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Short Contribution

The pachytene chromomere map of the oocyte of the Turkish hamster *(Mesocricetus brandti)*

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ABSTRACT A study of the chromomere maps of the sex and twenty autosomal bivalents of Turkish hamster pachytene oocytes was carried out. The average total number of chromomeres in early/mid pachytene autosomes was 280 with 91 on the p (short arm) and 189 on the q (long arm). The submetacentric X_1 chromosome had 20 chromomeres and the metacentric X_2 had 27. Comparisons of the number and location of oocyte chromomeres are made with the pachytene spermatocyte chromomere maps of this species.

KEY WORDS: *pachytene oocyte chromomeres*

The recently hypothesized relationship of replication phenomena, isochores and chromomeres (Bernardi, 1989), the possible correlation of the «macrocoil ladder» and replicative differentiation (Haapala, 1984) and discussions of genomic imprinting (Monk, 1990) have refocused attention on vertebrate meiotic structure. One of the most revealing stages of meiosis is pachytene where detailed chromomeric/interchromomeric patterns reveal much of the basic structure of the chromatin (Jhanwar *et al.*, 1982). Though studies of pachytene spermatocyte maps of many species including Cricetidae (Yerganian, 1959; Fredga and Santesson, 1964; Vistorin *et al.*, 1977) have been published, sparse data on oocyte maps (Luciani *et al.*, 1977; Jagiello and Fang, 1980; Fang and Jagiello, 1983) are available. Pachytene chromomere maps of Turkish hamster *(Mesocricetus brandti)* oocytes had not yet been produced, but were considered to be of particular interest since meiosis in this species is a post-natal event. Thus it was the purpose of the present study to map the pachytene oocytes of the Turkish hamster, and to compare these results with the spermatocyte map previously analyzed (Fang and Jagiello, submitted for publication). In addition to the time of occurrence of female meiotic prophase, the presence of sex chromosome and autosome #16 polymorphisms made this a unique species for study.

Twenty autosomal bivalents and one sex bivalent were found. All autosomal bivalents and the two X chromosomes were identifiable by a characteristic chromomere pattern. All were normally paired except the number 16 bivalent. Since in this species a normal 16 and a $16p^-$ were seen in analyses of mitotic complements (Fang *et al.*, submitted for publication), these variations were sought at pachytene. A normal $16^N/16^N$ bivalent was seen in oocytes of 87.5% animals studied, in 12.5% as $16p^-/16p^-$ and in none as $16^N/16p^-$. Similarly, the two forms of the X chromosome, a submetacentric designated X_1 and a longer metacentric X_2 were seen paired at pachytene. In 62.5% of oocytes the X_1X_1 was seen, and in 37.5%, the X_2X_2, with none paired as X_1X_2.

The number and location of chromomeres is presented in Table 1. The average total number of chromomeres in the early/mid pachytene autosomal complements was 280 with 91 on the p arm and 189 on the q. The X_1 chromosome contained 20 chromomeres and the X_2, 27 chromomeres. The $16p^-$ had 8 chromomeres identical to the normal 16q. Major chromomeres were comparable to the dark G bands seen in the mitotic metaphase complements of this species (Fang *et al.*, submitted for publication).

The distribution of the chromomeres in the oocyte complement is illustrated in Figs. 1 and 2. The large pale interchromomere regions of chromosomes 1 and 3, previously seen in pachytene spermatocytes (Fang and Jagiello, submitted for publication) and mitotic complements (Fang *et al.*, submitted for publication) were also found in the oocytes. Similarly, dark staining regions (? heterochromatin) were detected on the short arms of some of chromosomes 5,7,8,9,10,11,13,14,15,16 and some of the long arms of chromosomes 19 and 20.

Comparisons of the number and location of chromomeres seen in the present study with those previously observed in spermatocytes of this species are tabulated in Table 2. Data are expressed as number of chromomeres identified on specific bivalents of spermatocytes in excess of those seen in oocytes, or (in brackets) number of chromomeres seen in oocytes, not observed in spermatocytes. Ten chromomeres were found on the spermatocyte autosomal bivalent short arms and twenty-two chromomeres on the long arms not found on comparable oocyte autosomal bivalents.

Abbreviations used in this paper: p; short arm of bivalent; q, long arm of bivalent; N, normal.

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TABLE 1

CHROMOMERE NUMBER AND LOCATION OF EARLY/MID PACHYTENE OOCYTES OF THE TURKISH HAMSTER

Autosomal Bivalent	p arm	q arm	Total*
1	7	15	22
2	11	10	21
3	7	14	21
4	6	11	17
5	5	11	16
6	5	10	15
7	5	11	16
8	7	10	17
9	5	11	16
10	4	11	15
11	5	9	14
12	4	9	13
13	3	9	12
14	3	8	11
15	3	9	12
16	3	8	11
17	4	6	10
18	3	5	8
19	-	7	7
20	1	5	6
Total	91	189	280
X_1	6	14	20
X_2	12	15	27

*Centromere not included

Fig. 1. A representative Turkish hamster pachytene oocyte.

Three chromomeres on the p arms of oocytes and six chromomeres on the q arms were not found on corresponding bivalents of spermatocytes.

The present data on the pachytene oocyte chromomere map of the Turkish hamster are the third set of such observations to be published. Previous publications have detailed the chromomere map of the mouse (Jagiello and Fang, 1980) and human oocyte (Luciani *et al.*, 1977; Fang and Jagiello, 1983). Contrary to the findings in these two species, the chromomere maps of the oocytes of the Turkish hamster revealed a considerable deficit of chromomeres in the autosomal bivalents (280) compared with spermatocyte autosomes (304). In mouse oocyte pachytene maps, chromomeres exceed spermatocyte chromomeres (268 vs 184) at comparable stages (Jagiello and Fang, 1980) and similarly in human the additional chromomeres were found in oocytes (639 vs 386) (Fang and Jagiello, 1983). Interestingly, mitotic G-bands in the two sexes of Turkish hamster are comparable in number and location, as they are for mouse and man (Jagiello and Fang, 1980; Fang and Jagiello, 1983). One variable for Turkish hamster which might be considered in the reversal of the chromomere findings for oocytes vs spermatocytes in other species is the fact that entry into meiosis in the female Turkish hamster is, as in the Golden hamster (Challoner, 1974), a post-natal event, while in mouse and man it is prenatal (Baker, 1963). An obvious correlation of endocrine environment with chromomere characteristics is nevertheless not apparent, but must be considered as a possible regulator of the meiotic process (Byskov, 1986).

Consideration of parental imprinting of genes of the Turkish hamster in this unique circumstance is also provocative. Searle *et al.* (1989) has noted that genomic imprinting in the mouse takes place in a portion of the genome producing differential paternal and

TABLE 2

NUMBER AND LOCATION OF ADDITIONAL CHROMOMERES OF EACH AUTOSOMAL PACHYTENE BIVALENT OBSERVED IN SPERMATOCYTES AS COMPARED WITH OOCYTES

Bivalent	p arm Regions		q arm Regions			Total
	1	2	1	2	3	
1	1	2	3	0	1	7
2	1	1	0	1	1	4
3	(1)*	2	2	2	0	5
4	0	1	2	0	2	5
5	0	-	0	0	2	2
6	1	0	0	1	1	3
7	0	0	0	0	(1)	(1)
8	(1)	0	(1)	1	-	(1)
9	0	0	0	0	(1)	(1)
10	(1)	-	0	1	0	0
11	0	-	0	1	-	1
12	0	-	0	0	-	0
13	0	-	0	0	-	0
14	0	-	0	1	-	1
15	0	-	(2)	0	-	(2)
16	0	-	0	0	-	0
17	1	-	0	0	-	1
18	0	-	0	0	-	0
19	-	-	0	(1)	-	(1)
20	0	-	0	-	-	0

*Number in parenthesis indicates additional chromomere present in oocytes

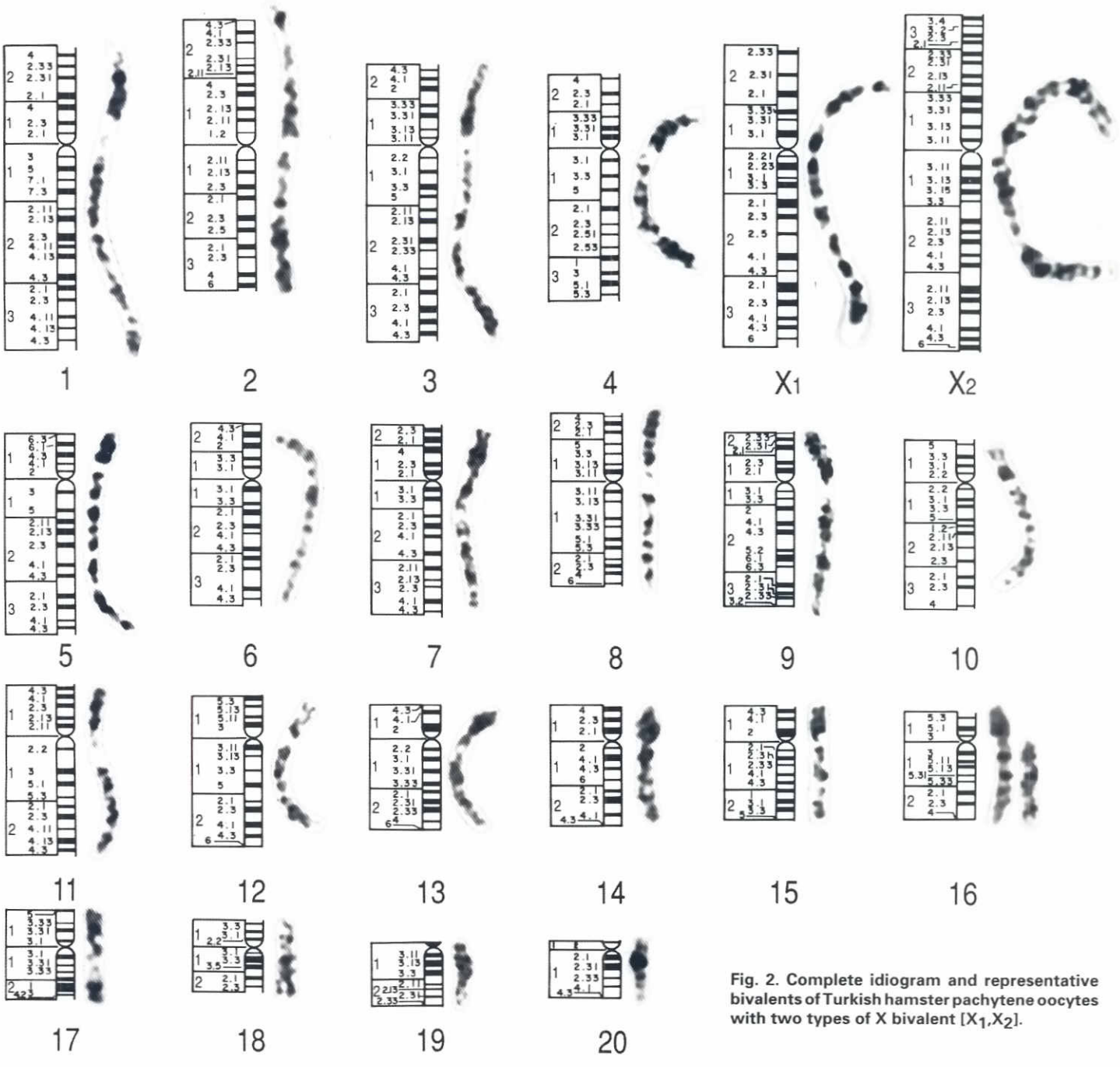

Fig. 2. Complete idiogram and representative bivalents of Turkish hamster pachytene oocytes with two types of X bivalent [X_1,X_2].

maternal transmission. Hall (1990) has suggested that pachytene is the meiotic stage where in mammals, imprinting, possibly methylation, might occur. Studies of genomic methylation, as a prime candidate for the imprinting mechanism, have indicated that the genomic alteration of germ cells does not survive to adulthood, with erasure early in gametogenesis (Monk, 1990). Genome differences in methylation between mouse oogenesis and spermatogenesis have been demonstrated and theorized to account for different regulation of parental genomes (Sanford *et al.*, 1987). If variable hormonal environment accounts for such sex differences (Byskov, 1986), tracking of specific sequence methylation through meiotic stages of both oogenesis and spermatogenesis could profitably be accomplished in a species such as the Turkish hamster where both phenomena occur soon after birth.

Experimental Procedures

Pachytene oocyte chromomere maps were obtained by analyzing ovaries from neonatal day 5 female Turkish hamsters. This day was selected from serial daily studies of meiosis in neonatal ovaries (Fang and Jagiello, unpublished observations). Eleven animals from eight different litters were sacrificed with chloroform, ovaries rapidly removed and placed into petri

dishes containing 0.05 M KCl solution for 45 minutes to 1 hour at 38°C. When the proper nuclear swelling was observed, ovaries were fixed with freshly prepared 3:1 absolute alcohol/glacial acetic acid. Each ovary was then cut into four pieces, squashed/smeared on a cold, clean wet slide which was air dried. Slides were stained with 1-2% Giemsa (Fisher). Intact, well-spread nuclei were selected for chromomere mapping (Fig. 1). Bivalents were classified into early/mid and late pachytene using the criteria of length and compaction of chromomeres. Drawings were made of selected complete well spread nuclei using a Zeiss 100X Neofluar lens and major and minor chromomeres were designated. The bivalents were identified by comparison with the previously developed mitotic G-band idiogram (Fang *et al.*, submitted for publication). Photomicrographs of each analyzed nucleus were taken and compared with the drawing and the original cell for verification of chromomere designations. Numbering of chromomeres was done using the International System for Human Cytogenetic Nomenclature (ISCN, 1985). One hundred twenty four oocyte nuclei were analyzed for the construction of the final idiogram (Fig. 2). Comparisons were made of chromomeric numbers and locations between previously analyzed spermatocytes (Fang and Jagiello, submitted for publication) and the present oocyte complements (Tables 1 and 2).

Acknowledgments

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