ISSN 0168-9525

TRENDS IN GENETICS

DNA, Differentiation & Development

REFERENCE EDITION

VOLUME 1

1985

ELSEVIER

TRENDS IN GENETICS

Published by:
Elsevier Science Publishers B.V. (Biochemical Division),
P.O. Box 1527,
1000 BM Amsterdam
The Netherlands.

Printed in the United Kingdom

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Editor S. Prentis, Elsevier Publications, 68 Hills Road, Cambridge CB2 1LA, UK

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Trends in Genetics

January 1985

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Trends in Genetics — a logical development

Trends in Genetics is being launched at an especially exciting time in biological research. Techniques and theories developed during the last few years are now generating a flood of information which is beginning to answer some of the most fundamental questions in biology. This first issue of *Trends in Genetics* addresses several of these questions: what controls early development in embryos? how is sex determined? which genetic processes are involved in evolution? what defects underlie genetic diseases? how do differentiated cells maintain their correct functional, spatial and temporal relationships? The full range of topics that will be covered by the journal cannot be demonstrated by this first issue alone, but these articles and the list of forthcoming titles below do indicate some of the 'trends in genetics' to be featured in the next few months.

A primary aim of *Trends in Genetics* is to provide researchers and students with concise and comprehensible reviews, commentaries and discussions. Each area of genetics has spawned its own specialized terminology which can make it difficult to decipher the wider significance of new information that is not closely related to one's own speciality. Yet an enforced retreat into increased specialization cannot be good for genetics or geneticists. We hope that *Trends in Genetics* will help readers to appreciate progress being made in a wide range of research programs and to see how it may relate to their own particular interests. To achieve this, authors have been invited to discuss each topic in terms which will be accessible to all geneticists.

There is, perhaps, one aspect of *Trends in Genetics* that merits special mention. By using the subtitle *'DNA, Differentiation and Development'* the journal has been given the opportunity to focus on issues in developmental biology which, at present, may have no well-defined genetic component. Given the greatly increased interest in developmental questions and the ever widening range of techniques available for studying them, it seems clear that *Trends in Genetics* can play an important role in providing a meeting ground for all scientists interested in development, whatever approaches they employ. Thus we hope that *Trends in Genetics* will not only facilitate communication between scientists in all branches of genetics, but also between them and related groups.

These, then, are some of the goals of this new publication, but everyone involved with the journal is very aware that its long-term success will only be assured if it reflects the interests of its intended audience. I hope, therefore, that readers will offer their comments on *Trends in Genetics* and the way in which it might develop. In particular, we will welcome direct contributions to three sections of the journal. In future issues there will be a place for letters to the editor where we shall publish correspondence on matters related to articles published in the journal or on any topic of general interest to geneticists and developmental biologists. Secondly, the Technical Tips section (p.6) is designed to offer the opportunity for readers to exchange useful experimental techniques. Finally, Genetic Jottings (facing p. XI) is available to anyone who wishes to publicize meetings, courses, workshops or awards.

Now that *Trends in Genetics* is launched I should like to thank all the authors for making the considerable effort of writing in a style which is suitable for the broad readership intended for the journal. This first issue is also the ideal place to acknowledge the invaluable contributions of the twenty members of the editorial board whose advice and comment has been vital in shaping the journal's future. Its sister publications from Elsevier's Cambridge office, which include *Trends in Biochemical Sciences, Trends in Neurosciences* and *Immunology Today*, have rapidly established themselves as essential reading for scientists in these disciplines. If *Trends in Genetics* can become as well regarded and useful, then its purpose will have been fulfilled.

Steve Prentis, Editor

Heterochronic mutations of *Caenorhabditis elegans*

their developmental and evolutionary significance

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V. Ambros and R. Horvitz have identified a set of genes that may control the timing of developmental events in the nematode *Caenorhabditis elegans*[1]. After hatching, *C. elegans* proceeds to the adult stage via four juvenile stages, each followed by a molt. Each juvenile stage is characterized by a distinct pattern of cell division and differentiation[2] called an S1, S2, S3 or S4 lineage pattern[1].

Ambros and Horvitz have found that the S1–S4 lineage patterns are modular; that is, a lineage pattern (including hypodermal, neuronal, muscle, and intestinal divisions and differentiations) characteristic of one stage can be expressed at a different stage. Each behaves like a complex lineage cassette that can be inserted into one or more of the available temporal slots.

Ambros and Horvitz have isolated mutations (which they call heterochronic mutations) that replace lineage patterns characteristic of one stage with patterns characteristic of a different stage. By analogy with homeotic mutations of *Drosophila*[3] and *C. elegans*[4] that substitute one spatial pattern for another, these are homeotic mutations that substitute one temporal lineage pattern for another.

Recessive mutations in the *lin-14* and *lin-28* genes delete particular lineage patterns and cause precocious expression of lineage patterns characteristic of later stages (see Fig. 1. re 1). For example, certain *lin-14* mutations delete S1, causing animals to undergo the sequence: embryo, S2, S3, S4, adult. The *lin-28* mutations and other *lin-14* mutations delete S2, resulting in the sequence: embryo, S1, S3, S4, adult.

Conversely, dominant *lin-14* mutations and recessive *lin-4* mutations[5] cause reiterations of particular lineage patterns (see Fig. 1). One *lin-14* allele causes the animal to express a reiterated series of S1 lineage patterns, while another causes the series: embryo, S1, S1 (or S2), S2, S2. The existence of these mutations suggests that the S1–S4 lineage patterns represent alternative developmental choices, and that at each stage a system of temporal control somehow decides between them.

Heterochronic mutations alter not only the type of lineage patterns expressed at each stage, but also the number of stages that occur during development. Mutations that delete lineage patterns often reduce the number of juvenile stages (to three instead of four), while mutations that reiterate lineage patterns often increase the number of stages (to five or six). This suggests that the selection of the correct lineage pattern is coupled to the process that determines the number of juvenile stages.

The heterochronic mutants are significant because they may eventually tell us how an organism can exert temporal control over developmental events. In principle, the expression of the correct lineage pattern at the correct stage could be determined by a sequential mechanism, each lineage pattern normally occurring as a result of expression of the previous lineage pattern. Alternatively, a particular lineage pattern could be expressed in response to temporal information that is independent of cell differentiation (a biological clock). The finding that S2 patterns need not follow S1 patterns, and S3 patterns need not follow S2 patterns suggests that such a system of temporal information may operate here.

The fact that single mutations can cause heterochrony has important implications for evolution. In *Ontogeny and Phylogeny*[6], S. J. Gould argues that heterochrony is a major mode of evolutionary change: 'There may be nothing new under the sun', he says, 'but permutations of the old within complex systems can work wonders'.

One type of heterochrony, called recapitulation, refers to the presence of traits that appear in later stages of ancestral development at earlier stages in the ontogeny of descendants. Recapitulation may allow new traits to be incorporated with a minimal disruption. Features might first appear in the adult form, where their developmental consequences are minimized, and could subsequently be shifted earlier by changes in developmental timing.

Other evolutionary sequences are best explained by postulating that changes in developmental timing have caused ancestral juvenile traits to persist in later ontogenic stages of descendants. This type of change (called paedomorphosis) may provide: (1) the opportunity to escape from an adult form no longer favored by its environment; (2) the ability to make a favorable juvenile niche available to the reproductive form; and (3) the possibility of remodelling ancestral adult genes whose functions are no longer required in the reproductive form of descendants.

An important issue in current evolutionary theory is whether heterochrony could have occurred by simple alterations in a developmental program. A classic example is the Mexican axolotl[7], which probably originated as a mutant with altered hormone metabolism. Unlike related salamanders, the axolotl does not undergo metamorphosis; instead its adult form resembles the larval form of its relatives. It is reported to differ from species that undergo metamorphosis by a single allele and, remarkably, it can be induced to undergo metamorphosis by treatment with the hormone thyroxin.

The *C. elegans* heterochronic mutants provide direct proof that both paedomorphosis and recapitulation can be caused by single mutations. The mutants show that a wide range of heterochronic changes are possible, including changes in the number of stages, the number of specialized cells and structures, and the type of cuticle produced. Ambros and Horvitz have also demonstrated that single mutations can change the relative timing of events in different tissues, the most striking examples being heterochronic *lin-29* mutations, which affect only hypodermal (skin) cells.

Evolution requires uninterrupted fertility. Thus it may be significant that the gonad is one of the few tissues not altered by *C. elegans* heterochronic mutations. Possibly selection has favored nematodes whose developmental programs uncouple gonadal from non-gonadal development. Such organisms should have greater access to the opportunities provided by heterochrony.

Given known spontaneous mutation rates in *C. elegans*[8] and the prevalence of *C. elegans* in nature, (R. Russell and T. Doniach, personal communication) one expects roughly several hundred heterochronic mutants in a square mile of fertile soil. Raw material for evolution? Ambros and Horvitz mention a nematode species that partially resembles a *lin-14* mutant of *C. elegans*. Perhaps during evolution this organism diverged from *C. elegans* or a common ancestor through heterochronic mutations.

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The elusive testis-determining factor

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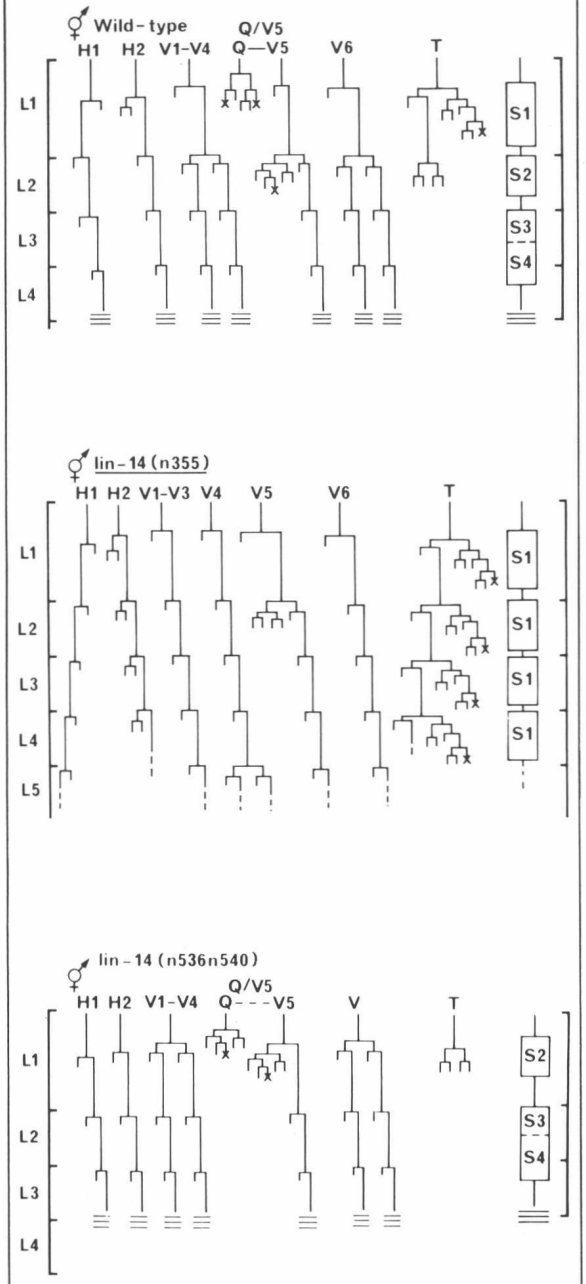

Fig. 1. *Patterns of cell divisions in* C. elegans *heterochronic mutants. H1, H2, V1–V4, Q/V5, V6 and T are ectodermal precursor cells located on the sides of the animal (anterior cells are drawn to the left). The vertical axis represents developmental time. The juvenile stages are called L1–L4/5, and the division patterns expressed at each stage are labelled S1–S4. The top panel depicts the lineage pattern of the wild-type hemaphrodite.* lin-14(n355) *is a dominant* lin-14 *mutation, and* lin-14(n536n540) *is a recessive* lin-14 *mutation. X indicates cell death; three stripes indicate that the cell produces adult-specific ridges in the overlying cuticle.* (Based on figure from Ref. 1).

The chromosomal basis of sex determination in mammals is clear: males have a Y chromosome, females do not. The genetic function of the Y chromosome in primary sex determination is to induce the undifferentiated fetal gonad to become a testis. Male secondary sexual characteristics are a consequence of having a testis. The genetic control and molecular processes of primary sex determination are obscure and the postulated testis-determining factor(s) has proved elusive. At a recent convocation* immunologists, geneticists and molecular biologists were forced to confront the partial demise of a favourite hypothesis.

For the last decade the dominant hypothesis of sex determination has been that the testis-determining factor is identical to the H-Y antigen[1]. Inbred female mice can reject male skin grafts from mice of the same inbred strain, but males can not reject female skin grafts. This graft rejection defines a minor histocompatibility antigen, controlled by the Y chromosome, called H-Y[2]. *In vitro* T-cell assays and serological assays have also been used to define this antigen[3]. However, considerable controversy surrounds the interpretation of the serological tests and it is not certain that all the assays define the same antigen.

The hypothesis of identity between the H-Y product and the testis-determining factor is based on circumstantial association, theoretical evolutionary arguments and *in vitro* antibody blocking experiments. None of the evidence is conclusive but the cumulative effect of the diverse data was seductive. Unfortunately, the hypothesis is no longer tenable without caveats because of an elegant series of collaborative experiments performed by a geneticist (A. McLaren, MRC Mammalian Development Unit, University College, London) and an immunologist (E. Simpson, Clinical Research Centre, Harrow).

These workers studied the inheritance of the testis-determining factor and H-Y in strains of mice carrying the sex-reversed *(Sxr)* mutation. Fertile male mice carrying the *Sxr* mutation possess an unusual Y chromosome (XY*Sxr*) in which a duplicated copy of the pericentric, sex-determining, region of the chromosome has been translocated to the distal tip of the chromosome. At meiosis, an apparently obligatory crossover between the ends of the X and Y chromosomes results in the transfer of this translocated region to one chromatid of the X chromosome[4,5]. Consequently half of the XX offspring inherit an abnormal X*Sxr* chromosome from their fathers and, although sterile, are phenotypically male with testes. Hitherto all such mice have also been H-Y positive when assayed by transplantation or for their ability to stimulate appropriate *H-2* restricted, *H-Y* specific T-cell clones. By crossing XY*Sxr* males to females carrying the balanced translocation T(X;16) 16H, abbreviated as T16H, fertile female offspring were generated which were T16H X*Sxr*. Obviously the forced inactivation of the intact X*Sxr* chromosome has extended to the sex-determining locus[6]. However, these females are still H-Y positive which might reflect a minor population of cells in which the *Sxr* region has not been inactivated or might represent a gradient of inactivation in the translocated *Sxr* region. By crossing T16H, X*Sxr* females with XY*Sxr* it is possible to construct X*Sxr* Y*Sxr* males, and it was in the progeny of one such male that an unusual event was noticed. The T16H, X*Sxr* daughters of an X*Sxr* Y*Sxr* male were H-Y typed. Most were positive but one was negative. The excep-

* The INSERM conference on *The molecular structure of the Y chromosome* held 23–26 September 1984 in Seillac, France.

tion was crossed to a normal male and gave rise to sex-reversed progeny and to XY*Sxr* carrier males in the expected proportions. All subsequent XX*Sxr* males and T16H, X*Sxr* females in this pedigree were H-Y negative. The inescapable conclusion of these results is that the gene coding for H-Y antigen expression in the *Sxr* chromosome segment can mutate or be lost without affecting the sex-determining ability of the *Sxr* region. This must raise a serious question about the role of H-Y in sex determination.

If H-Y is not the testis-determining factor then alternative approaches are needed to define this important molecule(s). The molecular biologists have suggested two approaches. Just as XX*Sxr* mice have inherited Y-derived sequences, some XX human males have been shown to have sequences derived from the Y chromosome. The groups of M. Fellous and J. Weissenbach (Pasteur Institute, Paris) and their collaborators have argued that each XX male should have inherited a variable amount of Y-derived material which will always include the testis-determining locus (*Td*). By investigating a large number of XX males with Y-derived probes it should be possible to define the region in which the *Td* locus is present[7]. DNA from the XX male with the smallest amount of Y-derived sequence in addition to *Td* would be a valuable starting material for cloning the *Td* locus. In one study 15 XX males were shown to have inherited variable amounts of the Y chromosome. The markers used could be arranged in a linear order, however, the *Td* locus appeared to be the terminal marker defined. As DNA from nine XX males investigated in the same study failed to react with all the probes used, it is possible that the nearest marker is still a considerable distance away from the *Td* gene. An additional complication is that some XX males may not have inherited Y-derived sequences but have mutations elsewhere in the genome.

The second approach proposed by the molecular biologists is to clone all those loci on the Y chromosome which are expressed. For the X chromosome or an autosome this would be a daunting task. However, apart from the postulated *Td* locus, the H-Y locus and a locus controlling expression of a cell-surface antigen[8], the Y chromosome is bereft of defined genes known to be expressed. C. Bishop (Pasteur Institute, Paris) has recently described two Y-encoded sequences, one from mouse and one from man, which are transcribed in testis. The mouse sequence was originally isolated from a library constructed from Y chromosomes which were flow-sorted, by M. Goldberg (Pasteur Institute, Paris) who ingeniously used Robertsonian translocation stocks of mice to remove contaminating chromosomes from the region of the Y peak. The transcribed mouse sequence is not present in the genome of XX*Sxr* mice and therefore cannot be related to the *Td* locus. The transcribed human sequence is also unlikely to be related to *Td* as it is absent from the DNA of XX males and maps to the long arm of the Y chromosome. Despite the failure of these initial experiments to find the *Td* locus this approach could be successful and, at the very least, will help define other functions of the Y chromosome.

Since the time of Aristotle humans have debated the origins of the difference between the sexes. It will be interesting to see if molecular biology can tell us the answer.

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Cell-free replication of SV40

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The characterization of the components and the mechanism of DNA replication has been largely dependent on the availability of soluble cell-free replication systems. For mammalian cells, the difficulty of analysing the synthesis of chromosomal DNA *in vitro* has been compounded by the lack of an identified sequence in the nuclear genome that serves as an origin of replication. This problem has been circumvented, in part, by using animal virus chromosomes as models for cellular chromosomes. For example, the development of cell extracts from mammalian cells that replicate adenovirus DNA faithfully[1] has led to insights into the mechanism of viral DNA synthesis. Perhaps more importantly, this cell-free system has provided a suitable assay for identifying cellular proteins involved in the replication process, as has been reported recently[2,3]. Thus, animal virus replication systems have proven useful for probing the workings of the normal cell.

In a recent publication, Li and Kelly[4] have introduced a new soluble cell-free system that replicates another animal virus, simian virus 40 (SV40). SV40 contains a small (5.2 kilobase pairs) circular DNA molecule complexed with cellular histones to form a minichromosome. The DNA molecule has a unique origin of replication and, in virus-infected monkey cells, SV40 DNA synthesis proceeds bidirectionally from the origin. Synthesis at the two growing forks occurs at a similar rate until they meet at a point approximately opposite to the origin on the physical map. The successful duplication of the SV40 minichromosome is not a simple matter: the highly supercoiled molecule must be relaxed near the origin of replication to allow the access of initiation components; the elongation of the two growing chains must proceed through the intertwisted parental strands and around the histone nucleosomes; and the two double-stranded circular products of the reaction, linked together in a topological knot, must be separated. All this takes place in a matter of minutes.

The system described by Li and Kelly reproduces all of these events in the replication of the SV40 genome. Their system has the following features: (1) replication of an exogenous DNA molecule in the extract depends on a functional SV40 origin of replication within the molecule; (2) the SV40-encoded protein, large T antigen, is required for DNA replication *in vitro*; (3) DNA polymerase α is the active synthetic enzyme in the extract; (4) DNA replication appears to initiate near the SV40 origin of replication and proceed bidirectionally but this has not yet been shown directly; (5) DNA synthesis generates intermediate structures similar to those seen during SV40 DNA replication *in vivo*; (6) complete daughter strands are synthesized in the reaction; and (7) replication products are resolved into double-stranded supercoiled molecules containing one parental and one daughter strand. These features indicate that the cell-free DNA synthetic reaction closely mimics the intracellular replication of the SV40 genome.

An earlier report from Ariga and Sugano[5] described a cell-free system for SV40 DNA replication that has many of the

properties of the Li and Kelly system. One important difference between these two procedures is the composition of the reaction mixtures. Ariga and Sugano prepared extracts from isolated nuclei from the human HeLa cell line and supplemented these extracts with SV40 T antigen, either in the form of an extract of virus-infected monkey cells or partially-purified T-antigen. The monkey cell extract alone fails to support the replication of the SV40 genome, although it is quite active in promoting repair synthesis.

The Li and Kelly method uses an extract of SV40-infected monkey cells or an extract of uninfected monkey cells supplemented with purified SV40 T antigen; no HeLa cell factors are required and hence the system may be somewhat simpler. The reason for these different results may be explained by the level of T antigen in the extracts; both groups used SV40-infected COS-1 cells for the monkey cell extract, but Ariga and Sugano carried out the infection with wild-type SV40, while Li and Kelly employed a cold-sensitive mutant of SV40 that overproduces wild-type T antigen. In support of this notion is the finding by Li and Kelly that extracts prepared from uninfected monkey COS-1 cells do not support the replication of SV40 DNA. This is surprising because these cells contain integrated SV40 sequences in the cell genome and produce enough SV40 T antigen to complement the intracellular replication of mutants of SV40 which do not encode functional T antigen.

While these systems will no doubt foster further detailed research into the process of viral DNA replication, the more significant aspect of this work may be that it provides a new way of studying the replication machinery. It will also allow a closer look at the actions of the SV40 T antigen, a multifunctional protein that stimulates the synthesis of both cellular and viral DNA and RNA, and is the causative agent in the malignant transformation of mammalian cells by SV40. How T antigen accomplishes these activities may become clearer as the biochemistry of these cell-free systems is dissected.

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Ethylnitrosourea as a mouse mutagen

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Mutations are invaluable for unravelling complex biological problems and the absence of suitable mutations can severely limit studies of biological phenomena. The sources of variation available to mouse geneticists are: (1) the naturally occurring variants in laboratory strains and wild-mice; and (2) mutations induced in laboratory mice by radiation, chemicals or retroviral insertion. Mutagenesis was not a feasible way of generating new mouse variants until the discovery by Russell *et al.*[1] of the extreme mutagenicity of ethylnitrosourea (ENU). This alkylating agent is the most potent known mutagen for mouse spermatogonial stem cells. In the mouse specific-locus test in which recessive mutations arising at seven loci of visible effect are scored, a single dose of 250 mg/kg ENU administered to male mice yielded 72 mutants in 10 146 offspring[2]. This is equivalent to one mutation at one locus per 1000 offspring. Similar results were reported by Ehling *et al.*[3] who found an average mutation frequency per locus of about 1 in 1200 offspring. The mutagenic effectiveness of ENU is also apparent when comparing the above data with results for other mutagens. The mutation frequency with ENU is 6 to 8 times greater than found with 600 R (the most effective acute dose of X-irradiation), 16 to 78 times the highest rate found with any other chemical and over 100 times the spontaneous mutation rate.

The potential of ENU for generating mutants of interest was illustrated at a recent meeting in Montpellier (Fourth International Workshop on Mouse Molecular Genetics, 18–20 September 1984). Mutants which provide models of genetic disease or which allow analysis of developmental pathways and complex genetic systems were described. Vernon Bode (Kansas State University) reported an experiment exploiting the high mutagenicity of ENU in which he isolated a mouse mutant that is a potential model of phenylketonuria (PKU).

Phenylketonuria is one of the more frequent genetic disorders in man, showing autosomal recessive inheritance. It is usually due to phenylalanine hydroxylase deficiency and, unless the patient receives a special low-phenylalanine diet, results in mental retardation. Bode used the Guthrie inhibition assay developed for screening human blood to screen offspring of mutagenized (C57BL/6 × CBA)F_1 males for high concentrations of blood phenylalanine. In general, when testing human blood the PKU heterozygote is not detected. In the mouse, no instances of a dominant hyperphenylalaninaemia were found in over 7000 offspring tested. A three-generation breeding programme was initiated to generate mouse mutants which are homozygous for this recessive trait. Nineteen male mice were mutagenized, mated to untreated females and the male offspring used for further testing. These male offspring were crossed to wild-type females and 2–4 daughters of this cross were backcrossed to their father. The offspring of the third cross were screened for high concentrations of blood phenylalanine by the Guthrie test. Backcrossing 2–4 daughters means that the chance of detecting the mutant gene is 75–94%. Among 105 male offspring of ENU-treated mice fully tested, one carrier of hereditary hyperphenylalaninaemia was identified. Investigations are in progress to find out if this mutant has phenylalanine hydroxylase deficiency. If so, then a potential model of phenylketonuria will have been induced by ENU.

Another ENU-induced mutant which is also a model of a genetically determined human disorder was described by J. Peters (MRC Radiobiology Unit, Harwell). The breeding procedure was simpler than that described by Bode since a mutant arising in a germ cell of a mutagenized parent could be scored in the first generation after treatment. In this experiment, males treated with ENU were crossed to females with different alleles at six loci which determine enzymes and proteins that can be analysed by electrophoresis. One of the offspring had a haemoglobin of altered electrophoretic mobility due to a Tyr → Cys mutation at β145 in the haemoglobin β-chain. The mutant haemoglobin is associated with polycythaemia, increased oxygen affinity and decreased haem–haem interactions. It is homologous to haemoglobin Rainier in man, which has the same amino acid substitution and similar physiological consequences.

The induction of mouse developmental mutations by ENU was described by W. Dove (University of Wisconsin) who searched for recessive mutations in the *T-H-2* region of chromosome 17. This also involved a three-generation breeding programme, but in this case chromosome 17 of the mutagenized mice was marked so that mutations arising on this chromosome could be recovered with high efficiency. Males homozygous for the chromosome 17 locus tufted (*tf*) were treated with ENU and crossed to *T/t* females (the outcross generation). The offspring were crossed to *T/t* and the wild-type mice which arose (*+tf/+t*) were either intercrossed or backcrossed to the *+tf/+t* parent (the inbreeding generation) to detect recessive lethals associated with the *tf* marked chromosome. The absence of *tf/tf* progeny indi-

cates that a recessive lethal linked to *tf* has been generated. In 105 fully tested gametes, two recessive prenatal lethals linked to *tf* were found as well as seven recessive visible mutations independent of *tf* or sex. One dominant visible was also recovered. Bode[4] has also used ENU to induce mutagenesis in the *T-H-2* region of chromosome 17 and reported the recovery of a mutation called *t-int* which interacts with *T* (Brachyury) to produce a tailless mouse. Interestingly, this mutation has been induced in wild-type chromatin whereas naturally occurring *t*-haplotypes involve stretches of abnormal chromatin which in a complete *t*-haplotype extend from *T* to the *H-2* complex. New mutants at the quaking (*qk*) and tufted (*tf*) loci on chromosome 17 have also been isolated. In a second experiment, *tf* and *qk* mutants were induced in the t^{w5}-haplotype, in *t*-chromatin.

The available information suggests that ENU-induced mutations are small intragenic changes e.g. a TAG → TGC transition was proposed for the haemoglobin β mutant (J. Schöneich, Central Institute of Genetics and Research in Cultivated Plants of the Academy of Sciences, GDR) described a point-mutation test which detects rat LDH-X (lactate dehydrogenase) specific antigenic determinants in mouse sperm using monospecific anti-rat LDH-X antibodies. The LDH-X polypeptide in mouse and rat differs only by two amino acids and this test is thought to detect base substitutions. ENU was found to induce mutations at doses above 60 mg/kg.

None of the ENU mutagenesis experiments required large numbers of personnel, and all used relatively modest numbers of mice. ENU appears to provide a feasible way of generating useful numbers of mutants. Whether ENU can regenerate mutants at all loci has not yet been established and it may induce mutations more frequently at some loci than at others. Ehling *et al.*[5] have suggested that the exceptionally high mutation frequency induced by 250 mg/kg of ENU in the mouse specific-locus test results from a disproportionate number of dilute and pink-eye mutants.

ENU is a powerful carcinogen and must be handled with care. With these provisos in mind, it still provides a viable method of generating genetically determined variants.

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Cloning Y chromosome DNA

Enrichment deletion is a general method for cloning Y chromosomal DNA. DNA from female BALB/c mice is sheared by sonication and DNA from male BALB/c mice is completely digested with *Mbo*1. A 100-fold excess of the female DNA is mixed with male DNA and the mixture is denatured, annealed and isolated. Three types of DNA hybrid form. In Type 1 hybrids (see Fig. 1) both strands would be derived from sheared female DNA. Type 2 hybrids contain one sheared strand from female DNA and one *Mbo*1-cut strand from male DNA. In Type 3 hybrids both strands are derived from *Mbo*1 cut male DNA. Single-stranded restriction fragments of male DNA that share no homology with female DNA self-hybridize to form Type 3 hybrids. Among this mixture of DNA hybrids only Type 3 hybrids can be easily cloned since only these hybrids contain the sequence GATC at both ends.

Lamar, E. E. and Palmer, E. (1984) Y-encoded, species-specific DNA in mice: evidence that the Y chromosome exists in two polymorphic forms in inbred strains. *Cell* 37, 171–177

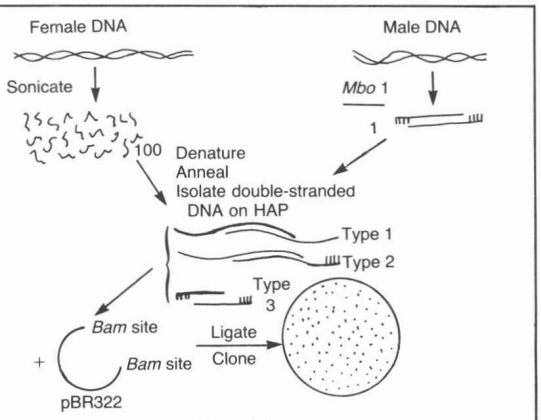

Fig. 1. *The enrichment deletion scheme for cloning Y chromosomal DNA.* (Based on a figure from Lamar and Palmer, 1984).

Cleaning up plasmid minipreps with lithium chloride

Ribosomal RNA can be removed from plasmid preps by precipitation with 2–2.25 M LiCl; tRNA, 5S RNA, DNA and polysaccharides remain in solution, and can be recovered directly from the supernatant by ethanol precipitation. The step works best with a concentrated solution: the nucleic acid from 10 mls of overnight culture can be taken up in 200–400 µl, mixed with an equal volume of 5 M LiCl, chilled on ice or at −20°C for 5 min and centrifuged. The residual low-molecular-weight RNA runs ahead of all but the smallest restriction fragments on gels. This is a particularly useful modification to the standard alkaline lysis method (Maniatis *et al.* (1983) *Molecular cloning: a laboratory manual*, Cold Spring Harbor Publications p. 368). Residual protein-SDS aggregates and other junk are cleanly removed, and the DNA is always digestible. Phenol extraction is not necessary. Incidentally, lysozyme can also be omitted from the alkaline lysis method.

Contributed by Hugh Pelham, MRC Lab. of Molecular Biology, Cambridge, UK.

Reduction of background on DNA and protein blots with dried milk

Solutions of dried skimmed milk (0.25–5%) are a cheap, simple and effective alternative to serum albumin, Denhardt's cocktail and other mixtures used to block non-specific binding of nucleic acid probes or antibodies to Southern, Northern and Western blots, and ELISA plate assays. It is particularly good for protein blots on nitrocellulose; once the filter is blocked, the milk can be omitted from further washes without a significant increase in background.

Johnson, D. A., Gautsch, J. W., Sportsman, J. R. and Elder, J. H. (1984) *Gene Anal. Techn.* 1, 3–8

Simultaneous mapping of biochemical and morphological traits

Electrophoretic isozyme analyses were combined with seedling assays for morphology in soybeans. Fifteen gene combinations could be tested for linkage in a single cross. Thick-slab gel methods were used so that the gels could be sliced into as many as seven slices and stained for a different enzyme.

Devine, T. E., Kiang, Y. T. and Gorman, M. B. (1984). Simultaneous genetic mapping of morphological and biochemical traits in soybean. *J. Hered.* 75, 311–312