

Histones of Mitosis and Meiosis in *Loxa flavicollis* (Hemipteran)

By HUDSON R. ANSLEY, Ph.D.

(From the Department of Biology, The Johns Hopkins University, Baltimore)

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ABSTRACT

In a study of chromosome pairing, the basic nucleoproteins of meiotic cells of *Loxa flavicollis*, a tropical bug, are compared with asynaptic cells of the testis and somatic cells. The normal meiotic cells are found to differ markedly from the other kinds, while only slight differences are found between asynaptic and somatic nuclei.

INTRODUCTION

Normal and asynaptic spermatocytes have been differentiated cytophotometrically in both a centipede and an insect by measuring the absorption of a stain selective for basic nucleoproteins, the latter presumably being histones of the chromosomes in close association with deoxyribonucleic acid (DNA) (3, 4). The asynaptic cells bind one-third more dye than the normal. It was not clear how to interpret this result, except to emphasize the exceptional character of the asynaptic cells. That such an emphasis might be misleading, however, is evident from the relative nature of cytophotometric data; there is no criterion of what is normal for ordinary mitosis. The data suggest, moreover, a difference in the amount of histone, while the actual difference may be qualitative. These uncertainties emphasize that a means of distinguishing changes in the composition of the histones is needed together with a thorough analysis of somatic cells to serve as a third standard of comparison. The results of such a test, presented here, indicate that histones of the asynaptic cells of the Hemipteran *Loxa flavicollis* Drury approach the condition of somatic cells both in amino acid composition and in dye-binding properties.

Materials and Methods

Gut and testis of the Hemipteran *Loxa flavicollis* Drury were sectioned at $9\ \mu$ and stained for histones by the method of Alfert and Geschwind (2). Alternate pieces of the ribbon were treated with acetic anhydride for 2 hours at room temperature, during which time the rest were held in 85 per cent alcohol.

Otherwise all sections were treated alike, *i.e.* kept together in the same coplin jar. Measurements were made by means of Pollister's (11) microspectrophotometric technique.

The apparatus, material, and details of the method, such as fixation, are described elsewhere (4, 5, 12, 13).

TABLE I
Mean Relative Values of Alkaline Fast Green Staining for Histones

Class of cells	Untreated	Acetylated	Difference	Per cent difference
Asynaptic	(N = 81) 2.70 ± .04	(N = 62) 1.42 ± .04	1.28 ± .06	47.4
Somatic	(N = 80) 2.55 ± .04	(N = 70) 1.32 ± .03	1.23 ± .05	48.2
Normal	(N = 67) 1.79 ± .04	(N = 52) 0.73 ± .03	1.06 ± .05	59.2
Normal corrected to scale (× 3/2)	2.68 ± .04	1.10 ± .03	1.59 ± .05	59.2

RESULTS

Three kinds of cells were compared: 1. normal spermatogonia and spermatocytes,¹ which will be classed together under the single designation *N* (for normal gametogenesis); 2. asynaptic spermatogonia and spermatocytes of the "harlequin" lobe of the testis, which will be classed together as *AS* (for asynaptic gametogenesis); and 3. diploid

¹For the purpose of the study of basic nucleoproteins, spermatogonia and spermatocytes of *Loxa* may be grouped together (4).

cells of the gut, which will be designated as *S* (for somatic nuclei).

The normal (*N*) nuclei of the testis have a mean relative value of $1.79 \pm .04$ for basic nucleoproteins (histones). The value for acetylated *N*

nuclei was $0.73 \pm .03$. The staining reaction was reduced 59.2 per cent by the application of acetic anhydride before staining.

The asynaptic (*AS*) nuclei of the testis have a mean relative value of $2.70 \pm .04$ for basic nucle-

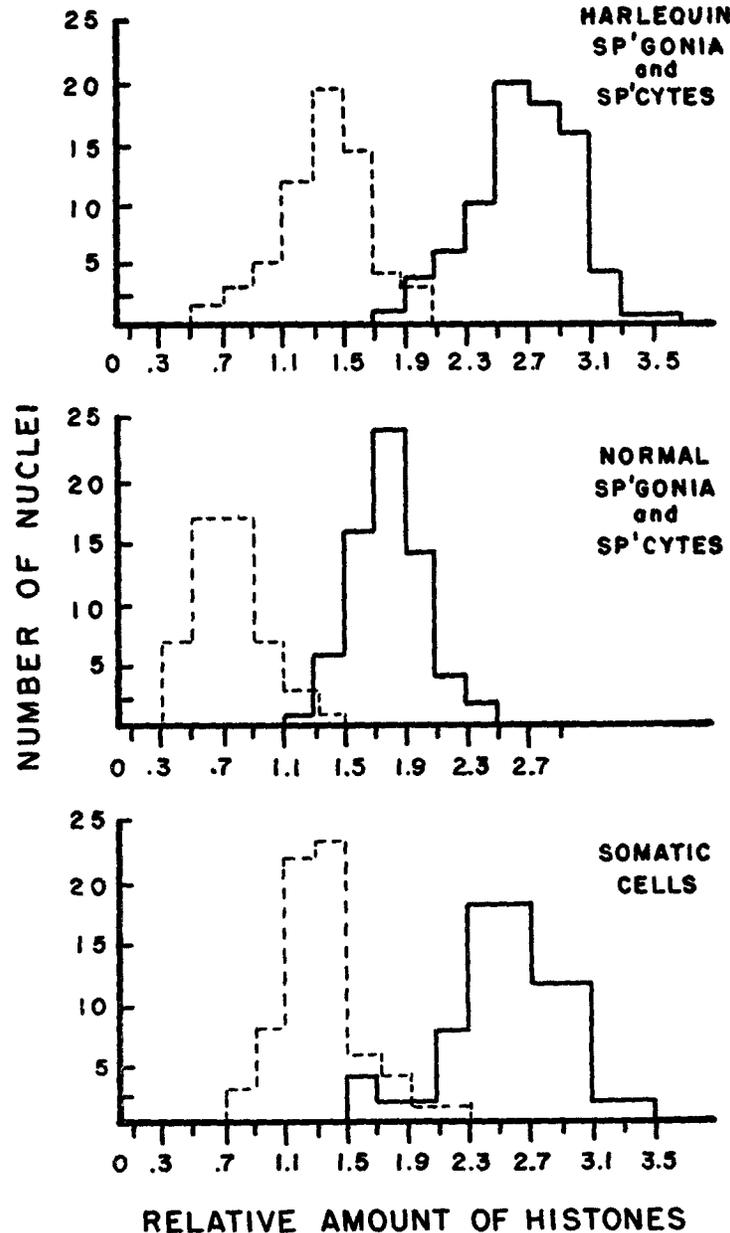


FIG. 1. The frequency distribution of the measurements of histones from the Alfert-Geschwind reaction are presented by means of histograms. In all histograms the ordinate represents the number of nuclei measured; the abscissa represents the relative amount of histone. Each pair of histograms is devoted to a separate class of cells, as indicated by the label. The solid line represents untreated nuclei; the broken line represents acetylated nuclei.

proteins. After acetylation the *AS* value is $1.42 \pm .04$. The reduction in staining amounts to 47.4 per cent. These values are close to the findings for the somatic (*S*) nuclei. Untreated *S* nuclei have a mean relative value of $2.55 \pm .04$. For acetylated *S* nuclei the value is $1.32 \pm .03$, showing a reduction of 48.2 per cent. These values are summarized in Table I, and the frequency distribution of the data is presented in Fig. 1.

The disparity between the normal meiotic (*N*) group of cells and both the *AS* and *S* groups is the most palpable result. Not only do the mean values, representing total nuclear dye content, uphold this disparity (of about one-third), but also the per cent reduction in the values after acetylation. In this latter respect the *N* cells show approximately an 11 per cent greater reduction than the *AS* and *S* cells. The *N* values may be brought into scale with those to which they are compared (*i.e.*, the *AS* and *S* values) by multiplying by $3/2$, which is the *AS/N* factor (4). Then the differences of the means may be tested directly. When this is done the 11 per cent difference is found to be significant at the 0.01 level of confidence. By contrast the 1 per cent difference between the *AS* and *S* groups is not significant.

Although the mean amounts of basic nucleoproteins of *AS* and *S* nuclei closely approximate one another, they also differ significantly, but so small a difference (5 per cent), even though it has statistical significance, cannot be stressed, owing to the error of the method, which is of about the same magnitude. Such a difference was noted before; histones of somatic nuclei of a centipede, *Scutigera forceps*, give somewhat lower values than asynaptic cells of the testis whether the Alfert-Geschwind or the Millon reaction of Pollister is used (3). This small difference might be accounted for by the fact that asynaptic cells do not behave like somatic cells, since a reduction in chromosome number and some sort of pairing occur in them. Equally good is the explanation that the chromatin of somatic nuclei is distributed differently from that of meiotic nuclei. In this regard, however, it is important to observe that no such difference is found with the Feulgen stain in the same preparations, and photographs of alkaline Fast Green and Feulgen preparations are indistinguishable (4).

DISCUSSION

The groups mainly responsible for staining by the Alfert-Geschwind reaction are the guanidine

groups of arginine and the ϵ -amino groups of lysine. Only about one-third of the α -amino groups and less than one-tenth of the imidazole groups of histidine can be expected to bind dye (1, 2, 8). Acetylation at room temperature primarily affects the ϵ -amino groups of lysine, but not the guanidine groups of arginine (10), and acts mainly to block the staining of lysine. The results suggest that the nuclei of normal spermatogonia and spermatocytes contain a greater proportion of lysine than do asynaptic or somatic nuclei, and correspondingly a smaller proportion of arginine. It is unlikely that the difference is so simple, however; differential association of histones with other proteins (*i.e.* masking) (7) or differential response to fixation would give the same effect.

Normal meiosis is thus correlated with a decrease in the amount of dye bound and probably with a change in the amino acid composition of the histones. Since this difference characterizes the spermatogonial generations as well as the spermatocytes, it is thus possible to distinguish gametogenic from somatic cells. Asynaptic cells of the testis approximate the somatic condition in *Loxa*. With respect to lysine and arginine, asynaptic and somatic cells appear the same, while with respect to the amount of dye bound they differ but slightly. That is to say, the asynaptic cells of *Loxa* develop, or rather keep, somatic histone properties.

The asynaptic cells of both *Loxa* and *Scutigera* exhibit a well regulated reduction and therefore pairing, thought to be of a touch-and-go nature (3, 4). It should be recalled that somatic cells under certain conditions can do the same (9). The lysine-plus, low dye-binding histones, if that is what has been found in normal meiosis, are characteristic of bivalent formation only and may not be essential for crossing over. Genetic studies of asynaptic mutants of maize and tomatoes show no reduction in the rate of crossing over (6, 14). It is not known what the state of the histones in asynaptic maize and tomatoes is, however, nor have the genetic consequences of somatic reduction been analyzed. Hence the genetic import of the low dye-binding histones cannot be postulated.

The involvement of histones in meiosis is aduced as a concomitant of those pairing maneuvers which result in the tetrad configuration of homologues, as opposed to touch-and-go pairing of chromosomes which are already condensed. It would appear that the histones of chromosomes have at least two "normal" states—the ga-

metogenic *N* state and the somatic *S* state. The latter perhaps includes the near somatic *AS* state. The change from the *S* to *N* state may be highly complex and accompanied by changes in non-histone protein and time of synthesis of DNA (4). A different kind of pairing and reduction is associated with each state, but the possibility remains that recombination is common to both. The change in pairing behavior with the associated change in histones may eventually provide the clue to the forces of attraction and repulsion among chromosomes.

It would be desirable to know which is the more primitive condition in chromosome evolution—the *S* or *N* state. The fact that recombination is present even in viruses once thought to be the size of genes, and likewise the fact that recombination occurs without chiasma formation in asynaptic cells, throw some further doubt on the chiasmate theory of recombination. Comparison of the basic nucleoproteins of the haploid and diploid generations of primitive plants and animals might further elucidate whether the classical “meiosis” is a recent development or whether it has existed side by side with sexual recombination throughout evolution.

SUMMARY

Normal meiotic nuclei differ from asynaptic and somatic nuclei in dye-binding capacity and apparently in amino acid composition. When

stained for basic nucleoproteins with and without acetylation, somatic and asynaptic nuclei respond in approximately the same way. One interpretation of the results might be that the proportion of lysine is about 11 per cent lower than in normal meiotic nuclei and the proportion of arginine correspondingly higher. The significance of this finding is discussed with respect to cytogenetics and evolution of chromosomes.

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