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Histone Transition During Spermiogenesis Is Absent in Segregation Distorter Males of *Drosophila melanogaster*

Abstract. *Males homozygous for the segregation distorter chromosome are often sterile or nearly sterile as a result of the dysfunction of virtually all their sperm. Spermatid bundles from such males do not exhibit the normal transition from lysine-rich to arginine-rich histones.*

Natural populations of *Drosophila melanogaster* typically contain a low frequency of certain second chromosomes designated SD (segregation distorter) (*1*). Heterozygous SD/SD$^+$ males produce mostly SD-bearing offspring because the sperm carrying SD$^+$ undergo dysfunction (*2*). Homozygous SD/SD males are sterile or nearly sterile as a result of the dysfunction of virtually all their sperm (*3*).

The developmental lesion leading to sperm dysfunction is not known. Motivated by suspicion that the lesion might involve the transition from histones rich in lysine to histones rich in arginine during spermiogenesis (*4*), we examined the histone transition in sterile SD/SD males. Here we report direct cytochemical evidence that the transition from somatic histones to sperm histones does not take place.

Two lines of circumstantial evidence focus attention on the histone transition. The first is a genetic correspondence. Segregation distorter chromosomes are known to carry two mutations, one called *Sd* (for segregation distorter), the other called *Rsp* (responder) (*5*). The *Sd* locus is on the left arm of the second chromosome near the locus of purple (2 : 54.5) (*5*). The histone locus is in region 39D-E of the salivary gland chromosomes (*6*), also close to purple. While an identity between the histone locus and *Sd* has not been shown, these observations suggest that the loci might be genetically or functionally related. A second line of circumstantial evidence implicating the histone transition is cytological. Heads of dysfunctional sperm often fail to condense; when they do condense, they appear to be unstable and frequently undergo a characteristic sequence of stages of degeneration (*7*). This, too, is consistent with the hypothesis that the developmental lesion involves a defective transition from lysine-rich to arginine-rich histones during spermiogenesis, since the histones rich in arginine are generally assumed to function in sperm head condensation (*8*).

Our results are shown in Table 1. Because SD-bearing chromosomes from nature carry recessive lethals unrelated to segregation distortion, SD homozygotes must be produced by combining SD's from different populations. The SD chromosomes listed in Table 1 were all isolated from natural populations in the midwestern United States (*9*), except for SD(NH)-2, which was isolated in Japan (*10*). Late spermatid bundles (*11*) were stained either for all histones (those rich in either lysine or arginine) or specifically for histones rich in arginine (*12*).

The first genotype examined was SD-72/SD(NH)-2. Histones rich in arginine were found to be absent in spermatid bundles, but total histones appeared normal. Since the males were 3 to 5 days old at the time of examination, the result could have been due to a delayed histone transition and not to its absence. Males

Table 1. Absence of arginine-rich histones in late spermatid bundles of homozygous SD males and correlations with male fertility. Fertility of males is taken from Hartl (*9*) except where noted in text. Cytochemical histone assay was modified from Alfert and Geschwind (*12*) and Das *et al.* (*4*). Testes of males dissected in Ringer were air dried, fixed with 10 percent formaldehyde for 6 hours, washed in tap water overnight, treated with 10 percent trichloroacetic acid for 30 minutes at 90°C, washed in 70 percent ethanol, washed in distilled water, and stained for 2 hours with 0.1 percent fast green at *p*H 8.1; the testes were then dehydrated in an ethanol series and mounted in Permount. Specific staining for histones rich in arginine was the same except for deamination of lysine residues with 5 percent $NaNO_2$ for 30 minutes after the treatment with trichloroacetic acid. In all cases, testes from wild-type males were placed on the same slides as those from experimental males to serve as controls. Testes from wild-type males were stained both for total histones and for histones rich in arginine. Each result is based on examination of testes from more than 60 males in at least two separate experiments.

Genotype of male	Fertility of male	Histones in late spermatid bundles	
		Lysine-rich plus arginine-rich	Arginine-rich
SD-72/SD(NH)-2	−	+	−
SD(M)-35/SD-72	−	+	−
SD(43)-1/SD-72	−	+	−
SD(M)-35/SD(NH)-2	−	+	−
SD(Berea)-110/SD(NH)-2	−	+	−
SD(43)-1/SD(NH)-2	−	−	−
SD(Berea)-110/SD-72	+	+	+

of this genotype were therefore examined at 2-day intervals from the time of eclosion to an age of 2 weeks. Arginine-rich histones were absent in males of all ages.

Table 1 shows that the defective histone transition is not a peculiarity of SD-72/SD(NH)-2. In all cases, sterile homozygotes produced late spermatid bundles lacking arginine-rich histones. Spermatid bundles from males of genotype SD(M)-35/SD-72 and SD(43)-1/SD-72 seem to have a slightly reduced amount of total histones as judged from the intensity with which bundles are stained; since the cytochemical assay is qualitative, not quantitative, this finding is at best only suggestive.

Two anomalies were encountered. Males of genotype SD(43)-1/SD(NH)-2 lack not only arginine-rich histones in their spermatid bundles, but also lysine-rich histones. However, the sterility of these males seems to be caused by a spermiogenic block that is unrelated to segregation distortion and which temporally precedes the block resulting from homozygosity for SD. Of 55 SD/SD genotypes examined by Hartl (*9*), SD(43)-1/SD(NH)-2 was the only one in which males never produced offspring. Other sterile SD homozygotes, including those in Table 1, produced a few offspring—less than 2 percent of the number produced by controls (*9*). Moreover, SD(43)-1/SD(NH)-2 males produce no motile sperm whereas all other SD homozygotes, even the sterile ones, produce large numbers of motile sperm (*9*). We therefore believe that the absence of lysine-rich histones in the late spermatid bundles of SD(43)-1/SD(NH)-2 males is not a result of homozygosity for SD.

The second anomaly in Table 1 concerns SD(Berea)-110/SD-72. In this case, arginine-rich histones were present in spermatid bundles. Since the fertility of males of this genotype had not previously been assessed, it seemed possible that such males might be fertile. Certain SD/SD genotypes are known to exhibit complementation at the *Sd* locus which leads to partial male fertility (*9*). Accordingly, males of genotype SD(Berea)-110/SD-72 were provided with groups of three virgin females at 2-day intervals for as long as the males produced offspring. Twenty SD(Berea)-110/SD-72 males produced an average of 222.8 ± 257.6 progeny per male; 19 control males produced an average of 1328.4 ± 249.5 progeny per male. The fertility of SD(Berea)-110/SD-72 males is therefore 17 percent of normal, a result which is well within the range of fertilities of complementing SD/SD genotypes (*9*). Thus, the presence of arginine-rich histones in late spermatid bundles of SD(Berea)-110/SD-72 males is accounted for by the large number of functional sperm that the males produce.

We conclude that sterile SD homozygotes have a defective transition from lysine-rich to arginine-rich histones during spermiogenesis. We must point out that the defective histone transition may be a secondary effect of homozygosity for SD; the primary lesion could be something quite different. Of interest in this connection would be an examination of individual spermatid heads in SD/SD$^+$ males. This is not possible with the cytochemical technique described here because the technique is not quantitative and because it is only sensitive enough to detect arginine-rich histones in entire spermatid bundles, not in individual spermatid heads. Nevertheless, our results strongly reinforce the suspicion that the developmental lesion in segregation distortion may be a defective transition from somatic histones to sperm histones.

Note added in proof: Ganetzky (*13*) has recently shown that *Sd* is probably between salivary chromosome bands 37D1-7 and 38B1-3, not near 39D-E, the locus of the somatic histones. Tokuyasu *et al.* (*14*) have studied spermiogenesis in heterozygous SD's with the electron microscope; the earliest observable abnormality presaging sperm dysfunction occurs in the chromatin in the latter half of nuclear condensation, corresponding in time with the histone transition. Evidently, *Sd* may be either a structural locus of sperm histones or a regulatory locus controlling the histone transition.

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1,25-Dihydroxyvitamin D in Biological Fluids: A Simplified and Sensitive Assay

Abstract. *A competitive binding assay for 1,25-dihydroxyvitamin D [1,25-$(OH_2D]$ in plasma has been developed in which intestinal cytosol preparations from rachitic chicks are used as the binding protein. A new method of extraction and two new chromatographic procedures are used for this assay. The method is sensitive to as little as 10 picograms of 1,25-$(OH)_2D$, and triplicate assays can be done on 5 milliliters of plasma. This assay shows that in the plasma of normal adult subjects there is a 1,25-$(OH)_2D$ concentration of 29 ± 2 picograms per milliliter, while none can be detected in the plasma of nephrectomized subjects and end-stage renal failure patients.*

Vitamin D requires hydroxylations in the 1 and 25 positions in order to express its biological activity in calcium, phosphate, and bone homeostasis. The resulting metabolite, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], has marked antirachitic activity and is active in the stimulation of intestinal absorption of calcium and phosphate (*1, 2*) and of bone resorption (*3*). This metabolite results from the sequential hydroxylation of vitamin D on the C-25 in the liver and the C-1 in the kidney. The renal 1-hydroxylation seems to be the major site of regulation of vitamin D metabolism in response to the calcium and phosphate status of the animal (*4, 5*). Concentrations of the metabolites of vitamin D in the tissue and blood have been studied almost exclusively by administering isotopically labeled vitamin D compounds to vitamin D–deficient animals (*5*). Recently, however, Haussler and his co-workers (*6*) used a competitive binding assay to measure 1,25-$(OH)_2D_3$ in human and rat plasma. Their assay, for which they use rachitic chick-