tion, although the observed absence of any lathyrogenic effect in the aortic tissue from aminoguanidine-treated normal rats in these experiments (Table 1) makes a significant effect of this type unlikely.

With the use of aminoguanidine it should now be possible to evaluate the role played by extensive glycosylation-induced protein cross-linking in the development of chronic diabetic complications and the sequelae of aging. The potential ability of aminoguanidine or related compounds to prevent reduced arterial elasticity, increased peripheral vascular resistance, arteriosclerosis, and capillary basement membrane thickening in diabetes and aging is an exciting clinical possibility that needs further investigation.

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High Resolution of Mouse Chromosomes: Banding Conservation Between Man and Mouse

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A detailed schematic representation of high-resolution G-banding patterns was prepared from elongated and finely banded mitotic chromosomes of the mouse. Such chromosomes can be obtained from both animal tissue and cell lines by a simple protocol, facilitating precise demarcation of breakpoints in chromosome rearrangements and aiding in the sublocalization of genes. Regions of subbanding homology were observed between human and mouse chromosomal segments known to have conserved gene assignments, an indication that, at the cytogenetic level, extensive regions of the mammalian genome may remain intact after 60 million years of species divergence.

CHROMOSOME BANDING PROCEDURES allow identification of individual mammalian chromosomes and, when combined with appropriate gene-mapping strategies, provide information on the location of genes. Recently, the analysis of human chromosome aberrations and comparative cytogenetics of humans and primates have benefited from high-resolution banding techniques (*1*). High-resolution cytogenetics provides precision in the delineation of chromosomal breakpoints and assignment of gene loci, greater than with earlier techniques, since analysis of late prophase subbanding reveals more than twice the number of bands seen at metaphase. Subbanding analysis of chromosomes has also become important in the study of the structural organization of chromosomes, with chromosomal DNA segments as small as about 1200 kilobases now being visualized at the light microscopic level (*2*). In mapping studies, the sublocalization of structural genes (and even DNA sequences whose function is not yet known) can be precisely established by in situ molecular hybridization of cloned DNA probes to high-resolution mitotic chromosomes (*3*).

In the past, the mouse has been a preferred mammalian species for in vivo genetic analysis, and numerous mouse cell lines have been established for the study of mutagenesis, gene regulation in vitro, and somatic cell genetics. Here we describe the use of actinomycin D in combination with acridine orange to induce excellent high-resolution mouse chromosome subbanding. Treatment with actinomycin D for 1 hour before the cell harvest and fixation procedure yields highly elongated chromosomes with an adequate number of mitoses for analysis (*4*). Acridine orange, when added for 20 minutes at the hypotonic step in the cell harvest procedure, holds chromosomes in an extended state, apparently by intercalating between DNA base pairs (*5*). We believe such pretreatment also induces more sharply defined chromosome subbanding.

Mouse chromosomes for high-resolution analysis were prepared from splenic lymphocytes of DBA/2 mice. Spleens were dispersed with a tissue screen, and the single-cell suspension (1×10^6 to 2×10^6 cells) was cultured in 10 ml of RPMI 1640 medium supplemented with 20% fetal calf serum, 2% L-glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml). Purified phytohemagglutinin (Burroughs Wellcome HA16, 0.3 μg/ml of cell culture) was then added and cultures were incubated at 37°C (*6*). After 72 hours, actinomycin D (3.0 μg/ml) and Colcemid (0.025 μg/ml) were added and incubation was continued for an additional hour. The cells were then centrifuged at 800*g* and gently resuspended in 9 ml of 0.075*M* KCl containing acridine orange (10 μg/ml). The cell suspension was then incubated for an additional 20 minutes at 37°C; the cells were then fixed in 3:1 methanol: acetic acid. Fixed cell suspension was then dropped onto ethanol-cleaned, dry slides from a distance of about 30 cm.

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Over 530 bands can be visualized in late prophase chromosomes of the mouse with these techniques, as compared to just over 200 bands seen with conventional metaphase techniques (Figs. 1 and 2). Band coalescence, as described for human chromosomes at increasing levels of chromosome condensation (*2, 7*), was also observed (Fig. 1a). Chromosome elongation and banding quality obtained with these modifications are comparable to results obtained with the cell synchronization techniques used for human chromosome preparations (*7*). We have had equivalent success with long-term cultures of L5178Y mouse lymphoma cells (*8*).

As gene linkage maps for both man and mouse have become more clearly defined in recent years, analysis of the linkage disruptions caused by chromosome rearrangements has favored the hypothesis that many long chromosomal segments may have been preserved since the divergence of man and mouse (*9*). For example, segments of up to 24 centimorgans have been reported to be conserved between human chromosome 1 and mouse chromosome 4 (*9, 10*). High-resolution comparison of chromosome subbanding in regions of supposed man/mouse genetic homology revealed a striking similarity in banding patterns at the late prophase stage of chromosome condensation (Fig. 3). Large segments of human chromosome 1 and mouse chromosome 4 spanning the known map positions for phosphoglucomutase-1 and phosphogluconate dehydrogenase had nearly identical banding patterns (Fig. 3a). Other extensive banding homologies were seen in the vicinity of the major histocompatibility complex loci (Fig. 3b), the galactokinase and soluble thymidine kinase genes (Fig. 3c), and segments spanning the immunoglobulin heavy chain gene loci and the α-1-antitrypsin gene (Fig. 3d). Other possible regions of genetic colinearity remain to be identified.

Subbanding homology demonstrated here for human and mouse chromosomes known to contain homologous genes indicates that, at the cytogenetic level, extensive regions of the mammalian genome may have remained intact after 60 million years of species divergence. The ability to identify cytogenetically homologous segments of the human and mouse genomes should increase our understanding of the evolution of mammalian chromosomes. In addition, sublocali-

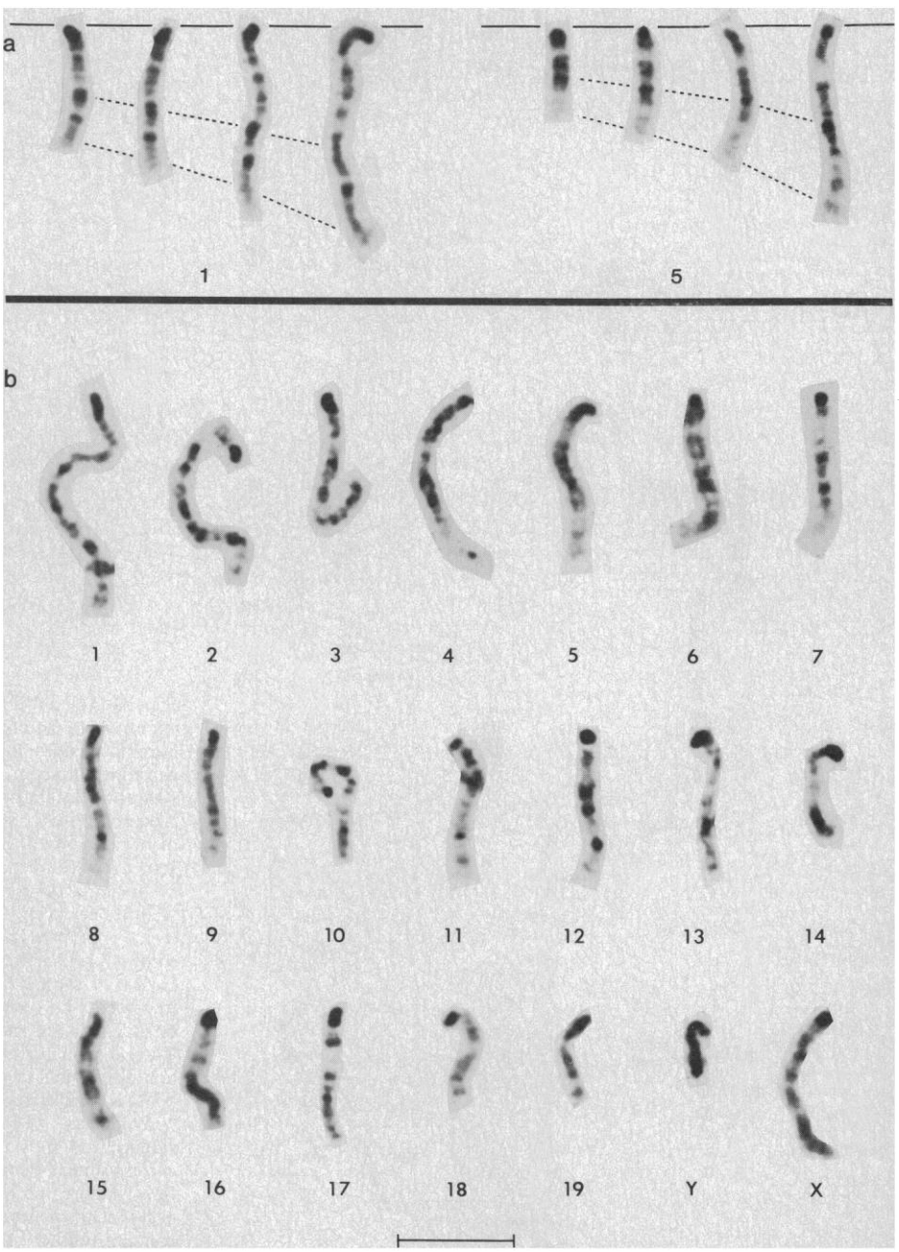

Fig. 1. (a) Examples of mouse chromosomes in decreasing stages of mitotic condensation. From left to right are shown representative chromosomes (numbers 1 and 5) at metaphase, early metaphase, prometaphase, and late prophase. Dashed lines indicate major band positions through the process of band coalescence (*2, 7*). The solid line indicates centromere position. Chromosome preparations were pretreated for banding by placing slides in 1*N* HCl for 20 minutes followed by a 90-minute incubation in 50% formamide and 2× standard saline citrate. The slides were then rinsed in tap water and dehydrated in 95% ethanol for 30 minutes. After dehydration, the slides were stained with a 3:1 phosphate buffer/Wright's stain solution. (b) Complete representative male haploid set of mouse chromosomes at late prophase. Chromosomes were prepared as described in (a). Scale bar, 10 μm.

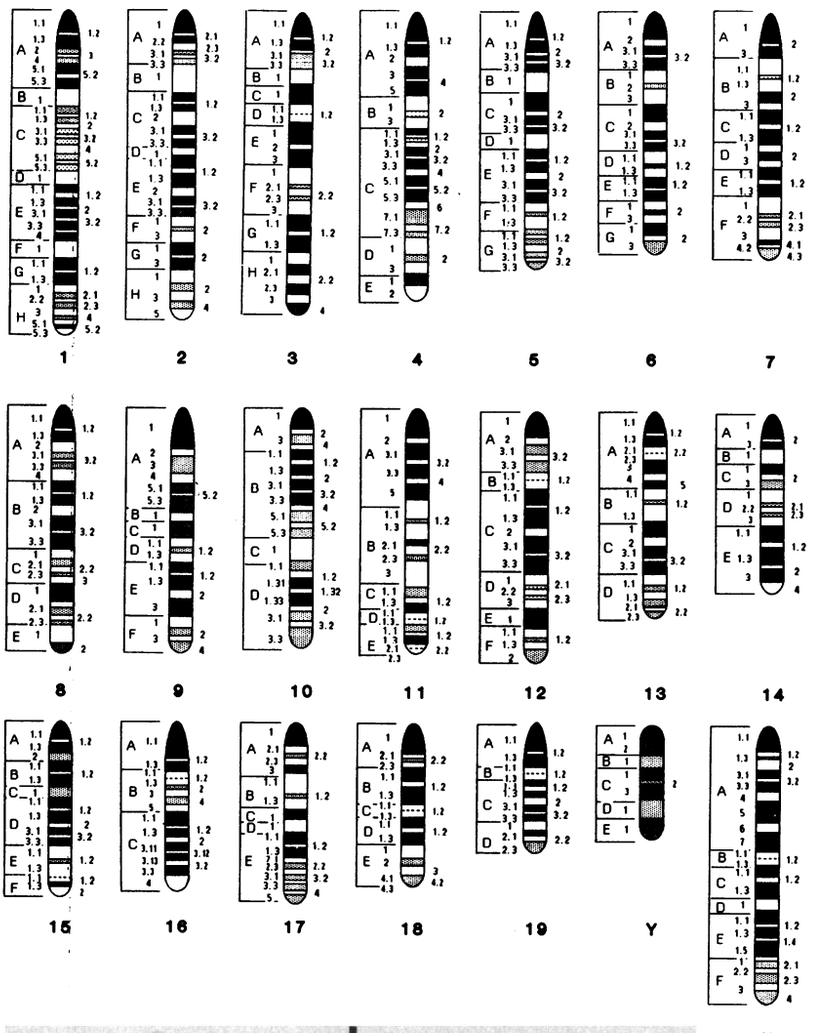

Fig. 2. Schematic representation of the late prophase chromosomes of the mouse. The ideogram represents relative position, size, and staining intensity of 530 bands. The nomenclature used here follows that originally proposed for mouse banded metaphase chromosomes (*11*) and the recommendations of the Committee on Standardized Genetic Nomenclature for Mice (*12*). Band maps were prepared by a procedure similar to that used in constructing high-resolution human ideograms (*4*, *5*). Briefly, five high-quality, well-banded chromosomes for each stage (metaphase, early metaphase, prometaphase, and late prophase) were enlarged approximately 4000× for measurement. Bands were measured and characteristic patterns were drawn for bands that appeared in the majority of chromosomes at each stage of condensation.

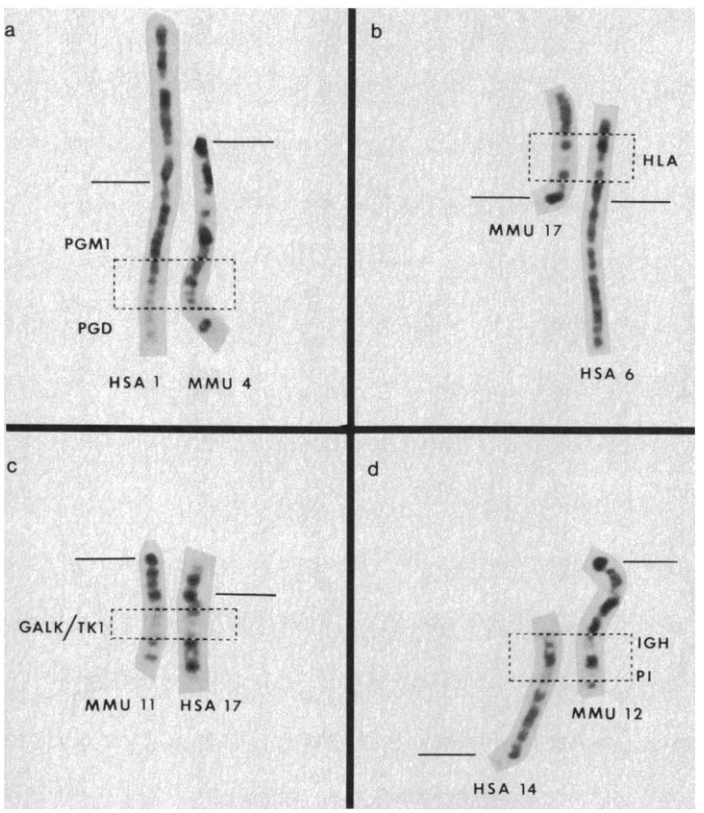

Fig. 3. Human (HSA) and mouse (MMU) chromosomes arranged to demonstrate homologous regions with high-resolution banding. Solid horizontal lines indicate centromere positions. Dotted boxes contain chromosome segments similar in banding pattern and staining intensity as well as of similar gene content and gene order relative to the centromere. Nomenclature for human gene loci is used throughout to show approximate positions of homologous mapped loci. (a) Human chromosome 1 shown on the left (inverted) and mouse chromosome 4 on the right. Phosphoglucomutase-1 (PGM1) and phosphogluconate dehydrogenase (PGD) have been assigned to either end of the homologous regions. (Box contains human 1p31.3-p35 and mouse 4C5.1-D2). (b) Mouse chromosome 17 on the left (inverted) and human chromosome 6 on the right. Genes for the major histocompatibility complex (HLA) have been localized to these homologous chromosome segments. (Box contains human 6p21.1-p23 and mouse 17A2.3-D1). (c) Mouse chromosome 11 on the left and human chromosome 17 on the right. The region of subbanding homology includes segments believed to contain the galactokinase (GALK) and cytoplasmic thymidine kinase (TK1) genes. (Box contains human 17q12-q21.3 and mouse 11B1.3-C1.3). Mouse TK1 was recently sublocalized by analysis of chromosome breakpoints (*8*). (d) Human chromosome 14 on the left (inverted) and mouse chromosome 12 on the right. The region of subbanding homology includes the immunoglobulin heavy chain genes (IGH) loci and the α-1-antitrypsin (PI) gene. (Box contains human 14q24.2-qter and 12D1-F1.3).

zation of genes on mouse chromosomes prepared for high-resolution analysis should more precisely delineate regions of genetic homology between man and mouse and should facilitate the use of the mouse as a model for human genetic disease.

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Viral Shedding and Transmission Between Hosts Determined by Reovirus L2 Gene

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Two reovirus isolates (type 1 Lang and type 3 Dearing) differ in their transmissibility between littermates of newborn mice. They also differ in the amounts of virus excreted by the gastrointestinal tract. With the use of reassortant viruses, these properties were mapped to the L2 gene. Thus environmental spread of reovirus is a genetic property.

MANY PATHOGENIC MICROORGANISMS have an obligatory stage in a eukaryotic host. Such a "parasitic" microorganism, whether virus, bacterium, or parasite, must develop a mechanism for efficient transmission from the infected host to another susceptible host. Transmission between animal hosts often takes place by defined routes such as respiratory, fecal-oral, or inoculation. The route of transmission is often related to the localization of the infecting microorganism. Thus, influenza, which localizes in the lung, is spread by respiratory secretions, whereas poliovirus, an enteric virus, is spread in stool.

Much has been learned in recent years about the mechanisms by which viruses localize in certain tissues, and hence about why viruses might select different strategies for transmission. There is also considerable information about the stability of viruses on release into the environment and the way in which properties such as droplet size, heat, and moisture might affect spread. Little is known, however, about how the genetic properties of viruses affect the capacity of viruses to be transmitted between hosts and to survive in the environment. Schulman (*1*) attempted to define differences in transmissibility between two strains of influenza that differed in virulence. He was able to show clearly that virulence and transmissibility were genetically determined, but the specific genetic basis for transmission was difficult to define (*2*). In fact, there is no example in which a specific gene (or genes) has been shown to play a clear role in transmission.

We used mammalian reoviruses to define the genetic basis of viral-host interactions. The reoviruses are widely distributed in nature, infecting humans, cows, mice, and many other species. By age 15, 50 to 80 percent of humans have antibody to reovirus (*3*). Although not important pathogens, they produce multiple pathologic changes in mice after experimental inoculation (*4*). The reovirus genome consists of ten segments of double-stranded (ds) RNA; there are three serotypes. Coinfection of L-cell cultures with different strains of virus results in reassortant virions (*5*). The relative ease of generating reassortants has enabled us and others to identify the functions of a number of genes [reviewed in (*4*)], including the gene responsible for receptor binding and serotype-specific immunity (S1), growth in differentiated tissue and protease sensitivity (M2), and initiation of persistent infection in cell culture (S4).

We used reovirus infection of newborn mice to define the gene responsible for transmission between mice and for enhanced secretion from the gastrointestinal tract. The gene that confers these properties is L2.

Initially we asked whether reovirus isolates could be identified that differed in their capacity to be transmitted between littermates. The standard laboratory strains, type 1 Lang and type 3 Dearing, showed marked differences in transmission under standard conditions (see Fig. 1). When a dose of 10^5 plaque-forming units (PFU) of type 1 Lang was given to two members of a litter (five litters inoculated), 57 ± 10 percent of the littermates were infected. Only one instance of transmission (five litters inoculated) was observed when type 3 Dearing was given at the same dosage (a transmission frequency of 3.7 ± 6 percent). The murine reovirus

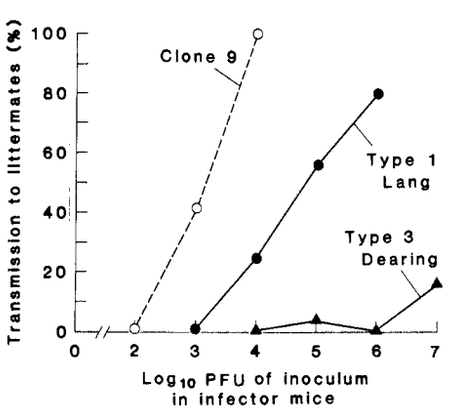

Fig. 1. Frequency of reovirus transmission among members of a litter as a function of inoculum given to infector mice. In standard transmission experiments (five replications), one litter of eight to ten suckling mice of the NIH/Swiss strain was placed in each cage. At age 1 to 2 days, two members of the litter were infected by orogastric intubation with a dose of virus in 30 μl of gelatin saline, as previously described (*6*). These "infector mice" were designated by clipping the tail and were replaced in the litter. At 10 days the littermates were killed and their intestines were removed (the site of primary replication for reovirus) (*14*). Intestines were placed in 1 ml of gelatin saline, frozen and thawed three times, and sonicated. The sonicate was diluted from 10^{-1} to 10^{-5}, and titers of virus were determined in the plaque assay previously described (*15*). Because of the toxicity of the intestinal contents to the L cells used in the assay, the 10^{-1} dilution wells were difficult to interpret. However, the assay detected the presence of virus at ≥1000 PFU per intestine. Presence of virus in the intestines of the littermates was scored as (+) transmission and its absence as (−) transmission.

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