CONSTITUTIVE HETEROCHROMATIN IN HUMAN MEIOSIS

by

Daniel J. Driscoll

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SUMMARY AND CONCLUSIONS

The objective of this project was to consider the behavior of constitutive (C-) heterochromatin in human gametogenesis. The study included 36 patients. Meiotic material was obtained from three sources: orchiectomies, testicular biopsies and oocytes obtained after prenatal or neonatal deaths. Somatic chromosomes were studied in 29 of the 32 male patients with G- and C-banding. Meiotic preparations were studied after G-11 and C-banding. One testicular sample was studied after *in situ* hybridization of satellite DNA II and III.

No significant differences could be found for the frequencies of C-band heteromorphisms between the orchiectomy ("normal" fertility) and the subfertile patients. Association of C-heterochromatin between nonhomologues was observed in 44.8 percent of the pachytene spermatocytes after C-banding and in 47.7 percent after G-11 staining. Nonhomologous associations were observed among all types of chromosomes (i.e., acrocentrics, metacentrics and the sex vesicle). Significant differences in NHA frequency were found among male patients after either C-banding ($p < .005$) or the G-11 procedure ($p << .005$). The patient with the highest frequency of NHA with both techniques had received extensive radiation exposure. No significant difference in NHA frequency was found between G-11 and C-banding. Further, no effects of age, fertility or C-band heteromorphism size could be found on NHA frequency. Results from hybridization with cRNA to satellite DNA III demonstrated a nonrandom distribution of grains in pachytene spermatocytes, but were inconclusive when cRNA to satellite DNA II was

used.

Bivalent 9 was often observed to be associated with the sex vesicle (12.6 percent frequency) or the nucleolus (14.6 percent frequency) in pachytene spermatocytes. The association of bivalent 9 with the nucleolus among male patients was not significant, but was significant ($p < .005$) for the association of 9 with the sex vesicle. No relation of heteromorphism size could be found on the associations of bivalent 9. NHAs and associations of bivalent 9 with the nucleolus were similarly found in oogenesis with the four patients having frequencies similar to that found in the males.

NHAs occur in a wide variety of species and are probably mediated by regions of homology on these nonhomologues. Common sat DNA is a likely candidate for these homologies. Our preliminary studies of sat DNA III support that thesis. The NHAs are a frequent and perhaps necessary cellular event in meiotic prophase. Possible functions and/or consequences of NHAs include: (1) initial alignment of chromosomes during leptonema and zygonema for pairing in pachynema; (2) establishing proximity between functionally related chromosomal regions (e.g., the NORs); (3) evolution of heteromorphisms; and (4) the dispersal of species-specific sat DNA. The frequent association of bivalent 9 with the nucleolus and the acrocentrics revives the question of whether chromosome 9 possesses nucleolar organizer capability or whether it is associated with the acrocentrics as a result of common sat DNA.

For seven male patients a total cell chiasma frequency of 49.42 was found. This agrees with the frequencies reported in the literature. A significant difference in total chiasma frequency ($p < .01$) among

patients was demonstrated. The chiasma frequency of bivalent 9 from six male patients was 2.23. A significant difference was found among individuals for the chiasma distribution in 9q when the heterochromatic region was not considered.

In a small percentage of spermatocytes a chiasma appeared in the 9qh region. A similar percentage of asymmetrical 9 dyads occurred at metaphase II lending support to the presence of a rare chiasma in the 9qh region. In the six males studied, 1/105 cells appears to demonstrate unequal crossing-over in the 9qh region. Due to the findings presented here, it would seem inappropriate to use the 9qh region as a single locus for linkage studies.

In the seven males studied the X and Y were paired in 81 percent of the diakineses/metaphase I's. No effect of heteromorphism size or amount could be found to effect total chiasma frequency or XY pairing.

The pictures and observations of this study underscore the great variability that the C-heterochromatic regions display during the progression of gametocytes through human meiosis.