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From a home-made laboratory to the Nobel Prize

An interview with Rita Levi Montalcini

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Why does one of our fingers grow only a few centimeters and not many meters or only a few millimeters? Why does a normal cell respect the boundaries of other cell territories and stop proliferating at the right time instead of invading the other territories as tumour cells do? The answer to these and to similar questions lies in a complex balance of factors, which regulate cell proliferation and cell interactions. A fundamental role in this control is played by polypeptide growth factors. The first one to be discovered among them was nerve growth factor (NGF). The discovery was due in the early fifties to Rita Levi-Montalcini, who was awarded the Nobel Prize in Medicine for this in 1986. As Rita says in her book entitled *The Saga of the Nerve Growth Factor* «the first attempts to obtain some knowledge of the control mechanisms of the nervous system date back to the beginning of the century» (1900). «In 1934, Viktor Hamburger (a student of Hans Spemann) explored the effect of limb bud extirpation, in 3-day old chick embryos, on spinal cord motor neurons in charge of the limb». The consequence was that the corresponding motor nerve cells, in the absence of their territories of innervation, failed to grow and differentiate. The author therefore hypothesized that under normal conditions «the pioneer nerve fibers, after reaching their target limb tissues, send signals to their centers which at this stage consist mainly of still undifferentiated nerve cells. Upon receiving the signal, they would be induced to undergo differentiation». A few years later Rita Levi-Montalcini reinvestigated the question in Turin, focusing her attention not on motor neurons, but on the sensory ones of the dorsal root ganglia and concluded that their failure to establish synaptic contacts with their end organs was responsible for the massive death of these cells. The differences in the interpretation of the results between Hamburger and Levi-Montalcini is due, in Hamburger's words, also to Rita's background in neurology: «She mastered the silver impregnation technique of Cajal-De Castro, which enabled her to distinguish between undifferentiated cells and neurons with axons». She belonged indeed to the school of the Italian anatomist Camillo Golgi, who invented the technique of silver staining for the nervous system by the use of a silver-chromium solution and, inspired by Cajal's work, she had decided to follow Cajal's new technical approach.

Unfortunately in July 1938, while the work of Rita was in its beginnings, the racial laws were promulgated by the Italian fascist government, which deprived the Italian Jewish citizens of all civil rights, including access to public schools and universities, and also barred the publication of non-Aryan articles, so that Rita's first results, except for an article published in the Belgian Archives de Biologie, had to be sent to the Pontificia Academia Scientiarum, which did not discriminate racially. Rita in 1939 spent a year at the

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Viktor Hamburger with Rita Levi Montalcini in 1970 at the Washington University in St. Louis, Missoury, USA.

Institut Neurologique in Brussels, but the threatened Nazi invasion of Belgium prompted her to return to Italy in December of the same year. There she created a small laboratory in a room of her house, where she continued her work in collaboration with her mentor, Giuseppe Levi. In the Spring of 1946, when the war was over, she received an invitation from professor Viktor Hamburger, Chairman of the Department of Zoology of the Washington University in St. Louis, Missouri, to reinvestigate together with him the effects of limb bud ablation on its innervating nerve cells. Three years later, in a joint paper the Rita Levi-Montalcini hypothesis was fully confirmed. In 1948 a former student of Hamburger, Elmer Bueker, reported that grafting fragments of mouse sarcoma 180 into the body wall of chick embryos resulted in the invasion of the sarcoma by the sensory nerve fibers emerging from the adjacent dorsal root ganglia. Rita reinvestigated this phenomenon and, while confirming Bueker's results, observed other effects indicating that the neoplastic cells released a soluble, diffusible agent which enhanced the differentiative process of developing sensory and sympathetic nerve cells. Between 1956 and 1958 two other most powerful sources of NGF were discovered in snake venom and in adult male mouse submaxillary salivary glands. The main target tissues were identified as neural crest derivatives. In 1968 Rita Levi-Montalcini came back to Italy and established a center of research on NGF under the sponsorship of the Italian National Research Council. In 1990 the modulatory role of NGF in homeostatic processes in the neuroendocrine-immune triad was discovered and became the object of intense investigation.

It would be impossible to describe in a few lines all the scientific activity of Rita Levi-Montalcini, but I cannot close this short preface without mentioning her human qualities, which one can appreciate reading the several fascinating, non-scientific books she has written and still writes, without mentioning her dedication to improving education of the youth through the action of the Levi-Montalcini Foundation. For a most interesting detail of her scientific activity the reader is advised to consult the book *The Saga of the Nerve Growth Factor* which she wrote in 1977.

Was the time ripe when you discovered the NGF or was it an unexpected breakthrough?

The discovery of Nerve Growth Factor (NGF) could in no way have been foreseen, nor was it in tune with the concepts that prevailed at the time. It was in no way possible, in fact, to foresee that a substance released by neoplastic tissues would upset the development of specific sectors such as the sensory and visceral peripheral nervous system. That is the reason why my first report to the New York Academy of Science aroused more scepticism and perplexity than enthusiasm, because it went against the generally accepted dogmas concerning the modalities of the development of the different components of the nervous system, which at the time were believed to be rigidly predetermined at the genetic level.

Do you believe you would have been able to carry out the same work in Italy at that time or was it necessary to leave the country to carry out the work, apart from political considerations?

There are two reasons for which I am certain that I would not have been able to make the discovery had I remained in Italy. The first is that, since I was working in total isolation as a result of the racial laws which precluded all access to any form of culture (universities, libraries, public professional activities etc.), I would never have been able to find out about the new experimental approaches that were being developed in the United States. The second reason is the result of the atmosphere of terrorism and persecution that loomed over citizens who had been defined as not belonging to the pure, or Aryan, race. As you see, one cannot say that politics played no part in the matter!

Do you think the conditions in Italy have improved since then, and how much compared to other European countries in the same economic situation?

Italian research suffers as a result of both scarcity of funds and the fact that whatever funds do exist, they are not allotted on the basis of merit. In fact, one must admit that the so-called baronial system still prevails in Italy. In other words, the research teams which get rewarded are not necessarily the ones that are the most

Rita Levi Montalcini and the king of Sweden on the occasion of the award of the Nobel Prize to Rita Levi Montalcini in 1986

worthy but rather, those that somehow belong to the dominant political parties. In this sense, researchers in Italy are at a disadvantage compared to those in other countries of a comparable cultural and scientific level.

I fully agree with your bitter consideration, but let me add a word of hope for the Italian scientific future: in the last few years a new procedure for selecting at least part of the research to be funded by Italian government has started. The applications are sent to a pool of international expert referees for peer review, and their advice is followed. Now let me ask you another question: What are the conditions, if any, that in Italy prepared or favoured your discovery?

The fortune of having studied with a person of great ethical and scientific character such as the illustrious histologist, Giuseppe Levi. It is no coincidence that three of his students, S. Luria, R. Dulbecco and myself, were all conferred the Nobel Prize for Medicine, albeit for research work that differed entirely from that of our great teacher and from each other's. In my case in particular, the study of the development of the nervous system under Giuseppe Levi's guidance, and of the *in vitro* culture technique which he was the first in Italy to apply, turned out to be enormously important.

What are your best memories of your Italian times?

The memories are many. The oldest have to do with my activities at the time in which I was preparing for my doctoral degree on the basis of the study of *in vitro* cultures. Then there are those relative to when at the Institute of Neuropsychiatry - with the late lamented, excellent physiologist and friend Fabio Visentìni - I worked on the development of the nervous system in chick embryos using both electrophysiological and biochemical techniques. My third set of recollections concern the activities I carried out in my small private laboratory, during the period of persecution in the wake of the racial laws, under conditions similar to those in which Robinson Crusoe had operated. In that period I made some díscoveríes, today considered pioneering, on the phenomenon of programmed cell death, now commonly referred to as apoptosis.

What have been the most important consequences of your discovery for Development Biology?

The discovery of NGF has demonstrated that the protein factors whích are released by al the cells in an organism play an essential role in the development of every system (in thi case of my factor, the nervous system).

Are there practical applications of NGF?

In the last decades, NGF has found unhoped-for possibilities of clinícal application to the most varied pathological conditions of a degenerative nature and to those belonging to homeodynarnic systems (nervous, immune and endocríne). A recent discovery has been that NGF may play an ímportant role in Alzheimer's disease, in disorders of viral origin such as Immune Deficiency Disorder (the HIV virus), and in afflictions of an autoimmune nature (multiple sclerosis). One clínical application with great potential for development -- which has recently been discovered by some young researchers working in my own group and in a team at the Venice Eye Clinic -- is the use of NGF as a collyrium with which to treat lesions of the cornea (of either traumatic, viral or toxic origin) that ìnevitably cause the cornea's destruction and, therefore, blindness. If administered topically, NGF induces the permanent regeneration of the damaged cornea and its total functional repair.

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