Review

IS HAPLOID GENE EXPRESSION POSSIBLE FOR SPERM ANTIGENS?

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The development of a spermatozoon (sperm) from a spermatid involves a complex process of differentiation during which a variety of new gene products appear. It has been generally assumed that no genetic transcription occurs after meiosis and, if this were so, that all the new sperm proteins would have to be transcribed from stored messenger RNA. However, the biochemical evidence suggests that there is no abrupt change in the rate of RNA synthesis during meiosis and that qualitative changes in RNA synthesis, to the extent that they are known, favor the likelihood of continuing messenger RNA synthesis. Experimental analyses of distorted transmission ratios of *t*-alleles and unbalanced chromosomal states in males also suggest that genes are expressed in haploid nuclei after meiosis. It is probable that spermatozoa are functionally equivalent in most respects because of intercellular bridges that create a continuous cytoplasm between developing spermatozoa, facilitating an exchange of most postmeiotic gene products. Plasma membrane proteins which are potential antigens might not be shared across the intercellular bridges but the evidence to date for haploid expression of sperm antigens is poor.

INTRODUCTION

Over the last several decades a number of papers have purported to show haploid expression of sperm antigens. If the phenomenon were true, it might provide a way for selecting spermatozoa of particular genotypes for breeding purposes, e.g. if H-Y antigen was expressed only on Y-bearing sperm, it should be possible to separate X- and Y-bearing sperm on this basis. However, these reports have met many objections. In what follows we will try to provide a perspective on these objections. By way of introduction, we describe the relevant genetic mechanisms. The two chief causes of departures from Mendelian genotypic ratios at the time of fertilization are meiotic segregation distortion, i.e. the lack of random chromosomal segregation, and gametic selection, i.e. an unequal competition between gametes which differ in their haploid genome. Gametic selection is most simply explained by invoking gene expression after meiosis since this can result in phenotypic differences which could influence gamete function. Non-Mendelian ratios occasionally occur in a variety of organisms. In plants, where one male pronucleus is activated during pollen formation, the occurrence of distorted transmission ratios is common, e.g. the sugary locus in maize (Brink and Burnham, 1927). As would be expected, the haploid

pollen tube nucleus is involved in RNA synthesis (Stanley and Linskins, 1974). A well-studied example of non-Mendelian segregation is *segregation distortion* in *Drosophila* (reviewed in Zimmering et al., 1970) but there is no definitive evidence for post-meiotic RNA transcription (Kettaneh and Hartl, 1976). There is also one very clear example (*t*-alleles; Gluecksohn-Waelsch and Erickson, 1970; Bennett, 1975), and several suggestive examples, of non-Mendelian ratios in mammals.

Gametic neutrality, which is almost universally observed, could be maintained in spite of post-meiotic gene expression by the sharing of post-meiotically expressed gene products among gametes. The fact that spermatozoal differentiation occurs in cells which partially share their cytoplasm (a quasi-syncytium) in mammals may provide an explanation for gametic equality as well as for successful fertilization by gametes which do not contain genes thought to be expressed post-meiotically (Erickson, 1973). As spermatozoa are highly differentiated cells, with many distinctive organelles and isoenzymes, it is obvious that a large number of genes must be involved in their development. The high frequency of intercellular cytoplasmic bridges between spermatocytes and between spermatids would facilitate equal sharing of messenger RNAs and proteins, while allowing exceptional spermatozoa with unbalanced chromosomal complements to receive gene products necessary for their development.

We hypothesize (1) that some genes are transcribed after meiosis in haploid, i.e. in genetically different, cells and (2) that the intercellular bridges do not always result in functionally equivalent sperm such that haploid expression of antigens would be possible. Recent advances in the biochemistry, immunology and genetics of spermatogenesis which illuminate these hypotheses will be discussed. We will review the evidence that RNA is transcribed after meiosis and that this RNA may include messenger RNA. We will discuss details of *t*-allele segregation distortion which argue strongly that the genotype of spermatozoa alters their function. We shall review the data on haploid expression of sperm antigens and information from other mammalian systems which suggests that haploid expression may be an occasional occurrence. Other aspects of spermatogenesis have been reviewed (Bishop, 1968; Fechheimer, 1970; Fritz, 1973; Turkington and Majumder, 1974; Bellve, 1979; Fawcett, 1979); Beatty's (1970) review on the genetics of spermatozoa has not been surpassed.

MORPHOLOGY OF MAMMALIAN SPERMATOGENESIS

Spermatogenesis is defined as the series of cellular events which result in the production of spermatozoa. Male germ cells (gonocytes) increase in number by mitosis during early male development. At puberty they assume a position along the basement membrane of the seminiferous tubule. Their histological appearance is modified when in this new position and, in this new guise, they are termed spermatogonia. Spermatogonial mitoses increase in number with an increase in luteinizing hormone and follicle stimulating hormones at puberty (reviewed in Midgely et al., 1978). The committed spermatogonium then undergoes a clonal expansion, marked by a series of mitoses with incomplete cytokinesis, resulting in cytoplasmic bridges, approximately 1 μm in diameter (Fawcett, 1972; Fig. 1). This clonal expansion typically results in 32 spermatocytes and four times as many spermatids but deviations from these numbers have been reported.
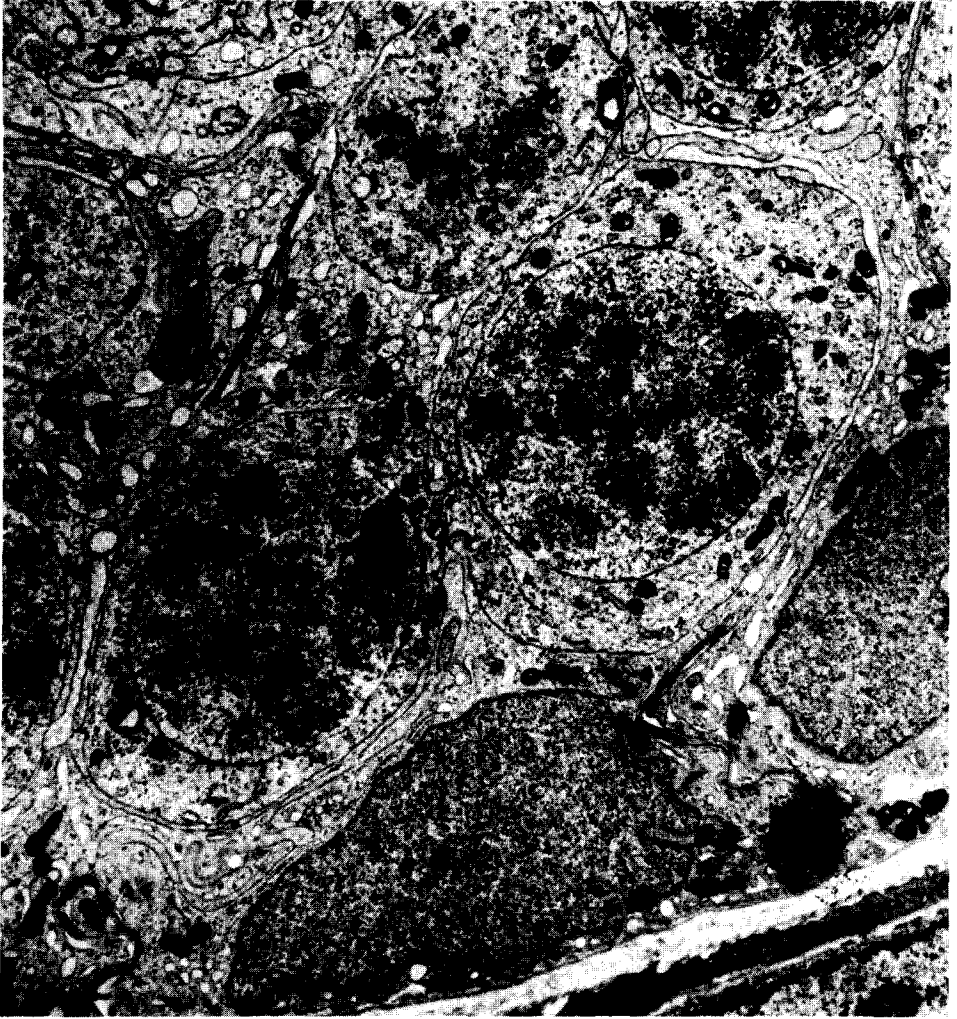
Fig. 1. Electron micrograph of an intercellular bridge between two spermatids from a rat testis. Boundaries of the bridge are continuous with the plasma membranes of the connected cells and are associated with electron-dense substance. (× 35,000). Courtesy of Dr. B. Gondos.

The number of mitoses involved in the clonal expansion and the percentage of spontaneously degenerating cells is not precisely known in any one mammalian species and varies between species. As many as 22 interconnected spermatocytes and as many as 74 interconnected spermatids have been counted in serial sections in rats (Moens and Go, 1972). Only linear, branching arrays of interconnected cells have been found in these studies; rings of cells, which would require fusion as their explanation, have not been detected (Moens and Go, 1972). These intercellular bridges seem to be maintained by specialized plasma membrane or submembrane structures (Dym and Fawcett, 1971; Beams and Kessel, 1976). The fate of these bridges during spermiogenesis (the differentiation of a
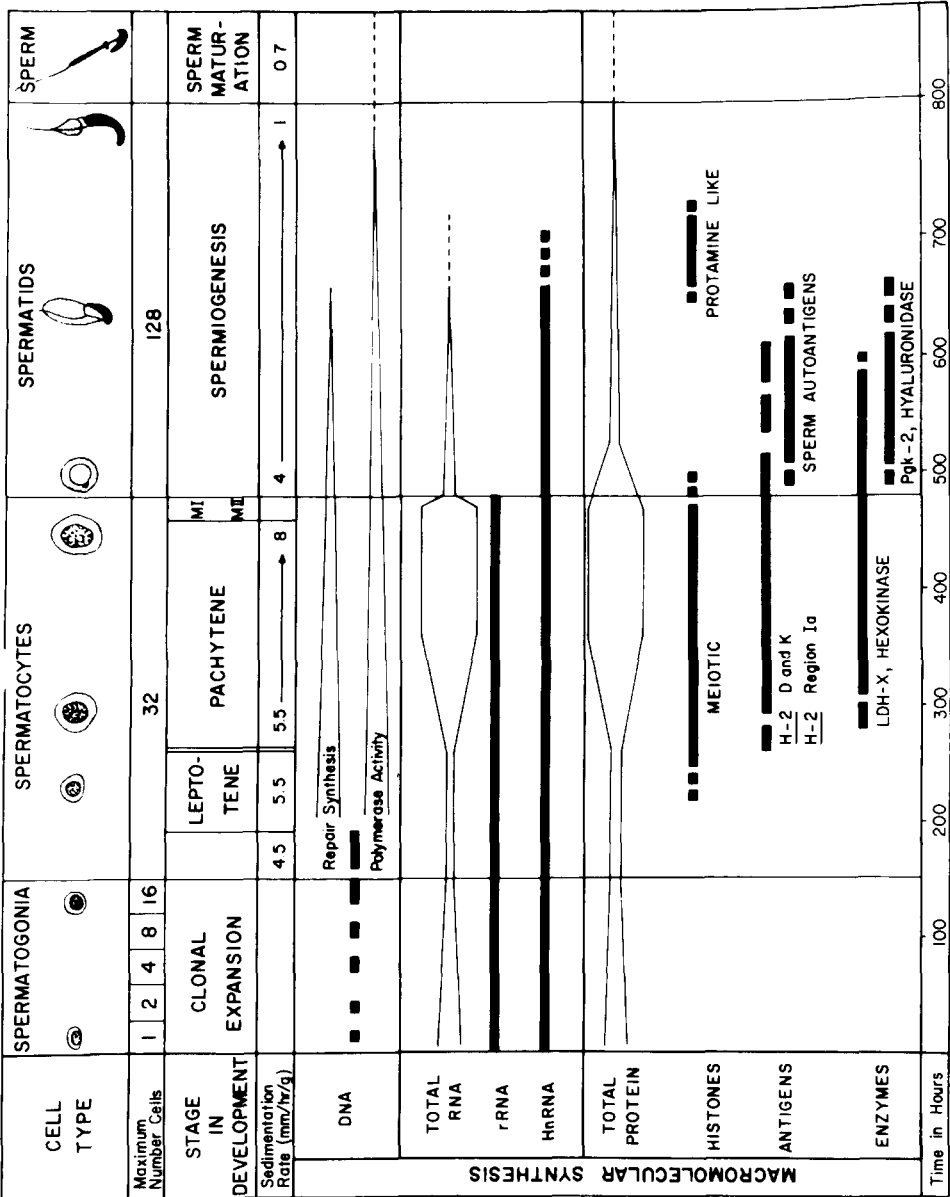
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Fig. 2. A schematic representation of spermatogenesis. Qualitative data for macromolecular syntheses are indicated by solid bars; uncertainties as to onset or duration of particular molecular syntheses are indicated by breaks. Quantitative variations are indicated by the relative heights of the margined areas; no comparison from one substance to another is intended.

spermatozoon from a spermatid) is less well known, but the spermatozoa are not inter-connected after their release into the seminiferous tubule. The intercellular bridges have been postulated to synchronize spermatogenic development (Schleiermacher and Schmidt, 1973). We believe that the cytoplasmic syncytia created by the intercellular bridges can provide for gametic neutrality despite post-meiotic gene expression.

The initial cellular product of the spermatogonial mitotic expansion, the spermato-cyte, initially resembles a spermatogonium, but it slowly increases in size as it undergoes DNA replication and extensive RNA and protein synthesis (Monesi, 1962). The configura-tion of the chromosomes provides the criteria for identifying the various stages of meiotic prophase (leptotene, zygotene, pachytene, diplotene, and diakinesis). It is important to note that the spermatocyte seems to undergo its maximal synthetic activity at the pachy-tene stage when loops similar to those in oocyte lampbrush chromosomes have been noted (Monesi, 1965). The reductional meiotic division is rapidly followed by the second, equational, division resulting in a round cell, the spermatid, which then slowly undergoes differentiation to form a spermatozoon. The sequence of cellular stages and the accom-panying macromolecular syntheses are summarized in Fig. 2.

DNA SYNTHESIS DURING SPERMATOGENESIS

Although the last major DNA synthesis occurs in pre-leptotene spermatocytes (Monesi, 1965; Kofman-Alfaro and Chandley, 1970), a low level of DNA synthesis occurs during meiotic prophase (Meistrich et al., 1975) which may be related to alterations in gene expression. Alternatively, this DNA synthesis might be involved in repair and/or meiotic pairing (Smyth and Stern, 1973). DNA polymerase activity declines to low levels during spermiogenesis, but does not decline further during sperm maturation (Daentl et al., 1977; Hecht et al., 1979). Thymidine and thymidilate kinase activities are also present in sperm (Daentl et al., 1977). The DNA polymerase activity present in spermatozoa has been thought to be mitochondrial in origin (Hecht, 1974) although histochemical and other techniques demonstrate a nuclear location (Chevaillier and Phillipe, 1976) with a ribonuclease sensitivity (Witkin and Bendich, 1977). Thus, the enzymes necessary for gene amplification, an important mechanism of gene expression in oogenesis, are pres-ent. The time of the increased DNA methylation, noted for many specific nucleotide sequences in sperm DNA (Kaputa and Sneider, 1979), is not known.

RNA SYNTHESIS DURING SPERMATOGENESIS

A large number of studies on the rates and kinds of RNA synthesis during spermato-genesis are relevant to the possibility of post-meiotic transcription. Histological studies using pyronin as the stain show little change in the amount of cytoplasmic RNA during the clonal expansion of spermatogonia (Daled, 1951). It was originally believed that lit-tle RNA was transcribed after meiosis since early mouse spermatids exposed to short pulses of [³H]uridine showed only a very small peak of uridine incorporation when exam-ined by autoradiography (Monesi, 1967). More recent quantitative autoradiographic studies showed, however, that the rate of RNA synthesis per cell decreased only 4-fold during meiosis in ram testis, i.e. the RNA synthesis/DNA ratio was essentially unchanged

(Loir, 1972). Furthermore, studies on mouse testicular cells separated by the Staput technique (a unit gravity sedimentation method capable of separating the cells of the various spermatogenic stages into populations that are roughly 70% pure populations — Lam et al., 1970; Meistrich et al., 1973) show that there are high rates of RNA synthesis in post-meiotic cell stages (Meistrich, 1972). In fact, in one study the highest level of [³H]uridine incorporation per cell was in early spermatids (Lee and Dixon, 1972). DNA-dependent, RNA polymerase has been shown to be active in early spermatids (Moore, 1971). These studies all depend on the incorporation of exogenous [³H]uridine into RNA. If the intracellular uridine pool size was greatly decreased in post-meiotic cells, added [³H]uridine would more quickly radiolabel the pool and label would be incorporated into RNA more rapidly, inflating the apparent rate of RNA synthesis during short pulses. However, pool size estimates indicate that the intracellular uridine pool is increasing, rather than decreasing, after meiosis (Geremia et al., 1977). Thus, although there appears to be significant RNA transcription after meiosis, the question is whether or not this includes messenger RNA (mRNA) synthesis.

In most cells about half of the newly synthesized RNA is ribosomal RNA (Harpold et al., 1979). Thus, a preliminary question focuses on whether or not ribosomal RNA synthesis is altered in post-meiotic cells. Three methods have been used to explore this question: (1) visualization of transcription units in identifiable cells, (2) characterization of newly synthesized (pulse-labelled) RNA from cells at different stages separated by differences in sedimentation velocity, and (3) studies on the time of synthesis of the ribosomal RNA which is found in spermatozoa. Kierszenbaum and Tres (1974a, b, 1975) have used whole-mount electron microscopic techniques, which allow direct visualization of the transcription process, on the spread chromosomes in combination with light and electron microscopic autoradiographic techniques, to compare RNA transcription in premeiotic and post-meiotic cells. RNA transcription in spermatocytes appears by these techniques to include both ribosomal precursor and heterogeneous nuclear RNA, but nucleolus-like, ribosomal RNA transcription patterns were not found after meiosis. Newly synthesized RNA from separated spermatogenic cells was thought to include ribosomal RNA when characterized by sucrose gradient centrifugation (Geremia et al., 1978), while electrophoresis of newly synthesized RNA suggested that ribosomal RNA synthesis decreased markedly in post-meiotic cells (Erickson et al., 1980a). Another group has found post-meiotic expression of ribosomal RNA cistrons using staining techniques (Schmid et al., 1977). However, this technique depends on the binding of silver by chromatin proteins (Bloom and Goodpasture, 1976) which are, of course, greatly altered during this stage of spermatogenesis. Studies on the time of synthesis of ribosomal RNA found in maturing mouse spermatozoa suggest that its synthesis is pre-meiotic (Betlach and Erickson, 1976).

Whether or not ribosomal RNA synthesis decreases significantly after meiosis, there is abundant evidence that heterogeneous nuclear RNA, considered to be the precursor of mRNA, is synthesized after meiosis. Early studies considered all the rapidly-labelled nuclear RNA of Chinese hamster testis to be the product of late pachytene RNA synthesis (Muramatsu et al., 1968) — heterogeneous short-lived RNA of 45—80 S with a high AU/GC ratio similar to that of liver extranucleolar nuclear RNA was found. It is true that isolated rat primary spermatocytes largely synthesize heterogeneous RNA which is

rapidly turned over (Grootegoed et al., 1977) but a variety of studies show that synthesis of heterogeneous RNA and RNA containing poly(A) tracts clearly continues in spermatids. Transcriptional patterns characteristic of heterogeneous nuclear RNA synthesis are found in spermatids (Kierszenbaum and Tres, 1974a, b, 1975). Sucrose gradient and electrophoretic characterization of newly synthesized RNA from spermatids demonstrate heterogeneous presumptive mRNA (Geremia et al., 1978; Erickson et al., 1980a). Some of this new RNA from spermatids is poly(A)-containing, whether determined with poly(U)-Sepharose (Geremia et al., 1978) or oligo(dT) cellulose (Erickson et al., 1980a). Similar results have been found for amphibian spermatogenesis (Kalt, 1979). Recent experiments using functional assays for mRNA (in vitro translation of specific proteins) have shown that mRNA for protamine-like histone and phosphoglycerate kinase-2 (see below) increase after meiosis (Erickson et al., 1980c). This result is most easily explained by postulating post-meiotic transcription of these messages.

A specific intracellular organelle, the chromatoid body, may be involved in the utilization of post-meiotic mRNA. This body appears during pachytene (Fawcett et al., 1970) but becomes prominent cytologically in early spermatids where it shows rapid movements between the nucleus and Golgi apparatus (Parvinen and Jokelainen, 1974). It incorporates [³H]uridine, but only after a 14-h delay, which suggests that the RNA has to be transported from the nucleus (Soderstrom and Parvinen, 1976). Actinomycin D treatment results in depletion of putative RNA in the chromatoid body of spermatids after a 12-h interval, further suggesting that the chromatoid body contains post-meiotically synthesized RNA (Parvinen et al., 1978). Alternatively, the chromatoid body may be formed by the extrusion of nucleolar components (Comings and Okada, 1972).

The questions these data raise are relevant to early mammalian development, not just spermatogenesis. If all the mRNA synthesis during spermatogenesis is pre-meiotic, then spermatogenic post-meiotic protein synthesis must occur through 'stored' mRNA. However, stored mRNA has not yet been generally found in mammals at any stage. Unlike the situation in sea urchin and amphibian embryos, the mammalian embryo does not seem to use many mRNAs synthesized during oogenesis. RNA synthesis commences almost with fertilization and expression of the paternal genome can be detected very early, at the 8-cell stage (Wudl and Chapman, 1976; Epstein, 1975). In later mammalian embryogenesis, messages for proteins characteristic of differentiated states have been found to be accumulated briefly prior to translation. For instance, the mRNA for myosin appears in cytoplasm some hours before its translation (Buckingham et al., 1974). The synthesis of some sperm specific proteins, such as the protamine-like histone, does not begin until more than a hundred hours after meiosis. Thus, the question as to whether 'stored' mRNA is used for spermatogenic post-meiotic protein synthesis and differentiation is important.

SPERMATOGENIC PROTEIN SYNTHESIS

It is widely accepted that there is active post-meiotic translation during spermatogenesis. A moderate level of pre-meiotic protein synthesis has been demonstrated autoradiographically in early spermatocytes; this synthesis increases further and then decreases prior to the meiotic divisions, in parallel with new RNA synthesis (Monesi, 1967; see

Fig. 2). After meiosis, autoradiography shows a peak of arginine incorporation late in spermiogenesis which most likely represents incorporation into protamine-like histone (Monesi, 1967).

Post-meiotic changes in protein composition of spermatozoa have been followed in many ways. For example, there is indirect evidence for changes in nuclear proteins as spermatogenesis proceeds. In the *Bovidae*, Fuelgen staining of nuclei decreases until the late spermatid stage, indicative of decreased accessibility of DNA to the stain (absorption at 265 nm remains constant, implying no change in the actual nucleic acid content of the nuclei). The Sakaguchi methyl green histochemical reaction, which is specific for protein-bound arginine, also increases until the late spermatid stage. There is some continuation of both these changes as the sperm progresses through the male reproductive tract, as has been found in rabbits, and with in vitro aging of bovine spermatozoa (Gledhill et al., 1966; Bouters et al., 1967; Salisbury et al., 1961). Amino acid analyses of protein hydrolysates have shown that the amino acid composition of bovine spermatozoa changes with passage through the epididymides so that some protein synthesis may continue in vivo in maturing spermatozoa (Lavon et al., 1971), although selective degradation of proteins could also explain some of these changes.

There is evidence for the post-meiotic synthesis of several sperm-specific enzymes and antigens. Among the sperm-specific enzyme with this potential are lactate dehydrogenase-X (LDH-X) (Blanco and Zinkham, 1963), hexokinase (Katzen, 1967; Sosa et al., 1972), a cyclic nucleotide phosphodiesterase (Christiansen et al., 1972), non-specific esterase (Meizel et al., 1971), an autosomally-coded phosphoglycerate kinase (Cooper et al., 1971; VandeBerg and Blohm, 1977), sperm hyaluronidase (Mancini et al., 1964; Borders and Rafferty, 1968), the acrosomal proteinase, acrosin (Stambaugh and Buckley, 1969), and testicular cytochrome *c* (Hennig, 1975; Kim, 1980). A number of other enzymes have not yet been shown to have spermatogenic-specific isoenzymes but major changes in their activities serve as markers for various stages of spermatogenesis: sorbitol dehydrogenase (Bishop, 1967), carnitine acetyltransferase (Vernon et al., 1971), α-glycerophosphate dehydrogenase (Posalaky, 1965), and succinic dehydrogenase (Ito, 1966). These changes in synthesis may require post-meiotic expression of regulatory proteins.

The developmental onset of enzyme activity during spermatogenesis is often instructive. For example, LDH-X activity is first found in 19-day-old male mice, which is when late pachytene spermatocytes are first observed (Goldberg and Hawtrey, 1968). Radio-pulse immunoprecipitation experiments performed on fractionated cells show that synthesis continues post-meiotically, with about half of the total synthesized in spermatids (Meistrich et al., 1977). Recent data suggests that the sperm-specific hexokinase also first appears during pachytene (Brock, 1975) while spermatozoal phosphoglycerate kinase does not appear until after meiosis (VandeBerg et al., 1976; Erickson et al., 1979b; Kramer and Erickson, 1981a). Studies on testicular hyaluronidase and immunohistological localization of 3 guinea pig spermatozoal autoantigens demonstrated that these proteins first appear in spermatids or, possibly, secondary spermatocytes (Males and Turkington, 1970; Radu and Voisin, 1975). In mice, the Ia antigen of spermatozoa makes its appearance at pachytene, as may also the *H-2* D and K antigens (Fellous et al., 1976; Erickson, 1977a, b).

Protamine-like histones have been documented in a variety of mammals (Bloch, 1969;

Calvin, 1976). Evidence for synthesis in late spermatids also exists in parallel with that evidence for lower vertebrates (Lam and Bruce, 1971; Bellve et al., 1975; Platz et al., 1975). A variety of meiotic histones, which replace the usual somatic histones until themselves replaced by protamine-like histones, have also been identified (Kistler and Geroch, 1975; Shires et al., 1975; Grimes et al., 1975). It is possible that these meiotic histones are 'pre-protamines' (Goldberg et al., 1977). Other proteins involved in the rearrangement of sperm chromatin may exist and may also be synthesized late in spermiogenesis: post-meiotic synthesis of non-histone, phenol-soluble acidic chromatin protein(s) has been suggested (Pipkin, 1975).

Another approach to the number of post-meiotically synthesized proteins involves electrophoretic analyses of newly synthesized proteins in separated spermatogenic cells or spermatozoa at various time intervals after labelling. One-dimensional gel electrophoretic analysis of sperm proteins demonstrated that insoluble sperm head proteins were synthesized in mid-spermatids in parallel with the synthesis of protamine-like histone (O'Brien and Bellve, 1980). Two-dimensional gel analyses of separated, spermatogenic cells by one group showed 11 spots unique to post-meiotic stages (Boitani et al., 1980), while our group found 10 cytoplasmic proteins specific to early spermatids, 1 cytoplasmic protein specific to late spermatids, 7 membrane proteins specific to early spermatids and 6 membrane proteins specific to late spermatids — a total of 24 post-meiotic appearing proteins (Kramer and Erickson, 1981b). These are, of course, minimal estimates.

t-ALLELE TRANSMISSION RATIO DISTORTION

Of the genetic variants in a variety of organisms which result in non-Mendelian ratios, perhaps the most interesting are mutations at the t-region of the mouse. In addition to unorthodox features of genetic transmission, t-alleles have remarkable effects on early embryonic development and differentiation (Glucksohn-Waelsch and Erickson, 1970; Bennett, 1975; Erickson et al., 1980b). The tail, from which the 'T' derives, has served as the indicator phenotype for mutations with more profound effects. The dominant T (brachyury) mutation causes a short tail in the heterozygous state. A large number of recessive mutations, t^n, interact with T to produce taillessness in compound heterozygotes (T/t^n). Such tailless mice are maintained via a balanced lethal system with the two homozygous types eliminated prenatally (both T/T and t^n/t^n are embryonic lethals). Males (but not females) heterozygous for a t-allele transmit it to many more of their progeny than the 50% dictated by Mendel's laws. Many t-alleles recently recovered from wild populations have segregation ratios of greater than 0.95, while laboratory populations are usually characterized by lower ratios. The ratios vary considerably between individual males carrying a particular t-allele as well as between different t-alleles (Braden, 1972); there are low distorters as well. However, it is now quite apparent that low (Dunn and Bennett, 1968) is a t-allele without T-interacting, lethal, or crossover suppression effects (Lyon and Mason, 1977). However, males which are compound heterozygotes for two different t-alleles which complement each other (for viability) are sterile.

Most of the known physiological effects of t-alleles can be interpreted by postulating that the gene product functions at cell surfaces; thus it has been suggested that the t-allele gene product might be detected as a cell surface antigen (Glucksohn-Waelsch and Erick-

son, 1971). A cell surface antigen determined by t-alleles might be less translocatable across intercellular bridges than a cytosol protein, and haploid gene expression could result in non-equivalent gametes and thus transmission ratio distortion (Erickson, 1978). If a putative t-antigen was not translocatable across the intercellular bridges, it should not be an essential component of spermatozoa, since it has been shown that spermatozoa deficient for the entire wild-type chromosomal T-region can successfully fertilize eggs (Lyon et al., 1972).

Evidence for the presence of an altered sperm-associated antigen determined by a dominant T allele was found subsequently with a mouse antiserum raised against spermatozoa from mice heterozygous for T (Bennett et al., 1972). However, the relevance of this spermatozoal antigen to the physiology of the T-region is questionable as T is silent in terms of sperm function. Further studies claimed partially distinctive antigens associated with several different recessive t-alleles, and produced evidence compatible with post-meiotic expression of a putative t-antigen in differentially viable t and wild-type spermatozoa (Yanagisawa et al., 1974a, b). Studies of anti-spermatozoal antibodies to spermatozoa from animals bearing various t-alleles, and analysis of the cross-reacting components, suggested that a complex antigenic system might be involved (Artzt and Bennett, 1977). Recent extensive experiments have been unable to confirm the existence of putative t-antigens on spermatozoa (Goodfellow et al., 1979; Gable et al., 1979).

Transmission ratio distortion can be accounted for by two different mechanisms, either unequal numbers of two kinds of spermatozoa or equal numbers of functionally different spermatozoa are produced. Selective cell death during spermatogenesis could be hypothesized to be involved in the generation of unequal numbers of functionally different spermatozoa. In such an hypothesis, unequal numbers of spermatozoa could result because some chromosomes preferentially segregate to cytoplasm that is destined to die (a hypothesis for segregation distorter in *Drosophila* — Peacock and Erickson, 1965). However, there is a deficiency of only about 13% of the cells expected on the basis of mouse spermatogenic kinetics, and these include pre- and post-meiotic cells (Oakberg, 1956a, b). Thus, non-Mendelian ratios of the magnitude seen with t-alleles cannot be explained by this mechanism.

Alternatively, there is evidence which suggests that the transmission ratio distortion controlled by t-alleles involves functional differences between spermatozoa bearing different t-alleles. Delayed mating, in which fertilization occurs as soon as the spermatozoa reach· the fallopian tube, compared to normal mating which requires that sperm remain in the female reproductive tract for several hours before fertilization, nullifies the transmission ratio distortion in the case of several t-alleles (Braden, 1958; Yanagisawa et al., 1961; Erickson, 1973; Fig. 3). The fact that the t-allele effect can be altered by changing the time of insemination relative to ovulation suggests that there are two classes of spermatozoa with unequal physiological characteristics, which could arise as the result of haploid gene expression. The transmission ratio distortions for the t^6 and t^{12} alleles found with in vitro fertilization were similar to those found with delayed mating (McGrath and Hillman, 1980, 1981). If verified for other t-alleles, this result would suggest that it is the physiological environment rather than the time element which is crucial.

The delayed mating experiments suggest that t-allele effects on transmission ratios could be mediated through alterations in spermatozoal metabolism, perhaps with sec-

LATE MATING

STANDARD MATING

ovulation

Insemination

eggs at fertilization site
in Fallopian Tube

Fertilization

Insemination

~1 hr.

Fertilization

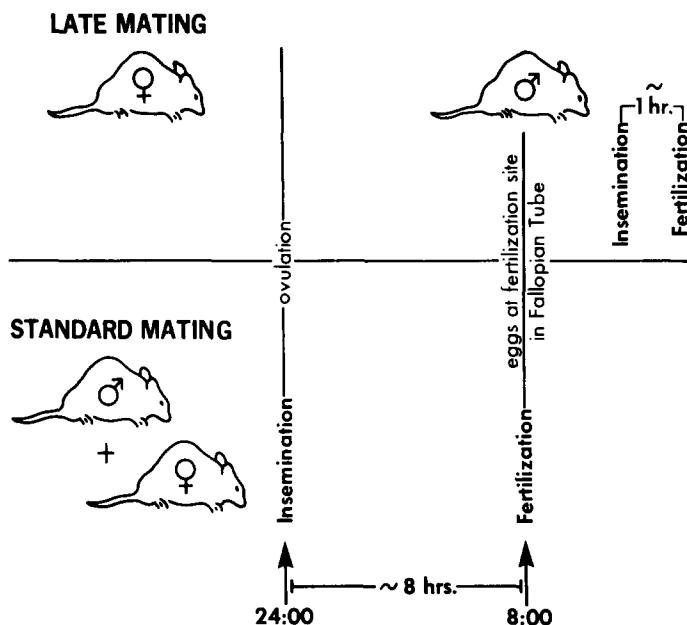├──── ~ 8 hrs. ────┤

24:00   8:00

Fig. 3. Delayed mating compared to standard mating. In standard mating, the male is continuously caged with the female which becomes receptive at the time of ovulation (about 24.00 hours) Since the eggs do not reach the fertilization site in the Fallopian tube until 7 or 8 h later, fertilization occurs after a 7 or 8 h sojourn by the spermatozoa in the female tract. With late mating, the male is not put into the cage until 08.00 hours, the eggs are already at the fertilization site, and fertilization occurs in about an hour.

ondary effects on sperm motility. These alterations could be related to the lethality of homozygous embryos, cause sterility in complemented males, and be beneficial in sperm in the presence of a wild-type allele. There is some evidence to suggest that t-bearing sperm differ in their ability to maintain motility (Yanagisawa, 1965; Katz et al., 1979). Ginsberg and Hillman (1974) found an inverse relationship between the transmission ratio and the NADH/NAD ratio of particular t-spermatozoa (from heterozygotes) while a direct correlation of transmission ratios with oxygen uptake was found. These results extend morphologic observations of early lipid accumulations and altered ATP turnover in several lethal t^n homozygotes (Hillman et al., 1970; Nadijcka and Hillman, 1975a, b; Ginsberg and Hillman, 1975). These metabolic defects could reside in mitochondria (Blake, 1977), under nuclear genome control, and, since mitochondria should be less likely to pass through the intercellular bridges connecting spermatids, the products of gene expression involving mitochondria might also not be shared by adjacent spermatocytes or spermatids. This hypothesis is further strengthened by the fact that mitochondria take on an enlarged role in the energy metabolism of sperm. Enzymes which are normally cytoplasmic are found to be purified with mitochondria (lactate dehydrogenase; De Domenech et al., 1970), localized in the midpiece histochemically (α-glycerophosphate dehydrogenase; Posalaky, 1965) or unusually difficult to solubilize (hexokinase; Harrison,

1971; glucose-6-phosphate dehydrogenase; Sarkar et al., 1977).

A further unifying hypothesis to explain the multiple effects of *t*-alleles might predict that antigens determined or controlled by some portion of the *T*-region are related to membrane transport, hormone receptors, adenylate cyclase or other membrane features affecting intracellular metabolism. This hypothesis gains some credence from the fact that Meruelo and Edidin (1975) have detected *H-2* determined differences in hepatic cAMP levels due to altered glucagon binding (Lafuse and Edidin, 1980). The possibility that *H-2* and *T* are functionally related has been suggested because of their linkage disequilibrium (Snell, 1968; Hammerberg and Klein, 1975). Neither *H-2* nor the *T*-region seems to have an effect on spermatozoal cAMP levels (Erickson et al., 1979a) measured under physiological conditions, although differences have been reported (Nadijcka and Hillman, 1980) in the presence of a phosphodiesterase inhibitor.

SPERMATOZOA DIFFERING IN CHROMOSOMAL CONTENT

Evidence for haploid gene expression is not limited to situations involving single genes. Spermatozoa differing in their chromosomal content may also be functionally different. For example, it is possible that spermatozoa bearing X- or Y-chromosomes express different phenotypes. Attempts to separate X- and Y-bearing spermatozoa by physical methods which do not depend on sperm motility (i.e. the sperm are passive) and those in which sperm motility plays a part (active) must be distinguished. It is frequently assumed that the difference in weight and/or density between an X- and a Y-chromosome would be large enough to allow passive separation. The slight difference in DNA content (about 3% (Sumner et al., 1971), which represents about 1% difference in total weight), is unlikely to allow passive physical separation of sperm (Roberts, 1972). In one study it was impossible to separate diploid spermatozoa more than slightly from haploid spermatozoa by density centrifugation (Beatty and Fechheimer, 1972). Thus, if X- and Y-bearing spermatozoa are separable by physical techniques, it may not be because of the slight difference in DNA content but because of differential gene expression in the two types of sperm.

A separation of X- and Y-bearing sperm under conditions in which each ciliary beat could provide a fractional separation based on the difference in DNA content, i.e. spermatozoal-active separation, is theoretically possible. In this case each ciliary motion provides a 'theoretical plate' for magnifying the minute difference in weight due to the difference between an X- and a Y-chromosome. Again, claims for successful spermatozoal-active separations may be interpreted in terms of differences in gene expression, as well as by differences in DNA content.

Recent data on the possibility of separating X- and Y-bearing spermatozoa have largely depended on staining a fluorescent body (F-body) believed to represent the Y-chromosome in fixed spermatozoa (Barlow and Vosa, 1970). This technique is only readily performed on human spermatozoa. The size of the spermatozoa affects the ease of detecting an F-body (Roberts and Goodall, 1976) but the technique seems well validated (Sumner and Robinson, 1976). Rodents and rabbits are more typical organisms for reproductive studies but evaluating separations by staining for F-bodies in human sperm is considerably easier than evaluation by artificial insemination with separated spermatozoal fractions in

these animals. Using F-body quantitation, a number of investigators have reported that Y-bearing spermatozoa migrate more rapidly in cervical mucus (Rohde et al., 1973; Goodall and Roberts, 1976; Beck et al., 1976), in a laminar flow set-up in semen extender (Bhattacharya et al., 1977) or in albumin gradients (Ericsson et al., 1973; David et al., 1977). However, two groups have presented negative data on X- and Y-sperm separation using the latter technique (Evans et al., 1975; Ross et al., 1975). The well known slight excess of males at birth is also seen early in pregnancy (Yamamoto et al., 1977), suggesting that this ratio is the result of preferential fertility rather than preferential mortality.

In summary, the many observations of preferential fertilization by Y-chromosome bearing sperm may be more readily explained by post-meiotic gene expression than by the very small difference in amount of DNA in Y- and X-bearing spermatozoa.

It is a general observation that males, both plant and animal, who are carriers of balanced translocations do not transmit the unbalanced state to their offspring nearly as frequently as do female balanced carriers (Hamerton, 1971; Sears, 1953). This result could be due to a difference in meiotic segregation in the two sexes or to a deficiency in fertilizing ability of sperm with unbalanced chromosomal constitution, i.e. post-meiotic gene expression in sperm. It is difficult to study meiosis in man but there have been a number of studies in other mammals which suggest that both mechanisms are at work in some species. In mice, where decreased transmission of translocations by the male occurs, meiotic nondisjunction was found to be markedly lower in the male than in the female according to one report (Oshimura and Tagaki, 1975). Furthermore, it has been reported that aneuploid sperm produced a significant number of aneuploid zygotes which died early in embryogenesis (Ford, 1972; Gropp, 1971). While these experiments did not provide evidence for post-meiotic gene expression, decreased transmission of radiation-induced deficiencies in male but not in female mice, seems to be explained by selective fertilization and not by differential nondisjunction in the male (Russell and Russell, 1960). In sheep, meiotic studies show a significant number of aneuploid secondary spermatocytes while aneuploid zygotes are not found (Chapman and Bruere, 1975). It therefore appears that aneuploid spermatocytes may preferentially degenerate or produce nonfunctional sperm and, thus, are influenced by their genetic content.

ANTIGENS ON SPERMATOZOA

Considering this background, what is the evidence for haploid expression of sperm antigens? Since the intercellular bridges connecting post-meiotic cells might limit sharing of membrane components (because of the membrane specializations which maintain them), we believe that cell surface antigens found on spermatozoa could show haploid expression. It is a moot point whether or not the human A, B, O and rhesus blood group antigens are expressed on spermatozoa. Thus, it is not surprising that there is even greater disagreement on whether or not there is haploid expression of such antigens. Gullbring (1957) reported the separation of blood group A spermatozoa from those bearing blood group B in AB individuals by agglutination. These results were apparently confirmed by a variation of this technique (Popivanov and Vulchanov, 1962) and by immunofluorescence (Shahani and Southham, 1962; Kerek, 1964) but not by electrophoresis of antibody-treated spermatozoa (Ackerman, 1969). This discrepancy might be explained by the

fact that a number of investigators have been able to find A or B substance on spermatozoa of secretors (individuals whose exocrine gland secretions contain soluble blood group substances) but not on the spematozoa of non-secretors. Spermatozoa from an O non-secretor can adsorb A or B substance from the seminal plasma of a secretor (Edwards et al., 1964; Boettcher, 1965; Parish et al., 1967; Rangnekar and Rao, 1970). Thus, ABO substance is adsorbed and could not show haploid expression. Detailed studies on the location of blood group glycosyl-transferases in the testis will be needed before the controversies concerning haploid expression of blood group antigens are solved.

Similar controversy surrounds the question of haploid expression of histocompatibility antigens on spermatozoa. It is not clear in all systems whether or not histocompatibility antigens are even expressed on spermatozoa, or whether they are simply adsorbed from seminal plasma. This is a problem encountered in studies of ejaculated human sperm, since seminal plasma does contain HLA antigens (Singal et al., 1971; Singal and Berry, 1972). It is not a problem for the studies of *H-2* antigens of mice since they are studied on spermatozoa from epididymides and ducti deferentes. The strongest evidence for the direct expression of histocompatibility-related antigens on spermatozoa comes from studies on the development of Ia antigen expression during spermatogenesis. The genes determining these antigens map within the *H-2* complex. Using mutant mice with germ-cell deficient testes, it was found that Ia antigens are not expressed on spermatogonia or testicular somatic cells but are expressed on primary spermatocytes and on cells at later stages of spermatogenesis (Fellous et al., 1976; Erickson, 1977a, b).

Most of the evidence, however, is against haploid expression of *H-2* antigens (Goldberg et al., 1970; Erickson, 1972; Johnson and Edidin, 1972). The original suggestion of haploid expression of histocompatibility antigens depended on the finding that a mixture of antibodies to each parental haplotype had an additive cytotoxic action on spermatozoa of heterozygous individuals (Fellous and Dausset, 1970). It seems equally possible, or perhaps more likely, that more sperm are killed when both anti-parental sera are used because more antibody molecules attach to the spermatozoa which are expressing both sets of parental antigens (Kerek and Afzelius, 1972; Fellous and Dausset, 1973). Recent reports that two classes of spermatozoa, each expressing only one parental HLA haplotype, may be separated in ejaculates of HLA heterozygous males (by treatment with antibody to one haplotype and complement, followed by removal of the dead spermatozoa on nylon wool columns) would seem to indicate haploid gene expression but need verification. The separated spermatozoa have then been typed for *cis* antigens (confirmed by family studies; Arnaiz-Villena and Festenstein, 1976) or used in a blast transformation test to demonstrate haploid expression of HLA-D (Halim and Festenstein, 1975, 1978). In the latter case it is quite possible that antibody blockage of mixed lymphocyte culture stimulating sites, independent of their haploid or diploid expression, was being detected. On the other hand, perhaps a difference in the mode of expression of the antigens on spermatozoa could turn out to be the first major difference in the HLA histocompatibility complex of man and the *H-2* histocompatibility complex of mouse. However, the putative HLA-D has very different properties on sperm than on lymphocytes (Levis and Dattner, 1979) and some workers find no more stimulation by allogeneic than autologous spermatozoa (Kurpisz and Szymezynski, 1979).

There has been a report that the percentage of males in mice can be decreased from

53.4% to 45.4% by treatment of sperm with antibody to the male-specific H-Y antigen (Bennett and Boyse, 1973), which might suggest that haploid expression of the H-Y antigen has taken place. Another paper presented a similar result using anti-cock sperm antibody on rabbit sperm (Hancock, 1978); however, the controls had an unexplained excess of males. F-body-positive human spermatozoa are preferentially stained by the lectin phytohaemagglutinin, which suggests haploid expression of some glycoprotein on Y-bearing sperm (Hegde et al., 1981). These observations need to be verified.

CONCLUSIONS

Although there is strong evidence for post-meiotic RNA synthesis during mammalian spermatogenesis, definitive proof of haploid gene expression of antigens or other markers is lacking. Transmission ratio distortion in *t*-allele-bearing males provides evidence for post-meiotic gene expression since delayed mating experiments argue strongly that there are similar numbers of functionally different spermatozoa in ejaculates of males carrying moderately distorting *t*-alleles. Other possible examples of transmission ratio distortion involve differences in chromosomal content of, or antigens on, sperm. In these instances, gene products from only one, or a few, loci could determine functional differences in the spermatozoa, the products of most other loci being equilibrated through the intercellular bridges. Thus, post-meiotic transcription of a number of loci may occur but the intercellular bridges connecting spermatids would usually maintain gametic equivalence. Membrane components such as cell surface antigens are likely candidates for the demonstration of haploid gene expression since the membrane specialization of the intercellular bridges would probably prevent lateral movement from one spermatid to another.

The fact that transmission ratio distortion has not been found in females can be explained by differences in the timing and physiology of gametogenesis in the two sexes. In contrast to spermatogenesis, where post-meiotic differentiation is extensive, the post-meiotic interval is very short in the maturation of the egg, in the higher vertebrates typically a matter of only a few hours. Thus, it is not surprising that there is a need for post-meiotic gene expression during spermatogenesis but not during oogenesis.

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