Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

In Praise of Complexity

THE *T* complex in the mouse has been a favorite target for molecular investigations in recent years, with the result that its area on chromosome *17* has become "one of the most completely analyzed regions of any mammalian chromosome" (LEHRACH 1988). It was 50 years ago last month that GENETICS published a paper by L. C. DUNN and myself that reported detailed genetic and developmental data on mutations at the *T* locus. This locus had come into the genetical spotlight in 1927 when the dominant mutation *T* was discovered by a cancer investigator, Madame DOBROVALSKAIA-ZAVADSKAIA, in the Pasteur Laboratory of the Radium Institute in Paris. The shortened tail in heterozygous mice (*T*/+) suggested the name of the mutation, *brachyury*. DOBROVALSKAIA-ZAVADSKAIA also reported the embryonic lethality of homozygotes (*T*/*T*). Furthermore, she obtained tailless progeny in outcrosses of short-tailed heterozygotes (*T*/+) to normal-tailed wild mice caught in the laboratory. Remarkably, these wild mice were heterozygous for a recessive *t* mutation that interacts with *T* to produce the tailless phenotype (*T*/*t*). These tailless heterozygotes bred true when crossed with each other because of the embryonic lethality of both homozygous types, thus constituting the first balanced-lethal system in mammals.

It is strongly to his credit that DUNN's power of intuition and scientific judgment led him to choose the *T* locus early in its genesis as the main focus of his research activities. Starting in the mid-1930s, DUNN and his colleagues became increasingly impressed by the numerous exceptional and unorthodox aspects of genetic behavior of *T*-locus mutations and their unwillingness to conform to the expectations of conventional genetics. Rules of Mendelian segregation, of allelism, and of allelic interactions were flaunted, and recombination appeared to be suppressed. DUNN fully realized the potential of this unusual genetic system for the analysis of a number of fundamental problems of gene transmission and expression. He did not share the naive assumption, held at that time by some, of a simple one-to-one relationship between a gene and its phenotype far removed from primary gene action, and he was aware of the great complexities of gene actions and interactions. Speculating about the state of developmental genetics at that time, DUNN (1939) gave credit to GOLDSCHMIDT (1938) for his attempts to deal with developmental effects of genes beyond the level of their transmission or structure. But DUNN felt that, in 1939, developmental genetics had not yet coalesced as a field. This is particularly interesting in view of the close relationship at the beginning of the century between embryology and the emerging science of genetics, as exemplified by the two eminent biologists E. B. WILSON and TH. BOVERI.

The *T* complex on chromosome *17* includes a range of mutations later discovered to cover about 16 cM (FRISCHAUF 1985). The relevant genes were shown to affect a variety of systems, thus creating a diversity of problems including those of genetic transmission, recombination, gene action, pleiotropy, evolution, genetic control of development and spermatogenesis. Such complexity of effects presented a unique situation as well as opportunity, and raised questions of gene structure, organization and expression, many of which have remained unanswered to this day.

The dominant mutation *T* causes complete absence of the notochord in homozygous embryos and severe abnormalities of those developmental systems that depend on interaction with the notochord during embryogeny (CHESLEY 1935). It was the causal analysis of these mutational effects that implicated the notochord in normal mechanisms of early mammalian development, analogous to its role in lower vertebrate embryos earlier elucidated by experimental microsurgical procedures.

Our 1939 paper describing the genetic aspects and various effects of mutations at the *T* locus dealt with the second instance of what was then called a recessive mutation, t^1, causing preimplantation lethality when homozygous. t^1 had been shown earlier to interact with *T* to produce the tailless phenotype (T/t^1) and to breed true in a balanced-lethal system. The 1939 paper also described the phenomenon of segregation

distortion that characterizes males carrying t^1. These regularly transmitted a highly significant excess of t^1 sperm over sperm carrying the wild-type allele. Earlier, DUNN had reported the existence of the recessive mutation t^0 that interacted with T to produce tailless mice (T/t^0). These bred true as a balanced-lethal system because t^0 also was lethal when homozygous. An embryological study of t^0 which I undertook at that time showed it to affect the formation and development of mesoderm in embryos 5 days after fertilization (GLUECKSOHN-SCHOENHEIMER 1940). Since T, t^0 and t^1 failed to recombine with each other, they were considered to be alleles at a single locus. However, even though t^0 and t^1 were lethal when homozygous, the compound t^0/t^1 proved to be viable and the majority of such animals were morphologically normal. Such complementation between supposed alleles raised early questions in DUNN's mind about their allelism and caused him to refer to T as a "complex" locus (DUNN 1954). Eventually, the recessive t "alleles" were shown not to be mutations at a single locus but rather to cover a considerable portion (12–16 cM) of the chromosome and to include closely linked normal and mutant gene sequences held together by inversions with consequent suppression of recombination (FRISCHAUF 1985); they were called t haplotypes. Their widespread distribution in natural populations was discovered and described by DUNN (1964) and is particularly intriguing in view of the earlier mentioned derivation of t^0 and t^1 from wild mice. The evolutionary dynamics of the different haplotypes causing segregation distortion in males and lethality of homozygous embryos has been the subject of much discussion (see, for example, BRUCK 1957 and LEWONTIN and DUNN 1960).

The 1939 paper represented a milestone in the analysis of the T locus, particularly in its relevance to developmental genetics. The identification of three mutations showing no recombination with each other, each affecting the same embryonic elements (notochord, mesoderm and neural tube) and causing early embryonic lethality when homozygous, seemed intriguing in terms of interpretations on both genetic and developmental levels. Because these mutations shared profound effects on early embryogenesis, they seemed for the first time to implicate genes (and, in this case, closely linked genes) in basic mechanisms of vertebrate differentiation. Therefore, the discovery of this mutant system in the mouse not only demonstrated the existence of developmental mechanisms in mammals analogous to those operating in lower animals (see below) but in addition raised the exciting prospect of providing an approach to the identification of specific genes controlling such mechanisms. SPEMANN in his work on embryonic induction in amphibians had totally ignored any role for genes in early development and instead considered a "vital force" to be instrumental in the action of the "organizer" and the induction of the neural tube by the notochord (*cf.* HOLTFRETER, as quoted by MOSCONA 1986). As a graduate student in the institute of SPEMANN, I had become acutely conscious of his failure to take into account genetic factors in the interpretations of his experimental results, and this only served to increase my own appreciation of the T locus and its effects as a potential tool in the causal analysis of vertebrate development and its genetic basis. This appeared particularly important at a time when the mammalian embryo had not yet become accessible to the experimental manipulations used in studies of amphibian embryos, and different means of analysis of developmental mechanisms had to be discovered.

The significance of the 1939 paper rests on its demonstration of a genetic system whose complexity has resisted final analysis to this day. On every level of phenotypic expression, mutations in and near the T complex are characterized by unexpected and previously undescribed effects. Developmentally, as mentioned above, inductive interactions in early embryonic stages, specifically between notochord-mesoderm and ectoderm, are affected by various mutations within and near the T complex. As a consequence, more or less severe abnormalities of the axial system (including those of the spine, spinal cord and headfolds) make their appearance and are frequently responsible for the embryonic lethality of homozygotes (GLUECKSOHN-WAELSCH and ERICKSON 1970).

The existence of a genetic basis for embryonic induction and cell-cell interactions, as demonstrated by the developmental analysis of T-complex effects, offers intriguing prospects in various directions, including the chance of finding a molecular basis for the mechanism of embryonic induction. As expressed by J. B. GURDON (1987), "The molecular basis of inducers and the inductive response remains almost totally obscure." Conceivably, a connection might be established between a particular gene sequence in the T-complex region and the effects of its product on neural tube induction, thus providing a clue to the molecular basis of induction.

Obviously, any number of molecules may be candidates for the role of transmitters of inductive signals. Recent studies of embryonic mesoderm induction in amphibians suggest tempting speculations. These involve molecules such as the transforming growth factor TGF-β_2 in the induction process (ROSA *et al.* 1988), perhaps operating by the modulation of effects of FGF (fibroblast growth factor) in the embryo (KIMELMAN *et al.* 1988). The latter authors speculate further about the possible role of FGF in mechanisms of signal transmission during gastrulation and neurulation. Growth factors appear to be promising candidates for

a prominent role in embryonic induction, and it would be fascinating to explore the possibility that the *T* complex might include DNA sequences that encode growth factors. If this turned out to be the case, it would not only serve to advance our understanding of the molecular basis of embryonic induction, but would also confirm the significant role of the *T* complex in the genetic regulation of mammalian development proposed almost 20 years ago in a *T*-locus review (GLUECKSOHN-WAELSCH and ERICKSON 1970). As stated more recently, present data are "compatible with the possibility that the relevant DNA sequences in the *T*-complex encode regulatory factors concerned with the expression of specific structural genes mapping elsewhere in the genome" (GLUECKSOHN-WAELSCH 1987). The principle of exploiting well defined mutations in a causal analysis of development, as applied for the first time with the *T* complex, was followed by numerous studies of other mutations in the mouse affecting different developmental systems GLUECKSOHN-WAELSCH 1983).

I do not intend to present here an integrated and analytical review of the present status of the *T* complex and its chromosomal neighbors in terms of molecular organization or gene expression; previous reviews include GLUECKSOHN-WAELSCH and ERICKSON (1970), BENNETT (1975), SILVER (1985) and FRISCHAUF (1985). Since the 1939 paper, progress has been particularly impressive on the molecular level of analysis, including the identification and cloning of many segments of the *T* complex and the *t* haplotypes (HERRMANN, BARLOW and LEHRACH 1987).

One of the exceptional phenomena expressed by many recessive *t* mutations (haplotypes) that has been explained is the apparent suppression of recombination between certain *t*-bearing and wild-type chromosomes. It was the failure of recombination that had indicated allelism between *T* and the recessive *t* alleles (DUNN and GLUECKSOHN-SCHOENHEIMER 1939). Later, these *t* haplotypes were shown to actively suppress recombination over a considerable stretch of chromosome *17*. Eventually, chromosome rearrangements such as inversions, suggested by DUNN and GLUECKSOHN-SCHOENHEIMER in 1943, were identified within certain *t* haplotypes, and the absence of recombination could be ascribed to a concrete form of "chromatin mismatching" (SILVER and ARTZT 1981).

The phenomenon of transmission ratio distortion in *t*-carrying males was shown to involve the interaction of "distorter" and "responding" genes (LYON 1984). Male sterility, caused by the interaction of certain *t* haplotypes in viable double heterozygotes, seems to be caused by a minimum of three sterility factors, probably identical to "distorter" genes (LYON 1986). In spite of much analytical progress, details of the mechanisms responsible for the various reproductive abnormalities as well as their biochemical or molecular basis have remained obscure.

The details of phenotypic effects of *T*-complex mutations in heterozygotes and homozygotes have been described exhaustively on various levels and impressive progress has been achieved in the molecular identification of gene sequences included in the *T* complex (BÚCAN *et al.* 1987). Nevertheless, a huge black box filled with unknowns remains between the molecular and phenotypic levels of analysis. Recent approaches inducing point mutations in the *T*-complex region may help to reveal the role of single-base changes in the causation of individual lethal embryonic effects that resemble those described earlier in *t*-haplotype and *T*-complex homozygotes (SHEDLOVSKY, KING and DOVE 1988). Attempting to dissect the *t*-haplotype region by point mutagenesis may reduce the complexity of this area by identifying individual effects. Prior to those studies, there were no indications of direct correlations between singe-base changes in the DNA of the *T* complex and developmental or other effects.

That analytical studies of developmental genetics depend strongly on the choice of proper model systems is demonstrated particularly well by the analysis of homeotic mutations in Drosophila that are able to change the prospective fate of particular cell types. These mutations were used by HADORN (1966) in studies of the genetic basis of cell determination, and particularly by LEWIS (1981) in his brilliant analysis of the *bithorax* gene complex. It was LEWIS who correlated the changes in morphogenesis brought about by these mutations with specific changes in the responsible genes and their organization. LEWIS's classical approach of a developmental geneticist who proceeds from the level of the mutant phenotype back toward the genome has been complemented beautifully by his collaboration with molecular biologists such as D. HOGNESS and W. BENDER. The subsequent identification of specific DNA sequences—homeoboxes—shared by different homeotic genes in Drosophila, and encoding regulatory proteins instrumental in the control of specific developmental genes, has moved Drosophila into the center of interest in developmental genetics. Attempts to extend this flurry of excitement about homeoboxes to the mammalian embryo have had limited success thus far, even though genes containing homeoboxes has been identified in the mouse and have been shown to express spatially restricted patterns during embryogenesis (HOLLAND and HOGAN 1988). As yet, however, it is not possible to assign a decisive role to these genes in normal or mutant developmental mechanisms.

In the intellectual history of genetics, studies of the *T* complex have played a significant role throughout the past 50 years or more. It is one of several prime systems responsible for the emergence of concepts

that do not focus on the gene as a single unit acting independently, but that consider the gene a member of a "field of higher order" in which several neighboring functional units may become integrated into one field of action, that is, a domain (GOLDSCHMIDT 1955; PONTECORVO 1958). This would apply in particular to the action of developmental genes. PONTECORVO considered the *T* locus in the mouse together with homeotic mutants in Drosophila as the "best known examples of such higher fields." He also speculated that MCCLINTOCK's controlling elements in maize might be further examples of "higher fields" and, in support of this, quotes MCCLINTOCK herself: "Controlling elements appear to reflect the presence in the nucleus of highly integrated systems operating to control gene action." Because it is difficult to paraphrase PONTECORVO's writings about the *T* locus, I would like to quote his own words: ". . . this is probably only a foretaste of what we are likely to find when we pass from the analysis of 'simple' genes like those providing the information for such a minor matter as the mere synthesis of an enzyme, to the genetic organization necessary to carry the information for morphogenetic processes" (PONTECORVO 1958).

Finally, I would like to refer once more to BARBARA MCCLINTOCK, who is quoted as having emphasized in a discussion with students the "hidden complexity" that continues to lurk in the most straightforward-seeming biological systems. It is her view that, following upon the "molecular" revolution in biology, analytical approaches to problems will require patience and must take into account the complexity of organisms (KELLER 1983). This certainly applies to the *T* complex in the mouse and the problem of eventually clarifying the complexities that characterize the correlation between the molecular identification of gene sequences in this interesting chromosomal region and their far-removed phenotypic expression.

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