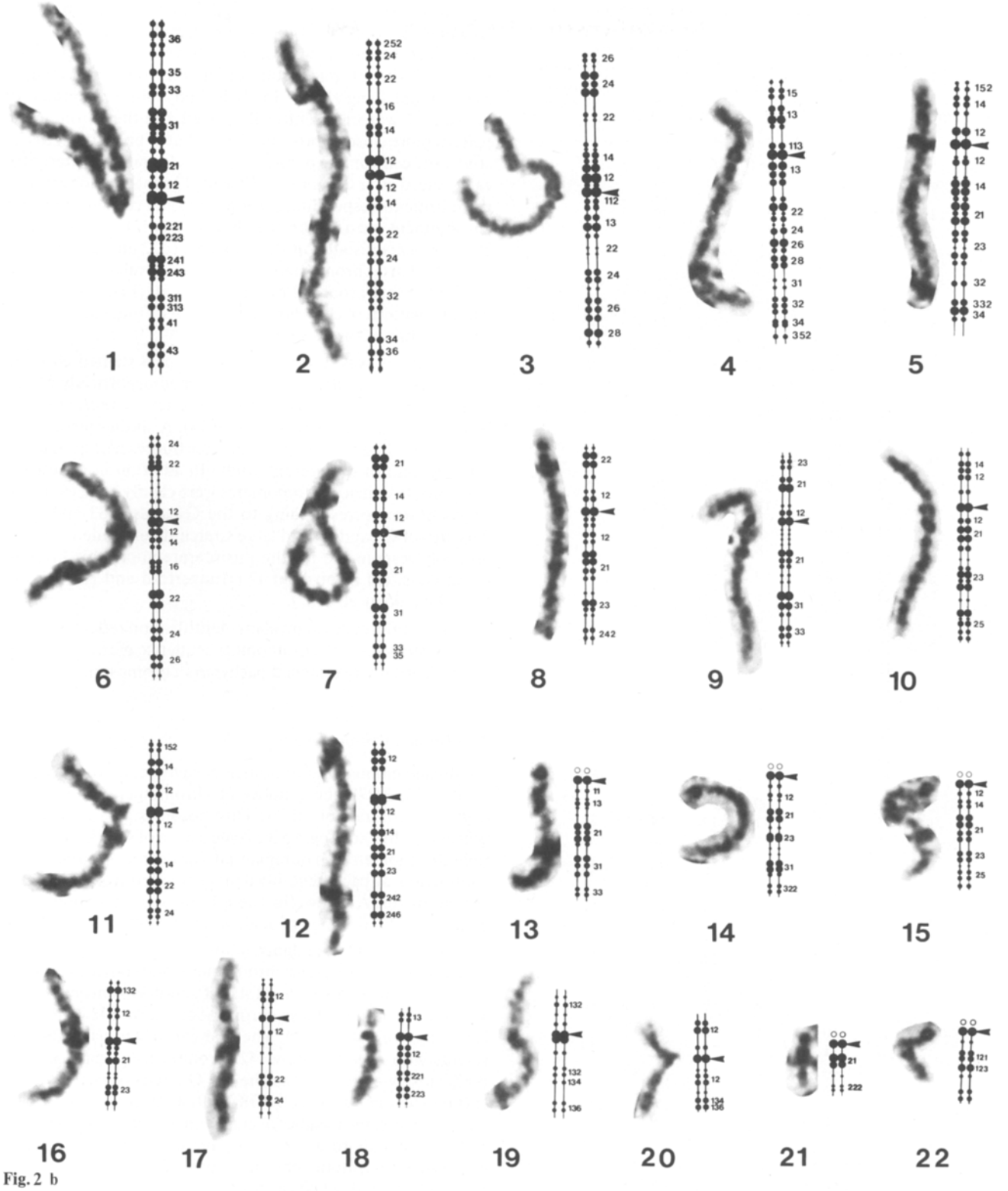

Fig. 2 b

6p21, 7q11, and 11q13, was found to be responsible for this discrepancy (Fig. 2).

Discussion

Technical improvements and identification of the bivalents

The technique of prolonged light hypotonic treatment combined with the short action of a 45% glacial acetic acid solution which we developed several years ago (Luciani et al. 1975) to obtain well-spread pachytene spermatocyte bivalents with well preserved chromomere structure, was used in this study. Similar techniques were recently used to give comparable results (Jagiello and Fang 1982; Jhanwar et al. 1982). These techniques have the advantage of allowing specific identification of each of the 22 autosomal bivalents within each cell selected for examination.

Table 1. Distribution of the number of chromomeres in human autosomal spermatocyte pachytene bivalents

	Condensed bivalents[a]			Decondensed bivalents[a]			Mid-condensed bivalents[a]
	p[b]	q[b]	p + q[c]	p[b]	q[b]	p + q[c]	p + q[c]
1	17[(1)]	20	36	21	24	44	
	19[(2)]	16	34	23	22	44	40[(3)]
2	12	19	30	17	24	40	
	12	19	30	17	24	40	30
3	12	13	24	16	16	31	
	14	14	27	18	21	38	28
4	7	16	22	10	22	31	
	8	19	26	11	24	34	27
5	6	16	21	7	22	28	
	7	14	20	8	21	28	26
6	8	15	22	9	20	28	
	9	14	22	11	19	29	30
7	9	14	22	11	19	29	
	10	12	21	11	17	27	26
8	7	14	20	10	16	25	
	7	13	19	10	19	28	19
9	7	14	20	9	20	28	
	8	11	18	8	14	21	21
10	6	13	18	9	15	23	
	7	14	20	8	17	24	20
11	6	12	17	9	14	22	
	8	12	19	9	14	22	17
12	6	12	17	9	17	24	
	6	13	18	7	17	23	18
13	0	15	15	0	18	18	
	1	13	13	1	16	16	17
14	0	14	14	0	18	18	
	1	16	16	1	18	18	18
15	0	12	12	0	16	16	
	1	15	15	1	19	19	20
16	6	8	14	7	10	17	
	8	10	17	10	11	20	13
17	5	10	14	7	12	18	
	6	9	14	7	12	18	15
18	5	10	14	6	11	16	
	3	9	11	4	11	14	11
19	5	6	10	6	8	13	
	6	7	12	8	10	17	10
20	4	7	10	5	8	12	
	5	7	11	7	9	15	14
21	0	7	7	0	9	9	
	1	6	6	1	7	7	11
22	0	7	7	0	9	9	
	1	7	7	1	9	9	11
Total			386[(1)]			499[(1)]	
			396[(2)]			511[(2)]	442[(3)]

[a] Pachytene bivalents classified as condensed, decondensed, mid-condensed, irrespective of the timing of the cell (early, mid, or late pachytene) in our study

[b] Centromere counted as one with each arm

[c] Centromere counted as only one for the whole bivalent

(1) Jagiello and Fang (1982)

(2) Present study

(3) Jhanwar et al. (1982)

The ability to compare the length and the chromomere pattern of each bivalent within its total complement might avoid errors in bivalent identification. However, concerning bivalents 17 and 19, our results differ from those reported by Jagiello and Fang (1982). In their study, the centromere of bivalent 17 was metacentrically placed and the chromomere pattern more closely corresponded to that of bivalent 19. In our study, the centromere of bivalent 17 was found submetacentrically placed after C-banding. Bivalent 17 appeared longer than the mitotic corresponding chromosome and carried one major chromomere centrally on the short arm (p12) and two major chromomeres distally on the long arm (q22 and q24) (Figs. 1 and 2). This chromomere pattern of bivalent 17 closely resembled that proposed by Jhanwar et al. (1982) and the G-banding pattern of chromosome 17 derived from late prophase somatic chromosomes (Yunis et al. 1979).

Bivalent 19 was described as a very pale stained chromosome with a large dark chromomere mediocentrically placed resulting from the fusion of centromeric heterochromatin with bands p12 and q12 (Luciani et al. 1975). A similar picture was reported by Jhanwar et al. (1982) after fluorescent quinacrine staining, and in the present study. In addition in contracted bivalents, two major chromomeres were clearly seen, everyone on each arm corresponding to the G-bands p132 and q132. Absence of C-banding and large stretching of bivalent 19 were probably responsible for the misinterpretation, this bivalent being classified as bivalent 17 (Hungerford and Hungerford 1978; Jagiello et al. 1982).

These differences in bivalent identification reflect the difficulty in analysing the chromomere sequence of these elongated and partially overlapped pachytene chromosomes.

Numbering of chromomeres

As already emphasized in human pachytene oocytes (Luciani et al. 1976, 1977), the number of chromomeres reflects the degree of contraction of the chromosomes, the contraction process leading to progressive coalescence of smaller and multiple chromomeres. In our material, the degree of variability in contraction of pachytene bivalents extended from 396 to 511 chromomeres. As shown in Table 1, this is in very good agreement with the findings of Jagiello et al. (1982): 386 to 499.

A striking correspondance in number and distribution was found between pachytene chromomeres and somatic G-bands. Contracted bivalents have a total of about 390 chromomeres, and mitotic prometaphase chromosomes have 378 bands (850 band stage of ISCN 1981). The mid-contracted pachytene bivalents with approximately 442 chromomeres (Jhanwar et al. 1982) closely correspond to the 423 G-bands of late prophase chromosomes (Yunis et al. 1978, 1979). In decondensed pachytene bivalents, the number of chromomeres increases up to 511 and corresponds to an intermediate stage between late and mid-prophase mitotic chromosomes. The high resolution achieved by mid-prophase mitotic chromosomes (798 dark Giemsa bands, Yunis 1981) is only reached by pachytene oocyte bivalents which are longer and more decondensed than those from spermatocytes. An estimation of the total number of chromomeres along pachytene oocyte bivalents calculated on the basis of our observations on bivalents 9, 13, 14, 15, 18, 21, and 22 (Luciani et al. 1977, 1978) could amount to approximately 830 G-bands. This strong correspondance between pachytene chromomeres and mitotic Giemsa dark bands observed at various degrees of contraction of meiotic and

mitotic chromosomes suggests the existence of a common mechanism in the process of chromosome contraction.

As chromosome contraction progresses with mitosis evolution, Jagiello and Fang (1982) closely correlated the progression of bivalent contraction with progression of the pachytene cells. Each cell was designated as early, mid, and late pachytene according to the length and chromomere pattern of its bivalents. This was based on the assumption of the occurrence of a progressive condensation of the autosomal chromatin from zygotene to late pachytene on. However, recent investigations on the behaviour of synaptonemal complexes in the human (Solari 1980) as well as in the mouse (Oud and Reutlinger 1981) have shown that chromatin becomes less condensed and diffuse in appearance at least during late pachytene. Thus, it is suggested that contracted pachytene bivalents could correspond to early/mid pachytene cells, meanwhile decondensed bivalents could correspond to the decondensation phase preceeding late pachytene despiralisation and pre-diffuse diplotene.

Stretching of specific pachytene bivalents

Frequently bivalents 16, 17, and 19 were found to be elongated in our observations based on the identification of pachytene chromosomes following trypsin treatment (Luciani et al. 1975) and the chromomere technique (present study). Difference in length was also reported for bivalent 22, which appeared longer than bivalent 21 (Hungerford et al. 1971). These cytological observations are enhanced by similar results obtained from synaptonemal complex measurements using the microspreading technique: bivalents 16, 17, 19, and 22 have significantly greater relative length than expected when compared to the mitotic chromosomes (Solari 1980). All the bivalents involved in this differential behaviour have much R-banded material. Thus, a correlation is strongly suggested between the amount of a chromosome in R-bands and its ability to lengthen.

Acknowledgements. We are grateful to Dr. A. Mattei and F. Benady for their kind cooperation in providing testicular material used in this study. This work was supported by a grant from the Caisse Nationale de l'Assurance Maladie des Travailleurs Salariés (C.N.A.M.T.S.) and from C.N.R.S., ERA n° 397.

References

Francke U, Oliver N (1978) Quantitative analysis of high resolution trypsin-Giemsa bands of human prometaphase chromosomes. Hum Genet 45 : 137–165

Hungerford DA, Hungerford AM (1978) Chromosome structure and function. IV. Pachytene chromomere maps of 16, 17 and 18; pachytene as a reference standard for metaphase banding. Cytogenet Cell Genet 21 : 212–230

Hungerford DA, La Badie GU, Balaban GB (1971) Chromosome structure and function in man. II. Provisional maps of the two smallest autosomes (chromosome 21 and 22) at pachytene in the male. Cytogenetics 10 : 33–37

ISCN (1981) An international system for human cytogenetic nomenclature: high resolution banding. Cytogenet Cell Genet 31 : 5–18

Jagiello GM, Fang JS (1982) Complete autosomal chromomere maps of human early and mid/late pachytene spermatocytes. Am J Hum Genet 34 : 112–124

Jhanwar SC, Burns JP, Alonso ML, Hew W, Chaganti RSK (1982) Mid-pachytene chromomere maps of human autosomes. Cytogenet Cell Genet 33 : 240–248

Luciani JM, Morazzani MR, Stahl A (1975) Identification of pachytene bivalents in human male meiosis using G-banding technique. Chromosoma 52 : 275–282

Luciani JM, Devictor M, Morazzani MR, Stahl A (1976) Meiosis of trisomy 21 in the human pachytene oocyte. Chromosoma 57 : 155–163

Luciani JM, Devictor M, Morazzani MR, Stahl A (1977) Pachytene mapping of the C_9 and acrocentric bivalents in the human oocyte. Hum Genet 36 : 197–204

Luciani JM, Devictor M, Boue J, Morazzani MR, Boue A, Stahl A (1978) Etude de la méiose ovocytaire chez un foetus trisomique 18. Comportement du chromosome surnuméraire et identification du bivalent 18. Ann Génét (Paris) 21 : 215–218

Oud JL, Reutlinger AHH (1981) Chromosome behaviour during early meiotic prophase of mouse primary spermatocytes. Chromosoma 83 : 395–407

Solari AJ (1980) Synaptonemal complexes and associated structures in microspread human spermatocytes. Chromosoma 81 : 315–337

Yunis JJ (1981) Mid-prophase human chromosomes. The attainment of 2000 bands. Hum Genet 56 : 293–298

Yunis JJ, Sawyer JR, Ball DW (1978) The characterization of high resolution G-banded chromosomes of man. Chromosoma 67 : 293–307

Yunis JJ, Ball DW, Sawyer JR (1979) G-banding patterns of high resolution human chromosomes 6–22, X and Y. Hum Genet 49 : 291–306

Received November 19, 1983