WHY ISN'T *a* MOUSE MORE LIKE *a* MAN?

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Jacques Monod was fond of saying that 'an elephant is like an *E. coli*, only more so'. Mouse geneticists have long felt, and partially justified their research in the belief, that 'a human is like a mouse, only more so'. Many excellent mouse models of genetic disease support this belief[1]. However, as more genetic models are identified in mice, more differences are being found between some of the mouse mutants and human diseases. Given the greatly increased ability to create desired mouse mutations[2], it is timely to review some of these mouse models and consider possible reasons why they do not always replicate the human disease associated with the same mutations. Three general mechanisms for the differences will be considered: (1) different biochemical pathways, (2) different developmental pathways, and (3) the possibility that some pathological processes occur at the same absolute rate in man and mice instead of being related to life span.

VARIATION IN BIOCHEMICAL PATHWAYS BETWEEN MOUSE AND MAN

The same genetic defect in mouse and man may result in different phenotypes because of differences in relevant biochemical pathways. For instance, the Lesch–Nyhan syndrome, recently reviewed in *TIG*[3], is a severe human disease caused by a deficiency of hypoxanthine guanine phospho-ribosyltransferase (HPRT). Mice have been made deficient in HPRT by selecting HPRT$^-$ embryonic stem cells, producing chimeric mice by injection of the HPRT$^-$ stem cells into blastocysts, and breeding HPRT$^-$ mice from gonadal chimeras[4]. However, these mice have apparently not developed some of the classical symptoms of Lesch–Nyhan disease, such as self-mutilatory behavior or symptoms of gout[3]. Inasmuch as mice, but not humans, can convert uric acid to allantoin by oxidation with urate oxidase, it is possible that mice are protected from potentially toxic metabolites of uric acid. Thus, it may be necessary to eliminate both urate oxidase and HPRT activity to create an animal model of Lesch–Nyhan disease. The recent cloning of the urate oxidase gene[5] should allow reverse genetic approaches to this goal.

Although most metabolic pathways do not differ between mouse and man, one major difference lies in the human inability to synthesize vitamin C, which could be relevant for some mouse models.

Sometimes different genetic defects result in a similar disease phenotype but then the animal model is not a precise one. Thus, the glucose-6-phosphatase deficiency discovered in mice carrying lethal albino mutations seemed a potential model of glycogen storage disease type Ia (Ref. 6). However, multiple other enzyme deficiencies were discovered in these mice and it now seems likely that the lethal albino deletions have eliminated a *trans*-acting factor necessary for the regulation of many unlinked genes. S. Gluecksohn-Waelsch has discussed these mutations in *TIG*[7].

A mouse model of hyperphenylalaninemia was discovered by screening the offspring of ENU-mutagenized mice by the Guthrie assay[8]. However, the mutation turned out to be the result of a deficiency in GTP-cyclohydrolase rather than phenylalanine oxidase activity and has a very different biochemical phenotype from phenylketonuria in man. Increased levels of phenylalanine similar to those in human phenylketonuria patients occur during the first 10 days of life but normal levels are found after 20 days of age unless a phenylalanine load is given.

VARIATION IN DEVELOPMENTAL PATHWAYS BETWEEN MICE AND MAN

A mouse is much smaller than a human and this, apparently, influences some developmental processes. One example concerns bone remodeling. Humans deficient

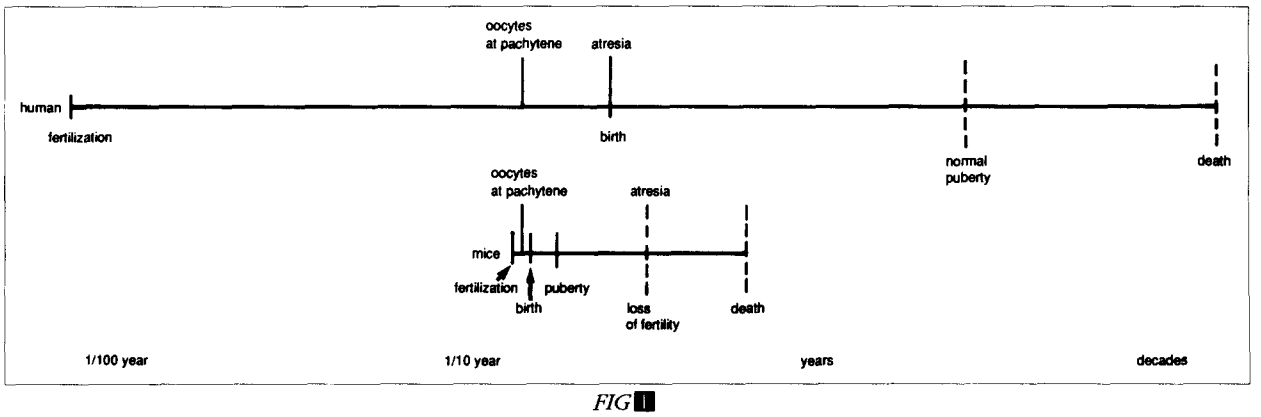

FIG 1

A COMPARISON OF OOGENESIS IN X0 WOMEN AND X0 MICE ON A LOGARITHMIC TIMESCALE (TO ENABLE THE DISCREPANT LIFE SPANS TO BE DISPLAYED IN A REASONABLE AMOUNT OF SPACE). THE TWO LIFE SPANS ARE ALIGNED AT THE POINT WHERE OOCYTES ENTER THE PACHYTENE STAGE OF MEIOSIS.

in carbonic anhydrase (CA) II suffer from osteopetrosis (marble bone), renal tubular acidosis (lack of ability to excrete normal amounts of hydrogen ion in their urine), intracranial calcifications, and mental retardation[9]. Mice deficient in CA II were recovered from an ENU mutagenesis/screening program[10]. A major difference between the results of CA II deficiency in mouse and man was the lack of osteopetrosis in mice; there was no sign of this even in old mice. The absence of osteopetrosis may be a consequence of the fact that rodents only remodel bone at the surfaces of their smaller bones and not internally, as larger mammals do[11]: they lack the Haversian canal system that is involved in bone remodeling in man. Thus, the lack of osteopetrosis in CA-II-deficient mice suggests the possibility that the function of CA II in osteoclasts is essential for internal, but not for surface, remodeling of bone.

The nervous system is very different between mice and man, and this variation reflects neurodevelopmental differences. Such neurodevelopmental pathway differences may be relevant to the usefulness of a number of neurological mutants in mice as animal models, although the lack of detailed knowledge about mechanisms of nervous system development makes comparisons difficult. For instance, the *jimpy* mutation in mice and Pelizaeus–Merzbacher disease in humans are thought to be homologous – they involve deficiencies in myelin proteolipid protein[12] and map to locally homologous regions of the X chromosome[13]. However, their phenotypes differ: tremor is prominent in the phenotype of the *jimpy* mutant while limb spasticity is more notable in humans suffering from Pelizaeus–Merzbacher disease.

Absolute time versus physiological time and rates of pathological processes

Physiological processes tend to occur faster in small animals than in larger ones[14] – for example, mouse heart rates are 10 times those of man. Thus, it is usually assumed that pathological processes occur at similar rates as defined by life span ('one year of a dog's life is equivalent to seven human years'). However, several examples suggest that some pathological processes are more closely related to absolute time. The C3H inbred strain of mice has low levels of β-glucuronidase, especially in liver[15], comparable to deficiencies that are symptomatic in many human lysosomal storage diseases[16]. However, stored material doesn't accumulate significantly until the mice are about a year old[17]. Thus, this rate of accumulation seems comparable to rates in the human disorders in which patients are normal at birth but typically develop symptoms in the second half of the first year of life.

A time-dependent rate of onset of symptoms may explain the mild phenotype of *mdx*, the probable mouse homolog of the dystrophin locus, the human X-linked locus at which some mutations cause a severe childhood muscular dystrophy (Duchenne) while others cause a milder adult muscular dystrophy (Becker) (recently reviewed in *TIG*[18]). The recent cloning of mouse dystrophin[19] appears to confirm the homology established by histology and map position (although some controversy remains[20]). The mild tremor and uncoordinated gait of *mdx* hemizygous mice[21] suggest that the mutation has resulted in either a Becker-like disease with life span-related onset or a Duchenne-like disease with an absolute time-related onset, since humans do not usually show symptoms until several years of age, a length of time greater than the usual survival of mice.

An absolute time-dependent rate of pathological processes offers an alternative or additional explanation to those given above for the discrepancies between mice and humans with HPRT and CA II deficiencies. Patients with Lesch–Nyhan disease are typically diagnosed at about a year of age – already a very old age for a mouse. CA-II-deficient humans do not have osteopetrosis at birth, but it has been detected at 3–4 months – in bones that are 25 times larger than their mouse counterparts and therefore allow earlier radiological detection of this disorder. In both cases, mice much older than the usual life span might show changes more typical of the human disorders.

Such time-related instead of life span-related differences in pathological processes may also explain differences such as that in fertility between X0 mice and humans. X0 mice are fertile, but oocyte production ceases prematurely at about 6 months of age[22] (there is variation dependent on genetic background). Patients with Turner's syndrome have normal numbers of oocytes early in fetal life but these oocytes die near the time of birth[23]. Thus, both mouse and human X0 oocytes have roughly similar survival rates. This is long enough for a mouse to be born, mature sexually and have several litters, but is barely enough time for a human to be born[24] (Fig. 1).

Absolute time-related onset of pathological processes could explain the lack of mouse mutations homologous to some common human diseases such as neurofibromatosis (NF). NF has one of the highest known mutation rates in man: 1×10^{-4} per gamete per generation[25]. Despite millions of mice examined annually at facilities such as the Jackson Laboratory, no homologous mutation seems to have been found [although transgenic mice with the *tat* gene of the human T-lymphotropic virus type-1 (HTLV-1) under control of its own long terminal repeat develop histologically similar tumors[26]]. *Cafe-au-lait* spots would probably not be visible under mouse fur; however, tumors should appear and be detected if their appearance is dictated by physiological time, but not if it is dependent on absolute time, since they typically start to appear during the second decade of life in humans.

Conclusion

Although there are many reasons why mice carrying mutations homologous to those causing human disease can differ from their human counterparts, even imperfect models can be useful. Studies evaluating the causes of differences should contribute to a better understanding of the human genetic disease. It is to be hoped that more genetic models, perfect and imperfect, and many such studies will be forthcoming.

REFERENCES

1 Leiter, E.H. *et al.* (1987) *Birth Defects Orig. Art. Series* 23, 221–257
2 Erickson, R.P. *Am. J. Hum. Genet.* (in press)
3 Stout, J.T. and Caskey, C.T. (1988) *Trends Genet.* 4, 175–178
4 Jackson, I.J. (1987) *Trends Genet.* 3, 119–120
5 Lee, C.C. *et al.* (1988) *Science* 239, 1288–1290
6 Erickson, R.P., Gluecksohn-Waelsch, S. and Cori, C.F. (1968) *Proc. Natl Acad. Sci. USA* 59, 437–444
7 Gluecksohn-Waelsch, S. (1987) *Trends Genet.* 3, 123–127
8 McDonald, J.D. and Bode, V.C. (1988) *Ped. Res.* 23, 63–67
9 Sly, W.S. *et al.* (1983) *Proc. Natl Acad. Sci. USA* 80, 2752–2756
10 Lewis, S. E. *et al.* (1988) *Proc. Natl Acad. Sci. USA* 85, 1962–1966
11 Marks, S. C. (1977) *Am. J. Anat.* 163, 157–167
12 Nave, K-L., Lai, C., Bloom, F.E. and Milner, R.J. (1988) *Proc. Natl Acad. Sci. USA* 83, 9264–9268
13 Willard, H. F. and Riordan, J. R. (1985) *Science* 230, 940–942
14 Calder, III, W. A. (1984) *Size, Function, and Life History*, pp. 139–161, Harvard University Press
15 Law, L. W., Morrow, A. G. and Greenspan, E. M. (1952) *J. Natl Cancer Inst.* 12, 909–916
16 Neufeld, E. and Munzer, J. in *The Metabolic Basis of Inherited Disease 6th edn* (Scriver, C.R., Beaudet, A., Sly, W.S. and Valle, D., eds), McGraw-Hill (in press)
17 Yatziv, S., Erickson, R. P., Sandman, R. and Robertson, W. van B. (1978) *Biochem. Genet.* 16, 1079–1084
18 Witkowski, J.A. (1988) *Trends Genet.* 4, 27–30
19 Hoffman, E.P., Monaco, A.P., Feener, C.C. and Kunkel, L.M. (1987) *Science* 238, 347–350
20 Grant, S.G., Mullins, L.J., Stephenson, D.A. and Chapman, V.M. (1988) *Genome* 30 (Suppl. 1), 222
21 Bulfield, G., Siller, W.G., Wright, P.A.L. and Moore, K.J. (1984) *Proc.Natl Acad. Sci. USA* 81, 1189–1192
22 Burgoyne, P.S.and Baker, T.G. (1981) *J. Reprod. Fert.* 61, 207–212
23 Jirásek, J.E. (1977) *Birth Defects Orig. Art.Ser.* 13, 13–39
24 Burgoyne, P.S. (1978) *Ann. Biol. Anim. Biochim. Biophys.* 18, 317–325
25 Crowe, F.W., Schull, W.J. and Neel, J.V. (1956) *A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis* (Thomas, C.C., ed.), pp. 1–181, Springfield
26 Hinrichs, S.H. *et al.* (1987) *Science* 237, 1340–1343

TECHNICAL TIPS

IN SITU HYBRIDIZATION USING BIOTIN-LABELED PROBES

A nonisotopic ***in situ*** hybridization technique which was developed to map genes on metaphase chromosomes of the nematode, ***Caenorhabditis elegans*** [1,2], has been applied to the mapping of genes on human metaphase chromosomes[3]. Biotin-labeled dUTP is incorporated into the probe DNA by nick translation[4,5] and the probe is then hybridized to metaphase spreads prepared by conventional methods. The site of hybridization is visualized by immunofluorescence using an anti-biotin antibody and a Texas red or fluorescein-labeled second antibody. The chromosomes are stained with Hoechst 33258. In order to record faint fluorescent signals from the hybridized probe a camera sensitive to low light levels (ISIT camera) is used. The images are time averaged for noise reduction and stored digitally. The fluorescent images from the hybridized probe and the chromosomes are aligned precisely by placing the signals from a heterologous ribosomal DNA probe, included in the hybridization mix, over the secondary constriction on the ends of the acrocentric (D and G group) chromosomes where the ribosomal genes are located.

Using this method it is possible to resolve the signals from hybridization of a probe to individual chromatids and to localize two probes simultaneously to alternate, but not adjacent bands. For the greatest sensitivity the ISIT camera is required. However, it is possible to see the hybridization signal from probes containing 3–4 kb of genomic DNA and therefore it would be possible to record such signals photographically.

Nonisotopic methods for ***in situ*** hybridization have obvious advantages over autoradiography: it is not necessary to use radioisotopes and the signal can be generated in a few hours, rather than days. In addition nonisotopically labeled probes can be hybridized to and then detected in thick specimens to look at the intranuclear location of DNA sequences or for applications where distortion caused by squashing or flattening of the tissue causes ambiguity. Fluorescent detection methods are particularly well suited to these applications, as optical sectioning using laser scanning confocal microscopy provides accurate resolution in three dimensions. In addition, two probes have been visualized in the same specimen by using a second labeling system other than biotin and appropriate fluorescently labeled antibodies. The low background from biotin-labeled probes should be useful for ***in situ*** hybridization mapping in systems (such as the mouse) where full karyotypic analysis is impracticable.

REFERENCES

1 Albertson, D.G. (1984) *EMBO J.* 3, 1227–1234
2 Albertson, D.G. (1985) *EMBO J.* 4, 2493–2498
3 Albertson, D.G. *et al.* (1988) *EMBO J.* 7, 2801–2805
4 Langer, P. *et al.* (1981) *Proc. Natl Acad. Sci. USA* 78, 6633–6637
5 Langer-Safer, P. *et al.* (1982) *Proc. Natl Acad. Sci. USA* 79, 4381–4385

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