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CYTOGENETICS OF THE MAMMALIAN X CHROMOSOME

PART A

BASIC MECHANISMS OF X CHROMOSOME BEHAVIOR

Editor

AVERY A. SANDBERG

Department of Genetics and Endocrinology
Roswell Park Memorial Institute
Buffalo, New York

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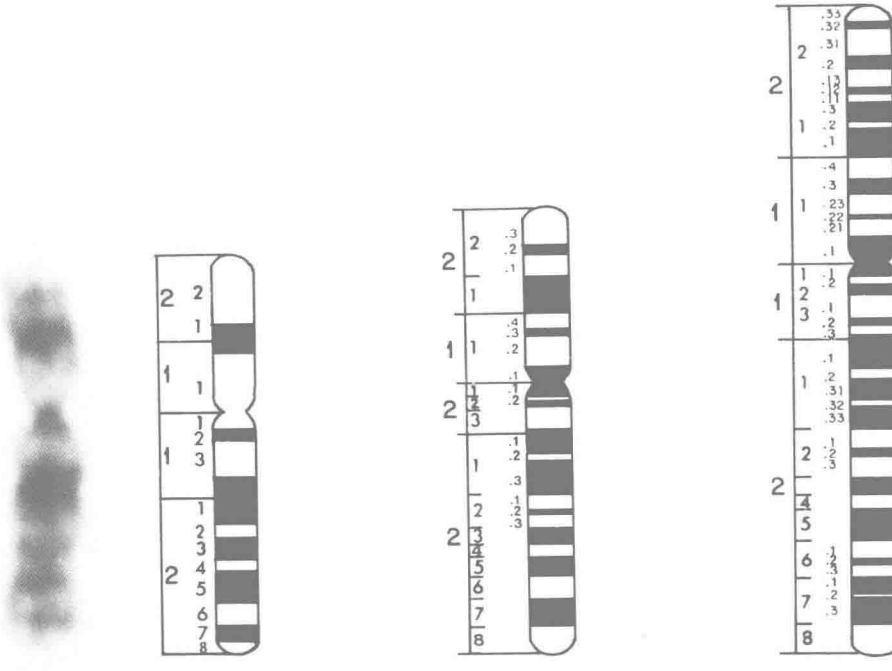
PART A

BASIC MECHANISMS OF X CHROMOSOME BEHAVIOR

Mapped Genetic Markers and Linked Genes on the Human X Chromosome (Listed Alphabetically)

Location on Chromosome	*Marker Symbol*	*Marker Name*
q28→qter	ALD	Adrenoleukodystrophy
q13→q27	BA2R	BALB/c 3T3 ts2 temperature sensitivity complementing
	C1HR	C1AGOH temperature sensitivity complementing
q	CBD	Color blindness (deutan)
q	CBP	Color blindness (protan)
	CGD	Chronic granulomatous disease
p11→q11	DHTR	Dihydrotestosterone receptor
p and q	DXS1–9	DNA segments
	DXZ1	
q21→q24	GLA	α-Galactosidase
q28	G6PD	Glucose-6-phosphate dehydrogenase
q	HEMA	Antihemophilic globulin A (factor VIII, hemophilia A)
q26→q28	HPRT	Hypoxanthine phosphoribosyltransferase
pter→p22.3	HYB	Y histocompatibility antigen, regulator
	HYC	Y histocompatibility antigen, receptor
p 22	ICH1	Ichthyosis factor
	MAOA	Monoamine oxidase
	MDB	Muscular dystrophy, Becker type
p 21	MDD	Muscular dystrophy, Duchenne type
	MIC2	Antigen identified by monoclonal antibody 12E7
	OA	Ocular albinism
	OTC	Ornithine transcarbamylase
q13	PGK	Phosphoglycerate kinase
q21→q27	PRPS	Phosphoribosylpyrophosphate synthetase
	RNN1	Nuclear RNA-1
	RS	Retinoschisis
q26→q28	S10-12	Surface antigens (X-linked) 1–3
q26→qter		
pter→p22.3	STS	Steroid sulfatase (microsomal)
cent→p13	TFM	Testicular feminization syndrome
	TATR	Tyrosine aminotransferase regulator
pter→p22.3	Xg	Xg blood group
	Xk	Kell blood group precursor
	XM	α_2-Macroglobulin
	XPAC	Fast kinetic complementation DNA repair in xeroderma pigmentosum, group A



G-Banded Human X Chromosome and Schematic Representations of It at Various Levels of Resolution, Showing the Regions, Bands, and Subbands.

Progress and Topics in Cytogenetics

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Avery A. Sandberg
Roswell Park Memorial Institute, Buffalo, New York

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Contributors

Jérôme Couturier, Institut de Progenèse, Centre National de la Recherche Scientifique, Equipe de Recherche Associée no. 47, F-75270 Paris Cedex 06, France **[375]**

Kay E. Davies, Department of Biochemistry, St. Mary's Medical School, Paddington, London, England **[479]**

Gordon W. Dewald, Cytogenetics Laboratory, Department of Laboratory Medicine, Mayo Clinic and Mayo Foundation, Rochester, MN 55905 **[405]**

Bernard Dutrillaux, Institut de Progenèse, Centre National de la Recherche Scientifique, Equipe de Recherche Associée no. 47, F-75270 Paris Cedex 06, France **[375]**

J. Egozcue, Department of Cell Biology, Faculty of Science, and Instituto de Biología Fundamental "Vicent Villar Palasí," Universidad Autónoma de Barcelona, Barcelona, Spain **[107]**

Charles J. Epstein, Departments of Pediatrics and of Biochemistry and Biophysics, University of California, San Francisco, CA 94143 **[51,341]**

Peter H. Fitzgerald, Cancer Society of New Zealand, Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand **[171]**

F. Giraud, Centre de Génétique Médicale et INSERM U. 242, Hôpital d'Enfants de la Timone, 13385 Marseille Cedex 5, France **[327, 449]**

Ingo Hansmann, Institut für Humangenetik, Universität Göttingen, Göttingen, West Germany **[131]**

Mary F. Lyon, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 ORD, England **[187]**

J.F. Mattei, Centre de Génétique Médicale et INSERM U. 242, Hôpital d'Enfants de la Timone, 13385 Marseille Cedex 5, France **[327, 449]**

M.G. Mattei, Centre de Génétique Médicale et INSERM U. 242, Hôpital d'Enfants de la Timone, 13385 Marseille Cedex 5, France **[327, 449]**

Ursula Mittwoch, Department of Genetics and Biometry, University College London, Wolfson House, 4 Stephenson Way, London NW1 4HE, England **[251]**

T. Mohandas, Division of Medical Genetics, Department of Pediatrics, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509 **[271, 299]**

Susumu Ohno, City of Hope Research Institute, Duarte, CA 91010 **[1]**

S. Pathak, Department of Cell Biology, The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Houston, TX 77030 **[67]**

Liane Brauch Russell, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830 **[205]**

Avery A. Sandberg, Department of Genetics and Endocrinology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263 **[xi]**

The number in brackets designates the first page number of the contributor's chapter.

Gloria E. Sarto, Department of Obstetrics and Gynecology, Northwestern University, The Medical School, Chicago, Illinois 60611 **[315]**

Larry J. Shapiro, Division of Medical Genetics, Department of Pediatrics, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509 **[271, 299]**

Nobuo Takagi, Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060, Japan **[21]**

Eeva Therman, Department of Medical Genetics, University of Wisconsin, Madison, WI 53706 **[315]**

Huntington F. Willard, Department of Medical Genetics, University of Toronto, Toronto, Ontario M5S, 1A8, Canada **[427, 463]**

Bryan D. Young, Beatson Institute for Cancer Research, Glasgow, G61 1BD, Scotland **[479]**

Alexander F. Zakharov, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow 115478, USSR **[357]**

Preface

No chromosome in the mammalian genome has received more attention, been studied more extensively, or shown a greater variety of behavior than the X chromosome. In the human, much of the accumulated material is concerned with a broad array of abnormal conditions associated with numerical X-chromosome abnormalities (among them the first clinical syndromes shown to be due to a missing or extra X chromosome, i.e. Klinefelter's syndrome, Turner syndrome), as well as with morphologic abnormalities of this chromosome and the expression of genetic material (genes) on it. It is not surprising that, to encompass such a wealth of information, it has been necessary to divide this work into two parts to avoid a large and cumbersome single volume. Part A covers the phylogeny, mapping, and cloning of the X chromosome; basic mechanisms of X-chromosome behavior and action, including DNA replication patterns; behavior in meiosis, mitosis, and development; and activation and inactivation of the X chromosome.

In each area covered, the subject matter is presented by a recognized world authority, in some cases a "historical figure" in his or her field. Every important facet of the X chromosome is reviewed in comprehensive, up-to-date fashion, with the background and historical aspects also given their due. One of the major reasons for undertaking this project was to bring together in one volume material dispersed over a wide variety of journals and other publications, thus making it readily available to students, researchers, and clinicians.

Undoubtedly, much remains to be learned about the X chromosome, but were we to know as much about other chromosomes as we do now about the X, there is little doubt that the field of mammalian cytogenetics (and genetics) would benefit immeasurably. This two-part volume constitutes, to my knowledge, the first attempt to gather together the available information on the X chromosome. Given the vicissitudes of planning, gathering, and editing a 49-chapter work by a large number of authors, this is not suprising. I am grateful to the contributors for their efforts and for the time expended in the preparation of their chapters; without their cooperation there would have been no book.

I wish to thank Miss Anne Marie Conti, Mrs. Cathy Russin, and Mrs. Diane Smith for clerical help, Mrs. AnneMarie Block for editorial assistance, and, in particular, my wife, Maryn, for her help in all facets of this volume.

Avery A. Sandberg, MD

THE X CHROMOSOME IN MEIOSIS, MITOSIS, AND DEVELOPMENT

Contents

Cytogenetics of the Mammalian X Chromosome, Part A
Basic Mechanisms of X Chromosome Behavior, pages 1–19

Chapter 1

Phylogeny of the X Chromosome of Man

Susumu Ohno

There are two alternative ways of minimizing a genetic disparity between the homogametic (XX or ZZ) and heterogametic (XY or ZW) sexes. One way is to maintain the X and the Y or the Z and the W in the homomorphic and genetically homologous state, in which case allelic differences at a few gene loci determine the sex. This is the means employed by most fish, amphibians, and reptiles. As YY males and WW females can be viable and fertile in this scheme, the male heterogamety and the female heterogamety are interchangeable within the same genus or even the same species. The other way is to let the Y or the W degenerate by miniaturization and heterochromatinization, until it retains only one or two pertinent genes in a functional state. This is the means employed by mammals and birds. As the switch from one means to the other has been accomplished exclusively at the expense of the Y or the W, the mammalian X and the avian Z have been conserved *in toto* since the time of their respective common ancestors.

Our recent DNA cloning experiment indicated that the female-determining gene residing on the ophidian W and the male-determining gene residing on the mammalian Y are one and the same, presumably governing the expression of H-Y plasma membrane antigen. Although conserved *in toto*, most of the mammalian X-linked genes have nothing whatsoever to do with sexual development, and a few that are involved in sex determination are expressed by males and females alike.

The very fact that true hermaphroditisms, of both synchronous and asynchronous types, are commonly encountered among teleost fish, and that the sex of turtles and alligators is rather haphazardly determined by incubation temperatures of fertilized eggs, tends to create an illusion that the chromosomal sex-determining mechanism is a modern, sophisticated device that arose late in vertebrate evolution. Nothing is further from the truth, for gono-

From the City of Hope Research Institute, Duarte, California 91010.

chorism is the rule in all members of the animal kingdom. It should also be pointed out that, in the plant kingdom, the sex chromosomes and sexual reproduction are most often found among members of the primitive phylum Bryophyta. Many of the mosses have an XX/XY scheme of sex determination. Some readers may be surprised to learn that the size difference between the large X and the small Y chromosome of mosses, such as *Sphaerocarpus donnellii*, is even more pronounced than that which we usually see between the mammalian X and Y chromosomes [1]. On the other hand, "modern" plants with beautiful blooms are, as a rule, hermaphrodites, the one flower bearing pollen on the stamens and ovules in the pistils. Thus, it may be construed that the true hermaphroditism that characterizes the "modern" plant is a secondary development. In a similar vein, hermaphroditisms of teleost fish and temperature-dependent sex determination of certain reptiles can also be viewed as a secondary development superimposed upon the preexisting chromosomal sex-determining mechanism.

SEX DETERMINATION BY THE LARGELY HOMOMORPHIC X AND Y, OR Z AND W, AND ITS FLEXIBILITY

By establishing the chromosomal sex-determining mechanism, organisms encounter one problem created by the heterogametic sex-specific occurrence of the Y or W chromosome. This should not create a great genetic disparity between the two sexes, for the simple reason that the progeny of the heterogametic sex inevitably include those of the homogametic sex. Whatever trait confers a selective advantage to the heterogametic sex must benefit the homogametic sex as well.

There are two ways of minimizing a genetic difference between the two sexes to the necessary minimum. It is worth remembering that, with regard to any gene locus, a mating between the heterozygote and the homozygote unfailingly perpetuates the same two genotypes in a one-to-one ratio, provided, of course, that only two alleles are involved. Thus, the minimal requirement for establishing the male heterogametic type of genetic sex-determining mechanism is to have two alleles at a single gene locus, homozygotes for the recessive allele developing as females and heterozygotes for the dominant allele developing as males. In this scheme of the chromosomal sex-determining mechanism, the X and the Y or the Z and the W can remain largely homologous to each other genetically and homomorphic to each other morphologically.

Indeed, in most of the gonochorist species of fish, amphibians, and reptiles cytogenetic identification of sex chromosomes is impossible. Yet, the male heterogamety or female heterogamety of a given species can easily be determined by sex-reversal experiments. These become particularly elegant if proper sex-linked marker genes are utilized. As an example, Yamamoto's ex-

periments with a small Japanese cypinodont fish, *Oryzias latipes*, are cited here. In this species, one of the body-coloration genes is closely linked to the sex-determining locus or loci. A recessive *r* (white) allele of the body-coloration locus is normally coupled with a female-determining gene on the X, while a dominant *R* (orange-red) allele is coupled with a male-determining gene on the Y. Thus, a stock can be constructed in which all the females are white (X^rX^r) and all the males are orange-red (X^rY^R). Because of the close linkage, cross-over orange-red females (X^rX^R) and white males (X^rY^r) are exceedingly rare (0.2%). Now if an appropriate amount of androgenic steroid is added to the aquarium water of hatchlings, all of the white X^rX^r females will be sex-reversed to functional males; a subsequent mating between such X^rX^r males and normal X^rX^r females naturally produces all female progeny of white phenotype, thus confirming the female homogamety. Conversely, an addition of oesterone produces sex-reversed X^rY^R red-orange females. By mating such sex-reversed females to normal red-orange males, a three-to-one sex ratio is obtained in the progeny; YY males:XY males:XX females = 1:2:1. If the YY males thus obtained, which are normal and fertile, are then mated to normal XX females, nothing but normal XY males result, thus confirming the male heterogamety [35,36].

It is the extensive genetic homology still retained between the X and the Y that confers the viability and fertility to YY males. In turn, the viability and fertility of YY males make a switch from the male heterogamety to the female heterogamety possible. If a new Y chromosome that, by a mutation, acquired a new female-determining allele dominant over the formerly dominant male-determining allele arises in an isolated population and spreads, the YY males now become homogametic ZZ males, and a new dominant Y becomes the female-determining W chromosome to produce ZW heterogametic females. Indeed, male heterogamety and female heterogamety often exist side-by-side in closely related species, and even in different populations of the same species, of gonochoristic fish, amphibians, and reptiles.

It now appears that the flexibility of this form of sex-determining mechanism is the foundation upon which secondary development of hermaphroditisms and the temperature-dependent sex-determining mechanism in numerous lower vertebrate species are based. In teleost fish, two forms of hermaphroditic reproduction are found: In the synchronous form, the same individual endowed with an ovary and a testis or ovotestes functions both as a male and a female in cross-fertilization or even self-fertilization. In the asynchronous form, which is age-dependent, it may be protoandrous (males when young) or protogynous (females when young). As to the temperature-dependent sex determination commonly found among turtles and alligators, the following observation made in pond turtles of the family Emididae is particularly pertinent. The key to our understanding is the heterogametic sex-specific

appearance of H-Y plasma membrane antigen in all vertebrates [32] and its relevance in determining the fate of embryonic indifferent gonads [33]. Among members of the family Emydidae, the morphologically distinct sex chromosomes of the XX/XY scheme have been reported only in the Asian black pond turtle *Siebenrockiella crassicollis* [4]. In *Emys orbicularis*, neither the X and the Y in male karyotypes nor the Z and the W in female karyotypes can be distinguished, and the nearly one-to-one sex ratio in this turtle species can be obtained only among the brood hatched within the narrow range of incubation temperatures. When extragonadal somatic cells (blood cells) and gonadal cells of such male and female turtles were serologically examined for H-Y antigen, all females and not a single male expressed H-Y antigen in either cell type, thus extablishing the chromosomal sex-determining mechanism of *Emys orbicularis* as the ZW/ZZ type of female heterogamety [37]. All *Emys orbicularis* hatched at high incubation temperatures of 30–30.5°C developed as females [27]. While their gonads (ovaries) uniformly expressed H-Y antigen, they were of two kinds with regard to the expression of H-Y antigen in their extragonadal somatic cells. Those who expressed H-Y antigen in extragonadal somatic cells too were obviously ZW genetic females. On the other hand, those with H-Y antigen-negative extragonadal somatic cells were ZZ genetic males that were sex-reversed to females by the high-temperature induction of H-Y antigen in their gonads [38]. The above series of experiments has been very instructive in revealing the temperature-dependent sex-determining mechanism operating in turtles and alligators as a mere secondary development superimposed upon the preexisting chromosomal sex-determining mechanism. The temperature-dependent sex determination is nothing but a form of sex reversal practiced by nature, and it relies upon the high-temperature-dependent induction of H-Y antigen expression in ZZ gonads as well as the low-temperature-dependent suppression of H-Y antigen in ZW gonads.

EVOLUTION OF THE HETEROMORPHIC SEX CHROMOSOMES EXCLUSIVELY AT THE EXPENSE OF THE Y OR THE W

Mammals, on one hand, and avian species, on the other, selected an alternative way as the means of minimizing a genetic difference between the two sexes. The means employed is to render the heterogametic sex-specific chromosome, the mammalian Y and the avian W, a genetic dummy retaining but one or a few genes that are exclusively concerned with the primary (gonadal) development of the heterogametic sex. It is extremely fortunate that one can readily follow the evolutionary transition from the genetically homologous, morphologically homomorphic Z and W to the grossly heteromorphic Z and W, exclusively due to progressive miniaturization and heterochromatinization of the W in snakes of the reptilian suborder Sepentes. By and large, diverse