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HISTORY OF SCIENCE

N. Dobrovolskaya-Zavadskaya and the Discovery of the T gene[1]

V. P. Korzh

The Institute of Molecular Agrobiology, 1 Research Link, National University of Singapore, 117604 Singapore
e-mail: vlad@ima.org.sg

Nadezhda Alexandrovna Dobrovolskaya was born in Kiev on September 13, 1878. She studied medicine in Saint Petersburg where in the early 1900s, she became one of the best known women surgeons. After the First World War started in 1914, she joined the Russian army and worked in military hospitals until 1917, when the revolution dramatically changed the fate of Russia and all Russians. At the end of civil war she was working in the hospital in the army of General Wrangel. After the defeat of Wrangel in 1920, Dobrovolskaya left Russia and went into exile. Like many other Russian emigrés, she came to Paris after fleeing Crimea via Turkey and Egypt. (Unfortunately, this period of the life of Nadezhda Dobrovolskaya is completely unknown to me.)

Here in Paris on October 1, 1921, at the age of 43, she joined Prof. Claudius Regaud in the Pasteur Laboratory. In France she is known as Nadine Dobrovolskaya-Zavadskaya. In the later literature, she sometimes is referred to simply as Zavadskaya. The Pasteur Laboratory was established in 1909 by the Institute of Radium (currently the Curie Institute) and the Pasteur Institute to study the biological effects of radioactivity (Fig. 2). The Pasteur Laboratory was one of the first research institutions committed to radiobiological studies. Eventually, Dobrovolskaya-Zavadskaya became well known for her major contribution to the field of radiobiology and radiology (Fig. 2). At the age of 76 she was invited to attend several conferences in Italy. On her way from Zurich to Milan, N. Dobrovolskaya-Zavadskaya became sick and died in Milan on October 31, 1954.

Relevant to the subject of my review is the fact that Nadine Dobrovolskaya-Zavadskaya made an important early contribution to the developmental genetics of the T gene, the founder gene of the T-box gene family. In 1923 she initiated one of the first genetic screenings of developmental mutants in mice by irradiation of testicles of males with X-rays.

In retrospect, perhaps the most important achievement of Dobrovolskaya-Zavadskaya (that is still remembered in developmental genetics) was the isolation of a robust strain of the mutant of the T gene otherwise known as *short tail* or *Brachyury* (*Bra*). She passed this strain to other laboratories. One of those interested in working with Bra was Boris Ephrussi, another Russian emigré and one of the pioneers of developmental genetics, who was working mainly in France. Boris Ephrussi established a culture of cells from T mice demonstrating that this lethal mutation affects only some cell lineages (Ephrussi, 1933, 1935). The second person who obtained the *Bra* strain from Dobrovolskaya-Zavadskaya was Prof. L.C. Dunn (1893–1974) in New York, USA.

There are several versions of the story of transfer of *Brachyury* mice to Columbia University. In 1928 L.C. Dunn moved to the Department of Zoology at Columbia University (Crampton, 1942). In 1930, Dobrovolskaya-Zavadskaya was invited to the USA to give a lecture for some Russian refugee organizations. On that occasion, she visited the lab of L.C. Dunn at Columbia and discussed with him the difficulties of understanding the *Brachyury* phenotype. L.C. Dunn asked Dobrovolskaya-Zavadskaya to provide him with Bra heterozygotes that, together with another line, *tailless*, arrived at Columbia in 1931 (Bennett, 1977). Despite the apparent frustration caused by a challenge to understand *Bra*, Dobrovolskaya-Zavadskaya worked on this subject for a few more years.

Fig. 1. Nadine Dobrovolskaya-Zavadskaya (1878–1954) in 1948.

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As a result of receiving *Bra* mice from Dobrovolskaya-Zavadskaya, L.C. Dunn together with his student Paul Chesley started an embryological study of them (Chesley, 1935). Actually, it was Chesley (and his supervisor L.C. Dunn) who in the conclusion of the second paper, when describing the analysis of *Bra* for the first time suggested that the T phenotype arises as a result of the notochord deficiency (Chesley, 1935). It was quite an insight, which was confirmed at the molecular level only very recently. In addition to the *Bra* mutant, Dobrovolskaya-Zavadskaya isolated and provided detailed characterization of *waltzing* and true *tailless* mutants and also described several other less characterized mutants.

Studies of Dobrovolskaya-Zavadskaya in mammalian genetics comprised 21 publications, including 16 short communications and 5 full-size papers (1927, 1929, 1933), in which the results of the genetic analysis of several mutations in mice were described. Her analysis revealed that *Bra* is caused by a Mendelian hereditary factor. In the heterozygous state, *Bra* behaves as a haplo-insufficient dominant character. In contrast to the opinions expressed in later literature, the analysis of her publications revealed that she correctly predicted the molecular nature of *Bra* mutation as a deletion (Dobrovolskaya-Zavadskaya, 1928; Herrmann *et al.*, 1990).

It was Dobrovolskaya-Zavadskaya who proposed the name *Brachyury*, from Greek. Later, she introduced the symbols [*T*— tail (English) or taille (French) for the mutant and *t* for the wild type gene] that are still in use today (Dobrovolskaya-Zavadskaya, 1933).

Starting in the late 1920s, she collaborated with several other former Russian scientists, including N. Kobozieff and, later on, Serge (Sergej) Veretennikoff, on *Brachyury*, as well as a number of other projects such as the analysis of effects of X-rays on ciliates (Dobrovolskaya-Zavadskaya and Kobozieff, 1930) and survival of mammals of different age after irradiation (Dobrovolskaya-Zavadskaya *et al.*, 1941). In respect of her *Bra* project, in 1930 in agreement with earlier reports, she confirmed that *Bra* homozygotes die *in utero* (Dobrovolskaya-Zavadskaya and Kobozieff, 1930). Further, Dobrovolskaya-Zavadskaya made a detailed morphological analysis of a skeleton of *Bra* heterozygotes demonstrating that a number of modifying factors are responsible for the observed variability in tail length (Dobrovolskaya-Zavadskaya *et al.*, 1934).

Also note that in 1928, she already correctly predicted the complicated nature of *t*-complex of genes, suggesting that *Bra* is only one of several genes in this complex, all of which may have their specific role in development. Apparently, the *t*-complex, characterized initially by Dobrovolskaya-Zavadskaya, turned to be "a golden mine" of genes that has puzzled geneticists since, and it was discovered due to the fact that she had already isolated mutants of two genes from this complex (*T* and *tailless*) in 1920.

Fig. 2. The Pasteur Laboratory in the Curie Institute (recent photo).

Her hypothesis on the complex nature of the *t*-complex was published slightly earlier than similar ideas of several geneticists working on *Drosophila* scute complex (Dubinin, 1929, 1932; Serebrovsky, 1929; Agol, 1930). In her later analysis, Dobrovolskaya-Zavadskaya drew parallels between the organization of both these complexes. Further, she extended her theoretical analysis of *t*-complex suggesting the existence of specific regulatory genes one set of which regulates the size and another, the structure of the tail. These regulatory genes could interact with other modifying genes (Dobrovolskaya-Zavadskaya *et al.*, 1934). This idea sounds very similar to a recent hypothesis based on the analysis of *Bra*-related "no tail" mutants in zebrafish suggesting that separate molecular mechanisms are involved in formation of the trunk and the tail (Griffin *et al.*, 1995).

The complex nature of the *t*-complex is still far from being understood. For example, another gene of the *t*-complex responsible for sex-ratio distortions has recently been cloned and analyzed (Herrmann *et al.*, 1999). As can be seen, the genetic analysis of *Bra* and the *t*-complex initiated by Dobrovolskaya-Zavadskaya in the late 1920s contributed to the development of a new scientific discipline - developmental genetics. In this respect, I need to mention that an important conclusion about

the organizing influence of the notochord with reference to the CNS and somites was made as a result of the embryological analysis of *Bra* homozygotes by Paul Chesley and, later on, Salome Gluecksohn-Waelsch (Schoenheimer) in Dunn's lab at Columbia University (Chesley, 1935; Gluecksohn-Schoenheimer, 1940).

Not all of Dobrovolskaya-Zavadskaya ideas are still valid. For example, she was mistaken in certain aspects of the nature of the interaction of X-rays and chromatin. Well, nobody is perfect…. Interestingly, until very recently, genes of the *t*-complex were actively studied in the Soviet Union (Malashenko, 1975; Baranov, 1982; Agulnik and Ruvinsky, 1989; Ruvinsky *et al*., 1991).

It seems that the most apparent reason for Dobrovolskaya-Zavadskaya to end her *Bra* project was to get away from the frustrating genetic puzzle, that would take another 60 years to start to understand, and back to the field of radiation oncology, where as a medical doctor, she was more inclined to work. It might be of interest to note that working in parallel on the genetic analysis of *Bra*, Dobrovolskaya-Zavadskaya almost singlehandedly developed several lines of laboratory mice early in the history of radiation oncology, some of which had low and others even lower incidence of cancer. She related these findings to the existence of a hereditary factor or factors in the genesis of cancer: her data were not in favor of a single dominant gene, but rather of several organ-specific recessive genes. These results led to an assumption that cancer is a multifactorial disease which develops through a specific conjunction of external and internal conditions (Löwy and Gaudilliere, 1998). (In my view, it is a good example of scientific versatility and an illustration of the wide scientific interests of Dobrovolskaya-Zavadskaya.) This explains to some extent why she shifted her attention from the analysis of *Bra*. This covers only one period in the biography of Dobrovolskaya-Zavadskaya, while other questions remain unanswered. Where was she educated? Who were her professors in Saint Petersburg? What was her background in genetics, if any?

Perhaps, somebody knows the answers and I will be grateful for any feedback. It seems to me that her results on *Brachyury* were in direct conflict with ideas (of other people and her own) that were dominating the field of genetics at the time. After all, we need to remember that developmental genetics started to gain on recognition only in the 1940s and 1950s (Sapp, 1987; Gilbert, 1991).

It seems to me quite appropriate to finish this historical note by citing one of the most active researchers of developmental role of *Brachyury* S. Gluecksohn-Waelsch: "If ever a history of ideas in developmental genetics were to be written…it would no doubt include as one of its most important chapters an account of the intellectual role that "inductive interaction" between the fields of genetics and embryology has played in the analysis of developmental mechanisms and their developmental control in higher organisms" (Gluecksohn-Waelsch, 1981). N. Dobrovolskaya-Zavadskaya by generating *T* mutants, characterizing them, and predicting the structure of *t*-complex and the nature of deficiency of chromatin in *Bra* made a fundamental contribution to the foundation of developmental genetics that completely reshaped the culture of embryological research and has dominated modern biological studies since. Her presence in the *Bra* field was very short and, perhaps for that reason, some of her ideas were forgotten or misinterpreted. This essay is the first attempt to restore an interest in the scientific heritage of N. Dobrovolskaya-Zavadskaya in the scientific community.

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