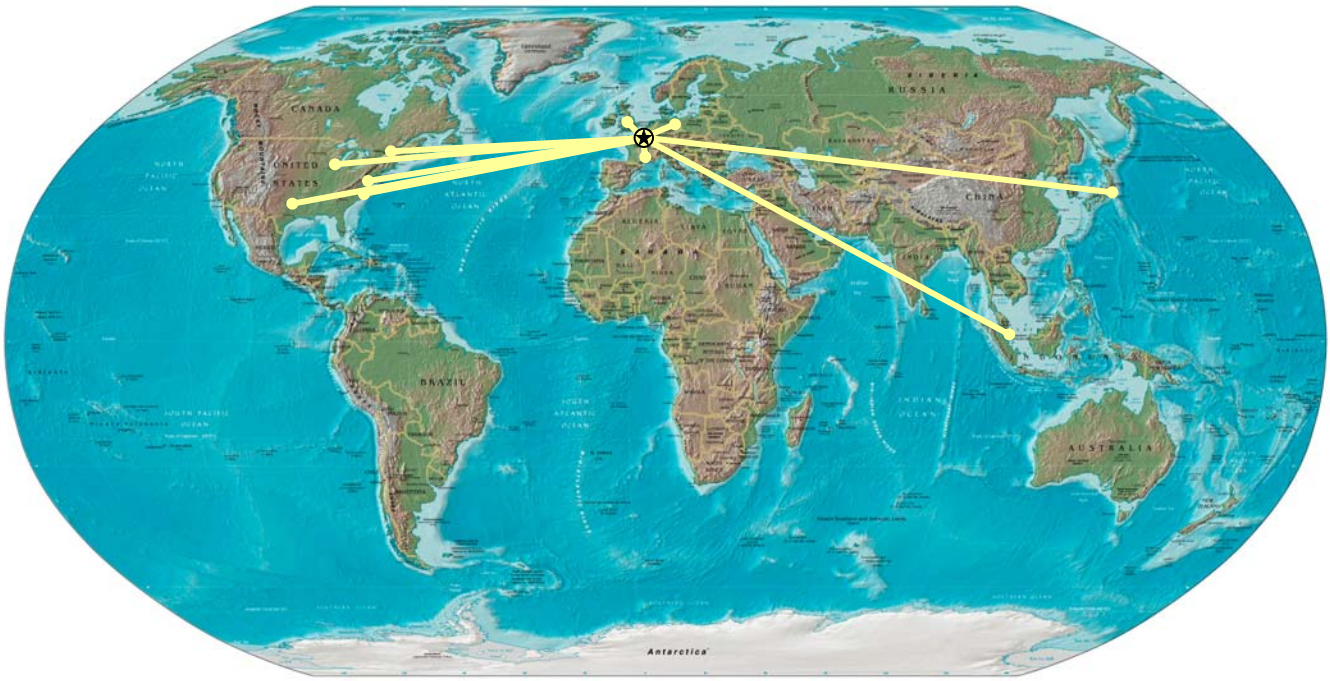


Projection du monde à la Guénet

A Tribute to Jean-Louis Guénet
November 10, 2005

Hommage à Jean-Louis Guénet

Min, Mom, and ROSA: A murine gateway into human cancer

William F. Dove
University of Wisconsin

I shall talk today about the efforts our laboratory is making to develop animal models for human colon cancer. I'll illustrate how the power of mouse biology and genetics combine to make progress. At the end, I'll discuss how the power of experimental analysis is growing rapidly across a broad range of mammals. In particular, the laboratory rat can now synergize with the laboratory mouse in the experimental analysis of human colon cancer. Experimental models are greatly needed for a myriad of cancers and for other aspects of human biology. My colleagues and I hope that the explorations that we are making for colon cancer will provide useful examples and cautionary tales. Drs. Corpet and Pierre of The School of Veterinary Medicine in Toulouse have recently published a review entitled "How good are rodent models of carcinogenesis in predicting efficacy in humans?". I'll return to this question at the end of my talk.

I first met Jean-Louis in 1975 when Alexandra and I worked at the Pasteur Institute in the unit directed by Francois Jacob that included a number of the members of today's audience including Phil Avner, Charles Babinet, Hedwig Jakob, and the young future leader of murine genetics, Jean-Louis Guenet. At the time, as a microbial molecular geneticist impressed by the work of Guido Pontecorvo and my colleague Bob DeMars, I was interested in exploring whether the use of mitotic recombination and co-dominant cell surface antigens could guide the development of the somatic cell genetics of the diploid embryonal carcinoma cell lines. We published this paper jointly with our Pasteur collaborators soon thereafter. At the time much of mouse genetics depended upon polymorphic strain differences – for example those at the *T* locus. The advent of DNA sequencing demonstrated that strain differences at a locus were far more complex than simple point mutations in single genes. This led both our laboratory and, in parallel, the laboratory of Vernon Bode to explore the use of point mutagenesis of the mouse germline by ethylnitrosourea. In this slide, Vernon, Alexandra and I talked about the progress that we had made in our university settings over the 1980's in developing the use of ethylnitrosourea to create point mutations in

A murine gateway into human colon cancer

How good are rodent models of carcinogenesis in predicting efficacy in humans?...

Corpet DE, Pierre F.
The School of Veterinary Medicine, Toulouse

Eur J Cancer. 41:1911-22 (2005)

Mouse genetics 1975

The genetics of teratocarcinoma transplantation: tumor formation in allogeneic hosts by the embryonal carcinoma cell lines F9 and PCC3

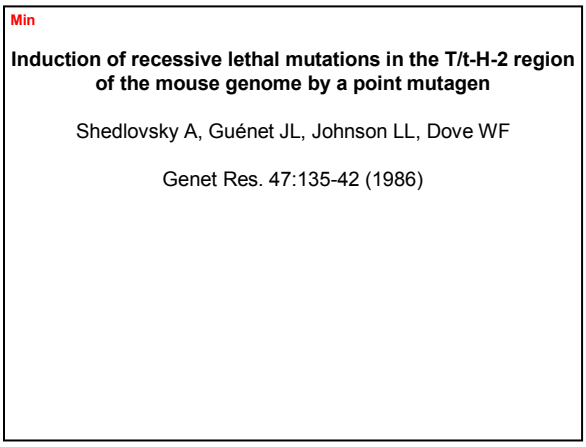
Avner PR, Dove WF, Dubois P, Gaillard JA, Guénet J-L, Jacob F, Jakob H and Shedlovsky A

Immunogenetics 7:103-115 (1978)

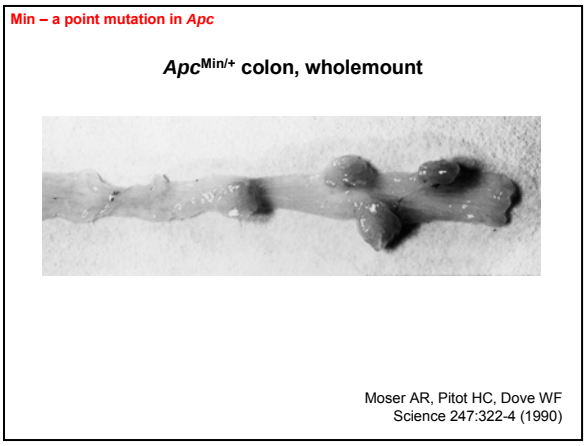
Min – point mutations in developmental genes

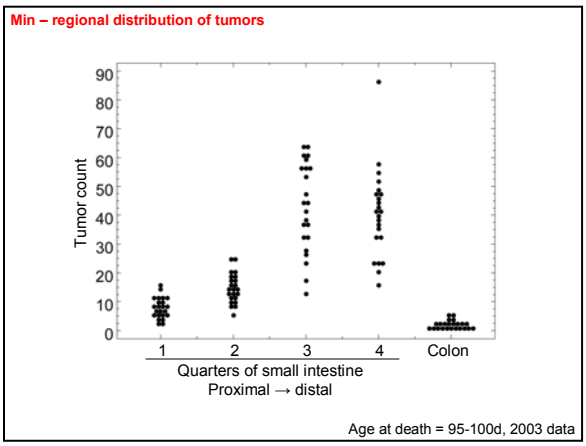
Vernon Bode, Alexandra Shedlovsky, and William Dove
From Phage Lambda to ENU mutagenesis of the mouse genome

individual developmental genes of the T locus and in the system controlling phenylalanine catabolism. Jean-Louis Guenet assisted in this gestational phase of ENU mutagenesis of the mouse germline, hosting Vernon on a sabbatical visit and Alexandra and me for several months in 1981, and collaborating on our first publication in this realm. Rudi Balling, Steve Brown, and Toshihiko Shiroishi are discussing today some of the worldwide growth of this mutational approach to mammalian genetics, growth that Jean-Louis has continued to foster.

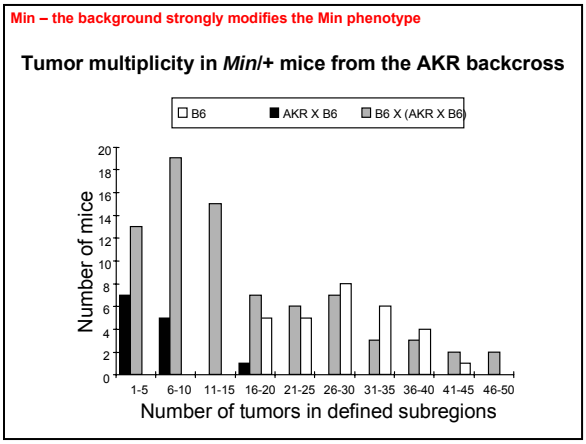


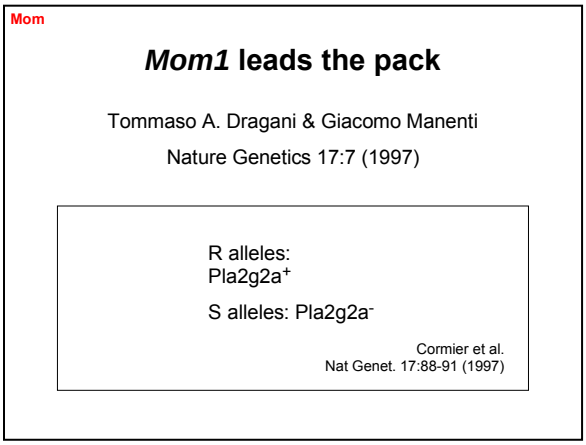
Our own research in mouse genetics became centered on colon cancer in the late 1980s when I our postdoctoral fellow Amy Moser, our pathologist Henry Pitot, and I discovered the ENU-induced mutant called Min - multiple intestinal neoplasia. This is a whole mount of the colon of a Min mouse, showing four adenomas. Working with the group of Ken Kinzler and Bert Vogelstein at Johns Hopkins, we showed that the Min mouse carried a single point mutation in *Apc*, the mouse homolog of the human gene adenomatous polyposis coli. This finding demonstrated the power of point mutagenesis of the mouse germline: a striking contrast to the polymorphic differences between strains where variation is found every few hundred base pairs, making it difficult to decide which change is responsible for the phenotype. The molecular concordance between the Min strain and human familial colon cancer has fueled hope that this mutant strain will provide an accurate experimental model for this common human disease. We must bear in mind, however, that the Min mouse is not an ideal model for human familial colon cancer. In this scatterplot of a set of Min animals, note that most of the adenomas that form in a Min mouse lie within the small intestine. In contrast to an average of two tumors in the colon, a total of around 100 tumors arise in the small intestine of the Min mouse. This very high tumor load leads to a very short lifespan in the range of 100 days, permitting cancer development only to the early adenoma stage. We'll return later to address whether one can obtain an improved murine model for colon cancer - - first, in which most of the tumors arise in the colon and, second, in which such the animal model lives long enough to develop the invasive stages of colon cancer that are so dire in the human disease.

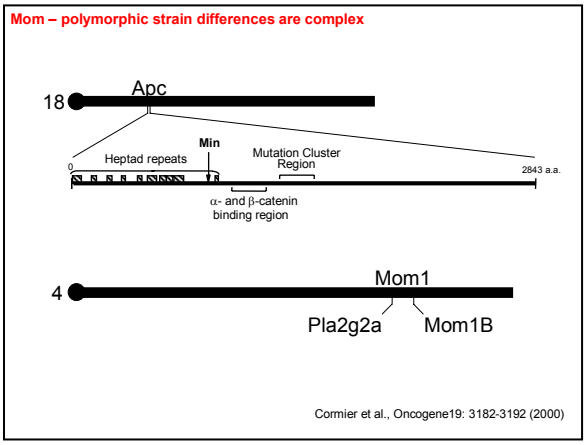




Most human colon cancer, familial or sporadic, involves mutations in the *Apc* "gatekeeper" gene or its immediate downstream target, Rolf Kemler's β-catenin. But the disease of familial neoplasia of the intestinal tract is not simply a matter of the Apc gatekeeper. The phenotype of animals carrying the *Min* mutation is greatly affected by the genetic background. For example, in this histogram, in white bars we see that the tumor multiplicity on the sensitive B6 background averages 30 on a defined sub-portion of the intestinal tract. By contrast, in an F1 made by crossing B6 Min animals to the resistant strain AKR, in the black bars we see that the average tumor multiplicity reduced by a factor of six. The genetic complexity of this dominant tumor resistance has been deduced by a genome-wide analysis of a set of animals in a segregating backcross generation, shown in the grey bars. Whereas the *Min* mutation lies on mouse chromosome 18, a region of the genome explaining a major portion of this modification of the Min phenotype lies on mouse chromosome 4. Min segregants with high tumor multiplicity are homozygous for B6 alleles in this region of the genome, while those with low tumor multiplicity are heterozygous for AKR alleles. This complex locus has been designated modifier of Min number 1, *Mom1*; it represents the first step in our discovery of factors in the genetic background that can influence the phenotype of animals carrying the predisposing *Min* allele at *Apc*. The *Mom1* region contains the gene for a secretory phospholipase, as noted by Art Buchberg and Linda Syracusa. This gene accounts for a portion of the Mom1 resistance. Strains carrying resistance alleles of *Mom1* express an active form of this gene, whereas sensitive alleles are inactivated by a frameshift mutation. We have shown that sensitive B6 animals are made partially resistant by the action of a transgene expressing this secretory phospholipase.

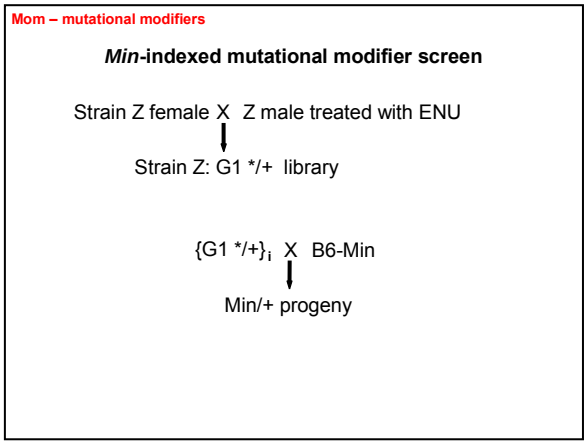


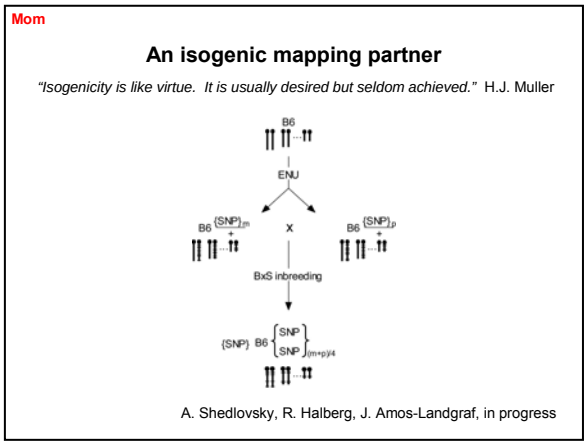




For a short while, it seemed that the secretory phospholipase would account entirely for the Mom1 phenotype. However, a recombinational analysis of the *Mom1* region at high resolution demonstrated that the region contains a second resistance factor, distal to the secretory phospholipase gene. This second component of *Mom1* is being actively pursued by our colleague, Robert Cormier at Minnesota. Finding that polymorphic modifiers can be

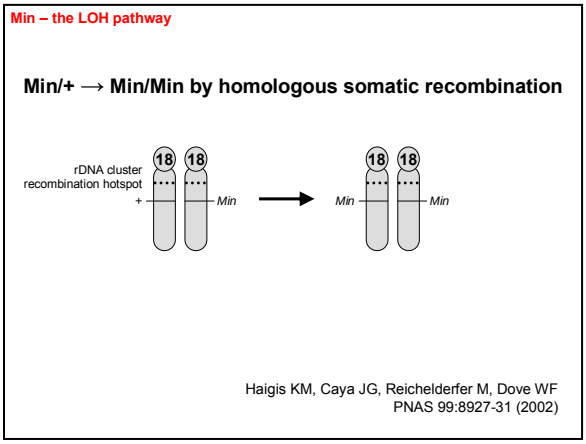
complex is not limited to our study of the *Mom1* region. Beverly Mock has discovered a cluster of modifiers of plasmacytoma formation in the mouse. Michael Gould has found that two of the loci that modify mammary carcinogenesis in the rat are complex. Indeed we are now aware that in the study of polymorphic genetic modifiers, there is an ascertainment bias in which the first regions to be found are the ones that contain multiple resistance factors. Furthermore, as recognized in the early promotion of ENU mutagenesis, the issue of identifying the polymorphic gene that is responsible for the phenotype is confused by the many differences in sequence between strains for any one region of the genome – a change every few hundred basepairs. For those two reasons, we've chosen instead to attempt to develop the genetics of mutagen-induced modifiers of the Min phenotype, again using ENU in several ways. We create a library of animals whose paternal genome has been heavily mutagenized. Then, as shown here, a member of the library is screened for dominant resistance or susceptibility modifiers by crossing with B6 Min and quantitatively scoring the phenotype in a set of Min testcross progeny. Now if the mutagenized strain, Z, is different from B6, any new modifier induced on Z can be mapped by crosses between Z and B6. However, this mapping process is obscured by the polymorphic modifiers segregating between strains Z and B6. If, by contrast, the mutagenized strain Z is B6 itself, then one avoids this complication of polymorphic modifiers segregating in the mapping cross. But how can one map the newly induced point mutation that modifies the Min phenotype? We are attacking this restriction by developing from B6 a set of isogenic mapping partners, called the B6-SNP strains. In short, we are attempting to carry out modifier genetics under quasi-isogenic conditions. We have created a set of these B6-SNP strains by ENU mutagenesis followed by a series of brother-sister matings within each line, to drive to homozygosity any newly induced point mutation. This process eliminates point mutations that are detrimental or lethal. Each fully inbred B6-SNP derivatives is being crossed to B6 Min to determine whether to ascertain whether the mapping partner is free of any dominant modifier of the Min phenotype.

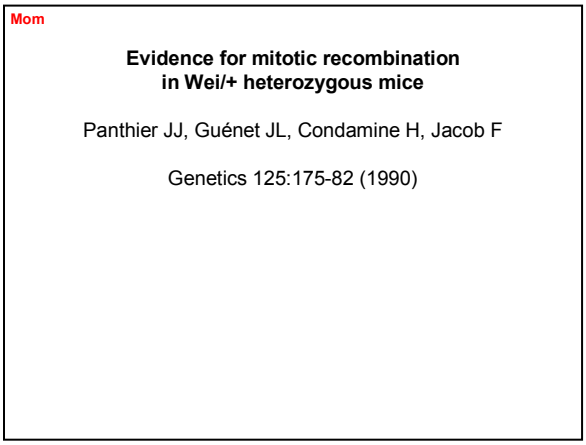




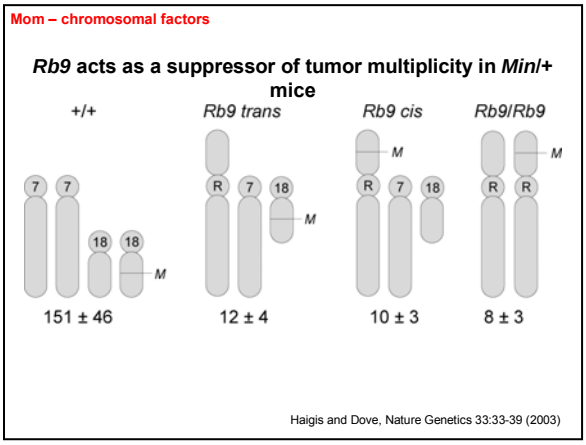
The remaining issue in our development of "isogenic modifier genetics" is also an outstanding challenge in contemporary mammalian genomics -- to find the new mutant sites in the genome of each B6-SNP mapping partner. If we can meet this challenge, we can strongly advance the exploration of the genetic factors that can modify the Min phenotype. We can move beyond the gatekeeper *Apc* into an understanding of tumor maintenance, growth, and progression.

Now, as we have continued to study the somatic genetics underlying loss of the gatekeeper function, *Apc*, we've discovered a class of modifiers distinct from the secretory phospholipase. We have found that chromosomal rearrangements also affect the probability of forming adenomas in Min mice. In the normal karyotype, *Min* lies near the centromere on the acrocentric chromosome 18. Tumors form when homologous somatic recombination converts a heterozygous constitutional genotype to a homozygous mutant genotype. This process seems to involve conservative homologous recombination, apparently not involving any chromosome genomic instability. A higher resolution analysis of the process is currently being pursued with the "optical mapping" team of our colleague David Schwartz at Wisconsin. As I have mentioned, the importance of mitotic recombination for mammalian somatic genetics was the basis for our maiden voyage into mouse genetics with Jean Louis in 1975. Jean-Jacques Panthier published with Guénet and Jacob some of the first evidence for mitotic recombination in mice.



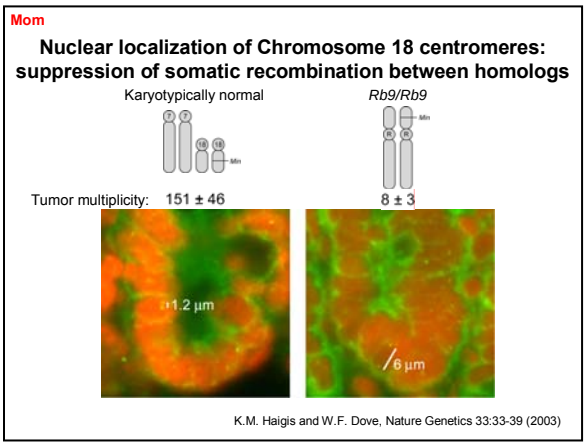


Our evidence for mitotic recombination in adenoma formation involved the construction of mice carrying Robertsonian fusions, to mark the centromere by joining mouse chromosome 7 to the *Min*-bearing chromosome 18. This Robertsonian allowed us then to follow the centromere of chromosome 18 and to show that, when tumors form, the centromere remains heterozygous, while the *Min* site became homozygous. When we studied tumor formation quantitatively in these Robertsonian fusion constructs, we found remarkably that, although the Robertsonian fusion did not change any known gene sequences, it did change by an order of magnitude the multiplicity (but not the net growth rate) of adenomas. In each of these rearranged configurations we find a great reduction in tumor multiplicity compared to the normal karyotype shown on the left.

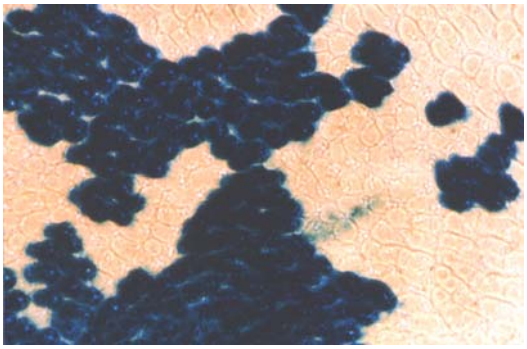


What is the explanation for this strong modifying effect of rearranging the karyotype? Performing *in situ* hybridization with a probe directed against the centromeric region of chromosome 18, we have observed that, in the normal karyotype the homologues are closely opposed to one another -- in this example lying 1.2 microns apart. By contrast, in the

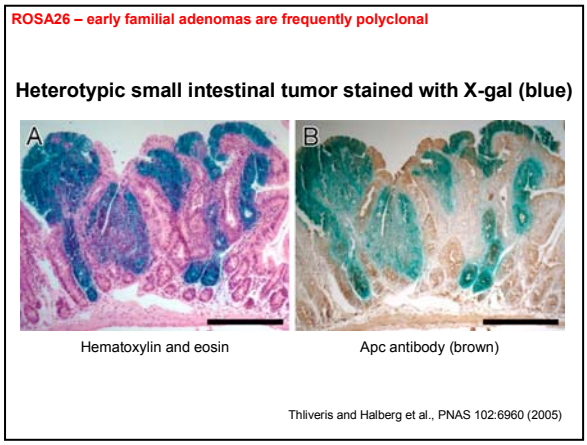
Robertsonian homozygote these two homologous regions of chromosome 18 lie much further apart. So the arrangement of genes within the mammalian nucleus seems to be a very important consideration in affecting somatic recombination.



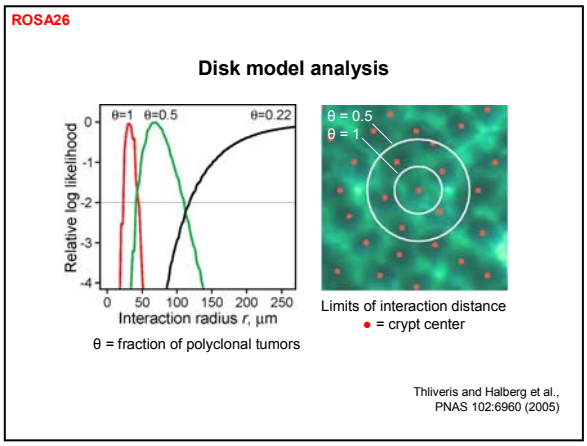
In summary, the genetic modifiers of the Min phenotype include both chromosomal effects on tumor initiation and genes affecting the net growth rate of emergent neoplasms, such as the secretory phospholipase. To fully understand the action of these modifying factors we have found it useful to analyze chimeric mice. Just as the chick/quail somatic lineage marker has been so informative in the research of Nicole LeDouarin and her colleagues in Paris, a clonal lineage marker for the mouse is the ROSA26 insertion of the β-galactosidase gene, *lacZ*. This marker, established by Philippe Soriano, is ubiquitously expressed in normal tissues and tumors of the mouse. Here is an XGal-stained whole mount of a colon from a chimeric animal made by fusion between a ROSA-positive and a ROSA-negative embryo. Note the small clusters of monoclonal crypts in this normal tissue. If the two components of the chimera differ in the *Mom1* modifier locus, one can ask whether there a long-range effect of the secretory phospholipase. We have shown that the secretory phospholipase has a very short range of action, not more than 1 crypt diameter away from its source. This modifier, however, is expressed by postmitotic Paneth or goblet cells surrounding the tumor, acting non-autonomously but locally to control the net growth rate of the tumor. The use of ROSA26 chimeras allows us to assess the distances over which genetic modifiers act.



Clonal marking in chimeras with ROSA26 gives us another novel insight into the biology of intestinal tumorigenesis - tumor clonality at various stages of tumorigenesis. In chimeras in which both the blue and white tissue carry the Min predisposition, we have found a remarkable result - - early familial tumors are very often if not always polyclonal, as shown in the next slide. In the left panel, the tumor section is stained with hematoxylin and eosin and with X-gal. The tumor has both white and blue components. When an adjacent slide from the tumor in the right panel is stained with Apc antibody in brown, normal tissue stains positive, but each clonal component of the tumor has lost Apc function. We have



demonstrated by molecular Apc genotyping that, remarkably, both components of such polyclonal adenomas have lost the wildtype allele of *Apc*, presumably by somatic recombination. Thus, even at very low tumor multiplicity, distinct clones cooperate in the formation of early tumors. A statistical analysis carried out by our biostatistician colleague, Michael Newton, indicates that the frequency of polyclonal tumors can be explained by a very short range of interaction between two to three crypts over a distance in the order of 50-100 microns. The cooperation then seems to involve nearest neighbor crypts, each undergoing loss of heterozygosity and then joining together to make the adenoma.



Does this study of the familial Min mouse model inform human colon cancer, familial or sporadic? Most of the investigations of the clonality of human colon cancer have involved X chromosome mosaicism. However, the intestinal patch sizes in X-chromosome mosaics are an order of magnitude larger than the patch sizes of the chimeras that we have analyzed. Because of the very short range of interaction implied by our analysis, the probability of detecting polyclonality is vastly reduced in human X-chromosome mosaics. One needs very fine grained chimeras or mosaics to detect the polyclonality of early intestinal tumors. In London, Marco Novelli and his colleagues have found a single human patient carrying both a mutation in the *APC* gene and a rearrangement of the Y chromosome. This individual displayed a significant degree of Y chromosome instability, leading to mosaicism of XO clones within XY colonic tissue. Novelli and his colleagues have observed a significant frequency of tumors that are karyotypically both XY and XO, as if they involve cooperation between two somatic lineages. However, the tumor multiplicities of this FAP individual were extremely high, making random fusion of distinct adenomas a possible alternative explanation. Furthermore, the instability of the rearranged Y chromosome made it conceivable that the mosaicism arose after rather than during tumor formation. Neither of these explanations holds for our experiments with chimeric Min mice, especially our recently analysis at low tumor multiplicity. Thus, the experimental mouse model for colon cancer provides an independent analysis at high resolution to investigate a claim made on the basis of the single available human patient. That early familial tumors of the colon involve clonal cooperation is apparently general.

Polyclonal origin of colonic adenomas in an XO/XY patient with FAP

Novelli MR, Williamson JA, Tomlinson IP, Elia G, Hodgson SV, Talbot IC, Bodmer WF, Wright NA

Science 272:1187-90 (1996)

X-inactivation patch size in human female tissue confounds the assessment of tumor clonality

Novelli M, Cossu A, Oukrif D, Quaglia A, Lakhani S, Poulsom R, Sasieni P, Carta P, Contini M, Pasca A, Palmieri G, Bodmer W, Tanda F, Wright N

PNAS 100:3311-4 (2003)

Now let's return to our opening question: how well do rodent models of carcinogenesis predict efficacy in the human? Note that the Min mouse, though it does display a few colonic tumors, develops most of its tumors in the small intestine and lives a very short time, precluding tumor progression. Does emergent mammalian genetics allow us to address these limitations?

All of you in the audience realize that the genetics of other experimental mammals is not nearly as well developed as that of the mouse. Most of Jean-Louis's work has been performed with the mouse. But not all. There is now a well developed map and genome sequence for the rat, some contributed by Jean-Louis! Michael Gould and his colleagues have found ways to make knockouts of particular genes in the rat, using ENU mutagenesis of the rat germline and screening for nonsense alleles by cloning particular segments of interest from mutagenized genomes into a read-through yeast vector. Working with the Gould group, we have obtained a rat carrying a knockout allele at position 1137 of the *Apc* gene of the rat, corresponding to the mutation cluster region in the human. This strain has been designated Pirc for polyposis in the rat colon – tumorigenesis is largely limited to the colon, at least at 90 days of age. Four tumors such tumors are shown in this wholemount of the colon of a Pirc rat. Thus, molecular genetics in the rat now provides a contrasting murine model for human FAP. One karyotypic feature the rat genome is that the *Apc* locus lies on a metacentric chromosome, facilitating the analysis of somatic recombination.

A murine gateway into human colon cancer

How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men

Corpet DE, Pierre F.
The School of Veterinary Medicine, Toulouse

Eur J Cancer. 41:1911-22 (2005)

"…rodent models roughly agree with human data, but do not predict accurately the efficacy of all chemopreventive agents in humans. Human beings will however not be able to find new ways to prevent cancer without the help of animal models."

A murine gateway

Rat gene mapping using PCR-analyzed microsatellites

Serikawa T, Kuramoto T, Hilbert P, Mori M, Yamada J, Dubay CJ, Lindpainter K, Ganten D, Guénet JL, Lathrop GM, et al.

Genetics 131:701-721 (1992)

A murine gateway

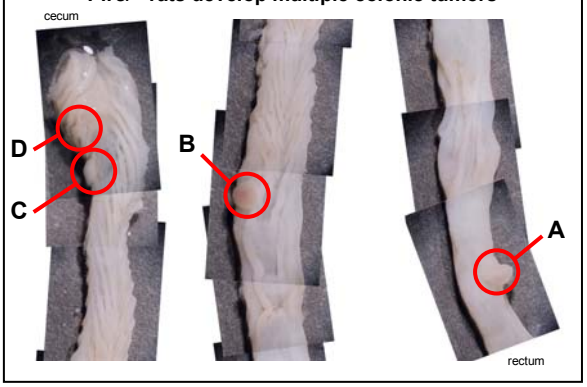
An ENU-induced Rat Knockout Model of Human Familial Adenomatous Polyposis

Lawrence N Kwong, James M Amos-Landgraf, Kaishun Chen, Jill D Haag, Jordy L Waller, Jane L Remfert, Yunhong Zan, Michael N Gould, William F Dove

International Mammalian Genome Society, Strasbourg 2005

A murine gateway

***Pirc*/+ rats develop multiple colonic tumors**



A murine gateway

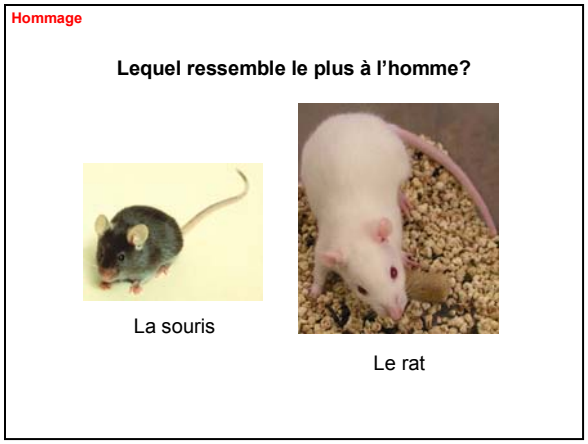
Human FAP and its murine models

	Tumor #	Location	Mutation	Chromosomes
Human FAP (codon 700-1500)	>100	Colon	APC	Metacentric
Min Mouse (C57BL/6J)	>100	Small Bowel	APC	Acrocentric
Rat + Carcinogen (Fischer-344)	~1	Distal Colon	Mainly β-catenin	Metacentric
Pirc Rat (Fischer-344)	**~5**	**Colon**	**APC**	**Metacentric**

My colleagues and I intend to explore the experimental dialog between the Min mouse and the Pirc rat in studying the biological, molecular, and medical issues involved in human colon cancer.

Today my tribute to Jean-Louis Guenet has developed the theme of l'homme et les animaux.

Lequel ressemble plus a l'homme?
La souris?
Le rat?



Non – **C'est un lion**