be generated via random chance in the N segment, DSP2 to J_H3 rearrangements (which lack RSA I sites) were amplified and digested with Rsa I and no cutting could be detected.

26. I am grateful to C. Hasemann, L. Van Dyk, J. D. Capra, R. Baer, and P. Lipsky for helpful discussions and comments on the manuscript. This work was supported by the Arthritis Foundation.

29 June 1990; accepted 21 September 1990

A Mouse Model of the Aniridia-Wilms Tumor Deletion Syndrome

TOM GLASER,* JOAN LANE, DAVID HOUSMAN

Deletion of chromosome 11p13 in humans produces the WAGR syndrome, consisting of aniridia (an absence or malformation of the iris), Wilms tumor (nephroblastoma), genitourinary malformations, and mental retardation. An interspecies backcross between *Mus musculus/domesticus* and *Mus spretus* was made in order to map the homologous chromosomal region in the mouse genome and to define an animal model of this syndrome. Nine evolutionarily conserved DNA clones from proximal human 11p were localized on mouse chromosome 2 near *Small-eyes* (*Sey*), a semidominant mutation that is phenotypically similar to aniridia. Analysis of Dickie's Small-eye (Sey^{Dey}), a poorly viable allele that has pleiotropic effects, revealed the deletion of three clones, *f3*, *f8*, and *k13*, which encompass the aniridia (*AN2*) and Wilms tumor susceptibility genes in man. Unlike their human counterparts, Sey^{Dey}/+ mice do not develop nephroblastomas. These findings suggest that the Small-eye defect is genetically equivalent to human aniridia, but that loss of the murine homolog of the Wilms tumor gene is not sufficient for tumor initiation. A comparison among *Sey* alleles suggests that the *AN2* gene product is required for induction of the lens and nasal placodes.

THE EYE HAS LONG PROVIDED A system for studying inductive interactions during embryonic development. In vertebrates, considerable insight into eye development has been gained through classical ablation and transplantation experiments, yet little is known about the underlying molecular events (*1*). In *Drosophila*, a key to understanding the morphogenesis of the compound eye has been the identification of mutations that affect various stages of eye development (*2*). A potentially comparable mutation in humans results in aniridia, an autosomal dominant disorder in which the iris is absent or malformed (*3*). It can occur as an isolated abnormality, affecting one in 60,000 people, or together with Wilms tumor in 25 to 33% of cases, as part of the WAGR $11p^-$ deletion syndrome (*4*). The phenotype varies from a nearly complete absence of iris tissue to a subtle thinning of the iris margin in an otherwise normal eye. Vision is often impaired because of concomitant hypoplasia of the fovea and optic nerve, and commonly deteriorates over a period of years as a result of cataracts, glaucoma, and corneal opacification (*3*). An aniridia gene (*AN2*) has been mapped within chromosome band 11p13 by analysis of overlapping WAGR deletions, reciprocal translocations in two aniridia families, and meiotic linkage in aniridia pedigrees (*5*). *AN2* forms part of the WAGR gene complex and is telomeric to the Wilms tumor susceptibility locus (*WT1*). We have isolated a set of recombinant DNA clones that are densely distributed throughout this region (*6*). We have now used these probes in a comparative mapping approach to define a mouse model for the WAGR syndrome.

The WAGR complex is flanked by genes that encode catalase (*CAT*) and the β chain of follicle-stimulating hormone (*FSHB*) (*5*), which are located on chromosome 2 in the mouse genome and are there denoted as *Cas-1* and *Fshb*, respectively (*7*). To map this region more precisely, we selected nine probes from the proximal short arm of human chromosome 11 that cross-hybridize with rodent DNA (Table 1). Probes *g2*, *f3*, *k13*, *f8*, and *o3* were derived from an irradiation-reduced somatic cell hybrid that contained a 3000-kilobase (kb) segment of band 11p13 as its only human DNA (*8*). The probes are interspersed within the WAGR complex and detect three transcribed genes (*6*), including one that has been directly implicated in Wilms tumorigenesis (*9, 10*). The gene order has been established by deletion analysis as follows (*5, 6*): *D11S14-CAT-g2-f3-(WT1, k13, f8)-AN2-o3-D11S16-FSHB*-11pter. Under conditions of reduced hybridization stringency, each probe detects a set of restriction fragment variants between *Mus musculus/domesticus* (the laboratory mouse) and *Mus spretus* (the western Mediterranean short-tailed mouse) (*11*). These restriction fragment variants were used to score a panel of 94 interspecies backcross mice (Fig. 1A). The nine probes from human chromosome 11p cosegregated in the center of mouse chromosome 2, between *ld*, a gene controlling limb development (*12*), and the glucagon gene (*Gcg*) (Fig. 1B). All other potential orders required several double and triple crossovers (Table 2). These results and similar multipoint linkage data of others (*13*) strongly suggest that *Cas-1* is not distal to the gene for β_2 microglobulin (*B2m*), as it appears in the consensus map (*14*). Furthermore, the results localized the murine WAGR region close to the semidominant mutation Small-eyes (*Sey*), the phenotype and inheritance pattern of which resemble human aniridia (*15*).

Individuals with aniridia and *Sey*/+ mice have similarly malformed eyes. Both suffer from a complete or partial absence of iris tissue, although the phenotype in each species varies considerably among carriers of the same mutant allele. In general, the ocular defects in *Sey*/+ mice are more extreme. In most cases, the eyes are less than half their normal size (microphthalmos), the anterior chamber of the eye is missing, the retina is abnormally folded, and the lens is absent or small and has anterior cataracts (*15*).

The *Sey* gene has been mapped in multipoint crosses 5 centimorgans (cM) proximal to *pallid* (*pa*), a region corresponding to Giemsa bands 2D or 2E (*15, 16*). Three mutant alleles have been described (*15*). The most deleterious are Sey^{Dey}, a spontaneous variant, and Sey^{H}, a mutation induced by x-irradiation of oocytes. Carriers of these alleles also have a white belly spot, have reduced pigmentation of their tails and feet, and are 10 to 20% smaller than their wild-type littermates. Fetal loss among heterozygotes is about 60%, and homozygous embryos die on or before the 6th day of gestation. The third allele, Sey^{MH}, produces defects in heterozygotes that are limited to the eye. Homozygous Sey^{MH}/Sey^{MH} embryos survive to birth but lack eyes and noses. On the basis of these findings, it has been proposed that Sey^{Dey} and Sey^{H} result from large deletions but that Sey^{MH} is an intragenic deletion or point mutation (*15*).

To investigate the relation between Small-

T. Glaser, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, and Harvard Medical School, Boston, MA 02115.
J. Lane and D. Housman, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

*To whom correspondence should be addressed.

Table 1. Genetic markers and polymorphisms from mouse chromosome 2. The human probes were derived from bands p11 or p13 of chromosome 11. The probes hybridize with specific mouse DNA fragments in Southern blots under low-stringency conditions (*40*). Restriction fragment length variants (RFLVs) were identified between partially inbred *M. spretus* (obtained from The Jackson Laboratory) and *M. m. domesticus* (C3H/HeJ or C57BL6/J). B, Bam HI; H, Hind III; P, Pst I; T, Taq I; FSH-β, β chain of follicle-stimulating hormone.

Marker	Gene symbol		Band	Probe			
	Human	Mouse		Name	Type	RFLV	Ref.
D14	*D11S14*	*D11S14h*	p11	pDS1	Human genomic	T	(*5*)
Catalase gene	*CAT*	*Cas-1*	p13	pC24	Human cDNA	H	(*42*)
g2	*D11S412*	*D11S412h*	p13	pE2	Human cDNA	P,B,T	(*6*)
f3	*D11S408*	*D11S408h*	p13	pf3.6-3	Human genomic	B,H	(*6*)
f8	*D11S411*	*D11S411h*	p13	pf8.10-3	Human genomic	B,H,P	(*6*)
k13 (Wilms tumor gene)	*WT1**	*Wt-1*	p13	pk13.28-1	Human genomic	B	(*6,9*)
o3			p13	po3.40-1	Human genomic	B,P	(*6*)
D16	*D11S16*	*D11S16h*	p13	p32.1	Human genomic	P,T	(*42*)
FSH-β gene	*FSHB*	*Fshb*	p13	pRB410	Human genomic	T	(*42*)
Limb deformity gene		*ld*		BP1.7	Mouse genomic	H,T	(*12*)

*Also known as locus *D11S413* (*6*).

eyes and aniridia, we used the DNA variants characterized above to analyze interspecies F_1 mice carrying the Sey^{Dey} mutation. Female laboratory mice (Sey^{Dey}/+) were mated with normal *M. spretus* males (+/+), and DNA from mutant and wild-type F_1 offspring was tested by Southern (DNA) blot analysis (Fig. 2). The absence of *M. m. domesticus*–specific DNA fragments in the mutant mice indicated that Sey^{Dey} is a chromosomal deletion. The deletion includes markers *f3*, *f8*, and *k13*, but spares *D14*, *Cas-1*, *g2*, *o3*, *D16*, and *Fshb* (Fig. 3). Additional polymorphisms detected between C3H and C57BL6 strains supported this interpretation and showed that the deletion is linked to the C3H haplotype (Fig. 2).

The deleted markers subtend a contiguous segment of the human WAGR complex that includes *AN2* and *WT1* (Fig. 3). If we assume that gene order within this segment is conserved between species, the Sey^{Dey} deletion must span all or part of the murine aniridia gene and all of the murine Wilms tumor gene. A complex rearrangement is unlikely in view of the density of markers tested and the pattern of marker loss. On the basis of pulsed-field gel electrophoresis data obtained with these probes and human cell lines (*17*), we estimate that the Sey^{Dey} deletion spans between 1370 and 2300 kb. Comparable submicroscopic deletions have been identified in a small number of individuals with the WAGR syndrome who appear karyotypically normal. We have positioned the *Sey* gene between *f8* and *o3* (Fig. 3), because no other configuration is consistent with the absence of ocular abnormalities in Wilms tumor patient DR, who has a constitutional 11p deletion (*18*). This comparison suggests that *AN2* and *Sey* are homologous loci. Although confirmation of this hypothesis must await the molecular cloning of the aniridia gene, further support is provided by the description of a presumed aniridia homozygote—produced from the mating of two unrelated individuals with autosomal dominant aniridia—who died after 37 weeks gestation and was lacking eyes and a nose (*19*).

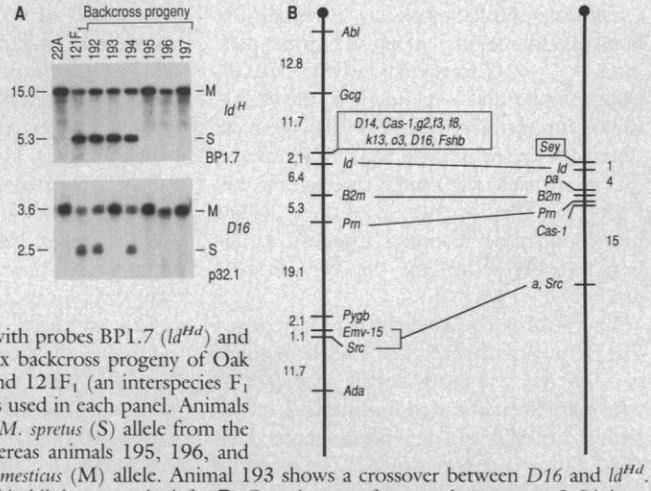

Fig. 1. Mapping the WAGR-homologous region on mouse chromosome 2. (**A**) Segregation of representative DNA polymorphisms in the interspecies backcross. N_1 backcross mice were produced by mating *M. m. domesticus* males with (*M. spretus* × *M. m. domesticus*) F_1 females as described (*41*). Genomic DNA was digested with Taq I and hybridized (*40*) with probes BP1.7 (ld^{Hd}) and p32.1 (*D16*). Shown are six backcross progeny of Oak Ridge strain 22A (male) and 121F_1 (an interspecies F_1 female). The same filter was used in each panel. Animals 192 and 194 inherited the *M. spretus* (S) allele from the F_1 female at both loci, whereas animals 195, 196, and 197 inherited the *M. m. domesticus* (M) allele. Animal 193 shows a crossover between *D16* and ld^{Hd}. Fragment sizes are indicated in kilobases on the left. (**B**) Genetic map of mouse chromosome 2. Linkage data from the interspecies backcross are summarized on the left, along with the percentage recombination observed in each interval (n = 94). The map combines results for 18 loci, including nine which have been previously typed in these mice (*41*). The loci have been ordered to minimize the number of multiple crossovers. The murine WAGR region is defined by nine markers, which correspond to clones from human chromosome 11p (indicated in the box). A portion of the consensus map (*16*) is shown on the right, with distances given in centimorgans. The maps are aligned by five reference markers, but differ in their relative orientation of *Cas-1*. The genetic distance between *ld* and *Src* is significantly greater in the interspecies cross than it appears in the consensus map (χ^2 = 11.6, $P < 0.001$); this difference can be explained by the generally higher frequency of recombination observed in females. Gene symbols: *a*, non-agouti; *Abl*, Abelson murine leukemia virus oncogene; *Ada*, adenosine deaminase; *Emv-15*, A^y-associated retroviral insertion; *pa*, pallid; *Prn*, scrapie prion protein; *Pygb*, liver glycogen phosphorylase; *Src*, Rous sarcoma virus oncogene.

During embryogenesis, the iris arises from elements of the anterior optic cup and surrounding mesenchyme, in close association with the developing lens (*20*). The dosage of the *AN2* gene is critical to this process. In Sey^{MH}/Sey^{MH} embryos, which appear to lack a functional aniridia gene, optic vesicles grow outward from the diencephalon normally but fail to induce lens pits in the overlying ectoderm (*15*). The nasal pits similarly do not form, and the optic and olfactory rudiments subsequently degenerate. All other craniofacial structures appear normal. These findings can be explained by a set of closely linked genes affecting the development of the iris, lens, and nose, or more simply, by a single gene (*AN2*) that controls a rate-limiting step in the induction of the lens and nasal placodes. We suggest that deficiency of *AN2* produces the aniridia or Small-eye phenotype by delaying or impairing lens induction.

This model is supported by several observations. (i) The lens pit appears 1 to 2 days later in Sey^{Dey}/+ embryos than it does in wild-type littermates, and the lens fails to separate from the cornea (*15*). (ii) Studies of many vertebrate species suggest that differ-

entiation of the iris, anterior chamber, and cornea depends on prior lens induction and is precisely coupled to lens growth and viability (*21*). (iii) A phenotype strikingly similar to Small-eyes has been produced in transgenic mice carrying ricin or diphtheria toxin genes under control of the αA- or γ2-crystallin promoter (*22*). The toxin genes are transcribed immediately after lens induction, and their products are restricted to, and specifically ablate, lens fiber cells. The resulting eyes demonstrate profound microphthalmia, aniridia, distortion of the anterior chamber, and folding of the retina, the severity of which is correlated with the level of transgene expression.

The difference between *AN2* and *Sey* phenotypes can be reconciled if both are viewed as quantitative defects in lens induction. When scaled according to eye volume, the mouse lens is five times larger than the human lens (*20, 23*). It may therefore depend more critically on the amount or timing of *Sey* gene expression for its early development. Although mutations in both *Sey* and *AN2* affect nearly every part of the eye, our results suggest that the iris is particularly sensitive to perturbations in early lens development. This effect appears to be limited to *AN2* gene deficiency, because duplication of *AN2* does not significantly alter the iris anatomy in individuals with trisomy 11p13 resulting from an insertional translocation (*24*). The extensive retinal folding observed in the toxin-producing transgenic (*22*) and *Sey*/+ mice is correlated with the degree of microphthalmia; it does not occur in individuals with aniridia, whose eyes are relatively normal in size. These observations are consistent with findings in chicks which demonstrate that growth of the retina, unlike other ocular structures, does not depend on lens size (*21*). However, because mouse retinas normally do not contain a fovea (*23*), the relatively poor foveal differentiation associated with aniridia cannot be assessed in *Sey*/+ mice.

The homology between *AN2* and *Sey* should facilitate analysis of other ocular disorders, including microphthalmia in mice (*23*) and humans, and a group of anterior chamber cleavage syndromes that resemble the Small-eye phenotype (*25*). A role for *AN2* in these disorders is also suggested by the existence of rare individuals with a specific anterior chamber defect (Peters' anomaly) and Wilms tumor or aniridia, and a family in which Peters' anomaly was inherited together with congenital absence of the nose (*26*).

Although several human cancers, including nephroblastoma, are inherited as mutations in tumor suppressor genes, this mechanism has not been demonstrated in any other mammalian species. Two findings suggest that Sey^{Dey} inactivates the murine homolog of the Wilms tumor gene (*Wt-1*). First, the Sey^{Dey} deletion envelops the region of homozygous loss in the WiT-13 sporadic Wilms tumor cell line (Fig. 3). This cell line contains two different submicroscopic deletions, one on each chromosome 11 homolog (*27*), which overlap by 140 to 345 kb (*17*). Second, the Sey^{Dey} deletion includes *k13*. This probe originates from the 3′ end of a gene that is expressed at high levels in fetal kidney and encodes a protein that contains four cysteine-histidine zinc fingers, a structure characteristic of nuclear transcription factors (*9*). Its identity as the 11p13 Wilms tumor gene (*WT1*) has been demonstrated by a 25–base pair (bp) internal deletion in a sporadic nephroblastoma, which disrupts the putative DNA binding domain but does not exist in the patient's germline DNA (*10*). In spite of these findings, and the rare occurrence of spontaneous nephroblastomas in mice (*28*), renal neoplasms have not been observed in Sey^{Dey} stocks maintained at The Jackson Laboratory (*29*) or in more than 60 Sey^{Dey}/+ mice examined at the Massachusetts Institute of Technology. The mice in the latter colony were closely monitored from birth for an average of 9 months and include the proge-

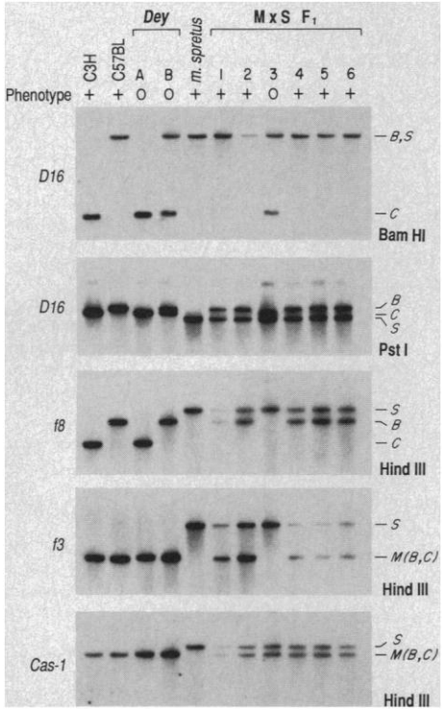

Fig. 2. Deletion analysis of the Sey^{Dey} mutation. Interspecies F_1 mice were produced by crossing Sey^{Dey}/+ *M. m. domesticus* (M) females and *M. spretus* (S) males. Genomic DNA from interspecies F_1, parental, and control mice was digested with the indicated restriction enzymes and hybridized (*40*) with probes p32.1 (*D16*), pf8.10-3 (*f8*), pf3.6-3 (*f3*), and pC24 (*Cas-1*). The Sey^{Dey} mutation arose in a C3H mouse (*15*) and is maintained on a B6C3 hybrid background. *Dey* A and B are two different *M. m. domesticus* Sey^{Dey}/+ females. The six F_1 mice shown are the progeny of female B and a wild-type *M. spretus* male. Mouse number 3 inherited the mutant phenotype (0) along with the C3H haplotype, whereas its five normal sibs (+) inherited the C57BL6 haplotype. The absence of *M. m. domesticus* alleles in mouse number 3 shows that *f3* and *f8* are deleted by the Sey^{Dey} mutation. Because mouse number 3 and female B are heterozygous, the deletion spares *D16* and *Cas-1*. The five wild-type littermates are heterozygous at all four loci. Alleles are designated as follows: *M*, *M. m. domesticus*; *S*, *M. spretus*; *B*, C57BL6/J; *C*, C3H/HeJ.

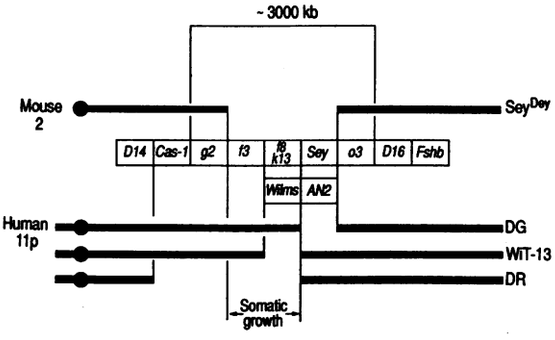

Fig. 3. Comparison of Sey^{Dey} and WAGR deletions. The horizontal bars indicate homologous portions of mouse chromosome 2 and human chromosome 11p. Nine conserved DNA markers are arranged in the center according to the gene order established in humans (*6*). The Sey^{Dey} chromosome lacks *f3*, *f8*, and *k13*, but retains the six surrounding markers. The positions of the aniridia (*AN2*) and Wilms tumor genes are defined here by deletions in three individuals with partial manifestations of the WAGR syndrome. The DG deletion is associated with aniridia only; it spans several markers between *f8* and *o3* (*6*) and has been transmitted as part of an unbalanced t(11;22) reciprocal translocation for five generations (*5*). The WiT-13 deletion represents the overlap of two different interstitial deletions in a sporadic Wilms tumor cell line (*27*); the region of homozygous loss does not include the aniridia gene (*6*). The DR deletion was identified in a child with Wilms tumor, genitourinary anomalies, and a catalase deficiency, but normal eyes (*18*). The arrows mark a region, the dosage of which appears to be critical for normal somatic growth in both species. It is bounded on the telomeric side by the distal endpoint in individual DR, whose height at 2 and 4 years was more than two standard deviations below the median (*18*). The growth phenotype can be separated from aniridia, because carriers of the DG deletion (*5*), individuals with isolated mutations in *AN2* (*3*), and mice heterozygous for the Sey^{MH} allele (*15*) are normal in size. The mouse centromere is oriented on the basis of a small number of recombinants observed between *Fshb* and *Cas-1* in other crosses involving *M. m. domesticus* and *M. spretus* (*13*).

Table 2. Recombination data localizing the murine WAGR region in relation to the glucagon (*Gcg*), limb deformity (*ld*), and β_2-microglobulin (*B2m*) genes. Alleles were determined with the probes and restriction fragment polymorphisms listed in Table 1. The typing of *B2m* and *Gcg* in these mice has been previously described (*41*). M, *M. m. domesticus*; S, *M. spretus*. SEM values are given for recombination fractions.

	Allele transmitted by F_1				No. of mice	Recombination fraction
	Gcg	*Fshb*	*ld*	*B2m*		
Nonrecombinants	M	M	M	M	28	
	S	S	S	S	47	
Recombinants						
*Gcg-Fshb**	M	S	S	S	2	11.7 ± 3.3
	S	M	M	M	9	
Fshb-ld	M	M	S	S	2	2.1 ± 1.5
	S	S	M	M	0	
ld-B2m	M	M	M	S	2	6.4 ± 2.5
	S	S	S	M	4	
Total					94	

**Fshb* represents nine markers—*D14*, *Cas-1*, *g2*, *f3*, *f8*, *k13*, *o3*, *D16*, and *Fshb*—among which no recombination was observed. These loci therefore map within 3.1 cM of each other (95% confidence interval).

ny of affected males and females. These results must be reconciled with the 60% penetrance of familial Wilms tumor in man (*30*) and the high frequency of bilateral and multicentric tumors in WAGR patients. In addition, the kidneys of affected mice appear histologically normal, with no evidence of focal dysplasia or persistent renal blastema. Such areas, collectively termed nephroblastomatosis, are thought to represent precursor lesions and are commonly found in kidneys of bilateral Wilms tumor patients (*31*). Finally, Sey^{Dey}/+ mice lack the diverse urogenital anomalies of WAGR patients, which involve the kidneys, gonads, genitalia, and mesonephric structures (*4*). This suggests that the underlying developmental events in mice are less sensitive to gene dosage or that the gene (or genes) responsible for urogenital defects lies outside the Sey^{Dey} deletion.

The apparent failure of Sey^{Dey}/+ heterozygotes to develop nephroblastomas can be explained in terms of Knudson's two-mutation model (*30*) by a smaller or more short-lived target cell population in mice than in humans. This would decrease the opportunity for the occurrence of postzygotic mutations in the remaining wild-type *Wt-1* allele that would result in malignant transformation. Indeed, loss of the metanephric blastema during the second half of gestation is the presumed basis for incomplete penetrance of the Wilms tumor trait in man (*30, 31*). Although the exact size of the target cell population in each species is unknown, it can be estimated in relative terms by the number of nephrons per adult kidney—1.2×10^4 in mice and 1.0×10^6 in humans (*32*). This difference predicts a significantly lower incidence of nephroblastoma among Sey^{Dey}/+ mice than WAGR patients, which may be difficult to detect in a setting of high perinatal mortality. Scaling effects could also explain why other embryonal cancers are rarely observed in mice. For example, familial retinoblastoma is transmitted in humans by mutations in 13q14 that inactivate or eliminate the *RB*-encoded protein $p105^{Rb}$ (*33*). An equivalent disease has been produced in transgenic mice by retinal-specific expression of the SV40 T antigen, an oncogene which inhibits $p105^{Rb}$ action (*34*). Nevertheless, comparable mouse mutations in *Rb-1* have failed to emerge from large-scale mutagenesis experiments in which chromosomal deletions, other forms of hereditary cancer, and numerous ocular abnormalities have been recovered (*15, 35*). If this interpretation is correct, it may be possible to specifically induce nephroblastomas in Sey^{Dey} carriers by chemical mutagenesis in utero or by transgenic expression of growth factors, such as insulin-like growth factor–II or N-*myc*, which appear to stimulate nephroblast proliferation (*36*).

Alternatively, homozygous mutations in *Wt-1* could be lethal to murine nephroblasts or may not be sufficient for tumor initiation. Although the Wilms tumor gene appears to control an essential step in nephroblast differentiation and has a similar tissue expression pattern in both species (*37*), its function may be relatively less important to murine nephrogenesis than other loci. In humans, mutations in at least three different genes appear to contribute to the etiology of Wilms tumor. In addition to *WT1*, these include loci in band p15 of chromosome 11—identified through studies of allelic segregation events in Wilms tumors and genetic linkage in families with Beckwith-Weidemann syndrome—and a non–chromosome 11p site identified through linkage analysis of Wilms tumor pedigrees (*38*).

A detailed comparison between *Sey* and WAGR mutations should reveal new phenotypic determinants in this region. These include genetic factors responsible for the reduced body size of Sey^{Dey}/+ mice and WAGR patients (Fig. 3); the murine homolog of a T cell leukemia breakpoint cluster region, *TCL2* (*39*), which is located between *g2* and *CAT* in man (*6*); and a locus responsible for the pigmentation defects in Sey^{Dey} and Sey^{H} mice, which may control the number or migration of melanocyte precursors. As an animal model of a specific human autosomal deletion syndrome, the Sey^{Dey}/+ mouse should improve our understanding of Wilms tumorigenesis and provide new insights concerning craniofacial development.

REFERENCES AND NOTES

1. H. Spemann, *Verh Anat. Ges* **15**, 61 (1901); J. W. McAvoy, *Differentiation* **17**, 137 (1980); R. M. Grainger, J. J. Henry, R A. Henderson, *Development* **102**, 517 (1988).
2. A. Tomlinson, *Nature* **340**, 510 (1989).
3. L. B. Nelson *et al*, *Surv Ophthalmol.* **28**, 621 (1984); H. M. Hittner, in *The Glaucomas*, R. Ritch, M. B. Sheilds, T. Krupin, Eds. (Mosby, St. Louis, 1989), pp. 869–884.
4. U. Francke, L. B. Holmes, L. Atkins, V. M. Riccardi, *Cytogenet. Cell Genet.* **24**, 185 (1979); N. E. Breslow and J. B. Beckwith, *J Nat Cancer Inst.* **68**, 429 (1982); C. Turleau *et al*, *Clin. Genet.* **26**, 356 (1984). WAGR denotes Wilms tumor (W), aniridia (A), genitourinary anomalies (G), and mental retardation (R).
5. WAGR deletions [V. van Heyningen *et al.*, *Proc Natl. Acad. Sci. U.S.A.* **82**, 8592 (1985); T. Glaser *et al.*, *Nature* **321**, 882 (1986)]; chromosome translocations disrupting *AN2* [K. O. J. Simola, S. Knuutila, I. Kaitila, A. Pirkola, P. Pohja, *Hum. Genet.* **63**, 158 (1983); M. Gessler, K. O. J. Simola, G. A. P. Bruns, *Science* **244**, 1575 (1989); L. M. Davis *et al.*, *ibid.* **241**, 840 (1988)]; meiotic linkage between aniridia and 11p13 [M. Mannens *et al.*, *Cytogenet. Cell Genet.* **52**, 32 (1989)].
6. T. Glaser *et al.*, *Cytogenet. Cell Genet.* **51**, 1005 (1989).
7. Catalase [R. C. Dickeman, R. N. Feinstein, D. Grahm, *J Hered.* **59**, 177 (1968); H. A. Hoffman and C. K. Grieshaber, *ibid.* **65**, 277 (1974)]; follicle-stimulating hormone β chain [T. M. Glaser *et al.*, *Cytogenet Cell Genet* **40**, 643 (1985)].
8. T. Glaser, E. Rose, H. Morse, D. Housman, C. Jones, *Genomics* **6**, 48 (1990).
9. K. M. Call *et al.*, *Cell* **60**, 509 (1990); M. Gessler *et al.*, *Nature* **343**, 774 (1990).
10. D. A. Haber *et al.*, *Cell* **61**, 1257 (1990).
11. B. Robert *et al.*, *Nature* **314**, 181 (1985); P. Avner, L. Amar, L. Dandalo, J. L. Guenet, *Trends Genet.* **4**, 19 (1988).
12. The limb deformity gene (ld^{Hd} allele) is identified in our study by BP1.7, a genomic DNA fragment cloned from the insertion site of a mouse mammary tumor virus (MMTV)–*myc* transgenic mouse; it is 3 kb to the left of probe A [R. P. Woychik, T. A. Stewart, L. G. Davis, P. D'Eustachio, P. Leder, *Nature* **318**, 36 (1985)].
13. R. van der Meer-de Jong *et al.*, *Genomics* 7, 270 (1990); L. M. Siracusa *et al*, *ibid.* **6**, 491 (1990).
14. M. T. Davisson, T. H. Roderick, A. L. Hilyard, D. P. Doolittle, *Mouse News Lett.* **81**, 12 (1988); J. Michaelson, *Immunogenetics* **17**, 219 (1983). *Cas-1* has been positioned on the genetic map primarily on the basis of two-point linkage tests between catalase and the *non-agouti* locus (*a*) (7); these results are consistent with our multipoint linkage data but are not sufficiently informative to specify gene order.
15. Sey^{Dey} allele [K. Theiler, D. S. Varnum, L. C. Stevens, *Anat. Embryol.* **155**, 81 (1978); K. Theiler and D. S. Varnum, *ibid.* **162**, 121 (1981)]; Sey^{H} allele [M. F. Lyon, R. J. S. Phillips, G. Fisher, *Mutat. Res.* **63**, 161 (1979)]; Sey^{MH} allele [R. C. Roberts, *Genet. Res.* **9**, 121 (1967); B. L. M. Hogan *et al.*, *J.*

Embryol. Exp. Morphol. **97**, 95 (1986); B. L. M. Hogan, C. M. Hetherington, M. F. Lyon, *Mouse News Lett.* **77**, 135 (1987); B. L. M. Hogan, E. M. A. First, G. Horsburgh, C. M. Hetherington, *Development* **103** (Suppl.), 115 (1988)]. The Sey^{MH} allele is denoted as *Sey* in some reports.
16. Linkage data [M. T. Davisson, *Mouse News Lett.* **75**, 30 (1986); T. Glaser, unpublished results]; cytogenetic correlate [A. G. Searle, C. V. Beechey, E. M. Eicher, M. N. Nesbitt, L. L. Washburn, *Cytogenet. Cell Genet.* **23**, 255 (1979)].
17. M. Gessler and G. A. P. Bruns, *Genomics* **5**, 43 (1989); E. A. Rose *et al.*, *Cell* **60**, 495 (1990). An example of a submicroscopic WAGR deletion is provided by patient No. 1 [Riccardi *et al.*, *J. Pediatr.* **100**, 574 (1982)] analyzed as cell line LCS036 [D. A. Compton *et al.*, *Cell* **55**, 827 (1988)].
18. C. Turleau *et al.*, *Hum. Genet.* **67**, 455 (1984).
19. S. V. Hodgson and K. E. Saunders, *J. Med. Genet.* **17**, 478 (1980).
20. V. Ozanics and F. A. Jakobiec, in *Ocular Anatomy, Embryology and Teratology*, F. A. Jakobiec, Ed. (Harper & Row, Philadelphia, 1982), pp. 11–96.
21. A. J. Coulombre and H. Hermann, *Exp. Eye Res.* **4**, 302 (1965); A. J. Coulombre, *Invest. Ophthalmol.* **4**, 411 (1965).
22. M. L. Breitman *et al.*, *Science* **238**, 1563 (1987); C. P. Landel, J. Zhao, D. Bok, G. Evans, *Genes Dev.* **2**, 1168 (1988); M. L. Breitman *et al.*, *Development* **106**, 457 (1989).
23. W. G. Robinson, T. Kuwabara, J. Zwaan, in *The Mouse in Biomedical Research*, H. L. Foster, J. D. Small, J. G. Fox, Eds. (Academic Press, New York, 1982), vol. IV, pp. 69–96.
24. C. Lavedan *et al.*, *Cytogenet. Cell Genet.* **50**, 70 (1989); R. J. Strobel, V. M. Riccardi, D. H. Ledbetter, H. M. Hittner, *Am. J. Med. Genet.* **7**, 15 (1980). One of these individuals has Brushfield spots in both irises, which is an anatomical variant occurring in 10% of normal eyes [D. D. Donaldson, *Arch. Ophthalmol.* **4**, 50 (1961)].
25. Microphthalmia [M. Warburg, *Int. Ophthalmol.* **4**, 46 (1981)]; anterior chamber syndromes [G. O. Waring, M. M. Rodrigues, P. R. Laibson, *Surv. Ophthalmol.* **20**, 3 (1975)].
26. Peters' anomaly (posterior corneal thinning with iridocorneal adhesions) has been described in individuals with Wilms tumor [R. Koster and A. Th. M. Van Balen, *Ophthalmol. Paediatr. Genet.* **6**, 1 (1985)], contralateral aniridia [R. A. Eifeman, *Ann. Ophthalmol.* **16**, 933 (1984)], and congenital arhinia [K. W. Ruprecht and F. Majewski, *Klin. Monatsbl. Augenheilkd.* **172**, 708 (1978)].
27. W. H. Lewis *et al.*, *Genomics* **3**, 25 (1988).
28. A. G. Liebelt, B. Saas, H. J. Sobel, R. M. Wermer, *Toxicol. Pathol.* **17**, 57 (1989).
29. M. T. Davisson, personal communication.
30. A. G. Knudson and L. C. Strong, *J. Nat. Cancer Inst.* **48**, 313 (1972); E. Matsunaga, *Hum. Genet.* **57**, 231 (1981).
31. K. E. Bove and A. J. McAdams, *Perspect. Pediatr. Pathol.* **3**, 185 (1976); R. L. Heideman, G. M. Haase, C. L. Foley, H. L. Wilson, W. C. Bailey, *Cancer* **55**, 1446 (1985). Roughly 10% of the Sey^{Dey}/+ mice examined did have polycystic livers. The cysts were first detected at about 12 weeks of age and were not found in wild-type littermates. In some mice, the liver contained more than 50 simple cysts between 1 and 3 mm in diameter, which were filled with bile-tinged clear fluid and lined with cuboidal cells. Although their relation to nephroblastoma and the WAGR syndrome is unclear, comparable hepatic cysts are observed in 40 to 90% of humans with adult polycystic kidney disease [J. Mitlinovic *et al.*, *Am. J. Med.* **68**, 741 (1980)].
32. Mouse [D. A. Rytand, *Am. J. Anat.* **62**, 507 (1938)]; human [E. L. Potter, *Normal and Abnormal Development of the Kidney* (Year Book, Chicago, 1972), pp. 72–73].
33. S. H. Friend *et al.*, *Nature* **323**, 643 (1986); W. H. Lee *et al.*, *Science* **235**, 1394 (1987); Y. K. Fung *et al.*, *ibid.* **236**, 657 (1987).
34. J. J. Windle *et al.*, *Nature* **343**, 665 (1990).
35. A. R. Moser, H. C. Pitot, W. F. Dove, *Science* **247**, 322 (1989); J. Kratachvilova, *J. Hered.* **72**, 302 (1981).
36. Mutagenesis in utero [L. B. Russell and C. S. Montgomery, *Mutat. Res.* **92**, 193 (1982); Y. Ohaki, *Pediatr. Pathol.* **9**, 19 (1989); T. Nakamura, M. Hara, T. Kasuga, *Am. J. Pathol.* **135**, 251 (1989)]; growth factors [T. Gansler *et al.*, *ibid.* **130**, 431 (1988); A. L. Brice, J. E. Cheetham, V. N. Bolton, N. C. Hill, P. N. Schofield, *Development* **106**, 543 (1989); C. J. Quaife *et al.*, *Endocrinology* **124**, 40 (1989); P. D. Nisen, K. A. Zimmerman, S. V. Cotler, F. Gilbert, F. W. Alt, *Cancer Res.* **46**, 6217 (1986)].
37. K. Pritchard-Jones *et al.*, *Nature* **346**, 194 (1990); A. J. Buckler, J. Pelletier, D. A. Haber, T. Glaser, D. E. Housman, in preparation.
38. Band 11p15 [I. Henry *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3247 (1989); M. Mannens *et al.*, *Hum. Genet.* **81**, 41 (1989); A. E. Reeve, S. A. Sih, A. M. Raizis, A. P. Feinberg, *Mol. Cell. Biol.* **9**, 1799 (1989); A. Koufos *et al.*, *Am. J. Hum. Genet.* **44**, 711 (1989); A. J. Ping *et al.*, *ibid.*, p. 720]; non–chromosome 11p site [P. Grundy *et al.*, *Nature* **336**, 374 (1988); V. Huff *et al.*, *ibid.*, p. 377].
39. T. Boehm, L. Buluwela, D. Williams, L. White, T. H. Rabbits, *EMBO J.* **7**, 2011 (1988); E. Champagne *et al.*, *Blood* **73**, 398 (1989).
40. Genomic DNA was isolated from mouse brains, digested with restriction enzymes, subjected to electrophoresis through 0.8% agarose, transferred to nylon membranes, and hybridized with ^{32}P-labeled insert DNA from plasmid clones as described (*41*). For low-stringency hybridization, the nylon filters were probed at 42°C in 35 to 40% formamide, 1 M NaCl, 50 mM tris-HCl (pH 7.4), 10% dextran sulfate, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (fraction V), and 0.5% SDS, and were washed four times (30 min each time) at 55°C in 2× SSC (0.3 M NaCl, 30 mM sodium citrate).
41. T. Glaser *et al.*, *Genomics* **5**, 510 (1989).
42. Catalase [G. A. P. Bruns, T. Glaser, J. F. Gusella, D. Housman, S. H. Orkin, *Am. J. Hum. Genet.* **36**, 25S (1984)]; *D11S16* [J. Feder *et al.*, *ibid.* **37**, 635 (1985)]; follicle-stimulating hormone β chain [A. Beck, V. Vellucci, K. Curry, *DNA* **4**, 76 (1985)]. Probe pRB410 contains two exons from the human *FSHB* gene, which were recombined to create an intact 410-bp coding sequence.
43. We thank L. B. Russell for providing the interspecies backcross mice; R. P. Woychik, P. Leder, G. A. P. Bruns, and A. Beck for providing probes BP1.7, pC24 and pRB410; T. Dryja and A. Buckler for discussion; and M. T. Davisson and B. L. M. Hogan for advice on breeding Sey^{Dey}/+ mice. Supported by grants from the NIH (GM27882) and the Medical Scientist Training Program (PHS NRSA award 2 T32 GMO7753-11).

23 April 1990; accepted 6 August 1990

Inhibition of the Complement Cascade by the Major Secretory Protein of Vaccinia Virus

GIRISH J. KOTWAL,* STUART N. ISAACS, ROBIN MCKENZIE, MICHAEL M. FRANK, BERNARD MOSS†

The complement system contributes to host defenses against invasion by infectious agents. A 35-kilodalton protein, encoded by vaccinia virus and secreted from infected cells, has sequence similarities to members of a gene family that includes complement control proteins. Biochemical and genetic studies showed that the viral protein binds to derivatives of the fourth component of complement and inhibits the classical complement cascade, suggesting that it serves as a defense molecule to help the virus evade the consequences of complement activation.

THE COMPLEMENT SYSTEM IS COMposed of more than 20 plasma proteins that participate in host defenses against infectious agents. The proposed antiviral mechanisms of complement components include virus neutralization and opsonization, lysis of virus-infected cells, and amplification of inflammatory and specific immune responses (*1*). Some viruses may have evolved defenses against the complement system. The envelope glycoprotein gC of herpes simplex viruses types 1 and 2 acts as a receptor for fragment C3b of the third component of complement and thereby modulates the alternative complement pathway in vitro (*2*). Epstein-Barr virus, another herpesvirus, also may regulate activation and processing of the third component of complement (*3*). The 35-kD major secretory protein of vaccinia virus, a poxvirus, contains conserved elements of the 60 amino acid repeating unit of structurally related eukaryotic proteins with the greatest similarity to a human protein (C4bp) that binds the C4b fragment of the fourth component of complement and inhibits the classical pathway of complement activation (*4*).

The classical pathway for activation of the complement system starts with the binding of the C1 complex via C1q to the Fc region of immunoglobulin (Ig). Activation of bound C1 leads to sequential cleavages of C4 and C2, forming C4b2a, the classical pathway C3 convertase. Cleavage of C3 by the bound convertase activates the terminal lytic mechanism that is common to both the

G. J. Kotwal, S. N. Isaacs, B. Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
R. McKenzie and M. M. Frank, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

*Present address: James N. Gamble Institute of Medical Research, Cincinnati, OH 45219.
†To whom correspondence should be addressed.