Mixed feelings about the Nobel Prize, 2011

Although I was expecting this news following rumours at the 2011 Toll meeting, I didn't expect it so early. In a way, I am glad the jury selected our story on "Toll in Drosophila immunity" (Lemaitre et al. Cell 1996) since the discovery path identifying TLRs (Toll-like receptors) as major regulators of immunity was complex and also involved other key findings. I am also happy for French science since such rewards are rare in my home country. Nevertheless, I also feel bitter about this award for the reasons I explain below. In short, I feel disappointed because at the time that I performed the critical work on Toll, Jules Hoffmann was not very supportive of the genetics approach I had undertaken. Subsequently, he has never been able to fully recognize my contribution, yet somehow it is he who is now collecting the honours for my work.

The main reason for this short text is to share this story with the "happy few" that might be interested since it tells us something about science as a deeply human and complex adventure.

A brief scientific autobiography

I did my PhD on the regulation of the P transposable element in Drosophila (supervisor: Dario Coen, Laboratory of Dominique Anxolabéhère, University of Paris, 1992). Although my PhD work did not yield exceptional results, it was a fruitful experience. I obtained a solid background in genetics just as these P elements were becoming central to Drosophila genetic studies, serving as the main "tool box" for manipulating the fruit fly's genome. Following my supervisor's advice, I learned how to use the fly community as a resource for my research, interacting with many other scientists. I was also among the first researchers to use the 'enhancer trap' technique (*P-lacZ* had just been discovered by O'Kane and Gehring, PNAS 1987), in my case to monitor the activity of the P promoter.

Enhancer traps revealed something unexpected at that time, namely the very complex expression pattern of Drosophila genes, including those developmental genes then thought only to be involved in the fly's development. At that time, scientists started to realize that in fact, they were not just "development" genes, but rather genes that could be re-used multiple times for other functions throughout the Drosophila's life cycle. Perhaps it was this research work that prepared my outlook for my future work on the immune function of the Toll pathway, a signaling cascade initially identified for its function in early embryonic development.

P elements are fascinating objects to study; they are relatively small DNA sequences of just 2.9kilobases but manage to code, in miniature, for all the complex regulatory mechanisms of the Drosophila genome. By the way, the question addressed by my PhD - to understand how this transposon is regulated (a state named "P cytotype") - has only recently been partially solved and found to involve complex regulation by RNA silencing and heterochromatin.

It was in 1991, at the French Drosophila meeting (Vichy), that I first heard about Drosophila immunity in talks by Jules Hoffmann and his colleagues. This topic interested me for several reasons. First, being a collector of insects and stones as a kid, I had come to sciences by the "natural history" route. Working on the mechanism used by insects to combat microbial infection was in some way a continuation of my childhood interest, being not too molecular for me.

Also, at that time, the "hot" topic in Drosophila research was embryonic development. Developmental genetics attracted the most talented and ambitious students and most talks were composed of photos of embryo cuticles or genes expressed in patterns. However I like doing things differently, so I was rather happy to avoid this crowded mainstream, preferring to work on more "functional" aspects of the Drosophila's life cycle.

Finally, my university lectures had given me the impression that "immunology" was "a mysterious discipline not without a certain esthetic". I thought maybe genetics, with its rigorous approach, could bring some new light to this discipline.

In November 1992, I moved to the research unit in Strasbourg, managed by Jules Hoffmann. During my first year there, I brought my own financial support with me, then Jules succeeded in obtaining a CNRS position for me. As an established French lab, it had many such permanent research positions. I worked in Jean-Marc Reichhart's sub-group with Jules as the overall research unit director.

The main question in the field was to understand the regulation of antimicrobial peptide genes that were strongly induced upon infection. It had just been established by several laboratories that the inducibility of these antimicrobial peptide genes was dependent on the presence of κB, a putative DNA binding site located in the promoter regions of these genes. This kB site is a target for NF-kB-like transcription factors, hence the search was now for the right NF-kB-like protein, and a good candidate appeared to be Dorsal.

Under the control of the Toll pathway, the Dorsal protein was known to play a role in the formation of the dorso-ventral axis in the developing fly embryo. When I arrived in Strasbourg, Jean Marc Reichhart had evidence that Dorsal was also expressed in the adult fly and that it could even be induced by infection.

My first year's research was devoted to a collective teamwork, led by Jean Marc Reichhart, to study Dorsal's function in immunity. At this time, I also ordered on my own initiative all the fly lines I could find that carried mutations affecting any of the genes involved in the pathways regulating dorso-ventral formation, including the famous Toll mutation.

However, our work on Dorsal turned out to be rather disappointing since its role in immunity was, in fact, minor (Reichhart et al., CRAS 1993). After this frustrating experience, from around mid-1993, Jules Hoffmann and Jean Marc Reichhart chose to direct the lab's most dynamic elements towards a biochemical identification of 'the' protein that must be binding to the kB sites. This tedious biochemical characterization lasted more than 5 years and it never succeeded - the transactivator, Relish, was eventually discovered much later in the lab of Dan Hultmark.

Meanwhile, I decided to persist with my genetic approach, analyzing the function of Toll pathway mutants in greater depth.

During this time (1993-95), neither Jules nor Jean-Marc Reichhart were very supportive of my genetics' work: none of the five students starting their PhDs in 1993 began projects involving any genetics **(See Supplementary Text S1 and Fig. S1).** Moreover, Jules was very far from the actual 'bench work'. I think it fair to say that he has always felt better among vertebrate immunologists than Drosophila geneticists.

Luckily, however, this situation gave me lots of freedom in my research work, as well as the possibility to attend fly meetings on my own and to establish my own networks in the Drosophila community. I was free to analyze the immune phenotypes of any fly line I could get my hands on that might have a possible link with immunity.

In early 1994, by chance, I found the *imd* mutation in the *Black cells* fly stock from the Bloomington Fly stocks Center (Indiana, US). A year later, after an intensive quest, I discovered Toll's function. Having both of these mutations in my hands was a unique chance since the phenotype of both mutations provided a framework for understanding the regulation of antimicrobial peptide genes. I will not go into the details of this part of the story here since it is already related elsewhere (**see Document 2**). The research work I did then was not spectacular in itself, it was just a good piece of genetics, in which open access to shared community resources played a significant role, together with some good luck and hard work.

In some ways, the main obstacles to our findings were all the preconceived ideas cluttering up the immunity field, such as the strange ways we used to infect flies, the fact that innate immunity was considered to be "non–specific", or the notion that immune pathways must either be essential for host survival or else redundant.

I performed this work with intensity, putting my family under pressure. But my work also benefitted from the environment of the whole research unit in Strasbourg – the antifungal peptide *Drosomycin* that appeared to be regulated by the Toll pathway had just been discovered by Pascal Fehlbaum in the team of Philippe Bulet and published. And later (1995), I received better support from technicians. However, my work on Toll was never a high priority for the laboratory's director. Although many of the lab's twelve technicians were involved in the attempt to biochemically purify the κb binding protein, I did not receive any assistance until mid-1994 when a lady initially involved in the laboratory cleaning became my part-time technician. She took care of the routine maintenance of my large fly stock collection with a strong motivation- a support that was critical for my research.

Jules never provided any ideas for my project, being very far from the realities of experimental bench work. This is why, for example, I still have all of my laboratory notebooks in my office with me – neither of my lab chiefs ever looked carefully at my data. In fact, Jules' time was mainly devoted to lab organization and communication, although he did help me a lot in writing the papers (at that time, my English was poor), and he did show an interest in my work. Nevertheless, I still wrote the first version of each of my scientific papers, for which I got feedback from my close colleague, Marie Meister. It would only then that I would start working on the final text with Jules.

Being naïve and young, I did not pay too much attention to the co-authorship issue **(See Supplementary Text S2 for author contributions to the 96 Cell paper)**. It did not seem important to me since I was obviously the first author. My second supervisor, Jean-Marc Reichhart, co-signed all of my papers despite contributing little to them - his interest at that time was firmly with the biochemical identification of κb binding proteins.

We successfully published our data in the journal Cell, showing the key role of the Toll pathway in the fly's defense against fungi, while showing that the role of the Imd pathway was essential in the production of antimicrobial peptides directed against bacterial infections. What I still appreciate about the 96 Cell paper is that it is a rather descriptive

paper. It analyzes the expression of many immune genes in many genetic backgrounds. Today, editors of top journals prefer a "twisted story" that turns around a single concept. Although such 'hot' papers are exciting on first reading, they often provide a biased view of reality, pushing their interpretation too hard. Descriptive papers have the advantage of providing a solid foundation to the field and they are less error-prone.

Globally, I had quite a happy time in Strasbourg. I maintained good relations with all of my colleagues, including Jean-Marc Reichhart and Jules, although I was never very close to them. In some respects, I was lucky to be able to work with so much independence in a big laboratory.

It is clear that during the years 1992-1995, Jules Hoffmann never really anticipated the power of genetics despite many signs that it was the most promising approach. His laboratory only finally made a real shift in this direction later on, a move probably influenced by Fotis Kafatos (who had just arrived at EMBL, Heidelberg) and the recruitment of Dominique Ferrandon. Administering far from the lab bench, Jules Hoffmann was directly influenced on scientific matters by Jean-Marc Reichhart, who himself, only became more committed to genetics when he started, not without courage, to develop a genetic approach on the *necrotic* mutant, a line given to him by Mike Ashburner and David Gubb (Cambridge).

In April 1998, I left on good terms Strasbourg to start my own small, but super-dynamic lab at Gif-sur-Yvette (close to Paris). I decided to direct my studies towards the fly's immune reaction in response to natural infection, together with a better characterisation of the Imd pathway which was still poorly defined. At the time, I did not immediately register what had not been predicted in our story: namely, the fact that mammalian TLRs (Toll-like receptors) fulfilled the concept of pattern-recognition receptors. Discoveries follow non-linear pathways! (See document 4 that repositions the 96 Cell paper in the context of the subsequent TLR discovery).

The tale(nt) of Hoffmann

What happened after I left the Strasbourg lab is, I feel, far more problematic. When "TLR" started to become a very "hot" topic, it became important to associate "heroes" to this complex discovery. Taking account of how this story unfolded, I feel disappointed with how Jules Hoffmann (unintentionally, or consciously) has devoted his communication skills to turning the discovery of "Toll" into a team work. He has never fully acknowledged my individual contributions, portraying the story as a joint effort **(See Supplementary Text S3)**. This is a statement that I consider to be entirely wrong. A deeper analysis of Jules Hoffmann's seminars and published texts reveals an unusual type of scientific discourse that offers a distant perspective of the work. It is often described as an "epopee" (an 'epic') involving many protagonists, but the work of key individuals (especially Philippe Bulet and sometimes myself) is not always mentioned. Acknowledgements and any attribution of credit goes in priority to lab members known to be fully devoted to the 'chief' and not to those researchers who actually did the work.

His talks usually done proceed with an extreme prudence, indicative of a significant distance from the experimental data. In a way, this is reminiscent of the story about the army general who always remains two men away from the scene of battle. This type of speech has probably a lot to do with the old French hierarchical system in science, but it

may also be observed with principal investigators (PIs) who have lost contact with the reality of lab work due to excessive travel or over-commitment to administration.

The network Jules subsequently built was very strong, especially among American vertebrate immunologists. Of note, I was not invited to either of the first two Toll meetings, nor to any meeting related to innate immunity organized in France or in America in the early 2000s (often co-organized by Jules Hoffmann). It was only in 2006 (a full ten years after the 1996 Cell paper!) that I was finally invited to the Toll meeting, probably due to the insistence of Neal Silverman (a drosophilist) **(See Supplementary Text S4)**.

In 2006, the Annual Review of Immunology contacted me to write a synthetic review on "Drosophila immunity", a dream for me since I love analyzing the literature. What a disappointment it was when I realized I was supposed to write this review with Jules Hoffmann as a joint invitation. It seemed it still wasn't possible for me to exist by myself, at least not at this level! Yet, throughout this period, my own lab team had produced a number of success stories, perhaps not always published in the 'star' journals (maybe too descriptive), but nonetheless with significant impact. I also received support for my work from the Drosophila and "host-pathogen" communities, as well as several immunologists, and in time I successfully established my own research network.

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After his ultra-classical talk on the Toll and Imd pathways at the 2011 Toll meeting, Jules again, in his very subtle manner, described the Toll story as a team-work. I sat there, nauseated.

In 2010, thanks to an EPFL colleague, I had just received a significant prize from the Bettencourt-Schueller Foundation. Jules now told me to thank him for his support in getting this prize (I had asked him for a recommendation letter). At that moment, I realized that I had never received any acknowledgement from him for his multiple prizes.

I became aware that I no longer felt well in his presence. No doubt an error I had made was never to have told him why I felt so exasperated. This interaction was toxic to me since it has reminded me of my intensive work on Toll and Imd, initially performed despite his indifference. Yet finally, nearly all the credit from my work has gone to him! At this meeting, I started to think that I should do something to clarify my position and discussed this idea with a few people.

Concluding remarks: turning the page

A characteristic of the scientific life is this obsessive quest to answer a precise question, carefully taking account of all its details. This experience can have a cost since it moves us away from everyday life, it seizes most of our attention and energy, sometimes to the exasperation of our family circle. Nevertheless, it is this experience, often transient in a human life, that gives a certain value and originality to scientific research: we are discovering something at the limit of human knowledge. Today, my head is still filled with code names used for labelling fly stocks of the Toll pathway, even though this is now useless information.

I still remember moments of enlightenment, kept to myself or shared with close colleagues, when we started to open the black box of Drosophila immune signaling pathways. This past intensive effort still helps me in running my laboratory, taking care of

details, trying to leave a space open to hazard, and also trying to give credit to individual initiatives which is not easy since we are all biased by our personality.

From this point of view, it is difficult for me to recognize Jules as a real scientist since he has never been close to the lab work. He did play a role in communication and networking, managing the laboratory, but he has never been directly involved in any key finding at the time when I was in the lab **(See Supplementary Text S4 and S2.xlsx)**.

In an ideal world, the problem I raised (that may also have affected the mammalian side of the TLR story), would not have existed if due credit to some of the key players had been better respected, and if there had been an honest recognition of the complexity of the discovery paths that brought these receptors into the spotlight. This may not necessarily have changed the choice of the Nobel prize laureates. Nevertheless, this lack of respect, all too apt to dismiss contributions by others (possibly unintentionally) has paid off. For those of you who prefer to keep a more naïve view of science, the 'take home message' of 2011's Nobel prize for TLRs could provide a mixed feeling.

This story tells us something about science and the power of communication. It is not a unique case and is probably not the most severe (see the interesting note on this topic from Lawrence Nature 2003 **415**, 835-836). It is no longer so much the science itself that matters, since science is by nature complex and hyper specialized, but rather the "buzz" around it. The Toll-TLR story is a good example in which some of the most successful scientists have been those with the capacity to simplify the story while displaying fewer scruples than the others. I realize that some personalities have more skills in communication, but good science also needs other, more interiorized natures. Perhaps it is a sign of our times that some personalities are benefitting from this emphasis on oversimplified communication. More globally, and outside the context of this story, the lack of control mechanisms in many aspects of science, provides an enormous advantage to less scrupulous people, but it also risks pushing many original minds away from the scientific field.

However, it is now time to turn the page and look at new topics that have arisen. After all, what has been amazing in the last 16 years is how immunology has changed. We started with a very narrow view of animal host defense. Immunity in Drosophila was mostly restricted to the response observed upon injection of a human bacterium, Escherichia coli. At university, I was taught that vertebrate immunologists had fun analyzing the response of mice to the injection of an antigen, ovalbumin, and they speculated a great deal.

When we first realized how important and complex innate immunity is, several of immunology's key words, like "adaptive" and "specific", were rendered blurred. Using the power of genetics on whole animals, rather than transfection in cultured cells, we realized that most components of the immune system have specific and not interchangeable roles. It was possible to build on this solid data to establish complex networks of proteins. Rather naively, we re-discovered the diversity of the "microbial world" and how different responses could depend on the mode of infection. The contaminated "LPS" solution purchased from Sigma was no longer the magic solution it had once been. There was an urgent need to go back to our old biochemistry textbooks to learn the complexity of microbial cell walls that had been masked behind the generic terms, "LPS" and "peptidoglycan". Integrating notions from cellular microbiology, it became clear that this immune response to a pathogen, with its virulence factor, is different to that of a non-pathogen, and that the immune response should be analyzed upon natural routes of

infection. This has also brought to light the notion of damage, and the host's complex repair response that often insults the host. The diversity of the immune reactions that occur in various organs and epithelia, such as gut and lungs, has been shown to depend on the route of infection. Opening our minds broadly, we realize today that "pathogens" are just one corner of the story and that dealing with our "beneficial commensal" microorganisms may well be as important for shaping the immune system. Finally, the evolutionary dimension has to be taken into consideration, revealing similarities in immune pathways between distant phyla, but also divergent adaptations between close species, like mouse and human, as revealed by the development of human genetics. We also realize today that some of our diseases might simply rely on this gap between the selective pressure that shaped our immune system in the past and our present lifestyle. It is these fabulous trips integrating many disciplines that make immunology such a fascinating topic. It is probable that the best discoveries still lay ahead of us.