

At the Crossroads of Developmental Genetics: The Cloning of the Classical Mouse *T* locus

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Summary

The discovery, more than 60 years ago, of a mutant mouse with a short tail led to the birth of the new field of developmental genetics. Over the years since, numerous investigators have probed the biology of the original short-tail mutation at the *T* locus, as well the naturally-occurring *t* haplotypes that were uncovered as a result of their interaction with this mutation. Although the *T* locus ranks among the best characterized developmental loci in the mouse, it was not among the first to be cloned. This situation has now been rectified with two recent reports from Herrmann, Lehrach and their colleagues. While the *T* locus is expressed uniquely in the embryonic tissues predicted from the mutant phenotype, the gene itself, as well as the predicted amino acid sequence of the *T* product, show no strong homology to any known sequence. For the moment, at least, the mystery behind the function of the *T* locus still awaits definitive resolution.

Introduction

While the mouse has long been the model system of choice for the majority of investigations into mammalian embryogenesis, this trend has become even more pronounced during the last decade with the development and utilization of increasingly more sophisticated tools to study and manipulate the mouse embryo. Genetic material from any source can be injected directly into the nuclei of one-celled zygotes⁽¹⁾; multiple cleavage-stage embryos can be teased apart into component cells and put back together again in new combinations⁽¹⁾; nuclei can be switched back and forth between different embryonic cytoplasms⁽²⁾; and embryo cells can be placed into tissue culture, where their genomes can be manipulated in a more controlled fashion, before they are returned to the embryo-proper⁽³⁾. Genetically-altered live animals can be obtained subsequent to all of these procedures, and these animals can transmit their altered genomes to their offspring. While none of these manipulations has yet been applied to human embryos, the mental image invoked is of a far more sophisticated technology than the so-called futuristic scenario of embryo farms described in Huxley's *Brave New World*.

Progress has also been made at the level of molecular

analysis within the developing embryo. The revolutionary polymerase chain reaction (PCR) protocol can be used to identify DNA and RNA sequences present within single cells⁽⁴⁾, and the somewhat older techniques of *in situ* hybridization and immuno-staining allow one to start with the fertilized egg and follow the patterns of individual gene expression through the four dimensions of space and time⁽¹⁾.

These powerful tools for analysis have led to the growth of a very large community of mouse developmental biologists. Nevertheless, progress in understanding the development of the mouse has lagged far behind that made in the other two major experimental animal systems used in developmental studies – *Drosophila melanogaster* and *Caenorhabditis elegans*. There are two major reasons for this lag. First, flies (*D. melanogaster*) and worms (*C. elegans*) are very small and produce large numbers of offspring; this makes it easy for individual investigators to screen and recover multiple mutations at any given locus or loci defined by phenotype alone. Second, the sophisticated genetics available in each of these systems, combined with their relatively small genomes, allows investigators to rapidly obtain DNA clones from any locus at which mutations exist.

Developmental Genetics in the Mouse: Current Status

The current experimental situation in the mouse is quite different in that two sets of so-called *developmental* loci have been identified which are, for the most part, non-overlapping. First, a large number of mutations with developmental phenotypes have been recovered over the last half-century that have yet to be cloned⁽⁵⁾. Second, a large number of genes with interesting patterns of expression during development have been cloned and characterized over the last decade, without the derivation of accompanying mutant alleles⁽⁶⁾. The actual number of loci at which both clones and mutant phenotypes are available is surprisingly small, but it is only with the combination of both sets of tools that it is possible to gain a full understanding of the mechanisms involved in the action of a particular gene on the process of development.

How does one go about deriving the missing half of the equation in the cases just described? There are actually two different problems with two different solutions. The emerging technology of homologous recombination in embryonic stem cells offers a potential solution to the problem of obtaining mutations at cloned loci of potential developmental interest. The actual replacement of wild-type genes with mutant alleles is carried out in tissue culture cells, which can then be incorporated back into chimeric embryos, and from there into the germ line^(3,7). Homozygous mutant mice can then be obtained through breeding, and the effect of the mutant allele on development can be

determined. Although this technology is still in its infancy, it clearly offers great promise.

The opposite pathway is the journey from a mutant phenotype to a DNA clone without any knowledge of the gene product or its function. This pathway is sometimes referred to as 'reverse genetics'⁽⁸⁾; however, most practitioners consider this approach to be directed genetics rather than any kind of reverse genetics. The basic premise is that through high resolution, classical genetic studies, it should be possible to map a particular mutant locus to a small region of the genome, which can then be reached molecularly by chromosomal walking from closely linked DNA markers. The identification of 'candidate genes' for the phenotypically-defined locus would then be pursued by systematic hybridization of cloned DNA fragments to RNA from appropriate tissues, by the demonstration of sequence conservation in distantly related mammals, or through the presence of clusters of particular types of restriction enzyme sites known empirically to occur frequently in what are called 'CpG islands' at the 5' ends of many mammalian genes⁽⁹⁾.

Although directed genetic cloning has had some notable success stories in mammals, this general approach has proven much more difficult to carry out in practice than in theory. One problem is that recombination events do not occur randomly along the mammalian genome, but rather are clustered at hotspots that can be located at distances of 500 kb or more from each other⁽¹⁰⁾. Consequently, it is often not possible to obtain a higher resolution mapping of a mutant locus by simply increasing the number of offspring scored, beyond a certain limit, in a recombination experiment. A second problem is that even with all of the tools that are currently at hand, one can never be certain that all of the genes in a large cloned region have actually been identified. The analogy to looking for a needle in a haystack is often not inappropriate.

workers describing the cloning of the mouse *T* locus^(11,12). The original *T* locus mutation was one of the first lethal mutations in mammals to be described. It was an X-ray induced mutation (although it wasn't recognized as such originally*) with a dominant shortening effect on tail length reported in 1927 by the Russian scientist Dobrovolskaia-Zavadskaia working in Paris^(13,14).

Through a fortuitous quirk of fate, the *T* mutation allowed the discovery in wild mice of an unusual genetic variant that expresses properties of a selfish chromosome, and is now known as a *t* haplotype^(15,16). Although *t* haplotypes by themselves have no visible effect on phenotype, in a double heterozygous genotype with a *T* mutation (*T/t*), they produce tailless mice, which are clearly distinguishable from the short-tailed mice that carry a *T* mutation alone (Fig. 1). *t* Haplotypes actually extend over a 15 cM length of chromosome 17 that includes the *T* locus, as well as the mouse major histocompatibility complex (*MHC* or *H-2*)⁽¹⁶⁾. They are maintained as intact genetic entities from one breeding generation to the next by a series of inversions relative to the wild-type form of the chromosome^(17,18,19,20). Although many *t* haplotypes carry recessive lethal mutations, and those that do not cause homozygous male sterility, these variant forms of the seventeenth chromosome are present in most wild populations of *Mus domesticus* at frequencies of 5–30%⁽²¹⁾. The reason for this is that in heterozygous +/*t* males, *t*-carrying haploid precursors to sperm cells are able to inactivate nearly all of their wild-type meiotic partners^(22,23). As a consequence, in the most extreme cases, over 99% of the offspring from such males may receive the *t*-form of chromosome 17, in a clear departure from Mendel's laws. This phenomenon is known as transmission ratio distortion.

Early on, the *T* mutation and *t* haplotypes were

The *T* locus and *t* Haplotypes

For these reasons, it was extremely gratifying to see the recent reports from Herrmann, Lehrach, and their co-

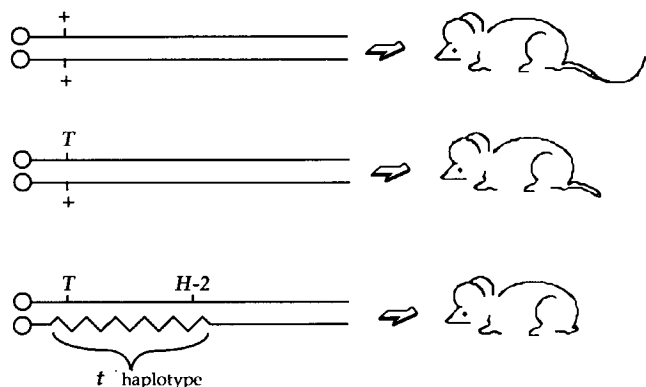


Fig. 1. *T/t* region genotypes and their corresponding phenotypes.

* Although the original *T* locus mutation was recovered in a short-tailed mouse having at least one X-ray-treated parent, Dobrovolskaia-Zavadskaia concluded that the X-rays themselves could not have been responsible for the mutation⁽¹³⁾. This conclusion has been accepted as a fact in all of the *T* locus literature published since that original article. But, how can Dobrovolskaia-Zavadskaia possibly have reached this conclusion? She certainly didn't have a clone of the *T* gene in hand. In fact, she couldn't have even known that the genetic material was composed of DNA. A re-reading of the original 1927 article allows one to follow the logic that she used. To paraphrase from the French: 'Is it possible that this mutation was caused by the action of the X-rays upon one of the parents? We think not for the following reason. Of the very large number of irradiated mice that have been used in breeding studies, only two have produced offspring with short tails. Therefore, the X-rays alone can not be responsible for this developmental defect'⁽¹³⁾. Obviously, Dobrovolskaia-Zavadskaia did not realize that the production of mutations at particular loci is a stochastic process. Instead, she seemed to believe that X-rays should function like a teratogen, giving rise to the same developmental defects over and over again. Since the short-tailed mice were so very infrequent, she reasoned, they were just chance events unrelated to the X-irradiation protocol. Our current understanding of mutagenic processes would say otherwise, and the demonstration by Herrmann and colleagues that the original *T* mutation is a deletion supports the likelihood of its induction by X-rays⁽¹¹⁾. The Dobrovolskaia-Zavadskaia paper was published on June 18th, 1927. Barely a month later, on July 22nd, Muller published his classic paper demonstrating the X-ray induction of mutations⁽³³⁾. For 63 years, no one returned to the original *T* locus paper to correct the mistake made by Dobrovolskaia-Zavadskaia.

considered to be different alleles at a single complex locus, and together they played a crucial role in the formation of a new field combining genetics and developmental biology, that of developmental genetics, which was pioneered by L.C. Dunn and his students Salome Walesch and Dorothea Bennett as well as others. Only in the last decade has it been appreciated that the *T* locus and *t* haplotypes are actually separate entities that are linked together only through their still-unexplained interaction to form a tailless mouse⁽²⁴⁾. Otherwise, the *T* locus appears to be a simple genetic locus, mutable in the laboratory, whereas *t* haplotypes are highly complex chromosomal entities that have evolved over millions of years in the real world. Nevertheless, each continues to be of interest in its own right to different types of biologists.

Although many other developmental mutations in the mouse have been described over the last 60 years, the *T* locus has always been a favorite among developmental geneticists, and as a consequence, its biological manifestations are well-described^(5,25,26,27). The *T* locus appears to encode a product that is essential for mesoderm formation and the subsequent morphogenesis of the notochord; homozygous mutant fetuses die about halfway through gestation at day 10. Interestingly, null alleles act as dominant mutations causing the shortening of tail length in *T*^{null}/*+* heterozygotes; this implies that the amount of *T* gene product expressed in the embryo must be critically regulated during normal development. Intense interest in *T* has led to the recovery and analysis of numerous mutations at this locus, and it is these that enabled Herrmann, Lehrach and their coworkers to demonstrate, beyond a doubt, that their newly-cloned, embryonically-expressed gene is indeed equivalent to the *T* locus.

Finding the Needle in the Haystack

The path to the cloning of the *T* locus began nine years ago, when Lehrach and his colleagues microdissected out the proximal portion of mouse chromosome 17 from metaphase spreads for the purpose of obtaining a series of anonymous DNA clones that could be used to identify genetic markers along this region of the genome^(28,29). When these markers were used by Herrmann in a high resolution genetic backcross with *T*-heterozygous animals, very tight linkage was observed between a locus defined by one clone (*D17Leh119II*) and the *T* locus⁽³⁰⁾. This marker was used as the starting point for walking and jumping along the chromosome toward the *T* locus⁽¹¹⁾. At this stage, the availability of five independently-derived deletions as well as a duplication over the *T* locus allowed its localization to a 110 kb region of DNA which began at a distance of 350 kb from the original starting point of the walk/jump. The 110 kb genomic region was screened for sequences that might be expressed in 8.5 day old mouse embryos and a single transcription unit was identified.

If the story ended at this point, Herrmann and his

colleagues would have had a good candidate for the *T* locus and not more, but the availability of one additional, recently-recovered, spontaneous *T* locus mutation (called *T*^{Wis}) allowed them to prove that the newly-discovered transcription unit and the *T* locus were one and the same. Molecular analysis of this last *T* mutation demonstrated the presence of a retroviral-like element that specifically disrupts the coding region of the cloned gene, preventing the expression of a normal product. The data demonstrate a causal link between the insertion of this retrotransposon and the production of the *T* mutation. For others attempting to clone genes whose products have not been identified, the lesson to be learned from the *T* locus is that multiple, independent mutations at the locus of interest can provide the missing tool needed for success.

As predicted, the *T* locus product is expressed in a highly specific fashion during embryogenesis. *In situ* hybridization studies localized its mRNA to both the early stage mesoderm as well as its epithelial progenitor; later during normal embryogenesis, mRNA becomes restricted to the notochord⁽¹²⁾. This expression pattern correlates exactly with the tissues affected in the homozygous mutant embryo. Interestingly, the gene appears to be highly conserved among vertebrate species. All of the data suggest that the *T* locus could play a crucial determining role in one aspect of mammalian development. Unfortunately (or fortunately for those anxious to work on this system), the *T* gene product shows no strong homology to any previously characterized sequence, and there is no clue, as yet, to the biochemical role that it might play in development. Hence, an understanding of its function, and of its interaction with *t* haplotypes, must await further analysis.

The Future

The cloning of the mutant locus that opened up the field of developmental genetics is a sign that one more barrier to the analysis of mammalian embryos has come tumbling down. Much important information is sure to follow from studies of the *T* locus as well as the many other mouse developmental loci that will soon surrender to the combined assaults of molecular biology and genetics. Indeed, the coming availability of YAC libraries of the mouse genome, with clones that have insert sizes of 200 kb or more⁽³¹⁾, will greatly increase the speed of chromosomal walks. In the more distant future, the large-scale genomic sequencing promised by the international human genome initiative should allow one to ferret out all hidden coding regions.

However, the primary question still remains unanswered. Will any of these studies lead us to the *Holy Grail* of Developmental Biology: a small set of *guiding principles* by which a complete human being develops from a single fertilized egg? Only time will tell if such 'non-trivial universals' actually exist or if, as Gunther Stent suggested a few years back, 'we are faced with a

near infinitude of particulars, which have to be sorted out case by case⁽³²⁾. Regretfully, I place my own bet with Stent.

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