Seminal Vesicle Formation and Specific Male Protein Secretion by Female Cells in Allophenic Mice

Abstract. *The relation of cellular sex genotype to phenotype was examined in seminal vesicles of adult allophenic mice with cellular sex chromosome mosaicism. Each animal originated from conjoined blastomeres of an embryo of female (XX) and one of male (XY) constitution, from different inbred strains. Cells of both sexes were detected in bone marrow and certain other somatic tissues; cellular sex of seminal vesicles was deduced from strain-associated electrophoretic variants of proteins coded for at autosomal loci. Seminal vesicles composed partly or entirely of female cells were found in male and pseudohermaphrodite individuals. In a pseudohermaphrodite, both allelic variants of the tissue-specific normal male seminal vesicle protein (*Svp-*locus) were present, signifying that female as well as male cells were synthesizing the protein. Male-determining factors on the Y chromosome are thus not required in cells that differentiate into functional seminal vesicles.*

We report a striking example of cellular developmental versatility in the mouse: differentiation of XX (chromosomally female) cells into seminal vesicles and synthesis by them of the organ-specific protein ordinarily found in males.

These observations were made in allophenic mice, animals that originate as artificial composites of blastomeres from two or more genotypically different embryos. The techniques for their production (*1*), recently reviewed (*2*), consist of aggregation of donor blastomeres in vitro at 37°C, after removal of the surrounding zona pellucida with Pronase, and subsequent surgical transfer of the composite to the uterus of a pseudopregnant surrogate mother. The majority of the embryos are viable.

Each tissue examined in allophenic animals derived from two embryos can contain cells of each genotype (*3*), for which unambiguous cell phenotypes are the indicators. Thus, every specialized cell population must arise from a minimum of two progenitor cells that form clones with tissue-specific functional genetic specialization. In a series of studies with appropriate allelic markers in the two cellular subpopulations, the actual clonal number, as well as the pattern of clonal deployment, has been found to be highly specific for each tissue. Clonal histories have been reconstructed for a number of tissues, including coat melanoblasts (*4*), hair follicles (*3, 5*), visual retina (*6*), pigment retina (*6*), liver (*7*), and vertebral column (*8*). The evidence suggests that differential gene activity and clonal initiation begin long before visible cell differentiation, and that developmental diversification is genetically controlled in clonal units (*3*).

Fortuitous combinations of blastomeres from XX and XY embryos occur. We have identified, by karyotype analyses, some 25 allophenic individuals that had both cellular sexes in one or more tissues. They comprise morphological males, females, and, infrequently, intersexes (*9, 10*), rather than exclusively males and intersexes as has been suggested in another report (*11*) based on three XX ↔ XY cases. Most of the XX ↔ XY animals are fertile. However, germ cell sex reversal does not occur; only the germinal cell strain that corresponds to the external sex phenotype undergoes complete gametogenesis and is functional (*9*).

In allophenic males with XY cells from two different inbred strains, both strains are sometimes present in the sex glands, seminal vesicles, and other accessory reproductive structures examined; thus each tissue must have a multiclonal orgin (*10*). In XX↔XY animals, both cell strains also sometimes coexist in various parts of the reproductive tract. Therefore, the somatic cells of at least some primordia of the mammalian tract apparently retain their ancient evolutionary developmental bipotentiality (*12*), although the germ cells do not. Dependence of secondary sex glands on fetal testicular hormone for their early development in the mouse (*13*) accounts for this lability. Three cases of adult XX ↔ XY mice with seminal vesicles composed partly or largely of XX cells are described below.

The first case (Table 1, animal A) has previously been mentioned (*10*). This was a sterile phenotypic male derived from the CBA/HIcr-*T6/T6* strain, bearing a cytologically detectable homozygous autosomal chromosome translocation, and the C57BL/6JNIcr (nontranslocation) strain. Its coat was *agouti*, like that of CBA. At autopsy (619 days of age), very small testes were seen. Chromosome examination of metaphase cells by standard procedures (*14*) after in vivo administration of colchicine established that the CBA-*T6/T6* cells were XX and the C57BL/6 cells, which lacked the translocation, were XY. There were 557 XX and 275 XY cells counted in bone marrow preparations, 48 XX and 26 XY cells in spleen, 59 XX and 60 XY in lymph nodes, and 25 XX in corneas. To ascertain the cellular chromosomal sex of other tissues, the supernatant fractions of the homogenized tissues were screened by separation in starch gel for allelic electrophoretic strain variants of isocitrate dehydrogenase (*15*). The procedure would allow a minor component of approximately 5 percent to go undetected. Liver, kidneys, and lungs were very largely of CBA, and therefore XX, type. In the reproductive tract, only the XX strain-type was found in the testes. It is likely that testicular differentiation may have been due to the presence of

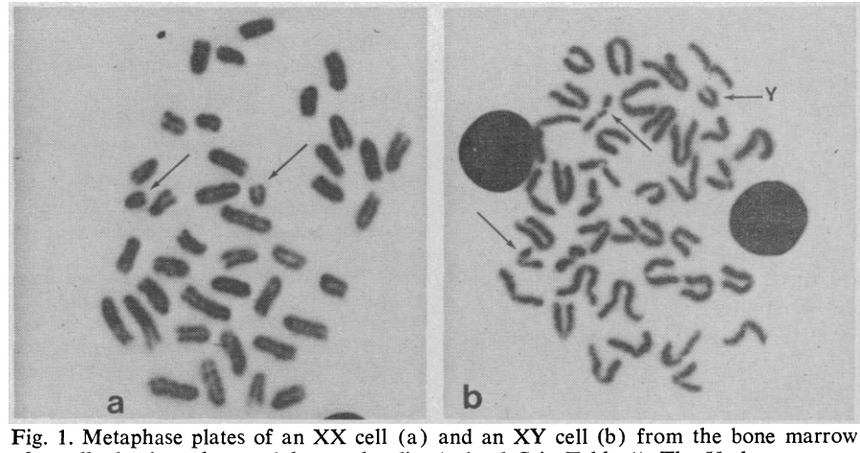

Fig. 1. Metaphase plates of an XX cell (a) and an XY cell (b) from the bone marrow of an allophenic male pseudohermaphrodite (animal C in Table 1). The X chromosomes are not distinguishable. An XX cell is identified by presence of the smallest (No. 19) pair of autosomes (unlabeled arrows) and the absence of the Y; an XY cell shows the two small autosomes and the Y chromosome.

Table 1. Female (XX) genotypic component in seminal vesicles and seminal vesicle protein of XX ↔ XY allophenic mice.

Animal	Strain combination	Sex phenotype	Chromosomal sex*		XX strain†	Seminal vesicle analyses	
			XX cells (No.)	XY cells (No.)		Marker‡	XX (%)
A	CBA-*T6/T6* ↔ C57BL/6	♂ (Sterile)	689	361	CBA-*T6/T6*	IDH	100
B	CBA-*T6/T6* ↔ C57BL/6	♂ (Not mated)	2622	75	C57BL/6	IDH	10
C	C3H ↔ C57BL/6	♂ Pseudohermaphrodite	7	25	C57BL/6?	SVP	25

* Sex chromosome constitution was analyzed in metaphase cells of some or all of the following tissues: bone marrow, spleen, lymph nodes, cornea. Cytological preparations were made from marrow of long bones, from approximately one third of the spleen, from both corneas, and from various lymph nodes. † The XX strain of animal C was not positively identifiable; the more conservative possibility is given here. ‡ Markers are: IDH, isocitrate dehydrogenase; SVP, seminal vesicle protein. Small minor components can go undetected with these markers.

an undetected, small but dominant, XY component in the sex glands, with XX somatic cells (but not germ cells) developing in a male direction (*10*). The seminal vesicles were morphologically normal but small; they also revealed only the XX strain, but a minor XY population may have been present.

The second case (animal B) was an ostensibly normal male of the same strain combination as animal A; it had not been test-mated. Its coat showed both *agouti* (CBA) and *nonagouti* (C57BL/6) hair follicle clones, in a finely banded pattern of transverse stripes interpreted as reflecting dermatome origin (*3, 5*); a relatively higher concentration of *nonagouti* (black) occurred on the crown and face. At autopsy, when the animal was 527 days of age, a normal male tract was observed. Metaphase studies permitted identification of the XX cell strain as C57BL/6 (nontranslocation) and the XY strain as CBA-*T6/T6*. There were 2488 XX and 34 XY cells tabulated in the bone marrow, 86 XX and 9 XY cells in spleen, 44 XX and no XY cells in lymph nodes, and 4 XX and 32 XY cells in corneas. Biochemical analyses of other tissues for isocitrate dehydrogenase variants showed the kidneys to be 80 percent of the XX strain, the lungs all (or largely) XX, and the individual liver lobes of variable composition, with a range of 11 to 80 percent of the XX strain-type. The seminal vesicles were largely of the XY strain with approximately 10 percent of the XX type.

The third case (animal C) was produced from the C3H/HeNIcr and C57BL/6NIcr cell strains. Its coat had both the *agouti* (C3H) and *nonagouti* (C57BL/6) strains visible in a hair follicle pattern similar to that of animal B. The external genitalia were intersexual, with a small penis, short genito-anal distance, and small vaginal opening ending in a cul-de-sac. At autopsy after colchicine treatment, at 93 days of age, a malelike tract with undescended testes was found; one gonad was slightly reduced, and the other was diminutive. The animal was classified as a male pseudohermaphrodite. Karyotype studies (Fig. 1) enabled both cell types to be recognized in bone marrow (3 XX and 12 XY cells) and spleen (4 XX and 13 XY cells). Although the female and male cells could not be identified as to their inbred strain of origin, the presence of cells of both inbred strains in any tissue would automatically signify the presence of both XX and XY cells. The liver was analyzed for allelic strain variants of malate dehydrogenase in the supernatant homogenate fraction, detectable by electrophoretic differences in starch gel (*16*); 95 percent of the C3H and 5 percent of the C57BL/6 variants were present.

Seminal vesicles of normal male mice secrete characteristic proteins not known to occur in any tissues of females. The seminal vesicle proteins (SVP) are electrophoretically separable in polyacrylamide gel. Some component proteins occur in two allelic genetic types, and inbred strains are classifiable according to their SVP electrophoretic patterns (*17*). The major bands of SVP are IV, V, and VI, according to the nomenclature of Platz and Wolfe (*17*). In strains homozygous for the autosomal Svp^a allele (for example, C3H), bands V and VI are relatively stronger, whereas IV and V are stronger in Svp^b homozygotes (for example, C57BL/6).

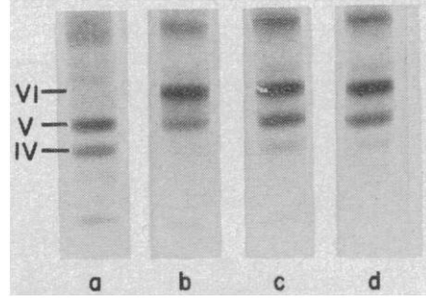

Fig. 2. Electrophoretic SVP patterns of a C57BL/6 control male (a), a C3H control male (b), and the left (c) and the right (d) seminal vesicles of the C3H ↔ C57BL/6 male pseudohermaphrodite with sex chromosome mosaicism (shown in Fig. 1). Each vesicle of the allophenic animal shows bands of both SVP strains, indicating SVP synthesis by XX and XY cells. Seminal vesicle fluid was dissolved in saline, and its soluble proteins were analyzed by polyacrylamide disc gel (7 percent) electrophoresis at *p*H 8.3 in tris-glycine buffer; the anode is at the bottom of the gels. Proteins were stained with amido black.

The seminal vesicles of animal C were small but contained ample fluid for strain diagnosis of SVP. The soluble proteins were separated electrophoretically (*18*), the gels were then stained with amido black, and densitometric tracings of the bands were obtained at 650 nm (Gilford model 2410 linear transport with model 2000 absorbance recorder and Beckman model DUR spectrophotometer). An equation has been developed (*19*) for calculation of the proportions of the two SVP phenotypes in an allophenic vesicle containing both types. The equation rests on the assumption that each cell synthesizes the same amount of protein, regardless of its SVP phenotype; calculations are based on the ratio of the strengths of bands IV : VI and V : VI, from the peaks for each band. Artificial mixtures of the two pure types were correctly diagnosed by these calculations. The presence of both SVP strains in the XX ↔ XY male pseudohermaphrodite is visible in the electrophoretic patterns (Fig. 2), in comparison with controls. Therefore, XX as well as XY cells must be secreting SVP. The proportion of the XX strain is either 25 percent, if C57BL/6 is XX, or 75 percent, if C3H is XX (Table 1).

Known dependence of seminal vesicle development on male hormone (*13*) indicates that one or both testes of the allophenic pseudohermaphrodite were producing androgen and that XX cells

in the fetus were able to respond to this stimulus by development in the male direction as seminal vesicles. A heritable form of male pseudohermaphroditism, not due to sex chromosome mosaicism, has been reported in rats; they secrete low levels of testosterone (*20*). The presence of a vagina in these pseudohermaphrodite rats as well as in an allophenic mouse such as case C could result from a subnormal amount of androgen in fetal stages.

From the fact that XO mice are females, it appears that the Y chromosome of the mouse is male-determining (*21*). Inasmuch as XX cells can develop into seminal vesicles and secrete SVP in the absence of any intracellular Y-linked factors, it follows that histogenesis of the vesicles and function of the *Svp* locus in the XY cells of normal males may also be independent of expression of male-determining factors on the Y in those cells.

BEATRICE MINTZ
MASAHARU DOMON
DAVID A. HUNGERFORD, JOHN MORROW*
Institute for Cancer Research, Philadelphia, Pennsylvania 19111

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* Present address: Department of Physiology and Cell Biology, University of Kansas, Lawrence 66044.

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Parasympathetic Ganglia: Activation of an Adrenergic Inhibitory Mechanism by Cholinomimetic Agents

Abstract. *Electrical stimulation of the sympathetic nerves to the urinary bladder or the intraarterial administration of the cholinomimetic substances acetylcholine or methacholine produced adrenergic inhibition in parasympathetic ganglia on the surface of the bladder. The inhibition appeared to be mediated, at least in part, via adrenergic inhibitory neurons located in the pelvic plexus. Atropine blocked the inhibitory response to injected cholinomimetic agents but did not alter the response to stimulation of the sympathetic nerves. Thus, the inhibitory neurons can be activated via both muscarinic and nonmuscarinic receptors, the latter being of primary physiological importance.*

Since the discovery by Marrazzi (*1*) that epinephrine depressed transmission in the superior cervical ganglion, there has been considerable interest in the possible role of the catecholamines as inhibitory transmitters in autonomic ganglia (*2*). The first electrophysiological evidence for such a role was obtained by Eccles and Libet (*3*). These investigators showed that repetitive stimulation of the preganglionic nerves to the curarized superior cervical ganglion of the rabbit produced a hyperpolarizing ganglionic potential (the P-potential) which was blocked by an α-adrenergic blocking agent or by atropine. They suggested that preganglionic fibers to the ganglion excited chromaffin cells, which in turn released an adrenaline-like substance. This substance was believed to act on the ganglion cells to produce the hyperpolarizing response. To account for the blockade of the P-potential by atropine, they proposed that transmission at the synapses on the chromaffin cells was cholinergic and was mediated via muscarinic receptors. The presence of chromaffin-like cells in close apposition to sympathetic ganglion cells (*4*) led to the proposal that the former might function as adrenergic inhibitory interneurons (*4*, *5*).

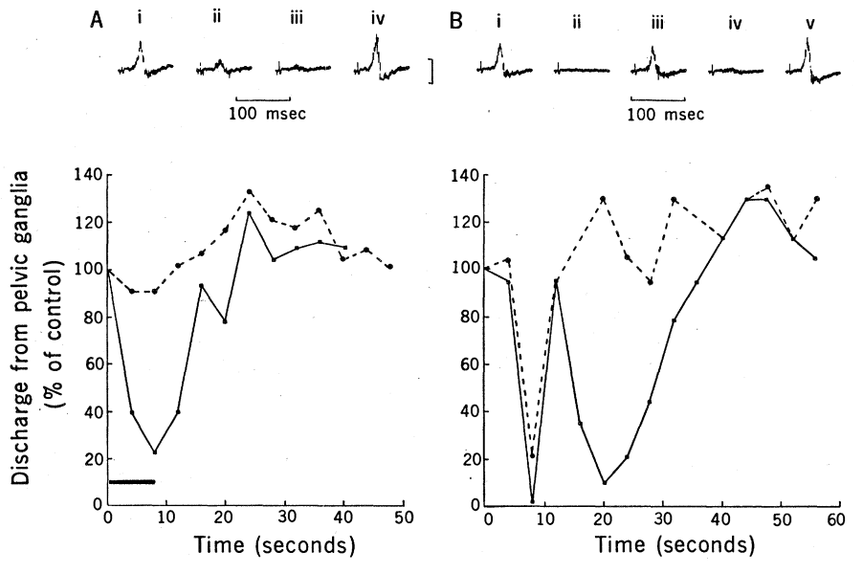

Fig. 1. Time course of the inhibitory effects in pelvic ganglia of (A) electrical stimulation (20 volts, 20 cycle/sec) of the hypogastric nerve (HGN-Stim) and of (B) injected methacholine. (A) Record i is the control discharge recorded on a vesical postganglionic nerve filament in response to submaximal stimulation of the pelvic nerve (2 volts, 0.5 cycle/sec). Records ii, iii, and iv are taken 4 seconds after HGN-Stim on, 2 seconds after HGN-Stim off, and 18 seconds after HGN-Stim off, respectively. Vertical calibration is 200 μv. Below is a graph of the depression by HGN-Stim (20 volts, 20 cycle/sec) of the discharge from pelvic ganglia. The bar indicates HGN-Stim. Dihydroergotamine (200 μg) was administered; the solid line shows data obtained before this drug was given, and the dashed line shows data obtained after it was given. (B) Record i is the control discharge, and records ii, iii, iv, and v are taken 8, 12, 20, and 44 seconds, respectively, after an injection of methacholine (5 μg). Vertical calibration is 200 μv. Below is a graph of the depression by methacholine (5 μg) of the discharge from pelvic ganglia. Dihydroergotamine (200 μg) was given; solid and dashed lines are as explained for A.