Genetic control of β-glucuronidase in murine spermatozoa

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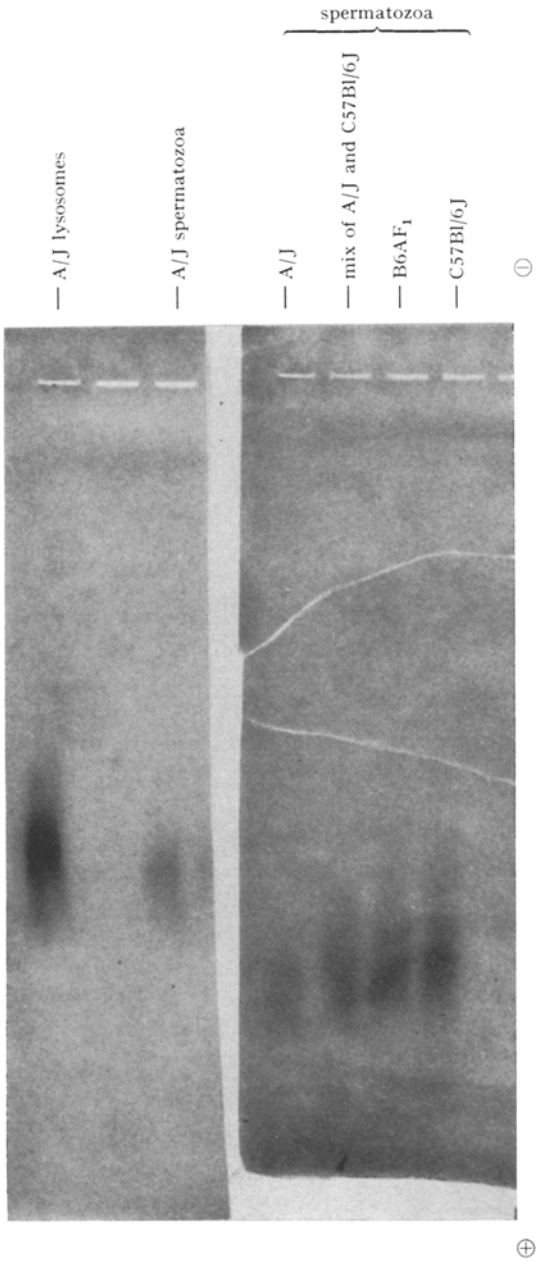
Summary. Electrophoretic studies of murine spermatozoal β-glucuronidase by acrylamide and starch gel electrophoresis demonstrated it to be the lysosomal form. There was no evidence for post-meiotic transcription of the structural gene for β-glucuronidase.

Many questions about the genetic control of spermatozoal structure and function should be conveniently answerable in Mus musculus. One important question is: are there more sperm-specific isoenzymes? We have performed electrophoretic studies of murine spermatozoal β-glucuronidase which demonstrated co-mobility with the lysosomal form. Spermatoza were like other tissues from heterozygotes for the fast and slow electrophoretic forms in showing a hybrid band, there was no evidence for haploid gene expression.



Starch gel electrophoresis of β-glucuronidase from A/J liver lysosomes compared to A/J spermatozoa (left) and of A/J (Gus^a), a 50:50 mixture of A/J and C57B1/6J, $B6AF_1$ (C57B1/6J × A/J, Gus^{ab}) and C57B1/6J (Gus^b) spermatozoal extracts (right). These are 2 different gels with the difference in motility of the A/J sperm extract on the 2 gels being explicable by variations in time of electrophoresis.

Spermatozoa from the epididymides and vasa deferentes of at least 8 week animals were harvested by slicing the tissue to approximately 1 mm slices in several ml of Beatty's solution (0.2 M glucose, 0.05 M NaCl, 0.02 M Na_2HPO_4, 0.0026 M KH_2PO_4) in which they were allowed to soak at least 5 min. The suspension was then screened through a No. 16 gauge screen which was rinsed with more solution. 10 min were allowed for tissue fragments to settle out and the supernatant was transferred to a centrifuge tube and centrifuged at 700 × g for 10 min at 4 °C. The pellet was resuspended in a similar volume (about 7 ml) and the centrifugation repeated. Microscopic examination revealed slight contamination of red blood cells and less than 2% contamination with other cells. The pellets were extracted in 5% Triton X-100 (Rohm and Haas) to maximize release of microsomal forms or in distilled water to recover maximally the lysosomal form; for comparison, liver was homogenized 10% wt/vol and extracted in a similar manner.

Extracts were electrophoresed on polyacrylamide gels in pH8.1 Tris-glycine according to the method of Swank and Paigen[2] or on 10% starch gels (Electrostarch Co.) with 0.008 M Tris (Sigma Chemical Co.), 0.003 M citric acid, pH 7.0 gel buffer and 0.223 M Tris, 0.086 M citric acid, pH 7.0 bridge buffer for 15–18 h at 150 v and 4 °C in a horizontal apparatus. After electrophoresis, the gels were pre-incubated for 30 min with a 0.2 M acetate, pH 4.6 buffer to adjust the pH downward and stained with naphtol AS-BI-β-glucuronic acid (Sigma Chemical Co.) and Fast Garnet GBC (Sigma Chemical Co.) according to the method of Lalley and Shows[3].

β-glucuronidase has a single lysosomal form and electrophoretically distinguishable multiple microsomal forms which have been designated L (lysosomal), M1...M4 (microsomal forms), and X (the 'free' microsomal form)[2]. The apoenzyme of these separable entities is controlled by the Gus locus while the M1...M4 forms have an added polypeptide controlled by a locus unlinked to the Gus locus. Polyacrylamide gel electrophoresis of 5% Triton extracts of liver disclosed M, X, and L bands while similar extracts of sperm demonstrated only the lysosomal form. Starch gel electrophoresis also demonstrated that the β-glucuronidase extracted from spermatozoa had an electrophoretic mobility indistinguishable from that found for lysosomal β-glucuronidase (figure). The electrophoretic difference between Gus^a/Gus^a (A/J) and Gus^b/Gus^b (C57B1/6J) was clearly seen in spermatozoa. Spermatozoa showed an F_1 hybrid band indistinguishable from that of other F_1 tissues while a 50:50 mixture showed the

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expected 2 bands. Evidence for lack of post-meiotic gene expression is also reported for glutamate oxalacetic transaminase[4]. Pre-meiotic synthesis of β-glucuronidase with persistence in post-meiotic stages has been suggested in rat testis[5]. However, as I have previously argued[6], the presence of intercellular bridges between spermatids provides a syncytial quality to the developing spermatozoa and would help to assure gamete equivalence.

Another conclusion this work provides is that the structural gene for spermatozoal β-glucuronidase is the Gus locus. The evidence for this comes from the demonstration by electrophoresis of the expression of the Gus^a and Gus^b alleles. This conclusion is interesting in that certain enzymes have been shown to have a sperm-specific isoenzyme (e.g. lactate dehydrogenase, hyaluronidase). A third conclusion is that spermatozoal β-glucuronidase shows the electrophoretic properties of the lysosomal form. This is not surprising as it is the lysosomal form which does not have attached peptides, but the finding is also relevant to the question of acrosome formation. The acrosome is known to contain many lysosomal enzymes and has been postulated to be a specialized lysosome[7], while the fact that acrosomal hyaluronidase and proteolytic activities are very different from those of lysosomes suggests less of an homology of acrosomes to lysosomes. If Mathur[8] is correct in asserting that β-glucuronidase is found in the mid-piece, then this lack of localization of lysosomal enzyme in the acrosome would argue further for the uniqueness of the acrosome in contrast to lysosomes.

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Evidence of the involvement of membrane-bound steroids in the photoperiodic induction of flowering in Xanthium

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Summary. Filipin, which interferes with sterol stabilization of phospholipid membrane layers, inhibits the photoperiodic induction of flowering in Xantium; the inhibition is reversed to a large extent by cholesterol.

The role of steroidal substances in plant flowering is a matter of controversy[2,3]. Application of oestrogenic substances, phytosterols and several other steroids have been reported to promote flowering of several plants and increased levels of oestrogen, oestrogen-like substances and sapogenins have been observed at the time of flowering or under photoinductive conditions. The relevant recent literature has been reviewed by Grunwald[4]. We report here our observations which suggest the involvement of membrane-bound steroids in flowering.

The polyene antibiotic filipin interacts specifically with sterols and inhibits the growth of those organisms which have membrane-bound steroids. Thus, it inhibits the growth of fungi and the inhibition is countered by sterols[5]; but species of Pythium which lacks sterol is completely unaffected by filipin and sterol treatment results in the conferment of sensitivity to filipin. Essentially similar results were obtained with mycoplasma laidlawii, which also lacks membrane-bound steroids[6]. Filipin also mediates permeability changes in erythrocytes[7]. Interaction of filipin with natural and synthetic membranes containing cholesterol has elucidated the mechanism of the interaction. The permeability effects are reversible with cholesterol[8,9].

Xanthium brittoni plants can be induced to flower with three photoinductive cycles. Photoperiodic effects on Xanthium buds are quantitative and bud growth from the vegetative condition to the development of influorescence primordium can be separated into 8 distinct stages numbered in an ascending order of development. Salisbury[10] has shown that the rate of floral bud growth can be most conveniently assessed from the stage number to which the developing floral bud belongs at any given time. The average stage corresponding to a particular treatment reflects the average advancement towards reproductive maturity. Filipin was applied on apical and the next 5 axillary buds of Xanthium at a concentration

Effect of filipin on photoperiodic induction of Xanthium and its reversal by cholesterol

Bud	Average of bud stages* Control	Filipin (100 μg/ml)	Filipin (100 μg/ml) + cholesterol (50 μg/ml)
Apical	8.00	6.00±0.30**	8.00
Axillary 1	7.67±0.19	3.50±0.34**	7.25±0.29
Axillary 2	5.33±0.20	2.37±0.30**	5.00±0.30
Axillary 3	5.00±0.20	1.00±0.20**	2.17±0.28**
Axillary 4	2.19±0.29	–	1.25±0.21***
Axillary 5	1.20±0.33	–	–

Each treatment was applied before each of 3 photoperiods. Scored after 10 days. * Bud stages scored according to Salisbury[10]. ** Significant at P = 0.01. *** Significant at P = 0.05.

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