Perspectives

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The Ascendency of Developmental Genetics, or How the T Complex Educated a Generation of Developmental Biologists

Virginia E. Papaioannou

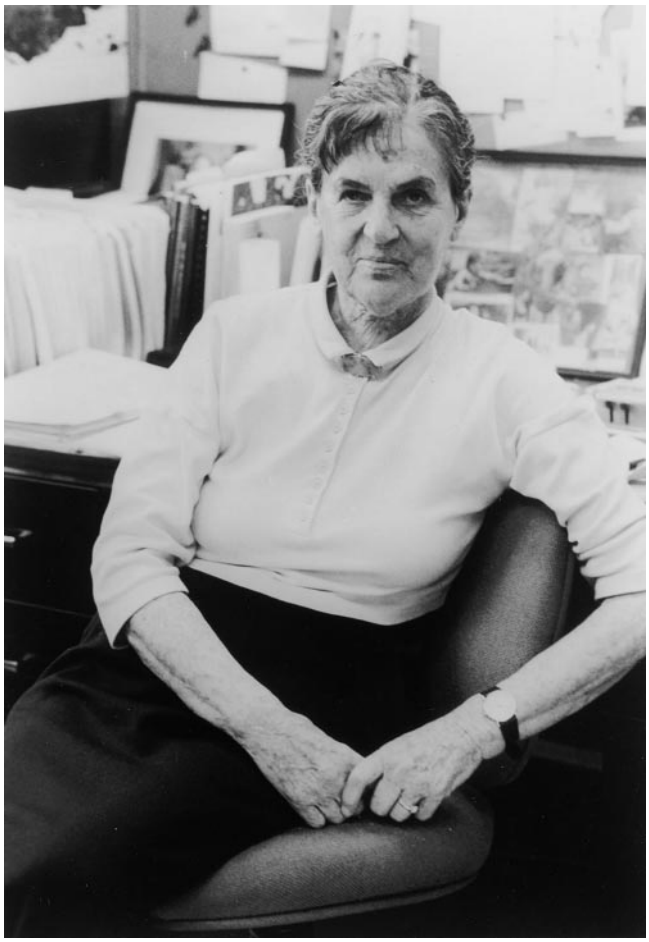
Department of Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, New York 10032

THIS past year marked the 60th anniversary of the publication in Genetics of a remarkable scientific paper from a remarkable woman, Salome Gluecksohn-Waelsch (then Gluecksohn-Schoenheimer), first describing the embryonic development of the tailless phenotypes resulting from the interaction of *T* (*Brachyury*) with *t* "alleles" (Gluecksohn-Schoenheimer 1938). In the historical context of the discovery and investigation of the T complex, that article does not represent the earliest beginnings; *T* and its interaction with *t* alleles was discovered more than 10 years earlier (Dobrovolskaia-Zavadskaia 1927), and the transmission ratio distortion characteristic of *t* alleles was already known (Chesley and Dunn 1936). Nor does it represent the first embryonic investigation of these genes; the embryonic lethality of *T*/*T* embryos had already been described (Chesley 1932). For me, its significance is more symbolic. The article reported early (and beautiful) work from one of several prominent women developmental biologists who were highly influential role models for aspiring women scientists, especially developmental biologists, throughout the following decades, and it deals with subjects (embryology and the *T* locus) dear to my heart. Perhaps most importantly, it investigates a phenomenon, the relationship between *T* and the tail interaction factor (also known as tct or *t c*omplex *t*ail interaction factor), of the *t*-bearing chromosomes. This remains an outstanding aspect of the T complex about which we have almost no clue and that we may not be much closer to understanding now than we were 60 years ago, a potent reminder that the T complex still has hidden secrets.

Salome has always been one to view scientific progress in a historical context and always has pertinent examples at the ready. Anyone who ever gave a research talk with Salome sitting in the front row expected, and possibly dreaded, the inevitable moment when she got to her feet with the first question and said something like "That is very interesting and reminds me of an experiment done by 'X' many years ago. . . ." Humility is not a usual characteristic of research scientists, but Salome had a way of fitting "new" discoveries into a historical framework that forced one to realize that the same biological issues have been around for a long time. The seduction of technology may have led us to believe that we were posing novel questions and discovering things that could lead to enlightenment, but that first question from the audience reminded us of our intellectual inheritance and the myriad contributions that simpler methods and inductive reasoning had made in leading us to our point of departure in a new study. And so, using Salome's 1938 article as a point of departure rather than a beginning, I offer some impressions on how the specter of the T complex affected a generation of mammalian geneticists, developmental biologists, and developmental geneticists.

The appropriately named T complex spawned an entire field of study that for years was plagued by complex genetics, bizarre interactions, contradictions, and misinterpretations. For those of you who might have been frightened out of the field early on, as I was for many years, and never had the time or inclination to get back to it, you will be happy to hear that the complexities have now been satisfactorily resolved by combinations of genetic, molecular, and evolutionary approaches, and the accepted view can be quite simply explained (Silver 1985, 1993). Apart from the fact that *t*-bearing chromosomes have some peculiar rearrangements that took a vast amount of mouse breeding and genetics to understand, the main problem that misled researchers for many years was the persistently pursued misinterpretation that *T* and the many small *t*'s discovered in wild mice were alleles of the same locus, despite ample evidence to the contrary. With that idea out of the way, the various aspects of T-complex phenotypes—effects on tail length, embryogenesis, fertility, transmission ratio, and recombination—can be explained as effects of many different linked genes, rather than pleiotropic effects of a single locus. The complex chromosome itself is what needs explanation, and this can be done in evolutionary terms.

To paraphrase the eloquent explanations of Lee Sil-



Salome Gluecksohn-Waelsch.

ver (who was never frightened by the problem; Silver 1985, 1993), the origin of the present-day *t*-bearing chromosomes, or haplotypes, can be explained by postulating the following steps in their evolution. First, several loci that cause transmission ratio distortion (TRD genes), favoring their own transmission to offspring and thus conferring a selective advantage to the chromosome, occurred *by chance* in a chromosomal region. With TRD gene linkage, selection would favor anything that kept the genes together on this "selfish chromosome." Second, chromosomal inversions occurred, which tended to keep the TRD genes together by suppressing recombination. The selective advantage of such inversions should have rapidly led to fixation of this chromosome, but the absence of fixation in modern mice is evidence for a counterbalancing negative selection, specifically, a detrimental effect of TRD genes on male fertility that renders *t*/*t* mice sterile. Third, selective pressure to counterbalance the male sterility effect of *t*/*t* mice—which would produce mice that were essentially evolutionary baggage, using resources without leaving offspring—favored the accumulation of unrelated, embryonic lethal genes that essentially got rid of this baggage before it was born (see Crow 1988; Lyon 1991). It would not matter how the sterile mice were eliminated—simply that elimination occurred at an early time; in fact there are many different lethal genes in different *t* haplotypes that have effects at various stages of embryogenesis.

Over more than a million years, additional TRD genes and additional inversions were recruited to the *t* haplotypes, effectively increasing the genetic expanse of the T-complex region. In terms of its contribution to the evolution of the T complex, only the tail interaction between different *t* haplotypes and *T*, a mutation on the non-*t*-bearing or wild-type chromosome, remains to be explained. However, the T complex is far from solved and we are now in the early stages of the difficult task of identifying specific genes responsible for the characteristic features of the T complex, assigning them to chromosomal positions and determining their molecular function.

With hindsight, it is easy to see why the T complex remained mysterious for so long. It was first described in the language of classical genetics at a time when the field of mammalian developmental biology and especially mammalian developmental genetics barely existed. It seemed to violate the most basic rule of inheritance, Mendel's first law, and consequently to wreak havoc with Hardy-Weinberg. The mechanisms by which the rules might be broken generated speculations that were hard to prove or disprove. At the time, the field of developmental biology was dominated by studies of lower organisms, and the role of genes in controlling developmental mechanisms was largely ignored. The discovery of the profound effects of *T* and the *t* alleles on developmental processes provided an early indication that genes might play a role in controlling developmental mechanisms in vertebrates, but ironically, misinterpretation and false assumptions led developmental biology down the wrong path for many years. It was at first an uneasy alliance between developmental biology and genetics.

Salome joined L. C. Dunn's lab in 1933 after training with Spemann in early amphibian development and began a career studying the complexities of gene actions and interactions and their role on developmental mechanisms in mammals. From the beginning, she and Dunn recognized the paradox that the complex phenotypic manifestations and the existence of complementation between the *T*/*t* alleles indicated separate loci, while other features, like the lack of recombination and the interaction affecting tail length, pointed to a single (complex) locus. Both possibilities were presented in a 1943 paper, but it was further stated that genotypically related effects could have a degree of independence in development and that a thorough study of development "might reveal common sources from which diverse effects arise" (Dunn and Gluecksohn-Schoenheimer 1943, p. 39). These further caveats clearly indicate, to my mind, that Dunn and Salome were making a conscious

effort to merge developmental and genetic thinking. As mouse genetics began to expand and the battery of mouse mutations affecting development increased, the T complex maintained a prominent place in the development of ideas about how genes control development.

An interesting, two-part article in 1964 by Dunn and Dorothea Bennett hints at the schizophrenia induced by the T complex. In part I, Dunn discusses the *t* alleles in terms of a juxtaposition of separate genes affecting related developmental processes that have persisted as a block of genetic material (Dunn 1964). In part II, Bennett, who became an influential and dynamic force in mammalian developmental genetics, also used the term "alleles" but considers the *t* alleles to be members of the same chromosomal locus, making an assumption, on the basis of this belief, that they are all related in genetic structure and therefore related in the processes they control (Bennett 1964). Meanwhile, however, the genetic evidence that the *t* alleles were not alleles at all but were different loci in an abnormal chromosomal region was accumulating (Lyon and Philips 1959; Lyon 1960). Mary Lyon, with her incredible insight, was making waves in genetic thinking with her explanation of the phenomenon of X-chromosome inactivation in female mammals that later became known as the Lyon hypothesis (Lyon 1961; Russell 1961), but her clear genetic evidence on the nature of the T complex was largely ignored at the time.

The 1960s and the following decade saw tremendous changes in the way vertebrate, especially mammalian, developmental biology was approached. The development of new experimental embryological techniques, such as chimeras (Tarkowski 1961; Mintz 1964; Gardner 1968) and new and improved culture techniques (Biggers *et al.* 1965; Brinster 1970), made the mammalian embryo more accessible than ever before. There was a strong impetus to apply the techniques and principles of molecular biology and to incorporate the insights of microbial genetics as a means by which to understand the developmental biology of more complex organisms with vastly more complex genomes. The accumulating wealth of information from experimental embryology along with the accumulating wealth of naturally occurring or induced developmental mutations in mammals, especially the mouse, opened many pathways to a more direct inquiry into the roles of genes in directing interrelated cascades of developmental events. Molecular genetic technology allowed the more direct examination of the link between gene expression and developmental mechanisms. Now, with the increased power of forward genetics and positional cloning, the classic technique of analyzing a mutation by working from its phenotype to the gene to uncover the primary effects on development could be supplemented by working in the opposite direction, from the gene to the phenotype (see Davis and Justice 1998). The establishment of transgenic technologies in the 1980s provided another major push toward this type of analysis (Papaioannou 1998). Genes can now be manipulated in a variety of ways, including overexpression, misexpression, and creation of specific mutations by targeted mutagenesis, so that the genetic analysis of developmental events can be approached at multiple levels in the complex organism with the ultimate goal of assigning a genetic basis to developmental mechanisms.

For many years after it was discovered, the T complex fostered the development of concepts of genes as interrelated functional units, moving the emphasis away from the gene as an independently acting unit and integrating ideas expounded by Hans Gruneberg on pedigrees of causes in development. On the basis of the assumption that the *t* alleles were structurally similar and specified membrane components, Bennett and colleagues proposed an elegant, unifying theory of the T locus as a master developmental locus, explaining the varying time of death caused by different *t* alleles as effects on cell-cell interactions at successive critical stages of development (Bennett 1964, 1975). This idea of the T locus unraveled, however, with the discovery of inversions and the consequent suppression of recombination. With the first fine structure mapping of the region (Artzt *et al.* 1982), attention shifted to the individual genes included within the 15 cM comprising the complex. To circumvent the problems associated with recombination suppression, ethylnitrosourea mutagenesis screens were used as a means of recovering new alleles of known loci in the region (Bode 1984) and of recovering the recessive lethal mutations (Shedlovsky *et al.* 1986). Gradually, the different early lethal phenotypes were all attributed to separate loci with no obvious structural or mechanistic connection apart from their linkage and their embryonic lethality. There are at least 16 different *t* lethal effects (Klein *et al.* 1984), but, as yet, no lethal allele has been cloned. The detailed phenotypic descriptions associated with many of these lethal mutations are a treasure chest of information awaiting the identification of genes.

On the other hand, a candidate gene for the tail interaction effect, called *T2*, has been identified and cloned in the laboratory of Karen Artzt (Rennebeck *et al.* 1998), a student of Dunn and Bennett and a longtime, influential expert in the T-complex field. This gene, identified by a transgene insertion, maps very close to *T*, has similar effects on axial mesoderm formation, but does not complement *T*. As there is no evidence for a direct effect of the transgene insertion on *T* or its regulatory regions, this second gene could be synonymous with the tail interaction factor, tct. Further analysis of this gene could soon get us closer to understanding this mysterious aspect of the T-complex and whether these separate genes act through similar mechanisms.

The key to *T* itself came with the cloning of the gene and the discovery that it encodes a transcription factor

(Herrmann *et al.* 1990; Herrmann 1995) that is part of a multigene family, the T-box family (Bollag *et al.* 1994). From this discovery, a new area of developmental genetics, that has come to dominate my own research in the past few years, opened up. Previously, my meager contribution to the study of the T complex consisted of a single forgotten paper (Papaioannou *et al.* 1979) resulting from a few weeks spent in Dorothea Bennett's lab teaching what I had learned from Wes Whitten about making aggregation chimeras and trying rather unsuccessfully to understand *t* haplotypes. Only years later did I venture back to the field, albeit in an area peripheral to the T complex itself, spurred by the possibility that the important developmental effects of *T* might herald a common feature of a conserved gene family with multiple family members in the mouse.

Uncovered on the basis of homology to the DNA-binding domain of the *T*-locus gene product, the T-box gene family is a novel family of transcription factors. T-box genes have been identified in the genomes of a wide range of metazoans from *Caenorhabditis elegans* to human. At last tally, 10 different genes, in addition to *T*, have been identified in the mouse and are found scattered through the genome. From all indications, the T-box gene family is implicated as playing critical roles in development: first, by the well-studied mutant phenotype of the proband of the family, *T*, then by the isolation of novel T-box genes from embryonic cDNA libraries and the demonstration of extensive embryonic expression, and finally, in addition to the *T* mutations known in many species, by the developmental phenotypes that result from other T-box gene mutations. Naturally occurring mutations in two T-box genes in humans are responsible for dysmorphic syndromes affecting the development of multiple organs (Bamshad *et al.* 1997; Basson *et al.* 1997; Li *et al.* 1997). A zebrafish mutant found during a mutagenesis screen has a mutation in a T-box gene that has drastic effects on the development of somites (Griffin *et al.* 1998; Ruvinsky *et al.* 1998). A targeted mutation of *Tbx6* in mouse results in a dramatic developmental switch from somite to neural development (Chapman and Papaioannou 1998). We are now able to apply all the power of reverse genetics to identifying and investigating the developmental roles of all the genes of this family. Working from gene to phenotype, using targeted mutagenesis and other powerful molecular techniques combined with the classic observational skills of embryology, developmental geneticists will be able to define the critical developmental processes affected by this gene family. One of the common features of the family, DNA binding and presumably transcriptional regulatory activity, provides the basis for understanding common mechanisms in what are likely to be myriad effects on development. Although the T-box genes may not be the master controllers of development once envisioned for the T-complex genes, they promise to be key players in understanding the complexity of the developing embryo.

In a Genetics Perspectives article written nearly a decade ago, Salome Gluecksohn-Waelsch discussed Guido Pontecorvo's and Barbara McClintock's ideas on biological complexity and their application to the T complex. She pointed out that "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" will require a great deal of patience and an analytical approach that takes into account the complexity of organisms (Gluecksohn-Waelsch 1989, p. 724). This is no less true today, when one considers that the cloning of the *T* gene led to the discovery of an entire gene family with many developmental genes, adding a new level of complexity to the attempt to elucidate the T complex.

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