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In memoriam

A tribute to Cécile Wandersman

Cécile Wandersman, Professor at the Institut Pasteur and a recognized expert in the field of bacterial secretion, heme transport and metabolism, passed away on December 21, 2014 at age 67. This tribute attempts to retrace her career by providing different perspectives on her contributions as a scientist, colleague and mentor.

The beginnings of a bacterial geneticist

By Maxime Schwartz

After having performed medical studies at Paris-6 University (MD in 1973), Cécile decided that she would not be a practicing doctor. Fascinated by the rapid progress of what was then called molecular biology, she joined the laboratory of Michael Yarmolinsky at Paris University and worked on bacteriophage P1. There, between 1972 and 1976, she obtained interesting results on the immunity system of the phage [53]. She might have continued this work but Yarmolisnky decided that he would return to the United States. Having been a member on the committee for Cécile's "Thèse de 3ème cycle", and impressed by her qualities, I gladly agreed when she proposed to join the Molecular Genetics Unit that I was heading at Institut Pasteur.

Our laboratory was then involved in the study of the transport of maltose and maltodextrins in *Escherichia coli*. We had found that an outer membrane protein, called LamB, which we had shown earlier to serve as a receptor for phage lambda, was a constituent of the transport system for these sugars. We also knew that a periplasmic protein, the maltose binding protein, was an essential component of this transport system. We wondered whether there was an interaction between these two proteins. Using a sophisticated technique of bacterial genetics, Cécile obtained evidence that this was indeed the case … and this was confirmed biochemically in collaboration with the laboratory of Hiroshi Nikaido, who worked at Berkeley [3,52]. This was the first demonstration of an interaction between proteins belonging to different layers of the bacterial envelope.

Another "first" came as a result of serendipity. Ulf Henning, from the Max Planck Institute in Tübingen, had isolated a bacteriophage, TuIa, using as a receptor an outer membrane protein then called Ia (later called OmpF). For some reason we asked for this phage and Cécile checked it, streaking TuIa phage suspensions across different bacterial suspensions. Why she did this, I do not remember, but the strains she used lacked either protein Ia, or protein LamB, or both. The one lacking only LamB was fully lysed, as expected since it had protein Ia, the TuIa phage receptor. However, unexpectedly the strain lacking protein Ia was not fully resistant, since rare plaques appeared on it. And no plaques appeared on the strain lacking both Ia and LamB. I still remember Cécile bringing me that plate! The conclusion was inescapable: the rare plaques growing on the strain lacking only Ia were phage mutants using LamB as a receptor instead of Ia, and these failed to grow when both proteins were missing. This phage mutant, that we called TPI, had retained the ability to use Ia as a receptor [51]. It was the first example of a phage able to choose between two proteins as its receptor! Several other examples were later found both for bacteriophages and animal viruses, including HIV, and this led to a different vision of the specificity in virus/receptor interactions.

A third "first" was a follow up of the study of TPI. Looking for single mutations rendering bacteria resistant to this phage, Cécile found mutations defining a gene that she called *tpo*. This gene mapped in or very close to a gene called *ompB*, already known to control the synthesis of proteins Ia and Ib (OmpF and OmpC). Unlike classical *ompB* mutations, *tpo* mutations also controlled the transcriptional regulation of *lamB*. With Felipe Moreno, then a postdoctoral fellow in our laboratory, Cécile showed that *ompB* and *tpo* were likely to constitute two adjacent genes [50]. These two genes were later

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renamed *ompR* and *envZ* respectively and shown to constitute the first two-component system ever described in transcriptional regulation. EnvZ is a membrane protein, which senses the extracellular osmolarity, and, by phosphorylating the cytoplasmic transcriptional regulator OmpR protein, modulates the expression of genes encoding outer membrane proteins. Two component systems were later shown to be widespread among bacteria. More generally, these bacterial two component systems now appear as simple models for the complex mechanisms whereby gene expression in animal cells responds to outside stimuli.

In 1982, once Cécile had completed her thesis I suggested that she join the laboratory of Harvey Lodish, to learn some biochemistry. Rather reluctantly, because all she liked was genetics, she obeyed … and it was not a great success. When she came back, one year later, we had started to study the secretion of proteins in Gram-negative bacteria, of which almost nothing was known at the time. I suggested that Cécile start working on her own system, and she chose the secretion of proteases in *Erwinia chrysanthemi*. Once she had started this project, she was soon rejoined by Philippe Delepelaire and Sylvie Létoffé and, later by Jean-Marc Ghigo and Rachel Binet and she became fully independent.

After that time, there is one event that I remember clearly, which turned out to be critical for the rest of her career. It was in the early nineties. I was the director of the Institut Pasteur and would only come to the laboratory once a week to participate in the lab meeting. At one of these, Cécile reported that they had cloned genes from *E. chrysanthemi* that allowed the *Serratia marcescens* proteases to be secreted in *E. coli* [48]. The question arose whether these genes might not allow the secretion of other proteins, in addition to the proteases. Cécile then found that a gene, mapping adjacent to the secretion genes, encoded a protein using this secretion system and was involved in the acquisition of heme from hemoglobin [32,33]. My contribution to this work was to give a name to this protein: HasA, for Heme Acquisition System! The study of this system was to become a major endeavor for the rest of Cécile's career.

More than anything, Cécile was a bacterial geneticist at heart. There was nothing she liked more than looking for mutants on Petri dishes! She thought you could solve any question in this way! In that, she resembled our good friend Jon Beckwith, from Harvard Medical School. Cécile was a character, well known in the whole institute. She was enthusiastic about her research, and immensely proud and happy when it led to a good paper. She was critical of her own work, but also, and perhaps more, of others. Her questions to speakers during seminars, although often justified, were not always well received! In other words she was not too diplomatic! She liked teaching, and did it both in France and abroad (Brazil). She was a very good scientist, and recognized as such in the international community.

The right model at the right time: deciphering type 1 secretion and its consequences

By Philippe Delepelaire

1. Protein secretion by gram-negative bacteria

The choice of the *E. chrysanthemi* model to study protease secretion was a happy accident that proved to be key to the study of the Type I Secretion System (T1SS). Analysis of the *Erwinia* protease secretion system reconstituted in *E. coli* revealed, in addition to the four metalloprotease genes and their specific thermostable inhibitor gene, three essential secretion functions [16,17,24,25,48]. In 1990, we showed that two of the protease secretion functions were analogous to those of α-hemolysin, and that *E. coli* TolC complemented the third *E. chrysanthemi* protease secretion gene and was also required for α-hemolysin secretion [47]. This was completely unexpected, and changing host and reconstituting the pathway in *E. coli* was therefore key to change the then current belief that only two functions were required for hemolysin secretion. The many phenotypes associated with TolC allowed the design of clever mutant screens, which shed light on T1SS functions [46,49].

These studies established the existence of different families in the T1SS field, and also showed the common existence of the so-called RTX motifs in those proteins. The minimal secretion signal was determined and its exposition in C-terminal position established [18]. At the same time a long-standing collaboration started with the lab of Muriel Delepierre at the Institut Pasteur in order to use structural biology to gain deeper insight in the underlying molecular processes involved in T1SS-dependent protease secretion (see below). Proteases secreted by other bacteria were also studied, including *Pseudomonas aeruginosa* and *S. marcescens* [19,26]. The incursion into *S. marcescens* (which resulted from a friendship between Cécile and Volkmar Braun in Tübingen) was especially rewarding. Cloning of the *S. marcescens* protease secretion function led to the discovery of a small secreted protein of unknown function and therefore called PsmX. Quickly identified as the first secreted heme-binding protein (a hemophore), PsmX was renamed HasA (for Heme-Acquisition System) [31–33]. The availability of various T1SS substrates enabled us to play with several T1SS reconstituted in *E. coli* to study the specificities of those systems. This led to the discovery that specificity of recognition of the substrate was given by its primary interaction with the inner membrane ATP-binding cassette (ABC) component of the T1SS [1,5,6]. It was also shown that HasA, besides its «true» hemophore functions, had quite exceptional properties, making it a great tool to study Type 1 protein secretion. Indeed, intracellular HasA would fold *in vivo* into the cytoplasm and inhibit the protease secretion by their dedicated transporters. Together with the heme-binding capacity of HasA, this would later allow the isolation of secretion complexes, which helped to define secretion steps and to show that the secretion complex was assembled on demand in an ordered fashion [27]. This work was a «first », which was later confirmed with α-hemolysin.

At the time the question of whether or not proteins were secreted folded or unfolded was a very hot topic, particularly for T1SS, as its secretion signal is at the C-terminus. HasA was the first substrate of T1SS shown to require a chaperone for its

secretion, so much that folded HasA was shown to inhibit its own secretion [9,12,13]. These results stood the test of time and the current picture is that so-called "anchoring sites" [40] help HasA secretion, together with the help of the SecB chaperone.

2. Heme acquisition

The identification of HasA as the first secreted heme-binding protein (hemophore) originated from two observations: *S. marcescens* can use heme as an iron source, and HasA expression and secretion are induced upon iron starvation. The final proof came with the demonstration that HasA was an heme-binding protein and that the inactivation of HasA made *S. marcescens* unable to grow at low heme concentrations, used as sole iron source [32]. The concept of hemophore was born, corresponding to a secreted protein avidly binding heme and transferring it to its membrane-bound transporter. The Has heme acquisition system was also reconstituted in *E. coli*, which led to the identification of HasR, the HasA dedicated and TonB-dependent receptor. The collaboration with structural biologists initiated on the C-terminal secretion signals was extended to characterize HasA, which was purified and its structure solved in both a heme-bound and heme-free state, by X-ray crystallography (with the lab of Richard Haser) [2] and NMR (Muriel Delepierre) [55] respectively. This enabled the identification of heme iron axial ligands and of their importance in heme-binding [14] and in conformational changes that occur upon heme-binding. Besides *Serratia*, the Has heme acquisition system was also identified in *Pseudomonas* and *Yersinia* [36,37,42]. Other labs also later found other types of hemophores with no structural similarity to HasA and this is now an active area of research, since heme is a major iron source for many commensal and pathogenic bacteria. The characterization of HasA prompted us to study its HasR receptor at structural and functional levels [15,35]. Initial *in vivo* studies showed that the isolated HasR barrel displayed heme-specific pore activities as well as HasA recognition properties, and that at least two HasA regions were involved in binding to HasR [23,30,38]. These *in vivo* studies paved the way for later *in vitro* studies on HasR−HasA interactions. The heme iron ligands in HasR were identified and it was shown that heme transfer from the HasA binding site to the HasR binding site occurred upon the formation of the HasA−HasR complex. Finally a collaboration established with the lab of Wolfram Welte in Konstanz led to the determination of the crystal structure of the HasA-HasR-heme complex, validating all previous data and leading to propose a detailed molecular mechanism for heme transfer from HasA to HasR [21,22].

The HasA−HasR system also gave birth to other interesting developments in several directions: first at the transcriptional level, the *has* locus comprises two genes respectively homologous to sigma and anti-sigma factors (HasI and HasS) of the ECF family (HasR has a N-terminal periplasmic signaling domain), subjecting the *has* locus to both a general F-dependent regulation and a specific and HasI dependent regulation. In contrast with the *fec* system studied in Volkmar Braun's lab, which is the archetype of those systems (where the inducer and the transported molecule are the same), in the Has system, the signal consists of heme in its binding site on the receptor, and of HasA bound to the HasR receptor [7,10,43]. Only 5 amino-acid residues from HasA are required together with heme to trigger induction. There is also no autoregulation of the sigma factor in the Has system, but the anti-sigma factor HasS is also regulated, therefore allowing quick adaptations to varying heme concentrations. Another interesting development concerned the different energy requirements for heme entry and HasA ejection, the latter being much greater than the former, which remains ill-understood at the molecular level [28]. It was also shown that a dedicated TonB analog, encoded in the *has* locus, HasB, displayed a different fold as compared to TonB [11] and could not cooperate with *E. coli* ExbBD and could therefore not complement TonB functions [41,4].

Although fully aware of what structural biology eventually brought to genetics, Cécile never forgot the purpose of those systems, i.e. allowing heme entry in the bacterium. This is why her last field of investigation addressed the question of heme fate once inside the bacterium. In *E. coli* K12, there is no specific heme permease, and two periplasmic binding proteins (DppA and MppA) were shown to be recruited by heme, together with the Dpp permease, to allow heme entry into the cytoplasm [29]. Finally, although there is no heme oxygenase in *E. coli* K12, a very astute screen showed that two proteins YfeX and EfeB were able to extract the heme iron atom from the protoporphyrin ring without breaking this ring open [34]. This is a completely new activity, never described before and whose mechanism is presently unknown. A similar protein, FepB was found in *Staphylococcus aureus* [44], also has *bona fide* heme oxygenases (IsdG and IsdI). Hence, Cécile's work came full circle and back to TolC, showing that the MacAB-TolC efflux pump was able to pump protoporphyrin IX out of *E. coli* cells [45].

When microbiology meets NMR

By Nadia Izadi-Pruneyre and Muriel Delepierre

Our story with Cécile started in 1992, when, while still in Maxime Schwartz's laboratory, Cécile was interested in studying type 1 secretion, a pathway used by proteins with a C-terminal secretion signal. At that time, no structural information about this C terminal secretion signal was available and it was not understood how this signal could be targeted to the secretion machinery. It was Philippe Delepelaire, who worked with Cécile, who proposed that we study the conformation of the secretion signal of PrtG, a protease secreted by *E. Chrysantemi* [54]. We then studied the C-terminal secretion signal of *S. marcescens* HasA, an entirely new type of heme-binding protein that had just been identified in Cécile's lab [32]. As structural biologists, we were of course very keen to solve the structure of HasA, although studying a protein of its size (19 kDa) by NMR was at the time quite challenging. In parallel to determining the structure of HasA, we also characterized the HasA heme-binding site [2,20,55].

Year after year, we progressively extended our collaboration with Cécile and studied other proteins of the Has system: HasR, the HasA membrane receptor, HasB the energy

transducer and very recently HasS the anti-sigma factor [8,11,21,39]. All in all, we have been working for more than twenty years in close collaboration with Cécile and people from her lab. While écile's lab produced all *in vivo* results, we determined the structures and characterized the molecular interactions between HasA, heme or other protein of the Has system. At first, we remember Cécile's mild disdain for structures, that she considered merely as beautiful color pictures used to decorate papers and talks. However, with time, due to constant exchanges of ideas and data, we ended up educating each other in microbiology, NMR and structural biology and we progressively succeeded to nurture Cécile true interest for structural and biophysical studies.

During our 20 year-long friendship, we have enjoyed stimulating and lively joint lab meetings, characterized by Cécile's sharp and quick wit. Thanks to our complementary approaches and more than 20 articles published together, the Has system is now considered among the most deeply characterized bacterial heme acquisition systems.

Passion under influence: discovering bacterial genetics with Cécile Wandersman

By Jean-Marc Ghigo

My encounter with Cécile and scientific research was a stroke of luck at a time of deep personal doubt. Trained in general biology to become a teacher, I was having second thoughts about my professional path, and, after one year of teaching in a high school, I felt that something, some spark, was missing. I then had the opportunity to take a leave of absence to follow a new multidisciplinary course that required a 6-month laboratory internship. I vividly remember snatching Cécile's offer to study *E. chrysanthemi* protease secretion off the information board and running to a pay phone to set up an appointment. This is how I joined Cécile's group in Maxime Schwartz's laboratory at the Institut Pasteur in 1990.

At the time, Cécile, Philippe Delepelaire and Sylvie Létoffé had already started to use *E. coli* as a heterologous host to study the secretion of *E. chrysanthemi* proteases PrtB and PrtC by a dedicated transport system, consisting of only 3 membrane proteins, including an ATP-Binding Cassette transporter. This ABC-dependent secretion system (now called Type 1 secretion system or T1SS) was used by proteins lacking the classical N-terminal signal sequence, such as *E. coli* α-hemolysin and Cécile had the foresight to choose *Erwinia* and *Serratia* metalloprotease secretion as model systems and use them to characterize their enigmatic C-terminal secretion signal and secretion mechanism.

The project Cécile first gave me was to study a gene close to *prtB* and *prtC,* and potentially encoding another *E. chrysanthemi* metalloprotease. I was her first student, and Cécile took my training to heart, while Philippe and Sylvie cocooned me so kindly that, after 6 months, I decided to further postpone going back to teaching and started a PhD thesis under Cécile's supervision - and I have never regretted it. During my time in Cécile's lab, we identified other T1SS proteins, which led to the genetic and structural analysis of their C-terminal signals with the NMR laboratory headed by Muriel Delepierre. We also identified a new secreted heme-binding protein (HasA), thus opening the way to a detailed characterization of what is now considered a widespread bacterial heme acquisition system.

Cécile always tried to keep doing experimental work throughout her whole career. For a long time, her bench and mine were side by side and I was constantly exposed to Cécile's truly eruptive enthusiasm and passion for science, her articulated (and non-negotiable) critics of the latest book or movie and her opinionated political positions. She was fun, joyful, witty and almost painfully imaginative, so much that I often felt exhausted after discussing ideas with her, desperately trying to keep up.

On the campus, Cécile was known to be very out-spoken. Her bluntness and very (very) expressive nods during seminars or meetings were famous, and, to some extent, interacting with her could be intimidating. She could indeed be very critical, and she had little tolerance for poor scientific grasp or ill-conceived experiments. Once, after weeks of failures and around the clock attempts to make a specific genetic construct and test some hypothesis, I finally got exciting results. I knew that I still had a few things to double check, but the essential information was there, and I was so happy that I could not wait to tell Cécile. It was Sunday morning, so I simply arranged the Petri dish and hand-written notes on her bench, hoping that she could share my joy when she would later come in the lab. However, all she did was to ask for more controls, with 3 exclamation points … She did, however, come to see me at some point with a big smile, but a compliment from Cécile was no small gift.

Nevertheless, many, on the campus and beyond, sought her advice and guidance. When puzzled by a result involving bacterial genetics, you could go knock at her door and she always seemed to have time on her hands to talk — or argue with you. I particularly admired and appreciated the way she had to mentor her students. She knew when to put her impatience aside and be kind and constructive when your project was at its lowest. She had this marvelous ability to put you back on track and to make sense of the most confusing results - or she just had the guts to advise you to cut your losses and move on.

Far from being directive, Cécile encouraged her collaborators to improve themselves and to explore their own ideas, loving nothing more than to engage them into discussing their data. You could then see the pleasure she took at juggling multiple scientific questions. Moreover, her remarkable scientific intuition often led her to propose decisive experiments, or helped new ideas emerge from these exchanges. She was so immensely happy and proud when she guessed right! On the other hand, what a glowing feeling to be able, during these sort of scientific battles, to convince her of the value of a new idea or of performing this or that experiment.

Working in Cécile's lab also meant being exposed to a very extended network of superb bacterial geneticists from all over the world. Cécile cherished her scientific family, seemingly united by a common love for genetic screens and the pride taken in addressing and solving difficult scientific questions - sometimes with no more than (preferably re-usable) wooden toothpicks.

Retrospectively, I feel extremely fortunate to have met Cécile. I could not have asked for a more passionate mentor, or a better scientific influence. I am immensely indebted to her for providing the spark I was longing for, and for showing me how exhilarating bacterial genetics could be (well, on some days).

Conflict of interest

There is no conflict of interest.

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